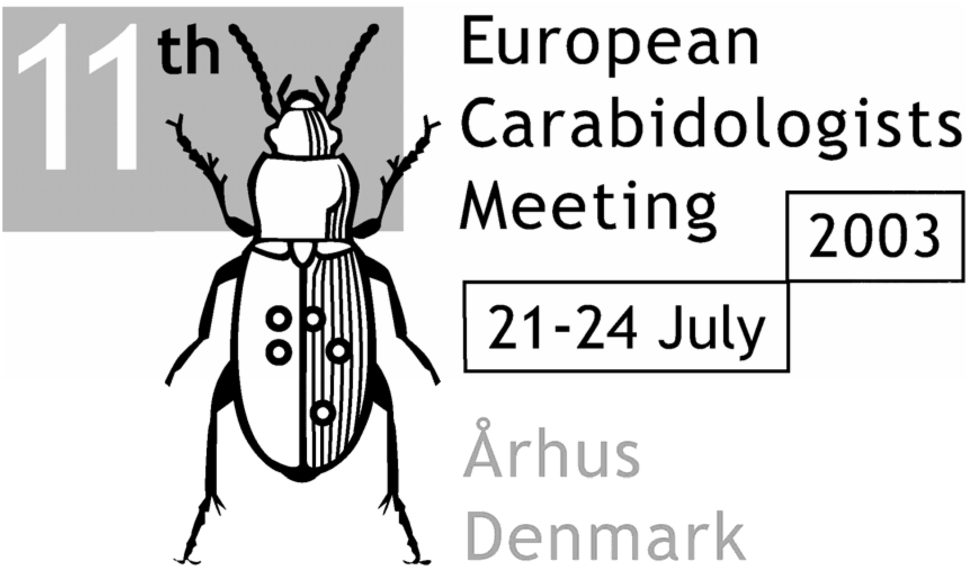
DIAS report

European Carabidology 2003

Procedings of the 11th European Carabidologists' Meeting

Gabor L. Lövei & Søren Toft (Editors)



Plant Production No 114 • January 2005

Ministry of Food, Agriculture and Fisheries
Danish Institute of Agricultural Sciences

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European Carabidology 2003

Procedings of the 11th European Carabidologists' Meeting, Århus July 2003

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The participants of the 11th European Carabidologists' Meeting:

First row, kneeling: T. Magura, R. Vermeulen, S. Venn, S. Vogt , B. Tóthmérész, M. Nielsen, Z. Elek, J-Y. Guo, A. Mazzei. *Standing:* M. Koivula, S. Toft, J. Kotze, T. Bonacci, F. Talarico, A. Taboada, K. Matveinen, J. Serrano, J. Sklodowski, M. Luff, S. Fawki, M. Telfer, F. Szentkirályi, M. Proksch, T. Basedow, Mrs. Weber, A. Hvam. *Standing, back row:* D. Mayntz, R. Pizzolotto, P. Saska, G. Szel, B. Hatteland, M. Bouget (partly obscured), H. Dhuyvetter, E. Gaublomme, R. Perez-Gomez, K. Desender, E. Arndt, W. Paill, T. Assmann, F. Weber, L. Møller. *Missing:* F. Kádár, N. Kamer, G. Lovei, D. Mossakowski, S. Navntoft, Z. Saghy, A. Spee.

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ISSN 1397-9884

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Preface

The 11th European Carabidologist Meeting was held between 21-25 July 2003, in Århus, Denmark. This followed just two years after the fine meeting held in Poland. At Tuczno, carabidologists decided that we should hold the European meetings every two years. We hoped that this would benefit students who otherwise may miss out completely on experiencing a mixing with fellow carabidologists. The significant participation of students in Århus is a heartwarming indication that these meetings would indeed attract more students in the future.

The logo of the meeting commemorates the work of early Danish carabidologists, who first used permanent elytral marks to study the ecology of ground beetles. The marking system is that of B. Schjøtz-Christensen, who studied ground beetles at the Mols Hills, where the conference enjoyed a fine field day and an informal conference dinner. The dots code the numbers 21 and 25, to mark the first and the last day of the conference, and 7, the month.

The current volume contains 33 of the lectures and posters presented at the meeting. Posters and talks were treated equally. We thank our colleagues who graciously spent time on reviewing submitted manuscripts, to authors for (mostly) timely responding to reviewers' and editorial remarks. We would also like to extend our appreciation for the volunteer students in Århus (Shams Fawki, Aino Hvam, Lene Møller Kragh, Maria Sloth Nielsen), to Ms. Marie P. Thyssen for the design of the conference logo, to P. Gajdos and his family for their help with the conference dinner, Mr. Viggo Mahler for leading the field excursion, Mr. Palle Pedersen for help with logistics and organisation, Ms. Sonja Graugaard and the Danish Institute of Agricultural Sciences, Flakkebjerg Research Centre for helping us to bring out these proceedings in a short time.

Gabor Lövei & Søren Toft
Proceedings Editors

Reproductive characteristics of *Carabus scheidleri* (Coleoptera: Carabidae) in Hungary

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Abstract

Seasonal activity, age structure and reproductive characteristics of *C. scheidleri* were studied by pitfall trapping and dissections in Hungary. The adults were collected from an abandoned field during 2000-2001.

Beetles were active between mid May and late September, with two peaks in the season. Both old and young imagines were present throughout the season. Ripe eggs were found in the ovaries during the whole sampling period. Peak egg numbers occurred twice in a season in synchrony with the female ground surface activity peak. The maximum number of mature eggs per gravid female was 22. The mean number of ripe eggs in the ovaries was 5.5 per females dissected. Ripe eggs were present in both young and old females.

Based on the preliminary results it seems that there are two reproductive periods of *C. scheidleri* in Hungary, and both imagines and larvae hibernate during winter.

Key words: Ground beetles, reproduction, ovaries, sex ratio, activity

Introduction

The ground beetle *Carabus scheidleri* (Panzer) is distributed across central Europe – Eastern Bavaria, Czech Republic, Austria, Southern Poland, Slovakia, Hungary, Northern Rumania and (uncertainly) Southwest-Ukraine (Hůrka, 1996, Turin *et al*., 2003). In this area it forms at least four subspecies which all have been reported from Hungary (Turin *et al.,* 2003). This species frequently lives in the forests, but also in fields, meadows and pastures (Hůrka, 1996). In Hungary, it is mainly reported from the beech forests from the hills (Turin *et al.,* 2003). It is the third most abundant species in the Pilis Biosphere Reserve near Budapest, Hungary (Kádár & Szél, 1999) and it is also common in lowlands where it prefers agricultural areas. Kromp (1990) found many specimens in Austrian potato fields.

Carabus scheidleri is a strictly protected species in Hungary. Being a polyphagous predator, this species can be an important natural enemy in agricultural areas, small gardens, and parks. As other *Carabus* species, *C. scheidleri* is very sensitive to changes in the environment, so it can be used as an indicator organism. However, we have little information about the population dynamics of this species. Thus, the aims of the present paper were the following: (1) to describe the pattern of seasonal activity, (2) the age structure, and (3) the sex ratio, and (4) to determine the reproductive characteristics of a population of *C. scheidleri* from Hungary.

Material and methods

The population dynamics of *Carabus scheidleri jucundus* CSIKI, 1906 was studied by means of pitfall trapping in the vicinity of Nagykovácsi, near Budapest, Hungary. The study area was an uncultivated field (1 ha), abandoned for more than ten years, bordered by an oak forest (*Querceto petreae-cerris*), an abandoned apple orchard in the hillside, shrubs-grassy areas near the forest hedge, and a mosaic of cultivated fields (lucerne, winter wheat, small vegetable gardens). The vegetation contained *Solidago* sp., *Arrhenatherum elatius* (L.), *Agropyron repens* (L.), *Melilotus officinalis* (L.), *Campanula glomerata* L., *Carlina vulgaris* L., *Picris hierarcioides* L., and several shrubs, mainly *Rosa* sp.

Samples were collected from the beginning of June to the end of August in 2000, and between mid-May and the beginning of September in 2001. Ten pitfall traps (plastic jars of 80 mm diameter, 300 ml volume, containing 4% formaldehyde as a killing agent and preservative, with a metal top above the traps) were installed in two rows. The rows were 10 m apart and the distance between the traps was 5 m. The traps were emptied weekly.

Collected beetles were sexed and aged. We distinguished three age-classes based on elytral hardness, condition and the number of bristles of the head and mandible wear (van Dijk 1972, 1979; Wallin 1989):

1. young beetles: intact bristles, sharp mandibles, soft or flexible elytra,
2. old beetles: broken and worn bristles and mandibles, hard and fragile elytra,
3. middle-aged beetles: transition between the two categories. Bristles and mandibles slightly worn, elytra hardened and dark but not yet fragile.

We examined the reproductive characteristics by dissection of the females, following the method of van Dijk (1972, 1979), Loreau (1985), Bousquet (1986), Wallin (1989) and Diefenbach et al. (1991). The developmental stage of the ovaries and the number of eggs were recorded. We separated females into three categories based on the physiological stage of the ovaries:

1. immature: undeveloped ovaries (pre-reproductive stage),
2. gravid: eggs of different stages of maturation present in the ovaries (reproductive stage),
3. old: beetles spent that i.e. past at least one reproductive phase (postreproductive stage).

The sex ratios between years were compared by the χ^2-statistic, and we used Student's *t*-test to compare the mean ripe egg numbers between years. We used the Statistica program package (StatSoft 2000) for statistical calculations.

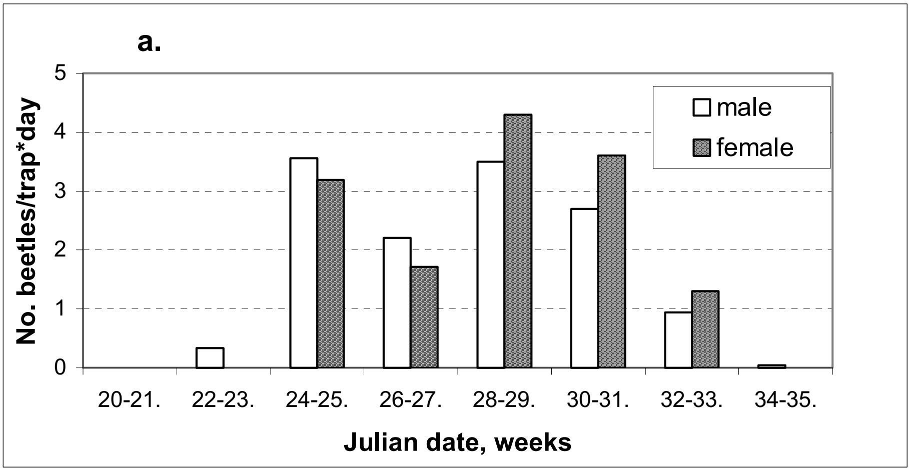
Results

During the period of experiments 578 individuals were trapped (Tab. 1). The observed sex ratio was not significantly different from expected equal representation of sexes in 2000 (females/males ratio=1.03; p=0.74), while significantly more females than males were caught in 2001 (females/males ratio=2.63; p<0.0001) (Tab. 1). The sex ratio differed significantly between the two study years (χ^2=11.35; d.f.=1; p=0.0008).

Table 1. Some characteristics of *Carabus scheidleri* population investigated at Nagykovácsi, central Hungary in 2000-2001.

Characteristics	2000		2001	
	females	males	females	males
Number of total catch	174	168	171	65
Number of individuals per age class (old/middle-aged/young)	26/45/103	41/21/106	10/21/140	-/-/65
Number of females with mature eggs	119	-	129	-
Total number of ripe eggs found	1029	-	804	-
Mean number of ripe eggs (mean±SD)	5.9±5.4	-	5.1±4.2	-

The activity period lasted from mid-May until the end of August, with seemingly two peaks in both 2000 and 2001 (Fig. 1). In 2000, the activity peaks occurred at the end of June and at the end of July. In 2001, there were also two activity peaks: the first one at the end of May and a second one in the end of July. The individuals of both sexes showed similar activity curves. There was a large inequality in the activity of the sexes in 2001 when three times more females than males were caught (Fig. 1b).



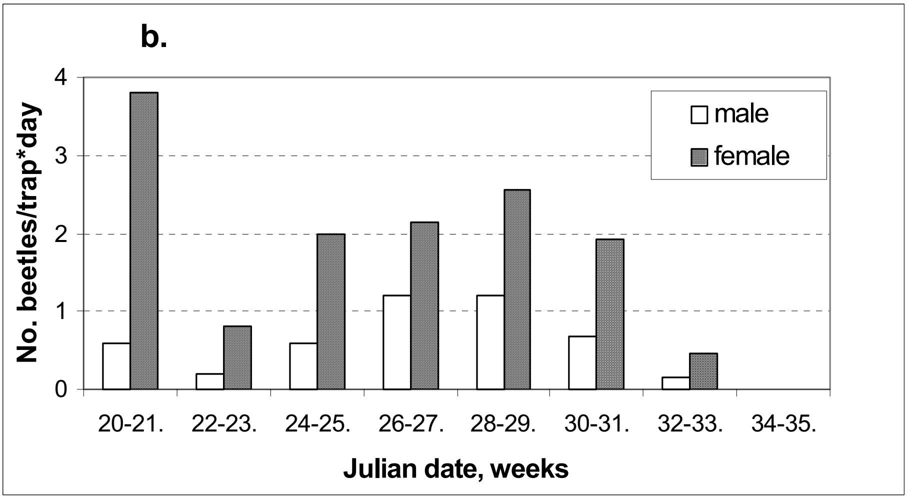


Figure 1. Seasonal ground surface activity of adult *C. scheidleri* collected by pitfall traps at Nagykovácsi, central Hungary in 2000 (a.) and 2001 (b.).

According to the data of the ageing based on the mandible wear the number of young beetles was similar between the two years, but more middle-aged and old individuals were caught in 2000 than in 2001 (Tab. 1).

The developmental stage of the ovaries showed that immature females were caught during the whole season in both years, with higher numbers at the onset of the season in 2001. Spent females were collected only in the end of the season in 2000, and both at the beginning and the end of the season in 2001. Gravid females were present during the whole season (Fig. 2.).

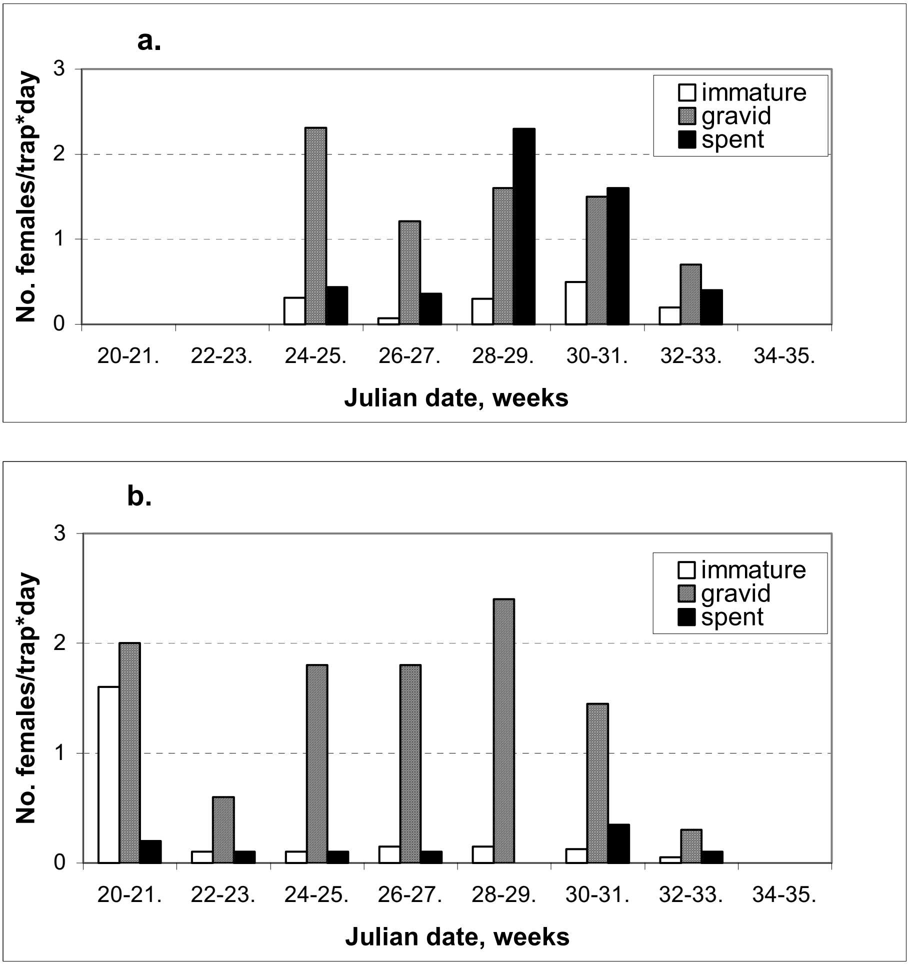


Figure 2. Seasonal activity pattern of the different aged *C. scheidleri* females collected by pitfall traps at Nagykovácsi, central Hungary in 2000 (a.) and 2001 (b.).

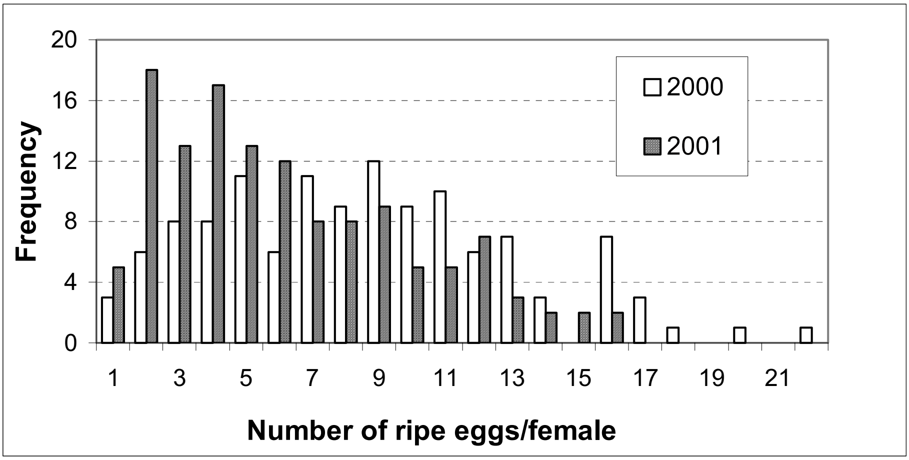


Figure 3. Frequency of number of ripe eggs per female collected by pitfall traps at Nagykovácsi, central Hungary in 2000-2001.

The mean number of ripe eggs was 5.9±5.4 per female (mean±*s.d.*, n=1029) in 2000, and 5.1±4.2 (n=804) eggs per female in 2001. There was no significant difference between the two years (Student's t-test, *t*=1.43, d.f.=314, p=0.15). The highest number of the ripe eggs in the ovaries was 22 eggs/female (Fig. 3); however, the most frequent number of eggs/female were 9 (in 2000), and 2 eggs (in 2001) (Fig. 3). Peak in numbers of eggs was found at the end of June in 2000, and in May and in July in 2001. Ripe eggs were found in both young and old beetles.

Discussion

Carabus scheidleri has wide preferences for habitats, as it lives in shaded (forests) as well as open habitats (fields, pastures) (Hurka, 1996; Turin *et al*., 2003). In Northern Hungary, it prefers the open areas, as the individuals were not found in the nearby forest, but they were numerous in the lucerne field which bordered the study area.

The seasonal activity over a period of 4-5 months is similar to the main activity period of its congener, *Carabus monilis* in Holland (Turin *et al*., 1977). The activity peak was not the same: *C. scheidleri* has two, while *C. monilis* has one activity peak.

We also found large differences in the activity between sexes. In 2001 the males had a lower activity. The reason for this unexpected, large difference is not known.

The size of the egg complement was not different between the two years, so we assume that food availability could not be the cause of this difference.

In this study we can describe the overwintering and ages of the adults using the method based on the mandible wear. Sparks *et al.* (1995) claim that mandible wear was not suitable for estimating the age. Our results, based on mandible wear, were well supported by the results of dissection, so this method can be more useful that claimed by Sparks *et al*. (1995). It seems that the majority of the males live for just one year, and the majority of females live for at least one year, and several of them longer. In general ground beetle females live longer than males (Hurka, 1973; Kreckwitz, 1980). This differential life span can explain the absence of middle-aged and old males in 2001.

According to the developmental stages of the ovaries and to the seasonal activity we suggest that this species overwinter as both larva and adult. Further, it seems that this population of *Carabus scheidleri* had two reproductive periods. During the first period, old beetles were reproductively active. These individuals reproduced at least once during the previous season and overwintered as adults. During the second period young individuals that overwintered as larvae and were fully developed in July reproduced for the first time. Several other *Carabus* species follow a similar reproductive dynamics (Rijnsdorp, 1980). Further studies are continously been done to clarify whether this species develops over several years, or the different generations overlap.

Acknowledgements

We are grateful to Á. Szentesi for the useful critical remarks and to G.L. Lövei for comments and improving the English. We are also grateful to an anonymous reviewer of an earlier version of this paper.

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GROUND beetles (Coleoptera: Carabidae) as CROWN beetles in a Central European flood plain forest

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Abstract

A mobile crane system was used to examine the invertebrate canopy fauna of a flood plain forest in Central Germany. The catch of Carabidae in the canopy was compared with samples of pitfall traps in the ground at the same site. Ground beetles are the dominating invertebrate predator group of the soil fauna in the examined flood plain forest. The guild is dominated by *Nebria brevicollis*, *Abax*, *Carabus*, and *Pterostichus* species. *Platynus assimilis*, with 183 trapped specimens, also comprised more than 8% of the total catch. But the catch of window traps and eclectors demonstrates that many carabid species are active in the forest canopy: 21 species were trapped on the ground, and 23 species in the tree crowns. Only two species were recorded in both strata (*Platynus assimilis* and *Loricera pilicornis*). *Dromius quadrimaculatus* was the most common carabid beetle in the canopy. A preference for certain tree species by carabid beetles could not be detected. Females generally prevailed in window traps (males:females = 1:1.8). The frequently expressed assumption that males migrate much more often than females could not be confirmed for most species. The only exception was *Dromius quadrimaculatus* with 4 times more flying males than females in the traps.

Key words: Carabidae, forest canopy, dispersal

Introduction

The large majority of animal species occurs in forests (Myers, 1990). Detailed research in tropical rain forests show tree crowns as areas of highest biodiversity (e.g. Basset, 2001; Basset *et al*., 2002; Erwin, 1988; Floren & Linsenmair, 2001; Gopal *et al*., 2000; Lowman & Nadkarni, 1995; Stork *et al*., 1997). Even the carabid beetles, generally known as active on the soil surface (= "ground beetles"), occur in the canopy of tropical rainforests in high diversity (Basset *et al.,* 2002; Erwin & Scott, 1980). It is unclear, however, whether there is a comparable high diversity of carabid beetles in the tree crowns of temperate forests.

We used the possibilities of a mobile crane system to examine the invertebrate canopy fauna of a flood plain forest in Central Germany. This contribution presents first results on "ground beetles" of this canopy project.

Methods and study site

The research area "Auwald Leipzig" is one of the rudiments of the formerly largest flood plain forest region in Central Europe along the streams of Elbe and Saale. Most of these forests fell victim to brown coal mining and urbanisation. The remaining forest areas are of greatest importance according to the EU Natura 2000 network (European Commission, 1995). The research area has a size of 5.6 km². *Quercus robur*, *Tilia* sp. and *Fraxinus excelsior* are the dominating tree species, with an average height of 30 m. The herb stratum is dominated by *Allium ursinum*.

A mobile tower crane of 40 m height was used to examine the canopy (Fig. 1). The crane could move along a 120 m long track and had a 45 m long derrick, covering 16.000 m². The fauna of the tree crowns was examined using 50 window traps (two in each of 25 trees) and 48 branch-eclectors (method after Behre 1989; four in each of 12 trees). Traps and eclectors were fixed at two levels (26 m and 22 m average), respectively. The traps were sampled at two weekly intervals from the end of March to September 2002. At the same time, 15 pitfall traps (3 rows, each with 5 traps) were used to examine the fauna at the ground. A stem-eclector (Funke, 1971) was installed on one tree of *Quercus robur*, *Tilia cordata,* and *Fraxinus excelsior*, respectively, to get an impression of the activity of climbing invertebrates.

In contrast to most of the formerly used methods of studying temperate canopies (e.g. cutting of trees, cutting of branches, knock down with pyrethrum), the use of a crane and the mentioned trap types are little destructive and allow continuous research of the tree crowns.

Results

Ground beetles were the dominating invertebrate predator group of the soil fauna in the examined flood plain forest. The guild was dominated by *Nebria brevicollis*, *Abax*, *Carabus*, and *Pterostichus* species. *Platynus assimilis*, with 183 specimens trapped, also comprised more than 8% of the total catch.

The window traps and eclectors demonstrated that many carabid beetles are active in the forest canopy: 21 species were trapped on the ground but 23 species in the tree crowns (Tab. 1). Only two species were recorded in both strata (*Platynus assimilis* and *Loricera pilicornis*, Fig. 2). *Dromius quadrimaculatus* was the most common carabid beetle in the canopy (window traps), only *Trechus quadristriatus* and *Platynus assimilis* were recorded in numbers as well. These three species were also trapped climbing in the tree crown with branch-eclectors. Larvae of Carabidae could not be detected with the window traps and eclectors used.

Table 1. Summarized numbers of specimens captured in window traps and branch eclectors (canopy) as well as in pitfall traps (ground). The species are listed according to their frequency.

Species	Canopy	Ground
Dromius quadrimaculatus	91	0
Trechus quadristriatus	19	0
Platynus assimilis	11	183
Amara aenea	5	0
Loricera pilicornis	2	1
Amara similata	2	0
Calodromius spilotus	2	0
Metophonus rufibarbis	2	0
Platynus dorsalis	2	0
Tachys bistriatus	2	0
Acupalpus dorsalis	1	0
Acupalpus flavicollis	1	0
Amara aulica	1	0
Amara communis	1	0
Badister bullatus	1	0
Bembidion lampros	1	0
Bradycellus verbasci	1	0
Calathus mollis	1	0
Demetrias monostigma	1	0
Dromius agilis	1	0
Harpalus tardus	1	0
Microlestes minutulus	1	0
Syntomus foveatus	1	0
Carabus nemoralis	0	453
Pterostichus oblongopunctatus	0	296
Abax parallelepipedus	0	292
Abax parallelus	0	246
Pterostichus melanarius	0	213
Nebria brevicollis	0	199
Carabus coriaceus	0	112
Pterostichus niger	0	111
Notiophilus biguttatus	0	34
Carabus granulatus	0	23
Badister lacertosus	0	19
Pterostichus strenuus	0	10
Asaphidion flavipes	0	3
Bembidion lampros	0	3
Stomis pumicatus	0	2
Cychrus caraboides	0	1
Leistus rufomarginatus	0	1
Platynus albipes	0	1
Pseudophonus rufipes	0	1

Figure 1. Study site with crane. Photography provided by the Botanical Institute, University of Leipzig.

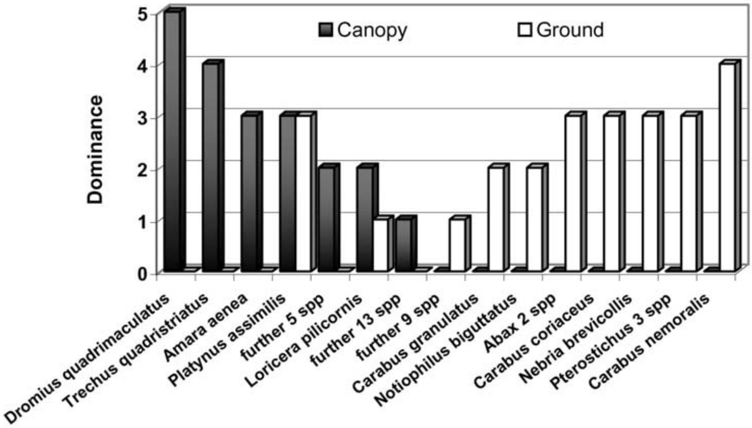

Figure 2. Ground beetles from the flood plain forest of Leipzig/Germany (March-September 2002). Canopy: window traps and branch-eclectors. Ground: pitfall traps. The species are listed in dominance classes (according to Engelmann 1978). 1 (subrecessive) <1.0%. 2 (recessive) 1.0-3.19%. 3 (subdominant) 3.2-9.99%. 4 (dominant) 10.0-31.99%. 5 (eudominant) ≥32.0%. The dominance classes are calculated separately for ground and canopy.

A preference for one or more tree species by carabid beetles could not be detected. As many adult specimens were climbing on *Quercus robur* with rough bark as on *Tilia* sp. or *Fraxinus excelsior* with a smoother bark. Only six individuals from six different species were recorded with stem-eclectors, indicating a weak soil-canopy-connection. Two of these species (*Pterostichus melanarius*, *Platynus assimilis*) also occured in pitfalls, five (incl. *P. assimils*) in window traps.

Other predator guilds in the examined tree crows were birds, spiders, ants, and neuropterans. The invertebrate predator species and particularly ants had a surprisingly low abundance.

Discussion

These first results suggest the occurrence of many carabid species in crowns of temperate forests. An assessment of carabid diversity "only from ground" seems to be inadequate in such forests.

The detected species number in the canopies exceeded that of the ground. The crown active guild was widely separate from the ground active guild. Part of species collected in the window traps are ruderal or riparian carabids and not typical of forests. Obviously they come from the rivers nearby, or even from urban areas within a mile from the site, and fly through the forest while dispersing to other suitable habitats. In the pitfall traps this kind of "edge effect" is only represented by *Pseudophonus rufipes* (1 specimen) and *Bembidion lampros* (3 specimens) (less than 0.2% of the total catch). In contrast, the proportion of open land species is 15.8% in the window traps (e.g. *Amara* 4 spp., *Bradycellus verbasci*, *Platynus dorsalis*, *Microlestes minutulus, Ophonus rufibarbis*). The trend is confirmed by the few individuals in the catches from stem-eclectors: three of these species are ruderal elements, probably climbing from the ground to the tree crown in order to fly away.

Females generally prevail in window traps (male:females = 1:1.8). The frequently expressed assumption that males migrate much more often than females cannot be confirmed for most species. The only exception is *Dromius quadrimaculatus* with 4 times more flying males than females in the traps.

Several small taxa (e.g. *Acupalpus* spp. and *Tachys bistriatus*) can be regarded as aerial plankton. It should be noted that *Tachys bistriatus*, an extremely rare species in Central Germany, was never before collected in the region of Leipzig at ground level, although a number of coleopterists were and are active there.

Acknowledgements

The project would not be possible without engaged assistance of several students. I thank particularly Carsten Schmidt, Claudia Jesche, and Pierre Angelo Cocco for sampling and sorting the canopy material, and Sebastian Winkler for his management of pitfall traps. We are indebted to W. Morawetz, P. Horchler, D. Bernhard, and M. Schlegel (University of Leipzig) for co-ordination of the crane project and financial support, as well as J. Adis (Plön) for helpful suggestions and discussions during preparation of the project. A. Floren (Munich) provided the branch-eclectors, M. Verhaagh (Karlsruhe) the window traps, and J. Adis the stem-eclectors. Martin Luff (Newcastle u.T.) and Soeren Toft (Aarhus) improved the MS which is gratefully acknowledged.

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Short-term effect of windthrow disturbance on ground beetle communities: gap and gap size effects

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Abstract

Windstorm is the main natural disturbance in temperate forests. Canopy perforation induces important ecological changes in terms of microclimate and ground microhabitats and creates patchy open areas in the forest mosaic. In managed oak-hornbeam forests storm-damaged in France in 1999, we sampled carabid beetles by pitfall and window-flight interception traps in 2001. I compared ground beetle assemblages in unlogged natural openings vs. closed forests. I studied short-term gap and gap size effects on carabid abundance, richness and assemblage composition (species and ecological groups based on habitat preference). Shortly after the disturbance, I observed a diversification of ground beetle assemblages in gaps at both air and ground levels in spite of a lower abundance in pitfall traps. The cumulative species richness for an equal sampling effort was greater in gaps (even in small ones) than in the closed forest. This richness increased with increasing gap area. Some forest species significantly declined in gaps, but none disappeared. Other forest species remained unaffected and several corticolous and arboricolous species were even favoured. Gap area did not significantly affect the forest group. Several open-land species appeared or increased in abundance in gaps. Their colonization was favoured by gap area. The assemblage composition, studied by NMDS and ANOSIM test, clearly differed between gaps (even small) and forest controls. Gaps larger than 0.3 ha were grouped according to the composition and colonization of open-land species. In uncleared gaps, the short-term community dynamics was dominated by colonization rather than local extinction processes.

Key words: Natural opening, colonisation, forest

Abbreviations: G = Gap, F = Forest control, SG = Small Gap, MG =Mid-size Gap, LG = Large Gaps

Introduction

Nature-based silviculture is a promising approach to meet the criteria for sustainable forestry. This brings natural disturbances into focus as a basic reference for forest management (Bengtsson *et al*., 2000). In most temperate deciduous forests, wind is the main natural disturbance (Emborg *et al*., 2000). By opening the canopy, windthrow causes a typical forest fragmentation called perforation (Forman, 1995). It results in a shifting mosaic of open early-successional patches in a forest matrix. The patch-gap analogy reverses the usual forest fragmentation perspective: opening size can be focused instead of woodlot size (Rudnicky & Hunter, 1993). Habitat patches can be considered in the light of island biogeography and landscape ecology concepts. Colonization and local extinction in habitat islands depend on patch characteristics (area, shape) and landscape. In community ecology, the relationships between disturbance, habitat heterogeneity and community dynamics is modelled by the synthetic Patch Dynamic Concept (Townsend, 1989).

Carabids have been studied in different forest openings: burnt (Holliday, 1991) or cut (Koivula, 2001) areas, but rarely in windthrow gaps (Duelli *et al*., 2002; Kenter *et al*., 1998). In western Europe, Lothar, the huge storm in 1999, gave us the possibility of a natural experiment. In the resulting gaps, carabid habitats were drastically disturbed in terms of ground cover, micro-sites, micro-climate and potential prey (Bouget & Duelli, 2004). In the present paper first I will assess whether and how carabid assemblages responded to the windstorm disturbance in the short term just one year and a half after the storm event. In other words, do gaps equate to habitat islands? Then, I will go on to appraise whether this response depends on gap area or not. Do changes in carabid abundance, richness and assemblage composition (species and ecological groups) depend on gap size? Patch area effects are related to the species-area relationship (Forman, 1995). A larger patch is more likely to have a greater habitat heterogeneity (habitat diversity hypothesis), a higher density of specific micro-habitats (density hypothesis) and a sharper micro-climatic contrast with the neighbouring matrix (edge effect).

The influence of gap isolation and the comparison between natural gaps and man-made openings are discussed in two other papers.

Sites, material and methods

Research area

Three lowland forests were under study: the state forests of Armainvilliers (1525 ha) and Crecy (a 1250-ha national block within a 5000-ha forest), and the Ferrieres regional forest (2890 ha) in the ‘Brie’ region. They are located about 50 km south-east of Paris and formed one forest block before fragmentation during the Middle Ages. They are currently being managed as coppice with standards under conversion to high forest and were severely storm-damaged in December, 1999. All three are oak-hornbeam forests (*Quercus petraea*, *Q. robur* and *Carpinus betulus*) with aspen (*Populus tremula*), birch (*Betula* sp.) and lime (*Tilia cordata*). Stand type in the study sites was controlled to avoid significant differences in structure, composition and soil.

Sampling design and study sites

A 50-plot sampling design was used to test the two effects quoted above. Twenty-four storm-created, unlogged gaps in 14 plots were selected within the study area. Gap perimeters and areas were mapped using the differential mode of a Global Positioning System (GPS). Gap shapes were irregular and a variable number of standing trees remained inside all the gaps. Study gaps ranged from 0.12 to 3.3 ha and were divided into three size classes: small (<0.3 ha, n_{SG}=8), medium (0.3-1 ha, n_{MG}=7) and large (> 1 ha, n_{LG}=9). To control for the environmental variation between sites (Underwood 1997), each gap was paired with an adjacent (25–50 m apart), closed-canopy control site (*n*=14).

Study group

Carabid beetles are widely recognised as potentially valuable indicators of environmental variation because they are a highly diverse taxon, can be easily sampled, and are sensitive to changes in the physical and biological environment (Lövei & Sunderland, 1996). All individuals were determined to the species level and assorted to ecological groups according to habitat preference (many references were used, especially Coulon *et al.* (2000), Desender (1986) and Turin (2000)). The nomenclature follows Freude (1976).

Sampling protocol

Ground beetles were sampled using window (for wing-dispersing species) and pitfall (for ground-dwelling species) traps. The parameter measured was the species activity-abundance but for the sake of brevity, hereafter I refer to activity-abundance as “abundance”. Pitfall traps were polyethylene beakers (85 mm in diameter x 110 mm in depth =0.55 L) half-filled with a 1:1 monopropylenglycol:water solution saturated with salt to kill and preserve the trapped arthropods. Acrylic glass covers (100 mm square) were positioned approximately 10 cm above each trap to prevent flooding by rain. Each window trap consisted of a transparent plastic pane (1 m^2) and a container below the pane. Salt water with ethanol was used for killing and preserving the beetles. A detergent was added in all the traps to reduce surface tension.

To maintain a minimum distance between traps, the number of traps per gap depended on gap size. One window and two pitfall traps were set up in forest controls and small gaps, one window and three pitfall traps in mid-size gaps, two window and four pitfall traps in large gaps. Traps were left in the field for one week prior to initial trapping, to reduce digging-in effects (Digweed *et al.,* 1995). The study focused on one sampling season during the second vegetation growth after the storm (2001). To cover the main period of carabid activity, traps were emptied and preserving fluid replenished monthly from mid April to mid October (for pitfall traps) or to late July (for window traps).

Data analysis

Pitfall and window trap datasets were kept separated. We compared the cumulative species richness between habitat classes using sample-based (and not individual-based) rarefaction calculations processed with EstimateS (Colwell & Coddington, 1994). Sample size was standardised at the least number of trap samples between habitat types. In each class, the expected number of species and standard deviation were then interpolated in the random sub-sample drawn for a larger sample (Magurran, 1988). Sampling order was randomized 100 times with replacement to eliminate sampling error and heterogeneity among the units sampled.

The other analyses were carried out using the computer package S-Plus 6.1. Linear mixed-model ANOVA (nested spatial variables as random effects: block, plot and site; fixed factors: habitat type, gap parameters, period) was used to test for differences in mean abundance and mean richness per trap of all carabids or ecological groups between forest and gap plots (Pinheiro & Bates, 2000). This model takes the configuration of the sampling design into account (e.g. the spatial pattern of traps over the research area). It is applied on ln (x+1) transformed data. Differences among means were investigated by multiple comparison tests (Sidak or Tukey post hoc tests).

As individual species abundances per trap did not comply with parametric assumptions, the non parametric pairwise Wilcoxon signed-ranks test (Legendre & Legendre, 1998) was used to compare the abundances between gaps and paired forest controls and to assess the species response to opening.

Three techniques were used to investigate assemblage composition (the first two methods include a log transformation of the data). Non metric multi-dimensional scaling (NMDS) based on the Bray-Curtis dissimilarity was used for pattern recognition in species composition (Clarke, 1993), pairwise ANOSIM procedures for testing for differences in assemblage composition amongst predefined groups (Clarke, 1993), and Indicator species analysis (IndVal) for detecting species indicative of particular habitats (Dufrêne & Legendre, 1997). The IndVal (Indicator Value) procedure is a useful method to find indicator species characterizing groups of samples. It combines a species' abundance with its frequency of occurrence in the various groups of samples. Samples were grouped using a hierarchical habitat typology derived from a hierarchical ascendant classification (UPGMA) of the Bray-Curtis similarity matrix.

Table 1. Gap and gap size effects. Mixed-model ANOVA of mean data per trap. Numbers are mean value in gaps, forest controls, small, mid-size and large gaps. N=abundance, S=richness, rel. N=relative abundance; letters indicate significant differences between means after a post-hoc Tukey or Sidak test. All F values are significant, *p*<0.01.

	All Species		Forest Species			Open-Land Species		
	N	S	N	Rel.N.	S	N	Rel.N.	S
Pitfall trap catches								
Forest	20.23	3.69	13.6	72.4	2.7	3.95	14	0.49
Gap	9.7	2.86	6.1	76.5	1.98	3.15	17.7	0.61
$F_{2,23}$	*234*	*456*	*174*	*1471*	*237*	*8*	*11.5*	*11*
Forest			13.64 *a*	72.4 *a*	2.70 *a*	3.95 *a*	14 *a*	0.49 *a*
Small gap			5.41 *b*	80.9 *a*	1.92 *a.b*	1.23 *a*	11.1 *a*	0.36 *a*
Medium gap			5.46 *b*	75.7 *a*	1.81 *b*	3.14 *a*	18.9 *a*	0.66 *a*
Large gap			7.11 *b*	74.5 *a*	2.16 *a.b*	4.39 *a*	20.7 *a*	0.74 *a*
$F_{4,21}$			*68*	*558*	*103*	*5*	*7.3*	*9*
Window trap catches								
Forest	2.31	0.91	0.28	18	0.17	0.28	27.5	0.26
Gap	5.28	2.8	0.46	15.4	0.34	1.61	35.2	1.18
$F_{2,23}$	*25*	*42*	*22*	*12*	*30*	*41*	*89*	*42*
Forest			0.28 *a*	18 *a*	0.17 *a*	0.28 *a*	27.5 *a*	0.26 *a*
Small gap			0.42 *a*	25.1 *a*	0.42 *a*	1.03 *a.b*	33.5 *a*	0.75 *a.b*
Medium gap			0.43 *a*	12.6 *a*	0.29 *a*	1.78 *b*	44 *a*	1.31 *b*
Large gap			0.50 *a*	12.5 *a*	0.33 *a*	1.81 *b*	31.1 *a*	1.33 *b*
$F_{4,21}$			*11*	*7.5*	*15*	*25*	*4.5*	*24*

Results

Sample overview

Over the seven monthly trapping sessions, the valid pitfall traps yielded 8427 individuals of 48 species. Seventeen species (35%) were represented by fewer than 5 individuals and 18 (37%) were open-land species. *Pterostichus madidus, Carabus auratus, Abax parallelepipedus, Pterostichus oblongopunctatus, Nebria brevicollis* were the dominant species. Over the four monthly sessions, the valid window traps yielded 875 individuals in 60 species. Thirty-four of these (57%) were represented by fewer than 5 individuals and 31 (52%) were open-land species. *Bembidion lunulatum, Acupalpus dubius, Bembidion dentellum, Amara similata* were the most abundant species. By adding the two data sets, the richness reached 80 species. Twenty-eight (35%) were trapped by both pitfall and window traps, 20 (25%) were only trapped by pitfall and 32 (40%) only by window traps.

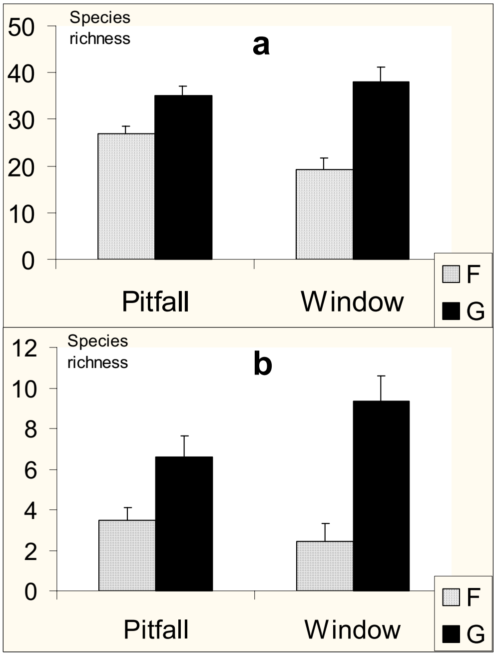

Figure 1. Sample-based rarefaction interpolation of total and open-land species richness in gaps (G) and forest controls (F) (100 sample randomisations with replacement; error bars are the corresponding standard deviations). Pitfall (n_{traps}=135), window (n_{traps}=36). (a): total species richness. (b): open-land species richness.

The two traps gave complementary insights on moving ground beetles in the air and at ground level. Window trap data seem to be very useful for studies on colonization.

Gap effect on the whole assemblage

According to the mixed-model ANOVA, significantly different numbers of individuals and species per trap were caught between closed forest and gaps, but the relationship depended on trap type. Pitfall traps caught more individuals and species of ground-dwelling carabids in forest controls whereas window traps caught more individuals and species of wing-dispersing individuals in gaps (Table 1). With standardized sampling effort, the sample-based rarefaction calculations showed that the cumulative species richness was higher in gaps than in closed forest, at both ground and air levels (Fig. 1a).

ANOSIM tests proved that gaps differed significantly in assemblage composition from closed forest at both ground and air levels (pitfall: ANOSIM statistics R=0.35, p<0.0001; window: R=0.42, p<0.0001).

Gap effect on species and ecological groups

Life history phenomena underlying the whole-assemblage response were briefly explored through the study of the colonisation of open-land species and of the persistence of forest species. At ground level, abundance and richness of forest species decreased from forest to gap, whereas the inverse trend was noticed at air level (Table 1, Table 2). Most forest species significantly declined in abundance immediately after the opening (paired Wilcoxon test, Table 2).These included *L. rufomarginatus, A. parallelepipedus, P. oblongopunctatus, P. assimilis,* and *P. madidus*. I did not observe any short-term disappearance of forest species. Some forest species, such as: *M. piceus*, *P. cristatus, A. parallelus,* were not significantly affected. Others were even favoured by the disturbance, including *D. quadrimaculatus*, *P. livens, and T. nana*.

Randomised accumulation curves showed that the ecological group of open-land species increased in abundance and richness in gaps (Fig. 1b). The abundance and richness of open-land species increased in gaps (Table 1, Table 2). Many open-land species appeared (*C. campestris, A. sexpunctatum, B. quadrimaculatum*) and others increased in abundance after the canopy opening, sometimes (*P. cupreus, P. versicolor)* but not always (*A. similata, L. pilicornis*) significantly (Wilcoxon test; Table 2). Eurytopic species with affinity to open areas also responded positively to clearing (*N. palustris, B. lunulatum). N. biguttatus,* an eurytopic species with affinity to forest environment, was negatively affected (Table 2).
The IndVal method identified roughly the same characteristic species as those sorted as gap sensitive by paired Wilcoxon tests**.** At air level (Fig. 2b), IndVal detected no characteristic forest species but several gap species, which are either open-land (*C. campestris, B. lampros, A. similata)*, eurytopic (*B. lunulatum)* or even forest species living under bark (*T. nana*, *P. livens*). At ground level (Fig. 2a), indicator species were rather different. Forest indicators were more numerous: *L. rufomarginatus, P. oblongopunctatus, P. assimilis, P. madidus, N. brevicollis*. Gap species were mainly open-land species: *A. sexpunctatum, P. cupreus*, *C. campestris, P. versicolor* (Fig. 2a).

Gap area effect on the whole assemblage

Gap area affects the cumulative species richness. At a standardised sampling effort, at air and ground levels, more species were caught in large gaps (Fig. 3a). All gaps, even small ones, showed a higher species richness than the closed forest. The two ordination biplots identify patterns in species composition (Fig. 4). The overall ANOSIM test was significant for pitfall but not for window data. From pair-wise ANOSIM tests, four differences were significant in the pitfall data set ($R_{SG\text{-}LG}$=0.23***,** $R_{F\text{-}LG}$ =0.33*, $R_{F\text{-}MG}$=0.49*, $R_{F\text{-}SG}$=0.42**). Large and mid-sized gaps were grouped into a single cluster, but mid-size gaps were not significantly different from small gaps. All gaps, even small ones, differed from closed forest. Only one difference was significant in the window data set ($R_{SG\text{-}LG}$=0.32*).

Table 2. Direction and extent of change in mean species abundance per trap from closed forest to gap (Wilcoxon signed-rank tests between abundance in each gap and in its paired forest control; ** $p<0.01$, * $0.01<p<0.05$, NS $p>0.05$).

Species/characteristic	Pitfall traps		Window traps	
	From forest to gap (%)	*p*	From forest to gap (%)	*p*
Forest species				
Absolute abundance	- 60	**	+ 64	*
Relative abundance	+ 6	**NS**	- 16	**NS**
Species richness	- 27	**	+ 115	*
Platynus assimilis Paykull	- 79	*	- 81	**NS**
Leistus rufomarginatus Dufts.	- 95	*		
Nebria brevicollis F.	- 93	**		
Notiophilus rufipes Curtis	- 87	**		
Pterostichus oblongopunctatus F.	- 83	**		
Abax parallelepipedus Piller & Mitter.	- 54	**		
Carabus nemoralis Müller	- 50	**		
Pterostichus madidus F.	- 48	**		
Pterostichus cristatus Dufour	- 27	**NS**		
Molops piceus Panzer	- 22	**NS**		
Abax parallelus Dufts.	- 22	**NS**		
Platynus livens Gyll.	+ 34	**NS**	+ 127	*
Dromius quadrimaculatus L.			+ 342	*
Tachyta nana Gyll.			+	**
Open land species				
Absolute abundance	+ 804	**	+ 886	**
Relative abundance	+ 109	**	+ 162	**
Species richness	+ 212	**	+ 757	**
Loricera pilicornis F.	+ 46	**NS**	+	**NS**
Poecilus cupreus L.	+ 1686	**	+ 600	**
Poecilus versicolor Sturm	+ 883	**	+	*
Cicindela campestris L.	+	*	+	**
Agonum sexpunctatum L.	+	*	+	*
Amara similata Gyll.	+ 167	**NS**	+	**
Bembidion quadrimaculatum L.	+	**NS**	+	**
Acupalpus flavicollis Sturm			+	**
Stenolophus teutonus Schrank			+ 526	*
Bembidion lampros Herbst			+ 931	**
Carabus auratus L.	- 39	**		
Eurytopic species				
Notiophilus palustris Dufts.	+ 650	**		
Pterostichus strenuus Panzer	+ 267	*		
Harpalus latus L.	+ 383	**NS**		
Acupalpus dubius Schilsky			+ 20	**
Bembidion lunulatum Fourcroy			+ 161	*
Pterostichus vernalis Panzer			+	*
Notiophilus biguttatus F.	- 54	*		

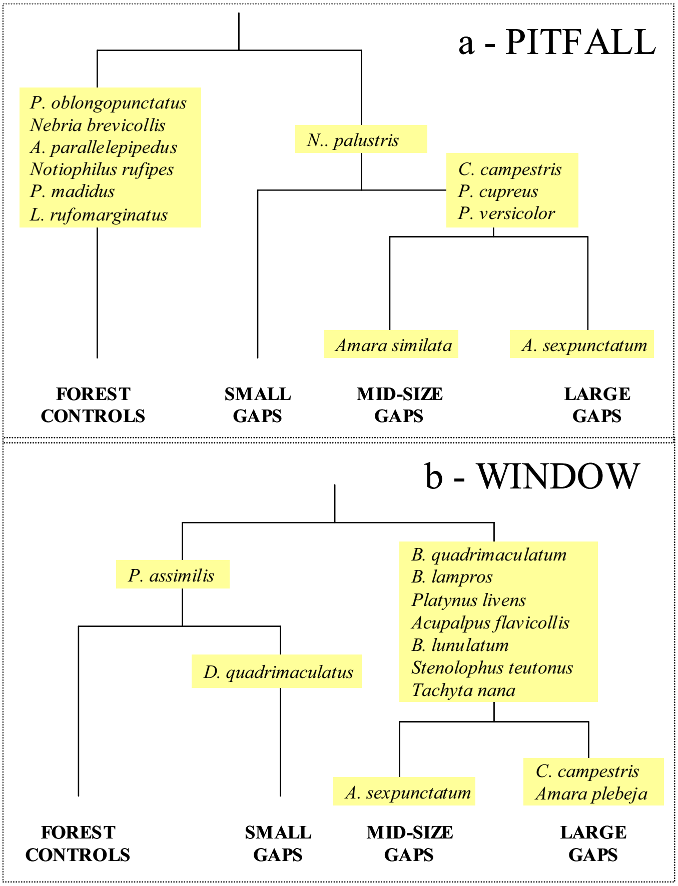

Figure 2. Characteristic species detected by the IndVal method (Dufrêne & Legendre 1997); (a): pitfall trap dataset; (b): window trap dataset. The process was based upon a hierarchical habitat typology from an ascendant classification (UPGMA) on Bray-Curtis dissimilarities. Only species with significant (p<0.05) and >25% Indicator Value are mentioned. When the Indicator Value of a species is significant at different levels, the species appear only at the level of its maximum Indicator Value.

Gap area effect on ecological groups

We did not observe any clear relationship between gap size class and abundance or richness of the forest species group (Table 1). No species abundance decreased in larger gaps. In contrast, data per trap indicated that richness, absolute and relative abundance of open-land species increased with gap area (even if pairwise differences in mean are not always significant; Table 1). At the ground level, more open-land species and individuals were caught in mid-size and large gaps than in small gaps and closed forest (these last two habitats being equal, Fig. 3b). At the air level, more open-land species and individuals were found in large than in small and mid-size gaps (the last two habitats being equal, Fig. 3b). Fewer open-land species and individuals were caught in forest than in gaps (whatever their area).

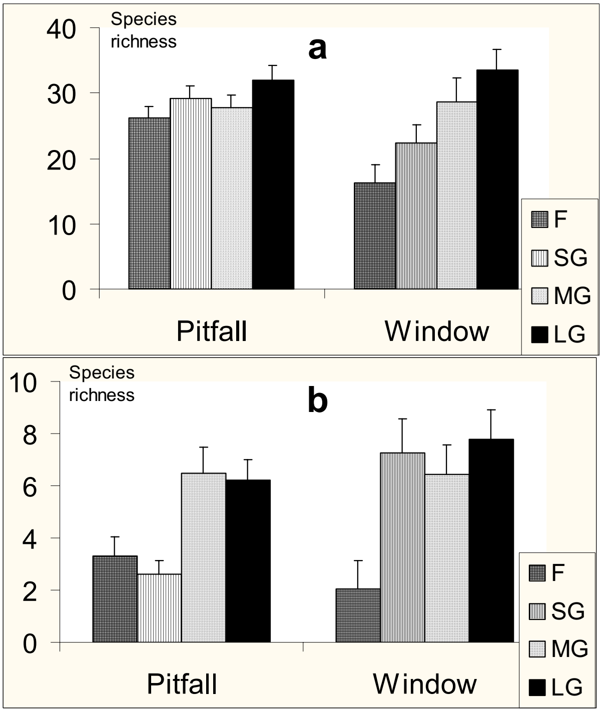

Figure 3. Sample-based rarefaction interpolation of total and open-land species richness in different gap size classes (from SG to LG) and forest controls (F) (100 sample randomisations with replacement; error bars are the corresponding standard deviations). Pitfall (n_{traps}=135), window (n_{traps}=36). (a): total species richness. (b): open-land species richness.

Discussion

Ecological determinants of windthrow gap effects are diverse. New micro-habitats (such as root plates, pits and mounds, fallen crowns) are created and some of them act as sheltering or overwintering sites. The density of grassy patches and coarse woody debris increase. The canopy opening strengthens micro-climatic contrasts and favours the development of the herb layer. Populations of xylophages and phytophages (i.e. potential prey) grow, but predation pressure by vertebrates can also grow.

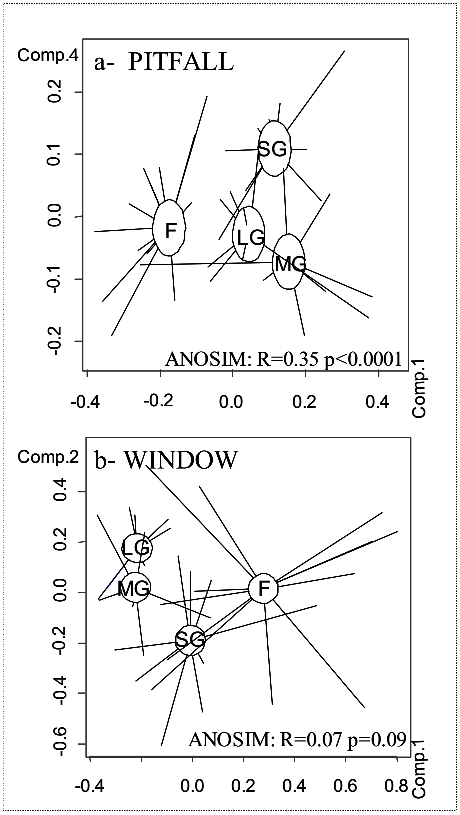

Figure 4. NMDS ordination plot of the Bray-Curtis dissimilarity matrix. The two axes with highest correlation to habitat type are represented. Stress values below 0.2 indicate a reliable representation (Clarke 1993): (a): 4dstress=0.091; (b): 4dstress= 0.138. From pair-wise ANOSIM tests (with Bonferroni correction of the significance threshold (Legendre & Legendre 1998): ** p<0.05/6=0.008; * p<0.01/6=0.002), four differences were significant in the pitfall data set (R_{SG-LG}=0.23*, R_{F-LG} =0.33*, R_{F-MG}=0.49*, R_{F-SG}=0.42) and only one in the window data set (R_{SG-LG}=0.32*). The differences in mean may be summarised in the following way ; pitfall: F(a,b), SG(b), MG(b,c), LG(c) ; window: F(a,b), SG(a), MG(a,b), LG(b).**

Gap effect: ground beetle response to opening

Shortly after the disturbance, I observed a gap effect, with the diversification of ground beetles assemblages, in spite of a lower abundance in pitfall data. A lower average catch was also observed on other natural clearings at ground level (Grechanichenko & Guseva, 2000; Saint-Germain & Mauffette, 2001; Martel *et al.*, 1991; Kenter *et al.,* 1998). In addition, species richness was higher in glades than in closed forest studied by Grechanichenko & Guseva (2000) and Duelli *et al.* (2002). In a spruce forest storm-damaged in 1990, richness was higher between 1992 and 1995 but higher in forest controls from 1996 onwards (Kenter *et al*., 1998). However, species diversity was lower at deciduous sites disturbed by an ice storm (Saint-Germain & Mauffette, 2001) or by canopy dieback (Martel *et al.,* 1991). In clear-cuts, in addition to carabids, the phenomenon of higher abundance and richness appears to be the rule for spiders, ants and butterflies (see references in Heliola *et al*., 2001).

The assemblage composition was altered from closed forest to gaps. Gaps are more dominated by colonisation than by local extinction processes (Walker & Chapin, 1987). Changes in forest species occur mainly at ground level, whereas changes in open-land species are more evident at air level (as colonisation occurs mainly by air dispersal).

The colonisation of gaps by open-land species

I have shown the importance of colonisation processes in gap community dynamics shortly after the opening disturbance. In spruce gaps, during the first three years after disturbance, richness and relative abundance of open-habitat species (*Bembidion lampros, Poecilus versicolor*, *Amara* sp.) grow at the expense of forest species (Kenter *et al*., 1998). After logging, gap conditions at the ground level are even more open: micro-climatic variations increase, the herb layer develops. This practice may strengthen the colonization of open-land species.

The persistence of forest species in gaps

The contrast between unlogged gap and forest was relatively low during the first years following the storm because the residual overstory and the living foliage of downed crowns formed a ground cover. This cover may enable the forest species to survive temporarily (Otte, 1989). Indeed, in the short-term, all the forest species persisted in gaps, although at a decreasing abundance. Some species remained unaffected. After regeneration felling in managed oakwoods, Richard *et al.* (2004) also distinguish unaffected or negatively affected (without disappearance) species. In clear-cuts, forest generalists show an abundance which increases with canopy openness (Koivula, 2001; Heliola *et al*., 2001), probably due to broader physiological tolerance and habitat requirements. In gaps, favoured species were arboricolous species foraging in fallen branches (*D. quadrimaculatus*), or subcorticolous species sheltering under the bark of fallen trees (*P. livens, T. nana*). The catches of forest specialists may represent remnant populations on their way to local extinction (Koivula, 2001). In Polish pine forests, Szyszko (1990) showed that the most radical changes in forest-species abundances occur three years after clear-cutting. However, in maple forests, *Synuchus impunctatus*, the dominant forest species, proved to be sensitive to the ice-storm disturbance (Saint-Germain & Mauffette, 2001). Nonetheless, Kenter *et al.* (1998) describe the revival of relative abundance of forest species four years after the storm in spruce gaps.

Gap area effect

The highest species richness was found in large gaps, in agreement with the species-area relationship predicted by patch-related concepts (Forman, 1995). Although the intensity of environmental changes is correlated with gap area, my results do not agree with the Intermediate Disturbance Hypothesis (the highest diversity in mid-size gaps). The size range covered by our study design was perhaps insufficient, and disturbance effects may not have been observed yet (delayed emigration of forest species).

Overall change in the carabid assemblages increased with gap area. Similarly, during the first years after clear-cutting, the carabid assemblages change much more in 2-ha clearcuts than in gap-felled stands (Koivula, 2001). Gap area did not affect the persistence of forest species. However, du Bus de Warnaffe (2002) showed that forest species survive in small openings (<0.5 ha) whereas they are threatened in clearings larger than 2 ha. Gap area favoured the colonisation of open-land species. Accordingly, many studies (e.g. Bauer (1989), De Vries *et al.* (1996), Magura *et al.* (2001)) reported that richness of carabid specialists of a fragmented habitat type respond positively to fragment size. Open patches in forest are perceived as habitat islands from 0.05 ha by open-land species (du Bus de Warnaffe, 2002).

Implications for forest management

Brawn *et al.* (2001) have pointed out that many bird species adapted to disturbance-mediated habitats have recently declined in North America, even more severely than old-growth species. Niemela *et al.* (1996) argue that a higher number of carabid specialists occur in early open habitats than in old-growth, and that the suitable period for these species is shorter than for closed-canopy species in the natural forestry cycle. From a conservation perspective, several gaps should be kept uncleared.

Acknowledgements

Members of the Ecosylv team are acknowledged, particularly F. Gosselin (for significant help with sampling design and data analyses), C. Moliard and G. Goujon (for technical assistance during the field experiment and the sorting stage). I am grateful to Gabor Lövei and Tibor Magura for valuable comments on the first draft of the manuscript.

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Cuticular hydrocarbon profiles of some ground beetle species (Coleoptera, Carabidae) and their possible role in predatory and antipredatory adaptation

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Abstract

In order to investigate relationships between the chemical composition of the cuticle and predatory behaviour and/or avoidance of predators in carabid beetles (Coleoptera: Carabidae), we analysed the cuticular hydrocarbon profiles of five carabid species, by means of solid phase microextraction collection followed by gas chromatography and mass spectrometry. The species were: *Siagona europaea* Dejean 1826, a species that preys exclusively on social insects (ants); *Brachinus sclopeta* Fabricius 1792, a species highly protected against predators and usually gregarious; *Carabus lefebvrei* Dejean 1826, *Poecilus cupreus* Linnaeus 1758 and *Pseudophonus rufipes* De Geer 1774, three species with unspecialized prey choice and no quinones in the defence glands, signifying a low level of chemical protection against predators.

Brachinus sclopeta and *Siagona europaea* possessed hydrocarbons with chain lengths from 21 to 30 carbon atoms. These were not found in the other three species. Since these molecules are responsible for nest-mate recognition in ants, we suspect that *S. europaea* uses them for temporary mimicry to avoid ant attacks. These hydrocarbons may be utilized by *B. sclopeta* for protection against predators or for kin recognition linked to gregarious behaviour.

Key words: Cuticular hydrocarbons, gas chromatographic analysis, carabid beetles

Introduction

Except of a few pioneering studies, little is known about the role of cuticular hydrocarbons in carabid beetles (Coleoptera: Carabidae). Recently, such hydrocarbons were found to act as semiochemicals in some taxa that prey on ants, including *Siagona europaea* (Zetto Brandmayr *et al.,* 2000a, b) and *Thermophilum* spp. larvae (Dinter *et al.,* 2002). By this chemical mimicry these carabid beetles seem to be protected from the attack by ants.
In carabid beetles, an amazing variety of chemical defence mechanisms against predators has evolved. The chemical composition of the secretion of pygidial glands has been studied by Schildknecht *et al.* (1961, 1968), Eisner *et al.* (1977, 1999), Aneshansley *et al.* (1969), Moore (1979) and Dazzini Valcurone & Pavan (1980). Thiele (1977) produced a review of the most common defence weapons in carabids, which run from hardly poisonous, weak acids such as isovaleric and isobutyric acids, to derived types of defence substances more or less highly toxic, as formic acid, m-cresol and quinones. However, except of a few pioneering studies (Dazzini Valcurone & Pavan, 1980; Moore, 1979), little is known about the role of cuticular hydrocarbons in carabid beetles.

The aim of this paper is to give further insight into the function of cuticular hydrocarbons in selected carabid species, to identify the more common components which constitute the cuticular profile of these species and to assess whether or not they are related to predatory behaviour or avoidance of predators.

Material and methods

Study species

We compared the cuticular profile of three groups of beetles:

- specialized ant predators. The species *Siagona europaea* is a highly specialized ant predator, which has the problem to enter ant nests without being recognized as intruder by the ants. *S. europaea* adults kept isolated (n=3) from ants for one week were tested to define the "innate" profile, while three individuals were tested after they killed and consumed ants. Ant predation in this species involves a strange capture behaviour of arching the body, and squeezing the ant over the beetle's back (Zetto Brandmayr *et al*., 2000b). The ants used as prey were *Tapinoma nigerrimum* Foerster 1855 (Dolichoderinae) (Zetto Brandmayr *et al*., 2000a);
- carabid beetles which are thought to be protected against predators since they possess chemical defence and aposematic colours. This includes species in the genus *Brachinus.* We used *B. sclopeta* in our experiments (n=5);
- individuals of carabid beetles with unspecialized prey choice and a low level of antipredatory responses (no quinones in the defence glands and no warning signals). Species examined in this group were *Carabus lefebvrei*, *Poecilus cupreus* and *Pseudophonus rufipes*.

All specimens of these species were collected in the field near Cosenza, Calabria, Italy, and tested soon after. In this phase we did not consider differences between sexes.

Gas chromatography

For the GC/MS analyses of cuticular components we used solid phase microextraction (SPME). For the analysis, a syringe needle was equipped with a polydimethylsiloxane fibre (Supelco® Inc.), which was introduced into a vial, where an individual was placed. The fibre was lowered and gently rubbed against the dorsal surface of the beetle for about 30 s, and then was withdrawn into the needle. Soon after, the syringe needle was inserted into the injection port of the gas-chromatograph, set in splitless mode, and the fibre was lowered for desorption for 3 min. Analysis was started simultaneously. Analyses were performed using a Varian (Walnut Creek, CA, USA) Saturn 2000 GC–MS ion-trap system in electron impact and positive chemical ionization modes, with acetonitrile as reagent gas, coupled to a Varian 3400 gas chromatograph (GC). The injection port was set at 250°C. The column was a 30 m Chrompack CP-Sil 8 CB low bleed/MS (0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas (1 ml/min flow). The oven was heated at 60°C for 2 min, and then the temperature was increased to 250°C at a rate of 16°C/min. The transfer line was set at 280°C. The ion trap temperature was set at 210°C with an ionization time of 2 ms, reaction time at 50 ms and scan rate at 1000 ms.

Results

The "normal" pattern of cuticular hydrocarbons of *S. europaea* was characterized by a number of compounds of carbon chain lengths from C21 to C29 (Table 1). This pattern was modified after the contact with ants: new components were recorded in the chromatogram, representing highly volatile substances. Table 1 also reports the retention times of the peaks and the percentages for each component between the two samples: the beetles kept separately from ants vs. ones that preyed on ants. The chromatogram of one sample of a *S. europaea* specimen after the contact with ants is depicted in Fig. 1, with the enlarged first part corresponding to the first 10 min of the GC run.

Compared to *S. europaea,* the chemical profile of *B. sclopeta* was more rich in compounds; including a high number of saturated and unsaturated hydrocarbons with chain lengths between C20 and C30. Most of the compounds responsible of peaks 1-12 had relatively short retention times (eluded in the first 10 min), indicating that they were very volatile. These compounds can possibly be smalled even when present in very small amounts.

Table 1. Peak identification of cuticular substances of *S. europaea* and their percentage representation.

Peak no.	Retention time, min	Name of the compound	Quantity in *S. europea*, %*	
			Without contact with ants	After contact with ants
1	2.80	2-methyl-4-heptanone	---	0.15±0.06
2	3.09	6-methyl-5-hepten-2-one	---	0.42±0.24
3	5.06	5-decanone	---	0.09±0.13
4	6.99	2-undecanone	---	0.26±0.17
5	8.70	iridomyrmecin	---	0.39±0.36
6	8.85	2-tridecanone	---	0.29±0.11
7	12.93	Heneicosane	6.57±5.96	1.31±0.62
8	13.36	Docosane	0.44±0.39	0.00±0
9	13.51	9-Docosene	0.93±0.43	0.74±0.65
10	13.78	9-Docosyne	0.76±0.58	0.99±0.09
11	13.93	9-Tricosene	23.90±17.83	3.71±1.43
12	14.06	Tricosane	18.48±6.75	17.60±1.53
13	14.19	1,5,7-Pentacosatriene[a]	3.71±3.62	7.69±3.34
14	14.34	1,5,7-Pentacosatriene[a]	37.55±21.62	59.26±6.07
15	15.08	9-Pentacosene	2.22±1.33	1.3±1.89
16	15.21	Pentacosane	1.23±0.34	1.16±0.54
17	16.61	14-Heptacosene	0.19±0.33	0.00±0
18	16.79	Heptacosane	3.25±1.45	2.56±0.48
19	18.90	Triacontene[b]	0.09±0.17	0.003±0.005
20	19.10	Nonacosane	0.67±0.67	2.07±1.76

[a] Mass spectra are very similar and almost super imposable either in EI or in CI with acetonitrile or isobutene. We think these compounds differ only for double bond substitution geometry.
[b] We were unable to identify double bond position
* Value are means±SD

The extremely simple carbon skeleton did not allow the correct isomer identification by library comparison (NIST98). However, they were identified by chemical ionization with acetonitrile (Zanetti *et al.*, 2001; Moneti *et al.*, 1997). In fact all hydrocarbons showed typical M+40 and/or M+54 ions. The same technique allowed the position of double bonds to be identified.

Finally, we analysed the chemical profiles of *Carabus lefebvrei*, *Poecilus cupreus* and *Pseudophonus rufipes* (Fig. 2). In these samples we were not yet able to identify the peaks. The compounds present eluded later than the components identified for *S. europaea* and *B. sclopeta*.

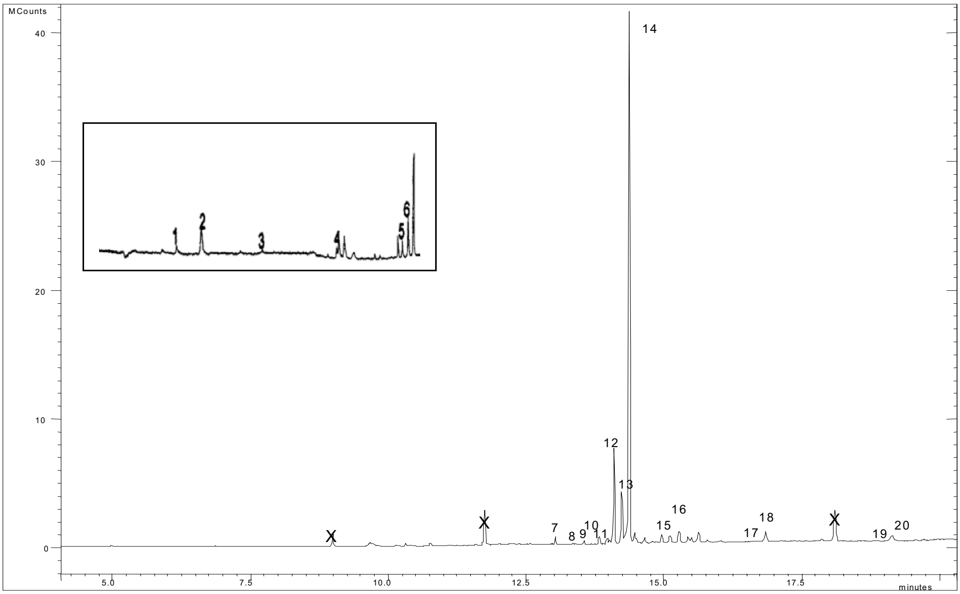

Figure 1. Chromatographic plot of *S. europaea*. In the insert the new peaks eluded in the first 20 minutes from *S. europaea* after ant predation.

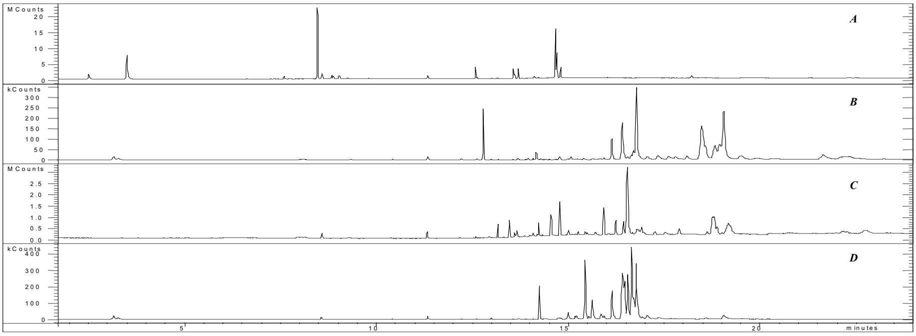

Figure 2. Chromatographic plot of *Brachinus sclopeta* (A), *Carabus lefebvrei* (B), *Poecilus cupreus* (C), and *Pseudophonus rufipes* (D).

Table 2. Peak identification of cuticular substances of *Brachinus sclopeta.*

Peak	Retention Time (min)	Compound
1	2.55	p-benzoquinone
2	3.55	2-methyl-p-benzoquinone
3	7.64	Tridecane
4	8.50	1-(2,5-dihydroxyphenyl)-ethanone
5	8.51	Pentadecane
6	12.03	Eicosane (C_{20})
7	12.53	7-heneicosene (C_{21})
8	12.63	Heneicosane (C_{21})
9	13.07	8-docosene (C_{22})
10	13.20	Docosane (C_{22})
11	13.62	9-tricosene (C_{23})
12	13.65	7-tricosene (C_{23})
13	13.75	Tricosane (C_{23})
14	14.16	8-tetracosene (C_{24})
15	14.27	Tetracosane (C_{24})
16	14.71	9-pentacosene (C_{25})
17	14.75	7-pentacosene (C_{25})
18	14.85	Pentacosane (C_{25})
19	16.07	9-heptacosene (C_{27})
20	16.23	Heptacosane (C_{27})
21	17.36	Squalene (C_{30})
22	18.27	Nonacosane (C_{29})

Discussion

We investigated several species of carabid beetles belonging to three groups according to predatory habits/avoidance of predators, in order to give a contribution to the knowledge of the chemical cuticular profile. In *S. europaea* and *B. sclopeta* we found a wide group of saturated and unsaturated hydrocarbons with chain lengths from 21 to 30 carbon atoms. These compounds are present also in the chemical profile of the ant species used as prey in this study (Talarico, 2002). Out of these highly volatile components there are five ketones which are well known as pheromones produced by ant glands: in particular, 2-undecanone and 2-methyl-4-heptanone originate from the pygidial gland, 5-decanone from the mandibular gland and 2-tridecanone from an anal gland (Dufour's glands) (see for review Hölldobler & Wilson, 1990).

These beetles are either myrmecophagous or chemically protected. In the third sample, consisting of three "normal" carabid species *Carabus lefebvrei*, *Poecilus cupreus* and *Pseudophonus rufipes*, none of these components were detected, but hydrocarbons of greater molecular mass were found.

Cuticular hydrocarbons with chain lengths of C23 to C32 are responsible for nestmate recognition in ants (Bonavita-Cougourdan *et al.*, 1987; Lahav *et al.*, 1999; Lenoir *et al.*, 2001). Many myrmecophilous or myrmecophagous arthropods have these molecules in their cuticular profiles to achieve chemical congruency with ants (Vander Meer and Wojcik, 1982; Howard *et al.*,1990; Allan *et al.*, 2002; Dinter *et al.*, 2002). The chemical mimicry may be realised by biosynthesis of the semiochemicals and/or by camouflage, resulting from passive or active cuticular absorption (see for review Lenoir *et al.*, 2001).

It is likely that the myrmecophagous *S. europaea* uses a scent related to that of the ants. This scent is composed of cuticular hydrocarbons partially shared plus volatile substances from ant glands transferred to the beetles. These substances are usually not considered as colony cues, but they may play a role in colony odour (Dani *et al.*, 1996). Moreover, as most of the myrmecophagous insects (Howard *et al.*, 1990), *S. europaea* has multiple host capability. This is possibly allowed by the stereotypical preying behaviour which allows a fast change in the cuticular profile.

The presence of these compounds in *B. sclopeta* probably serves as a complex odorous profile parallel to a complex visual display (warning colours) to build a template easy to be learned by predators. In addition, they can be important for the recognition of the aggregation site (Wautier, 1971).

The other three species of carabid beetles do not need these predatory or antipredatory strategies and the presence of such semiochemicals would be of little biological significance.

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Theory versus reality: a review on the ecological and population genetic effects of forest fragmentation on wild organisms, with an emphasis on ground beetles

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Abstract

We review the assumed and observed effects of forest fragmentation on plant and animal populations, with an emphasis on ground beetles in temperate regions. Studies on forest fragmentation are much influenced by spatial and temporal scales and were mostly done on plants and large animals. Assumed genetic and ecological processes predict that populations occurring in small, isolated habitat fragments will end up in a so-called extinction vortex: a negative spiral towards extinction, initiated by habitat fragmentation.

Observed effects of forest fragmentation are illustrated with ground beetle case studies, at assemblage and population level. However, results obtained thus far emphasise the difficulties of finding general, species-, site-, or region-independent rules and therefore show the impossibility of proposing generally valid solutions. In many cases, empirical results deviate from theoretical expectations or emphasise the need for more detailed sampling, including the simultaneous study of forest carabid assemblages combined with possible edge effects. Genetic results are also unequivocal and stress the need to simultaneously study multiple model species, including at least a forest generalist and a forest specialist species.

Forest fragmentation appears to give rise to context-specific effects. Suggested fragmentation remedies therefore cannot readily be applied without risks to different natural and highly fragmented situations. Population genetic and ecological effects of restoration measures should be monitored, preferentially for several model taxa. These should include rare as well as more common species, as these show, at least in ground beetles, different ecological as well as genetic responses towards forest fragmentation. Forest ground beetles therefore will continue to play an important role is such future studies.

Key words: Scaling, extinction vortex, edge effects, connectivity

Introduction

The most important threats to global diversity result from direct and indirect consequences of habitat destruction and fragmentation. Fragmentation means that remaining habitat of a fixed surface is located in ever smaller and more isolated discrete patches or fragments. Effects of habitat fragmentation are:

- reduced suitable habitat size, causing reduced population sizes of animals and plants
- the formation of a “matrix habitat”, usually unsuitable for species living in the fragment, causing increased isolation between populations inhabiting fragments of a particular habitat type
- deterioration of habitat quality due to increased edge effects, as influenced by size and shape of the remaining habitat patch (smaller fragments contain less core habitat and hence population sizes are even more reduced).

In this paper I review the assumed and observed effects of temperate forest fragmentation on wild organisms, with an emphasis on ground beetles. First, I shall further introduce the topic, including the importance of scaling, give an overview of scientific papers related to the subject as well as a short review of the main ecological theories that have been proposed as conceptual framework to predict the effects of habitat fragmentation and the role of habitat corridors in species persistence. A description of the assumed ecological and genetic processes involved in forest fragmentation is followed by a summary of observed effects on wild organisms, with an emphasis on ground beetle studies from European forests. Results related to reduced patch size, increased spatial and temporal isolation and increased edge effects will be presented. Finally, a summary is given on the effect of decreased connectivity of the intermediate matrix and the controversy of creating corridors as a proposed remedy to counteract the effects of increased isolation.

Scaling in studies on forest fragmentation

Scaling of forest patches and their distribution in space and time over the landscape are of great importance in studies on the impact of fragmentation. Scaling largely influences specific animals or plants and interacts with their body size, population density, reproductive strategy, home range and dispersal abilities.

First, there is a spatial aspect. The scale of perception of habitat patterns varies among different organisms. For example, large bird species perceive a forest on the scale of tens of hectares, a ground beetle on the scale of tens of square meters. Due to extreme forest fragmentation that occurred in many European regions, effects on smaller organisms can still be studied on relevant (small) scales, while this is no longer appropriate for large organisms.

Secondly, scaling also has a temporal aspect. Forest fragmentation can have a short or very long history, depending on the region. Western European forest experienced a long and severe fragmentation in several waves starting with the Roman period. A first regression took place

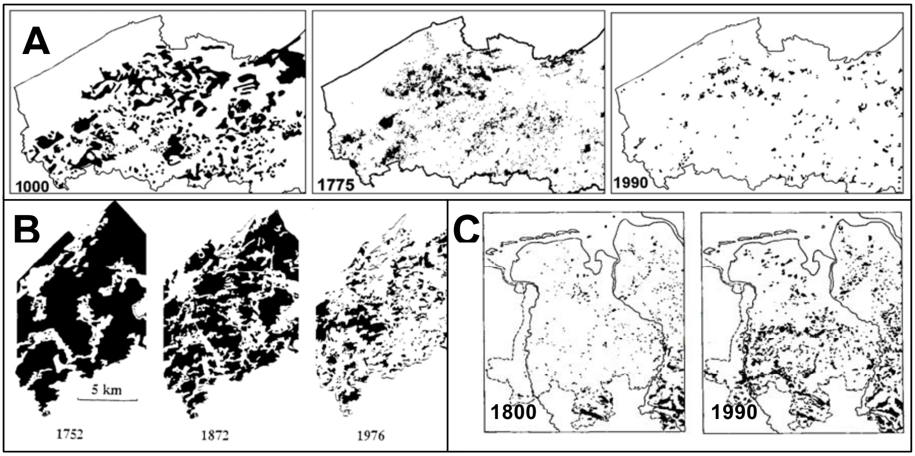

Figure 1. Time scaling of forest fragmentation in different regions of Europe: (A) Western and Eastern Flanders: left map: situation around 1000 AD, before extensive Medieval deforestation (after Tack *et al.*, 1993, modified); central map: de Ferraris (1775); right figure based on a recent map (after Desender *et al.,* 1999); (B) Southern Finland (after Halme & Niemelä, 1993); (C) NW Germany (after Assmann, 1999).

until the end of the Roman period (around 1000 A.D.), followed by a partial forest recovery during early Medieval period. There was renewed deforestation from the late Middle Ages onwards, followed by a large-scale deforestation in the 19th century (Tack *et al*., 1993; Verhulst, 1995) (Fig. 1A).

As a result, forests in many regions of Europe are now extremely reduced in size and highly fragmented, especially in the lowlands of the Netherlands and Flanders (Fig. 1A). In other areas, such as Southern Finland (Fig. 1B), forest area has been seriously reduced only during the past 150 years. In NW Germany, forests today cover a larger area than 200 years ago (Fig. 1C). Large variation in the spatial and/or temporal scaling of fragmentation constitute a first reason for being cautious when extrapolating to devise conservation measures as remedies against fragmentation (see further).

Scientific studies on habitat fragmentation

During the last decade, the number of scientific papers on habitat fragmentation has very much increased. More than 1200 papers were published since 1990 (Fig. 2; ISI, Web of Science, search since 1990, keywords 'habitat fragmentation'). In 2002, more than 10% of all papers published in two leading conservation journals (Conservation Biology; Biological Conservation) deals with this topic and shows that fragmentation has become a major conservation issue. Forest fragmentation has received most attention in these studies. In animals, there has been a very strong emphasis on vertebrates, especially birds and mammals.

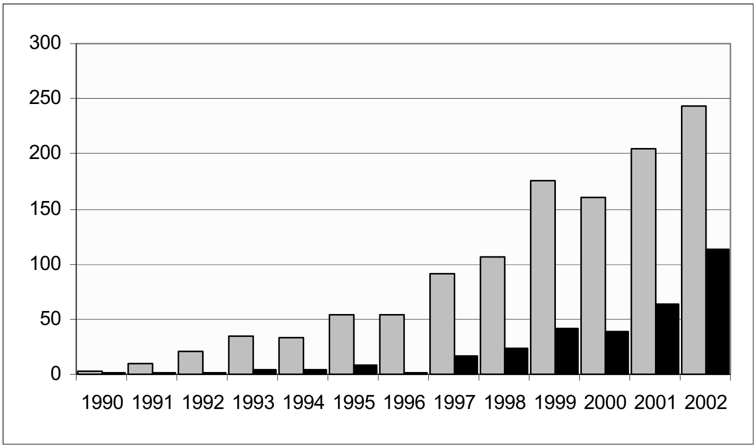

Figure 2. Number of scientific papers on habitat fragmentation since 1990 (ISI, Web of Science); black collumns: publications in Conservation Biology and Biological Conservation.

Insects as well as invertebrates in general are much underrepresented, especially when compared to their much higher species diversity. Among insects, ground beetles have been relatively well studied (some 60 papers). Further, there is a pronounced bias towards non-genetic (species-level and species assemblage) studies, while population genetic and population ecological papers constitute only about 10% each.

Predictions on the outcome of habitat fragmentation and the role of habitat corridors in species persistence: ecological theories and their shortcomings

Most ecological processes depend on spatial scales much larger than a single habitat patch. Consequently, spatial patterns and ecological processes have to be connected at a landscape scale. Within the smaller habitat fragments that remain, reduced populations are more isolated, leading to increased local extinction and decreased rates of gene flow and re-colonisation. The surrounding, human-altered matrix may influence fragments via invasive or predator species. In order to successfully conserve the natural wildlife of habitat fragments several aspects of habitat geometry have to be taken into account. Habitat geometry and its effect on species diversity has long been studied. One important generalisation is that the species richness of an island or a habitat patch is strongly correlated to its area. MacArthur & Wilson (1963, 1967) proposed in their theory of island biogeography (=equilibrium theory) that only island area and isolation determine the species number of habitat isolates through immigration (colonisation) and extinction. These ideas were soon also applied to human-fragmented habitats and to nature reserve design leading to the so-called ‘SLOSS’-debate (Doak & Mills, 1994; Lomolino, 1994). Since the eighties, the theory has been increasingly under fire and criticised as inapplicable to nature conservation problems (Cook *et al*., 2002; Haila, 1986; Hanski, 1999; Hoopes & Harrison, 1998; Lomolino, 1994).

Total species number in nature reserves as well as on islands, indeed, cannot be reduced to the simple factors area (habitat size) and isolation alone. Habitat fragments start with relic biota and are as such also fundamentally different from most true islands starting mostly *de novo* without any life (see also Watson, 2002). The theory also assumed no survival in the matrix (between 'habitat islands'), whereas habitat fragments are clearly influenced by the surrounding landscape. But maybe most importantly, the equilibrium theory did not take into account the idiosyncratic nature (autecology) of most species. Only species number was predicted. Nevertheless predictions from this theory are still used in conservation biology.

In the last decades, the theory of island biogeography has been largely replaced, within the context of habitat fragmentation, mainly by the ecological theories of metapopulation, source-sink and disturbance dynamics. Instead of looking at the total number of species, these theories are emphasising the patch occupancy of individual species and propose that species diversity and abundance depend on spatial and temporal characteristics of habitats.

Metapopulation theory (Levins, 1969; Hanski, 1999) examines the dynamics of sets of semi-independent populations connected by dispersal, and therefore concentrates on the persistence of spatially distributed populations in spatially structured habitats. As a consequence of habitat fragmentation, populations of animals and plants may become divided into sub-populations acting more or less as part of a metapopulation, if individuals are still sufficiently moving between these fragments. The general idea is that long-term survival of populations being part of a good functioning metapopulation is much enhanced as compared to independent populations with local extinction and recolonisation. Studies focus primarily on the effects of patch area and connectivity of suitable habitats. In these studies, there has been an emphasis on bird and butterfly species, mainly for practical reasons, and because populations of such species seem to correspond most to the assumptions of metapopulation modelling. Metapopulation theory predicts that corridors between fragments should increase the regional persistence of species by reducing isolation and enhancing colonisation probability. This viewpoint seems so self-evident that the ideas, mainly developed from modelling, have been implemented already in many regions as the primary emphasis of conservation plans. Very unfortunately, most of the necessary empirical ecological field data on this matter are still lacking.

The evidence on metapopulations in nature is mixed (Simberloff, 1998). The functioning of a metapopulation is very much scale-dependent, depending on the size, home range and dispersal power of the species in question. A given network of nature reserves and corridors therefore is unlikely to be able to support a functional metapopulation for many different species. The question thus arises if metapopulation models can be reliable sources for many cases of specific predictions (generalisation problem) and anyway they need a large amount of field data, usually difficult to obtain. Metapopulation models nevertheless can be useful for weighing different conservation strategies and suggesting critical areas for further study, but at the same time they could be misused by using spatial models to justify further habitat loss, ‘easily’ to be restored or compensated for by creating networks.

Source-sink models are an elaboration of a metapopulation approach and examine the dynamics of populations in habitat patches of different qualities. The theory proposes that populations exist in heterogeneous habitats including areas where surpluses are produced (sources), migrating to other areas where the population cannot replace itself without immigration (sinks) (Pulliam, 1988). Although this theory draws the attention of conservation managers to the very important aspect of variability of habitat quality, there are only few case studies that have been able to validate source-sink models. Yet, as metapopulation models, these are to a high degree dependent on a number of assumptions and rarely based on sufficient or adequate empirical data. As such they are again most suitable for general insight instead of specific conservation actions. A possible misuse of the theory is to justify further habitat reduction by the claim that low-density patches (sinks) could be of no use or even harmful for the viability of a species.

Disturbance dynamics have been looking at assemblages in spatial and temporal mosaics and pertain that appropriate levels of disturbance, such as natural fires, maintain diversity. An example is the ‘intermediate disturbance hypothesis’ (Connell, 1978): intermediate levels of (natural) disturbance are suggested to promote the coexistence of a larger number of species in a spatial mosaic of patches in different stages of succession. These correspond somewhat to the conditions which are met with in our heavily cultivated European landscape mosaics (human-induced disturbance). Inherently, there is the potential misuse of this theory to justify further enhancement of such a mosaic pattern at the expense of larger habitat entities, indeed possibly carrying much lower total species richness, but possibly being composed of species with much higher conservation values. Management strategies of many nature reserves are now based on this disturbance principle in order to mimic natural disturbances that are no longer active or historical human disturbance that created many of these mosaic landscapes at the expense of large ancient forests. The management is considered appropriate simply because of the high total species diversity found. Within the context of forest fragmentation, this theory could distract the attention from the problems of small populations by promoting or sustaining fine-scaled spatial and temporal mosaics.

A general conclusion of this short overview of ecological theories is that appealing, trendy theories should always be used with great caution and after they have withstood a whole series of well-conceived empirical verifications (Hoopes & Harrison, 1998; Lomolino, 1994).

Assumed processes involved in habitat fragmentation: 'the extinction vortex'

The assumed processes likely to be involved in species extinctions as consequences of habitat fragmentation have been summarised in the so-called 'extinction vortex' (Frankham *et al.*, 2002; Gilpin & Soulé, 1986). We have somewhat adapted this scheme (Fig. 3).

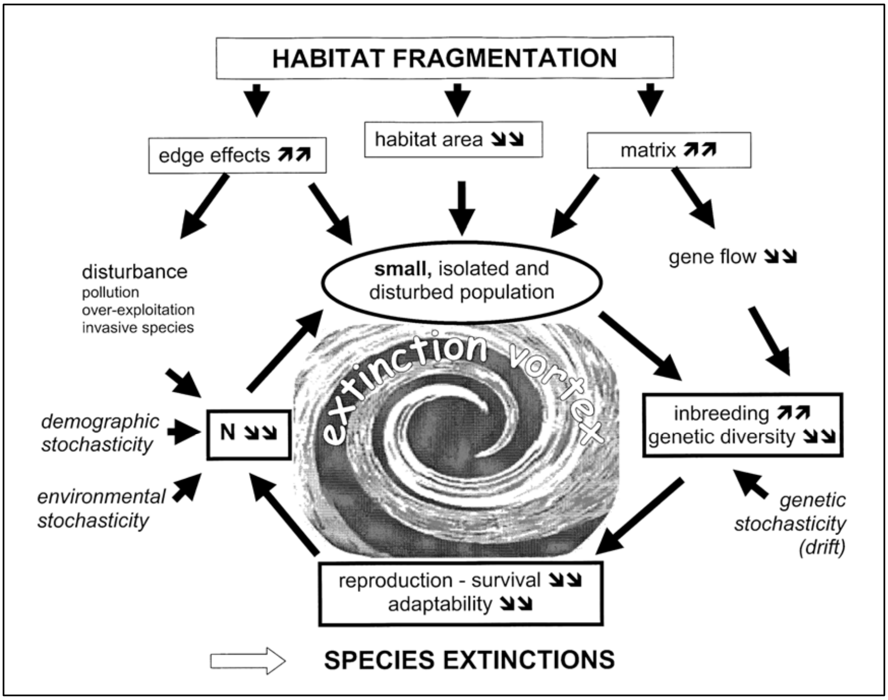

Figure 3. Assumed processes involved in human-induced habitat fragmentation: the 'extinction vortex' (modified, after Frankham *et al.*, 2002).

Small-sized wild populations, occurring in habitat fragments, face threats from increased inbreeding (short-term genetic effect), further loss of genetic variability due to genetic drift and lowered evolvability or adaptability (long-term genetic effect of fragmentation). Inbreeding, the mating between close relatives, increases the chances of obtaining individuals holding deleterious alleles (which are normally recessive) in homozygosity. These individuals can have a lowered reproduction and/or be less viable or unviable. Inbreeding thus leads to lowered reproduction and reduced survival, i.o.w. lower mean fitness ('inbreeding depression') of a population. Random genetic drift (genetic stochasticity) leads to further loss of genetic diversity (genetic erosion). This is a relatively slow process in large populations, but much more pronounced in small populations. Especially in the absence of immigration, in the absence of gene flow between populations and in the presence of strongly differing selection regimes between these isolated populations, interpopulation differentiation may occur. If not yet extinct, genetic impoverishment renders the populations of a species less evolvable and therefore again more vulnerable. This lower adaptability of populations is a long-term effect of fragmentation.

These processes cause further reduction of populations, which become more and more prone to environmental and demographic stochastic effects and increased major disturbing factors (partly also a result of fragmentation-induced increased edge effects), such as over-exploitation, pollution, and effects of invasive or introduced species. The result is a further reduction in reproduction and survival, and population size.

On the whole, population genetic and population ecological theory predict that reduced populations occurring in small, isolated habitat fragments will end up in a so-called extinction vortex: a downward spiral towards extinction, initiated by habitat fragmentation.

Observed effects of forest fragmentation on ground beetles

In the foregoing section, a short account was given on the assumed consequences of habitat fragmentation related to the ecological, demographic and genetic problems of reduced and isolated populations. To what extent these expected patterns have been observed in real empirical studies, is the subject of this section. We review observed consequences of fragmentation (mainly from studies on ground beetles) based on (a) species richness or assemblage level studies and (b) population level studies, including genetic investigations.

Increased forest fragmentation is expected to entail one or more of the following effects: (1) reduced area/patch size, (2) increased spatial and temporal isolation, (3) increased edge and shape effects and (4) decreased connectivity of the intermediate matrix.

Reduced area/patch size

Theory predicts patch area-dependent stochastic extinction processes as a result of shrinking habitat size and related to increased extinction risks of species as a consequence of reduced population size (‘extinction vortex’). At the assemblage level this leads to the prediction of a (1) reduced species diversity in smaller patches. At the population level, important predictions following from genetic processes in the ‘extinction vortex’-scheme are (2) reduced genetic diversity in smaller populations, (3) inbreeding depression in small populations, (4) higher population extinction probability as a consequence of lower genetic diversity and (5) lower adaptability (evolutionary potential) as a consequence of inbreeding. How far have these predictions been confirmed by empirical observations in ground beetles?

Reduced carabid species richness in smaller forest patches?

Several case studies recently have been conducted on ground beetle diversity of forest fragments. In Finland (a.o. Halme & Niemelä, 1993; Niemelä *et al.,* 1988) and in Hungary (Magura *et al*., 2001a), carabid species richness mostly increased in smaller forest patches, which contained more open-habitat species. Burke & Goulet (1998) on the contrary showed the lowest ground beetle species richness in a small isolated forest fragment in Canada, but in this study isolation effects were possibly confounded with patch size effects. Assmann (1999) studied 79 sampling sites in ancient as well as recent forests in NW Germany and obtained a higher carabid species richness in small forests, independently for two types of forest habitat.

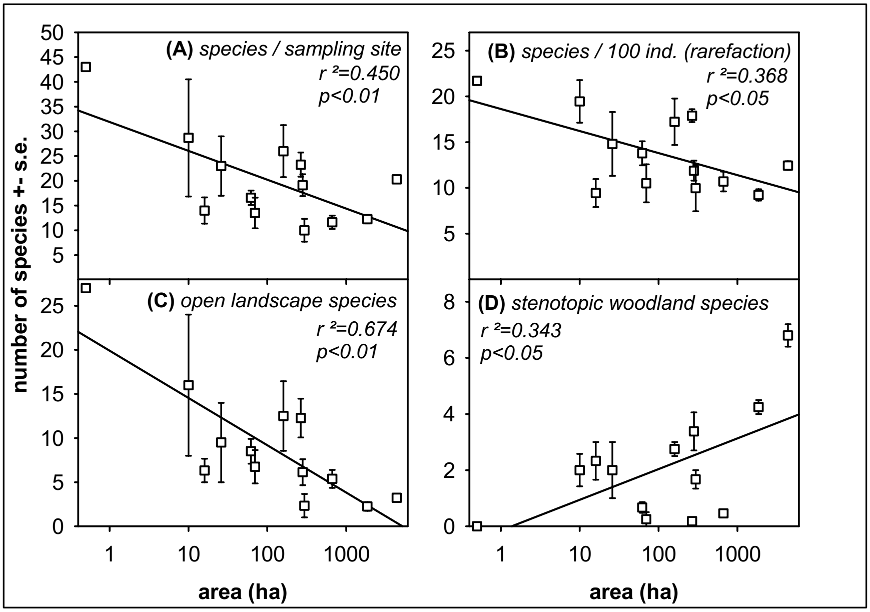

Figure 4. Mean species diversity versus log(woodland area) in Flanders for (A) total carabid species per sampling series, (B) number of species per 100 ind. (rarefaction), mean number of (C) open landscape species and (D) stenotopic woodland species (after Desender *et al.*, 1999).

Forest carabid species behaved indifferently to forest area, but more forest species, with a lower dispersal power, were found in ancient forest.

In the region of Flanders, we compared some years ago data from 100 year cycle samples from 13 forests differing in size and history (Desender *et al*., 1999) to a unique archaeological (late Roman) forest ground beetle dataset (based on more than 1000 individuals.) from the same region. Total carabid diversity in general was higher in smaller forests (Fig. 4), but this was mainly due to an increased number of open landscape species, whereas the opposite trend was observed for stenotopic forest species. These mostly constantly wingless ancient forest species were much more numerous in larger than in smaller forests (based on similarly sized samples). Exactly the opposite was true for open landscape species, edge species or species invading from surrounding habitats, which were mostly constantly winged and thus with a high dispersal power (Fig. 5). In addition, the archaeological sample from the same region showed a still higher number of stenotopic forest species per sample, suggesting that our forest core assemblages, that are nowadays considered as representative for species-rich ancient forest, already might be impoverished to some extent. In recent years, this dataset has been further enlarged (cf. Desender *et al.,* 2002a) and by now includes 250 sampling sites from some 80 ancient and more recent forests in Flanders. We have now also documented in detail the history and landscape-ecological context of all sampling sites (GIS-environment, including historical maps of forest), enabling simultaneous study of area, age, edge and shape effects. Preliminary analyses (Desender, unpubl.) have confirmed the previous results, i.e. total number of carabid species and non-forest ground beetle species appear to be best explained by edge effects (see also chapter 3.3), whereas the most specialised forest carabids occur only in continuous and ancient forests.

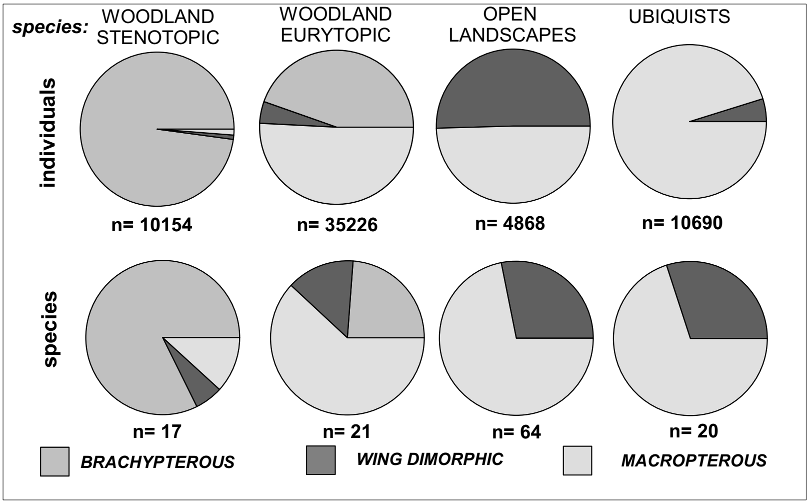

Figure 5. Dispersal power and habitat preference in woodland inhabiting ground beetles, based on data for 13 flemish woods (more than 60.000 carabids, belonging to 122 species). Species are classified into 4 habitat preference categories; dispersal power clearly increases from left to right (after Desender *et al.*, 1999).

To conclude, results from ground beetle studies show context-specific results and highlight the importance of species-specific responses towards forest fragment size reduction. A meta-analysis based on 25 animal studies (Bender *et al.*, 1998), mostly based on density data, led to similar conclusions: patch size effects on density appeared (1) strong and positive for habitat interior (core) species, (2) strong and negative for edge or invading species, but (3) negligible for generalist species.

The relationships between patch size and population density thus largely differ between species, due to varying habitat preference, immigration behaviour or dispersal capacity and life style of different taxa (see also Bowman *et al*., 2002; Matthysen *et al*., 1995).

Reduced genetic diversity in smaller carabid populations?

Until now, relatively few studies have investigated whether genetic erosion can be observed in forest fragment carabid populations. Allozymes (the alternative protein forms encoded by different alleles at the same enzyme loci) from three generalist forest carabids (*Abax ater* (Villers, 1789), *Pterostichus oblongopunctatus* (Fabricius, 1787) and *Carabus nemoralis* Müller, 1764) were studied by Butterweck (1998) but showed no obvious effects of genetic impoverishment in smaller forests. Drees (2003) on the other hand observed an increased allelic richness of microsatellites (highly polymorphic DNA markers consisting of short tandemly repeated sequence motifs) of *Carabus auronitens* Fabricius, 1792 in larger German forests (with larger beetle populations). In Switzerland, Keller & Largiader (2003) also found indications for genetic erosion, while studying barrier affects of roads on the forest ground beetle *Carabus violaceus* Fabricius, 1787: a lower microsatellite allelic variability was observed in smaller and more isolated forest fragments.

In Flanders, we have so far investigated 14 forest ground beetle species in this respect and results have been reported for four species (Desender *et al*., 1999, 2002b,c, 2004). Some examples are illustrated in Fig. 6 (Desender, in prep.). For a majority of the studied stenotopic forest carabid species, there was a more or less pronounced positive relationship between genetic diversity and habitat area (~population size). However, there were also several cases where small to very small fragments showed no indications of genetic erosion or even a higher genetic diversity as compared to larger areas, especially in more generalist forest species. Suggestions to explain these unexpected observations are the presence of relic populations in such small fragments or the possibility of local adaptation in small patches as a response to increased environmental heterogeneity (Desender *et al*., in prep.). For the majority of stenotopic forest species, however, empirical observations lead to the conclusion that genetic variation is related to population size.

To conclude, there is reduced genetic diversity (= genetic erosion) in smaller populations of many stenotopic forest carabid species (for other taxa see also: Frankham, 1996; Frankham *et al.*, 2002; Soulé, 1976), but responses are species-specific. Empirical results on forest ground beetles show the need to study multiple model species: at least one specialist and one generalist forest species in a given region.

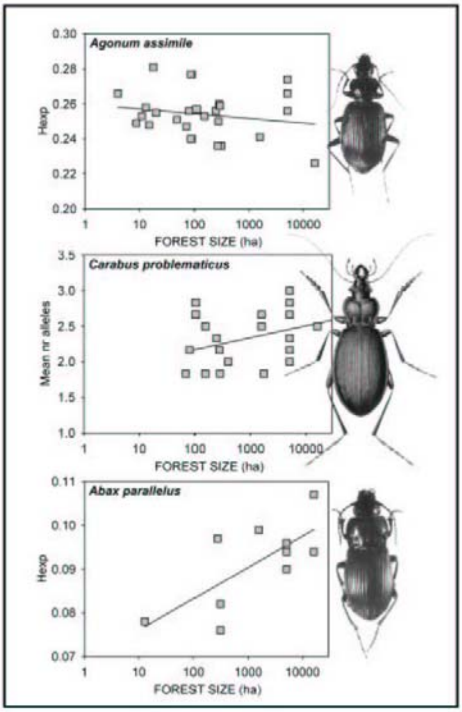

Figure 6. Genetic diversity estimates (expected heterozygosity or mean number of alleles per allozyme locus) and forest fragment area in some ground beetle species in Flanders (forest generalist winged species *Agonum assimile* (Paykull, 1798), wingless stenotopic forest species: *Carabus problematicus* Herbst, 1786 and *Abax parallelus* (Duftschmid, 1812) (Desender, unpubl.).

Inbreeding depression in small populations?

Although it is generally expected that small, isolated populations will suffer reduced fitness (inbreeding depression) due to inbreeding, there have been no thorough ground beetle studies investigating the relation between population characteristics, inbreeding and fitness. In other animals, there are many empirical studies, directly or indirectly confirming this effect. A review on 44 mammal species compared the mortality of inbred versus outbred populations and demonstrated inbreeding depression in nearly all species (Ralls & Ballou, 1983). Empirical results thus are consistent with the hypothesis that inbreeding can significantly reduce the fitness of natural populations (cf. Frankham *et al*., 2002 for a recent review).

Higher population extinction probability as a consequence of lower genetic diversity?

Lande (1988) argued that random demographic and environmental events would drive small wild populations to extinction before genetic factors come into play. Recently, more emphasis has been put on the importance of genetic factors in the extinction vortex, and the current view is that all factors, genetic, demographic and environmental stochastic are important. One reason that this view has changed only recently is that it is extremely difficult to directly observe or empirically prove a higher extinction probability as a consequence of lower population genetic diversity. The first demonstration of this phenomenon (Frankham & Ralls, 1998; Saccheri *et al*., 1998) was based on empirical field data of patch occupancy and genetic variability of the Glanville Fritillary butterfly in Finland. In this study, population genetic data were first gathered from a high number of populations (patches). Patch occupancy probabilities were modelled independently (for a much higher number of habitat patches) based on ecological variables. The year after, a number of butterfly populations had gone extinct and decreased genetic heterozygosity appeared to have contributed highly significantly to these observed extinctions, as compared to ecological variables. Until now, no such studies have been performed with ground beetles.

Lower adaptability (evolutionary potential) as a consequence of inbreeding?

Even more difficult is the issue to show empirically that there is a lower adaptability (evolutionary potential) as a consequence of inbreeding. No such studies have been done yet with (forest) carabids. Recent (experimental) work showed how continuously inbred *Drosophila*-lines can only weakly adapt to changed environmental conditions (changed salinity concentrations) as compared to outbred lines, which are much better at adapting to newly imposed and more severe environmental conditions (cf. Amos & Balmford, 2001; Frankham *et al*., 1999, 2002).

Increased spatial and/or temporal isolation

Increased isolation in space and time as a result of habitat fragmentation processes is expected to influence, directly or indirectly, several of the processes of the ‘extinction vortex’. First, simply by an assumed lowered colonisation probability, there is the prediction, on assemblage or community level, of a (1) reduction in species diversity due to lowered chances for colonisation as compared to local extinction in more isolated habitat patches (reduced ‘rescue effect’). At the population genetic level, increased isolation entails a reduced gene flow and thus a lowered level of genetic exchange between populations. As a result, it should become more difficult or even impossible to compensate for losses in genetic variability due to other fragmentation-induced processes (mainly inbreeding and genetic drift) in reduced populations. If this prediction holds true, (2) increasingly isolated populations (in space and time) are expected to show increased genetic differentiation due to the lack of gene flow. As dispersal power can vary considerably between species (see higher), this prediction can also be investigated by comparing genetic differentiation between related species differing in vagility.

Reduced forest carabid diversity due to lowered colonisation probability?

One study (Gruttke, 1997) has addressed this question in forest carabids. Fewer forest carabids were observed in more isolated old forests, even after mathematically correcting for area differences. This effect appeared to be mainly due to species-specific dispersal capacities, being highly reduced in ancient forest carabids.

Increased genetic differentiation between carabid populations due to lack of gene flow? Several studies on forest ground beetle genetics have confirmed the expectation of increased genetic differentiation between populations due to lack of gene flow, but results again appear to be highly species- and region-specific (on forest *Carabus* species: Assmann, 2003; Assmann & Günther, 2000; Brouat *et al.*, 2003; Desender *et al.*, 2002c, 2004; Keller & Largiader, 2003; Rasplus *et al.,* 2001; on other forest carabids: Desender *et al.*, 1999, 2002b, unpubl.). In Flanders, empirical results on levels of genetic differentiation between different forests (based on allozyme studies) have been compared for an array of 13 forest ground beetles with known dispersal power (~gene flow) (Fig. 7: Desender, unpubl.). All three winged (macropterous) species invariably show a lower Fst-estimate (a measure of population genetic differentiation) as compared to all investigated constantly wingless (brachypterous) beetles. Within the group of wingless species, however, there is a very high variability in

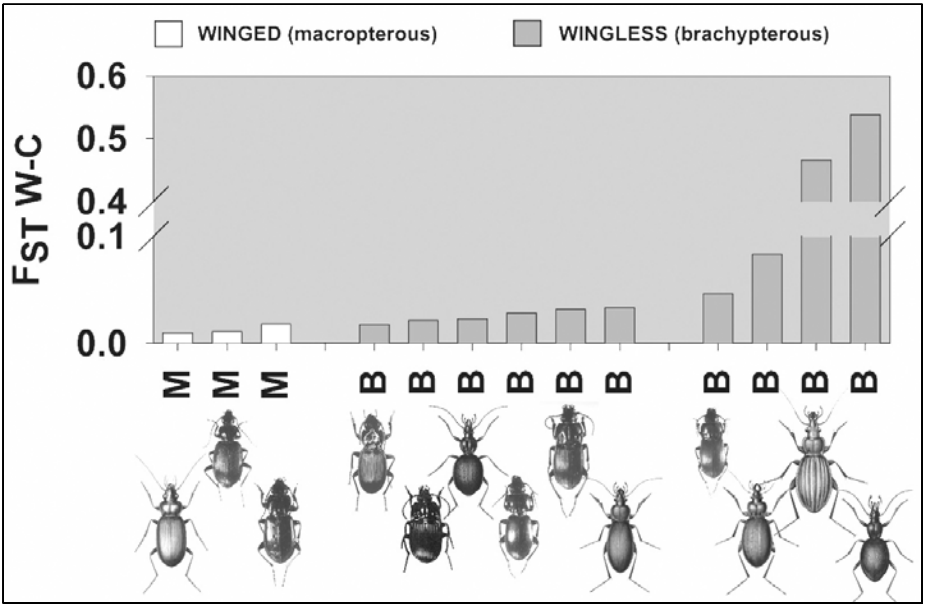

Figure 7. Population genetic differentiation estimates for 13 forest ground beetle species in Flanders (Fst-values, Weir & Cockerham estimates, all statistically highly significant). Species grouped into winged (M) (from left to right: *Nebria brevicollis* (Fabricius, 1792), *Agonum assimile, Pterostichus oblongopunctatus*) and wingless (B) (*P. cristatus* (Dufour, 1820), *Abax ovalis* (Duftschmid, 1812), *Cychrus attenuatus* (Fabricius, 1792), *P. madidus* (Fabricius, 1775), *A. ater, Carabus violaceus, A. parallelus, C. problematicus, C. auronitens, Cychrus caraboides* (Linnaeus, 1758) (Desender, unpubl.).

observed degree of population genetic differentiation, and extreme values are observed in some stenotopic species from large ancient forests, regularly found in sparse populations only.

The prediction of increased genetic differentiation between populations due to the absence of gene flow is confirmed, but only to a certain level, showing that other factors (related to species ecology, biology and abundance) also must play an important role. Again, autecological mechanisms appear to be crucial when interpreting effects of habitat fragmentation on populations of wild organisms.

To conclude, negative effects of increased isolation due to fragmentation are species- and habitat-specific (dynamic versus more stable habitats) and, in our region, are most pronounced in ancient forest species. This relates especially to the habitat-related dispersal capacities of plants and animals, which appear to be highly reduced in old forest species.

Increased edge and shape effects and the decrease of habitat quality in small fragments

Edges are the interface between structurally differing habitats. Most obvious examples concern forest edges in a surrounding agricultural or urbanised landscape. Such forest edges mostly are, more than core forest habitat, expected to be affected by external influences, including disturbance from the surrounding matrix. Observed effects of edges can be grouped in several categories: abiotic effects, direct and indirect biological effects (Murcia, 1995). Abiotic effects of edges involve changes in environmental conditions due to natural (microclimatological: wind, temperature, relative humidity, light) or anthropogenic factors (e.g. nutrients or pesticides). Obvious examples are observed steep microclimate gradients perpendicular to forest edges. These gradients also depend on edge age or management history, physiognomy, orientation, and matrix type. In many studies, influences observed in environmental conditions disappeared over the first 50 m into a forest fragment, and were less pronounced at north facing edges than at south facing edges (Hunter, 1999; Honnay *et al.*, 2002).

As a result of increased fragmentation, there is a relative increase of habitat edges, dependent on fragment size and shape. This non-linear effect on the amount of edge versus interior or core habitat as a function of fragment area and shape is illustrated in Fig. 8. The examples compare the percentage of core versus edge habitat of differently sized and shaped fragments, under the assumption of a 50 m wide edge zone. Within the range of relatively small fragments, core habitat quickly disappears, especially when fragments are more elongated and less regularly shaped (rectangle, compared to circle or square). As a consequence, small irregular forest fragments in our cultivated landscape are relatively much more expected to be negatively influenced by edge habitat (and thus by the surrounding matrix) as compared to large patches. A similar sampling effort therefore is expected to show higher species diversity estimates in smaller forests, although the species quality can be very much reduced due to a lower incidence or complete absence of forest core species. Carabid data (similar sampling efforts) obtained for forests in Flanders and elsewhere confirm these expectations (Assmann, 1999; Desender *et al*., 1999; Niemelä, 2001a). Few ground beetle studies however have directly taken fragment shape phenomena into consideration. More forest ground beetles were found in fragments with an increasing shape complexity (Niemelä, 1997). Usher *et al.* (1993) showed positive shape effects on carabid assemblages of very small 'farm woodlands' in the UK, whereas Magura *et al*. (2001a) failed to find an influence of the shape of forest patches on total species or forest species richness. However, distance of sampling site to forest edge, as another possibly influencing factor, was not studied separately or independently in these papers or in most of the earlier-mentioned studies.

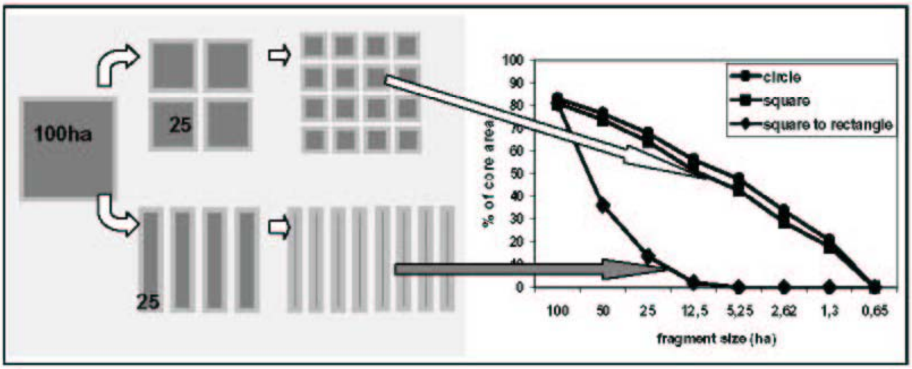

Figure 8. Percentage of core forest habitat in fragments of different shape and decreasing size.

Edge effects on carabid beetles have been studied mainly within two different contexts. First, several papers have looked at the carabid abundance and species diversity of 'old forest-grassland transitions' in Hungary (Magura, 2002; Magura & Tothmeresz, 1997, 1998; Magura *et al*., 2001b). A significant edge effect on ground beetles was detected in most of these investigations with a higher carabid diversity at forest edges as compared to forest interior. This increased diversity was attributed to the presence of carabids from adjacent habitats as well as of typical 'edge species' (though these were defined by separate analysis in each study and sometimes also classified as forest species in another study). Kotze & Samways (2001) failed to find such a 'biological edge effect' for carabids of South African grassland-montane forest transitions. A second set of ground beetle studies investigated transitions from forest edges to recent clear-cut areas, mainly in Finland (a.o. Heliölä *et al*., 2001; Koivula & Niemelä, 2002) and Canada (Spence *et al*., 1996). No edge specialist species were observed in these studies. Results from such samplings however can be more difficult to interpret because they address questions of edge effects in the first phases of establishment. Moreover, trapping results in recent clear-cuts are possibly also influenced by (1) escape activity of forest carabids, which are suddenly confronted to a major change of their habitat, and/or by (2) a certain resilience time before typical forest species have disappeared from recent clear-cuts. It is therefore not surprising that some of these studies failed to find edge effects: e.g. Koivula &

Niemelä (2002) observed forest specialist carabids in more or less equal numbers along a transect from forest to clear-cut, whereas forest-habitat generalists were trapped in even higher numbers in the clear-cut. Spence *et al.* (1996) on the other hand concluded that edges had an adverse effect on forest specialists with a negative influence up to 80 m in forest. Again, most of the studies showed highly species-specific reactions.

Indirect edge effects involve changes in species interactions as a result of increased disturbance, e.g. increased nest predation (Chalfoun *et al.*, 2002), brood parasitism, herbivory, insect pollination, plant competition, or seed dispersal closer to habitat edges. There are many scientific papers on this matter, empirically showing such effects, but to our knowledge none have included ground beetles.

In general, we can conclude that edges have deleterious consequences for organisms that remain in (forest) fragments, but species respond in many different ways (Murcia, 1995). Edge effects clearly also have been observed in several forest carabid studies, but there is some controversy on the existence of forest edge carabid species (though possibly less relevant for conservation; forest edges moreover mostly are hard boundaries without transition zone). Direction and magnitude of edge effects on forest ground beetles also appear to be species-, site- and context-specific. This topic needs further and more elaborated study in ground beetles, also in view of the complete lack of scientific papers on population genetic studies within this context.

Decreased connectivity of the intermediate matrix and the controversy of newly created corridors

Can corridors in the intermediate matrix, linking forest fragments, functionally reduce habitat isolation to a sufficient degree to prevent extinction of populations? No studies on carabids have directly answered this question, though many have made the suggestion (see Niemelä, 2001b for a recent review). In what follows, we have therefore summarised supposed positive and negative effects of the creation of corridors on forest fragment organisms, including ground beetles. We refer to other papers for more comprehensive accounts on the large number of carabid studies that have been performed in hedges (Burel, 1996; Thiele, 1977).

Modelling and empirical studies suggest that corridors can increase species richness of connected fragments, by creating movement pathways for animals of various taxa, mainly mammals and birds. There are many papers from hedges showing the occurrence of (generalist) forest ground beetles (e.g. Thiele, 1977; Niemelä, 2001a,b). Whether this increases the species richness or population persistence in connected fragments has not been studied in carabids or has only involved (very) generalist forest species, which are not of primary conservation concern. The above-mentioned contrasting population genetic results for common as opposed to more specialised forest-interior carabids warn against such an approach. Simulation models and (few) laboratory and field experiments in other animal taxa suggest a (slight) increase in species richness or population persistence in connected forest patches as compared to isolated patches (Hobbs, 1992; Tewksbury *et al*., 2002). There is limited evidence (none for ground beetles) that corridors as such enhance the persistence of populations occurring in connected patches.

However, corridors may be not effective, as has also been suggested by many other authors (e.g. Haila, 2002; Hunter, 2002; Niemelä, 2001b). Reasons for this include: (1) Corridors are not always easily found by moving forest organisms. (2) Also, it is well known that dispersal in critical forest organisms (ancient forest carabids) mostly is very low (Assmann, 1999; Desender *et al*., 1999). (3) Corridors are mostly 'low quality habitat' ('edge' instead of 'core' habitat) and as such not suited for specialised organisms. As a result, few species may perceive and use linear strips of vegetation as movement pathways, and maybe only vertebrates can benefit.

Creating corridors even might involve risks and have serious negative effects, especially when linking forest fragments with a long history of isolation. These include: (1) Low quality corridors could act as sinks, decreasing the size of a (meta)population. (2) Corridors may help spreading disturbance, predators, diseases or catastrophes (cf. Hale *et al*., 2001; Simberloff *et al*., 1992). (3) Linking fragments can entail the possibility of outbreeding depression (lower fitness of mixed populations, as a result of outbreeding, when brought into contact). Critical species potentially have been locally adapted during a possibly long evolution of population fragmentation. The underlying process is the disruption of co-adapted gene complexes, possibly resulting in genetically intermediate populations adapted to neither of the linked sites, microhabitats or habitats. There are some documented cases of this process (Frankham *et al*., 2002). Within European ancient mosaic landscapes, there are indications for such risks derived from population genetic studies on ground beetles (cf. Desender *et al.,* 1998, 2002c, 2004). (4) If populations (fragments) to be linked differ to a large extent in relative size (area), there is an increased risk for swamping (hybridising out of existence) of locally adapted small populations, resulting in the loss of rare or unique alleles or genotypes. Such risks seem especially high if populations of very small (e.g. relic or old) fragments are to be connected to those of larger (but genetically different or poorer, e.g. more recent) habitats. Again, there are already some empirical genetic data for ground beetles in Flanders (Desender et al., in prep.), indicating that such risks could follow from the creation of corridors.

Therefore, it is important to (1) stop or reverse habitat loss: e.g. enlarge rather than connect forest fragments (in order to reduce the basic 'extinction vortex' processes) or to adopt a strategy of a network of unconnected habitat patches (stepping-stones). It is also better to spend resources on appropriate management and thus to (2) stop or reverse habitat deterioration. Of course, existing corridors, such as old hedges, have a nature value, as habitats on their own, especially in our old cultivated mosaic landscapes, where they have been present for a very long time (Burel, 1996). As such, existing hedges and the entire landscape have to be managed more completely in favour of wildlife, including ground beetle assemblages.

Conclusions

Forest fragmentation gives rise to species- and context-specific effects, difficult to generalise and regularly different from theoretical predictions. Results obtained thus far emphasise the difficulties of finding general, species-, site-, or region-independent rules and therefore show the impossibility of proposing generally valid solutions. It is therefore not at all expected that suggestions derived from current popular ecological theories could be readily applied without risks to different natural and highly fragmented situations.

Our knowledge of the effects of forest fragmentation on animals and plants in general, or on carabid beetles in particular, is still very inadequate and uneven, characterised by a general lack of sufficiently detailed population genetic and population ecological studies and nearly complete absence of genetic monitoring programs. These are surely needed because they can make region-specific inferences about the relative importance of the different ecological and genetic processes acting as a consequence of habitat fragmentation.

To conclude, assumed and observed effects of forest loss and decreased forest habitat quality (edge and shape effects) show that, in order to improve survival prospects of endangered species, we must stop, even reverse if possible, habitat loss and deterioration, plus increasing efforts in habitat restoration. Current emphasis in conservation biology on habitat spatial pattern, i.e. the creation of corridors, may be misplaced. One generalisation that can be made, however, is that small-sized populations are always at risk due to population genetic and ecological processes. Any measure helping small and endangered populations to increase should be a conservation priority. The population genetic and ecological effects of the applied restoration measures should be monitored, preferentially for several model taxa, including rare as well as more common species. These species, at least in ground beetles, show contrasting ecological as well as genetic responses towards forest fragmentation. Forest ground beetles therefore will continue to play an important role future studies.

Acknowledgements

The organizers of the ECM at Aarhus are acknowledged for their invitation and support to present this review during the meeting. This review benefited from discussions with many colleagues, in particular J.P. Maelfait (IN), A. Ervynck (IUAP), O. Honnay (KUL), and B. De Vos (IBW), although views expressed in this paper are of course the responsibility only of the author. Special thanks also to A. Drumont, E. Gaublomme, H. Dhuyvetter, V. Versteirt & P. Verdyck (Coleoptera Research Group, RBINSc) for their substantial help in recent genetic studies on forest ground beetles. Regional and national Belgian nature conservation and forestry authorities allowed us to sample carabids in their forests. J.P. Maelfait (IN), L. Baert, P. Grootaert, W. De Coninck, W. Heirbaut (RBINSc), M. Pollet, H. Segers, D. De Bakker and F. Hendrickx (RUG), S. Thys and L. De Bruyn (RUCA, IN), B. De Vos, K. Vandekerkhove, P.

Van De Kerckhove and D. Van Den Meerschaut (IBW), D. Maddelein and D. Gorissen (AMINAL), are acknowledged for their help in forest sampling and support in various ways. Th. Assmann, C. Drees, I. Keller, J. Kotze, J. Niemelä, T. Magura, B. Tothmeresz & J.Y. Rasplus kindly provided last-minute reprints, preprints or unpublished data on their forest carabid studies. Our ecological and genetic forest carabid studies have been supported mainly by the RBINSc (Dept. Entomology) and the Institute of Ecology (RUG). Additional financial support was obtained from the Flemish Government (AMINAL/Natuur/ projects VLINA 96/01 and 00/015; AMINAL, Dept. Bos & Groen, projects B&G/15/96 and B&G/29/98) and the Belgian Federal Government (DWTC, project MO 36/006). Our investigations are also carried out within the framework of the Network of the Fund for Scientific Research-Flanders (Belgium) FWO.010.97N ('Ecological genetics: patterns and processes of genetic variation in natural populations') and FWO.017.02N (Ecological genetics: a new approach).

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Interaction between regional forest history, ecology and conservation genetics of *Carabus problematicus* in Flanders (Belgium)

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Abstract

Allozyme and microsatellite markers were used to compare population genetic characteristics of 11 populations, and allozymes alone of 24 populations of *C. problematicus* from four eco-regions in Belgium. *C. problematicus* differs in habitat preference, commonness, degree of isolation between populations, indicator value for ancient forests and supposed nature conservation value in general between these eco-regions. Overall, the genetic data showed a high level of structuring. Allelic distributions are illustrated for two allozyme and two microsatellite markers and suggest a variety of underlying evolutionary processes resulting from forest fragmentation as well as isolation by distance. These results are of particular conservation genetic interest against the background of region-specific forest history. The conservation genetic value of *C. problematicus* populations differs dramatically according to the region and on a relatively small geographic scale. *C. problematicus* therefore can be considered a powerful model species with applications in conservation studies at different levels of perception.

Key words: Forest fragmentation, population genetics, allozymes, microsatellites

Introduction

Recently there has been a growing interest in forest history and biodiversity, stimulated by the IUFRO (Int. Union of Forest Research Organisations) and reflected in recent symposia and books specialised on this subject (Kirby & Watkins, 1998; Vera, 2000; Honnay *et al.,* 2004). Especially in Western Europe, the low amount of remaining ancient forests has reached alarming proportions. In Belgium, as in many other parts of Europe, forests have been subjected to about 7000 years of human influences. Their history in general is one of woodland destruction, fragmentation and degradation, although locally (e.g. in Flanders between 1300-1800) there were also periods of forest rehabilitation and expansion (Bloemers & Van Dorp, 1991; Tack *et al*., 1993; Tack & Hermy, 1998). Around 1850, the woodland area reached its absolute minimum in this region, while subsequent reforestation was mainly by pine and poplar stands, which are biologically rather poor. The decrease in forest area has thus been accompanied by a serious decline in forest habitat quality. Forests nowadays cover about 20% of Belgium, but there are large differences between regions, with less than 10% of forested area in Flanders (Hermy *et al.*, 2003).

There is a growing need for quality assessment in biodiversity and conservation studies. Species richness is not a sufficient criterion and can be very much misleading in the evaluation of forest conservation value, especially in fragmented temperate forests (Desender, 2005). Alternative questions are related to the nature of the species (e.g. 'are ancient forest species present?'), to their population genetic diversity, morphological and genetic identity, and population viability. Recent developments in forest research have stressed the importance of both historical and actual ecology in shaping the diversity and evolutionary potential of woodland plants and animals, especially in Western Europe (Assmann, 1999; Desender *et al.*, 1999, 2002a; Niemelä, 2001). Numerous studies point to qualitative differences in species composition between ancient and recently established forests (on plants, cf. Godefroid & Koedam, 2003a,b; Graae *et al.*, 2003; Hermy *et al.*, 1999; Matlack, 1994; on invertebrates, cf. Assmann, 1999; Desender *et al.*, 1999). These differences are supposed to be attributed to the limited (re)colonisation capacity of many species characteristic of ancient forests. Whether morphological and/or genetic changes (impoverishment) accompanied the decline of ancient forests species was seldom investigated. Two studies suggested that forest history is of major importance for the ecological and genetic constitution of ancient forest beetle communities, and in the long run for their micro- and macroevolution (Assmann, 1999; Desender *et al.*, 1999, 2004).

Genetic diversity has become one of the keywords of scientists who are concerned about the sustainable management of forests. Behind this concern is the assumption that high levels of diversity provide a guarantee for the perenniality of forests. In theory, reduced woodland area and a lower age are both expected to reduce genetic variability of forest organisms, through chance effects such as genetic drift and reduced population viability as a consequence of inbreeding (Desender, 2005). Other processes, such as local adaptation by natural selection, also can have profound effects on the genetic constitution of forest organisms, and, in theory, could increase population differentiation between isolated ancient populations. There is therefore an urgent need to combine the analysis of several kinds of biochemical markers when possibly unrelated phenomena are likely to leave their footprints on the genetic structure of populations (Arnaud-Haoud *et al.*, 2003).

Invertebrates, e.g. ground beetles, combine a number of features of high interest for such studies and, without doubt, are the most diverse component of woodlands. Ground beetles (Coleoptera, Carabidae) mostly show a high species richness and many species have a pronounced habitat preference for forest interiors. These beetles are very well documented in Belgium, as can be derived from a large amount of distribution data since about 1850 (Desender *et al.*, 1994, 2003b), as well as from archaeological data (Desender *et al.,* 1999;

Ervynck *et al.*,1994). Most stenotopic woodland carabid species are constantly wingless or never develop functional flight musculature (Assmann, 1999; Desender, 1989; Desender *et al.*, 1999).

Carabus problematicus Herbst, 1786 is a wingless beetle, occurring in Western Europe from Finland to southern France (Turin, 2000). In an earlier contribution, we presented ecological as well as preliminary genetic data on this forest ground beetle. The ecology and genetic diversity of this species were linked to both forest age and size (Desender *et al.*, 2004). In Belgium, situated near the centre of the current geographic distribution area of *C. problematicus*, there appears to be a remarkable diversity in abundance as well as in preferred habitat of this beetle according to the eco-region (Desender *et al.*, 2004). In the South, especially in the forested regions of the Ardennes, *C. problematicus* occurs abundantly in many woodlands, with a preference for acidic soils (Baguette, 1993). In the northern Campine region, the species is also common but increasingly found (in low numbers) in heathlike habitats. This corresponds to the general trend of its habitat preference shifting to more open habitat types towards northern Europe (Houston, 1981; Rijnsdorp, 1980) or in cooler mountain areas at high altitude (Buse *et al.*, 2001; Butterfield, 1996; Dennis *et al.*, 2002; Sparks *et al.*, 1995; Turin, 2000). The beetle apparently rather successfully colonised many recent forests in the Campine area, probably through its sporadic occurrence in heathlike habitats in between forests. In the central and especially the westernmost part of Flanders, however, *C. problematicus* has become increasingly rare and is now a strong indicator of ancient forests. Archaeological data show that *C. problematicus* once occurred at other sites in that region (Desender *et al.*, 1999). The beetle probably disappeared mainly as a result of the negative consequences of forest fragmentation. Forest history (ancient versus recent forests) and forest area both appeared to be important for the current ecology, distribution and genetics of *C. problematicus* populations in Flanders (Desender *et al.*, 2004).

In this paper, we present additional population genetic analyses and results for *C. problematicus* from different eco-regions in Belgium. Patterns of allelic distribution will be illustrated for several individual markers in order to derive suggested underlying processes responsible for observed conservation genetic values in different regions. Dendrograms will also be constructed based on genetic distance between the studied populations in different eco-regions.

Material and methods

Sampling for population genetic studies mainly took place during 2000-2002. For each population, some 30 to 40 individuals were studied, if available. Detailed digital forest data, used within an Arcview GIS-environment, are available from the ‘Digital version of the forest reference layer’ (MVG, LIN, AMINAL, afd. Bos en Groen, edition 2001, OC-GIS Flanders).

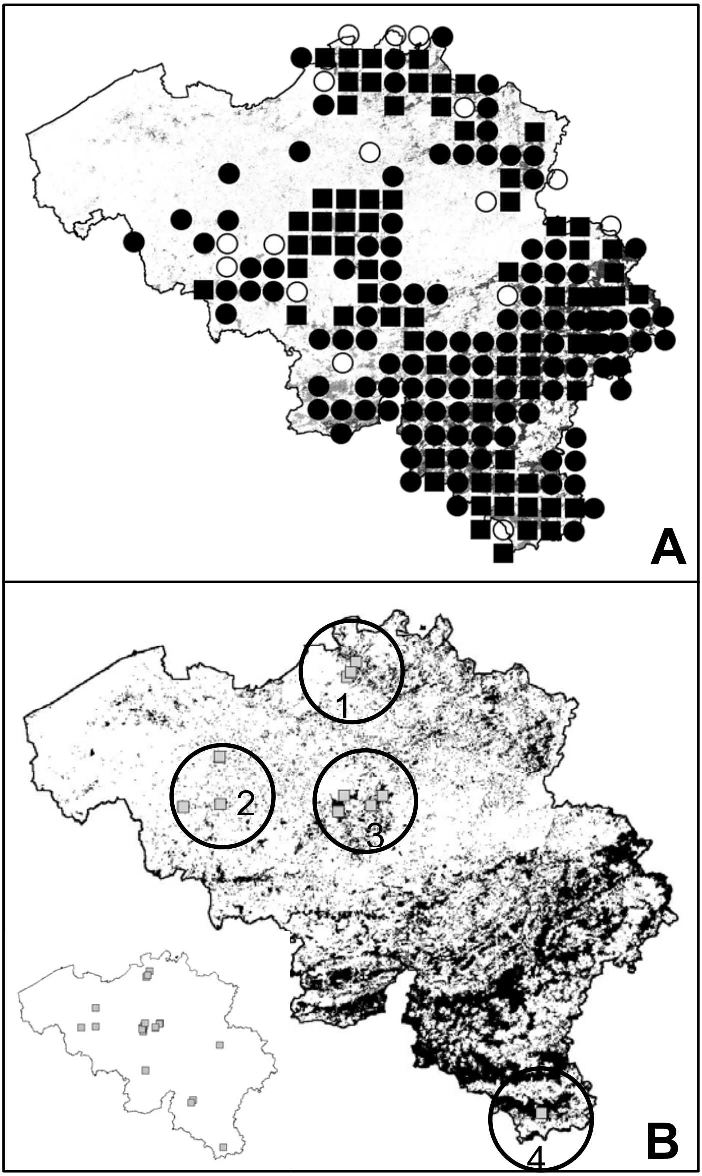

Figure 1. (A) Distribution of *Carabus problematicus* in Belgium (open symbol: only data before 1950, black circle: data since 1950, black square: data from both time periods) and (B) forest map of Belgium with localisation of 11 sampling sites used for microsatellite and allozyme study in different eco-regions: 1. Northern Campine region, 2. Western Belgium (species occurring only in very few isolated ancient forests), 3. Central Belgium loam region, 4. Southern Belgium Gaume region (inset map shows 24 sampling sites from allozyme dataset, with several additional sites in the Ardennes, region 4).

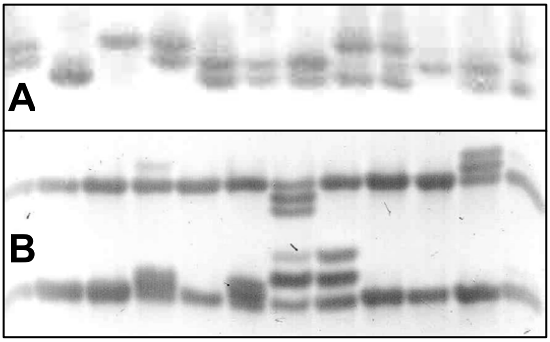

Figure 2. Examples of cellulose acetate zymograms for *Carabus problematicus* : (A) PEP-Z, monomeric enzyme and (B) GPI, dimeric enzyme. Genotypes read (from left to right): (A) BC, AA, CC, BC, AB, AB, AB, AC, AC, BB, AB, AC (B) lower line: BB, BB, BB, BC, BB, BC, BD, BD, BB, BB, BB, BB; upper line: BB, BB, BB, BB, BB, BB, AB, BB, BB, BB, BC; BB.

In a preliminary data set, genetic variability and differentiation were studied with allozymes and microsatellite DNA markers on 350 beetles from 11 populations (Desender *et al.*, 2004). Recently, we added some 400 more individuals from 13 additional populations to the allozyme dataset. Fig. 1 shows the sampling locations for these two sets of populations, with reference to the geographical distribution of *C. problematicus* in different eco-regions of Belgium.

Cellulose acetate electrophoresis was applied to study variability at enzyme loci (Hebert & Beaton, 1989). After a pilot study on 27 loci, 6 polymorphic enzymes were routinely screened (AAT, G6PDH, GPI, IDH1, IDH2 and PEP-Z). These loci were chosen because they could be easily interpreted and scored. Fig. 2 illustrates cellulose acetate zymograms for two of these variable enzymes of *C. problematicus* along with their genotypic interpretation. For electrophoresis, two legs of each beetle were crushed in 50 µl of distilled water, while the rest of the body was kept in absolute ethanol for future DNA extraction or for morphometric studies. The same populations were also typed with an automated sequencer for 4 variable microsatellite markers in DNA-samples extracted from one leg of each beetle. We recently developed these markers (Gaublomme *et al.*, 2003) in co-operation with the INRA (Centre de Biologie et de Gestion des Populations, Montferrier-sur-Lez, France). Microsatellite primer sequences and polymerase chain reaction protocols are given elsewhere (Gaublomme *et al.*, 2003). For more details on field sampling, electrophoresis and standard software for population genetic analyses, we refer to Desender & Verdyck (2001) and Desender *et al.* (1998, 2004). Hierarchical 'Analysis of Molecular Variance' (AMOVA), implemented in Arlequin 2 (Schneider *et al.*, 2000), was used to partition and test the genetic variance between eco-regions, between populations within regions and within populations. To this end, the southernmost Belgian population was omitted from the analysis, because only one population was available from that region. Genetic distance between populations from different eco-regions was visualised in a UPGMA dendrogram, based on Nei's 78 unbiased distance (Nei, 1978).

Table 1. AMOVA-results based on all enzyme and microsatellite loci of 10 populations of *C. problematicus* in three eco-regions in Flanders (Campine, Brabant and E&W Flanders).

Source of variation	*d.f.*	Sum of squares	Variance components	Percentage of variation	Significance
Among eco-regions	2	45.138	0.08331	4.68	$p<0.004$
Among populations within regions	7	41.550	0.07124	4.00	$p<0.000$
Within populations	596	969.458	1.62661	91.32	$p<0.000$
Total	605	1056.147	1.78115		

Results

Genetic differentiation of *C. problematicus* between populations and eco-regions

The population genetic data showed no deviations from Hardy-Weinberg equilibrium and no linkage disequilibrium, which means that the studied enzyme as well as microsatellite loci can be used as independent markers. Overall, we observe a high degree of genetic structuring between the studied populations of *C. problematicus.* Genetic differentiation estimates between 11 populations yield an overall F_{st} (6 variable enzymes) of 0.076 ($p<0.0001$) and an overall R_{st} (4 microsatellites) of 0.106 ($p<0.0000$) or F_{st} of 0.130 ($p<0.0000$). As much as about 10% of the total genetic variation can thus be attributed to differences between populations. Allozyme differentiation based on results for 24 populations is 0.069 ($p<0.0001$), which is comparable to the F_{st} obtained for the smaller dataset.

Table 1 shows the results of a general AMOVA based on all enzyme and microsatellite loci for 10 populations of *C. problematicus* in three distinguished eco-regions in Flanders (single southernmost Belgian population omitted for this hierarchical analysis of variance). There is highly significant genetic variation between regions, between populations within regions and within populations. Overall, a somewhat higher part of the total genetic variation (4.7%) originates from differences due to eco-region as compared to 4% of the genetic variation between populations within regions, but both values are highly significant.

Patterns of allelic variation obtained for individual markers

Fig. 3 illustrates different patterns of allelic distribution obtained for the enzymes PEP-Z (four alleles) and IDH2 (two alleles) and for two microsatellites: Cpro97 with four alleles and the hypervariable Cpro98 with 17 alleles. The observed patterns are projected against a map of Belgium with the schematised distribution area of *C. problematicus*.

For the enzyme PEP-Z (Fig. 3A), we observe a pattern, typical for overall ‘isolation by distance’ effects (larger genetic difference at a larger geographic distance), obvious because of the highly differentiated Gaume population (Southern Belgium), with completely different allelic frequencies. In addition, one of the isolated ancient forests in the westernmost part of

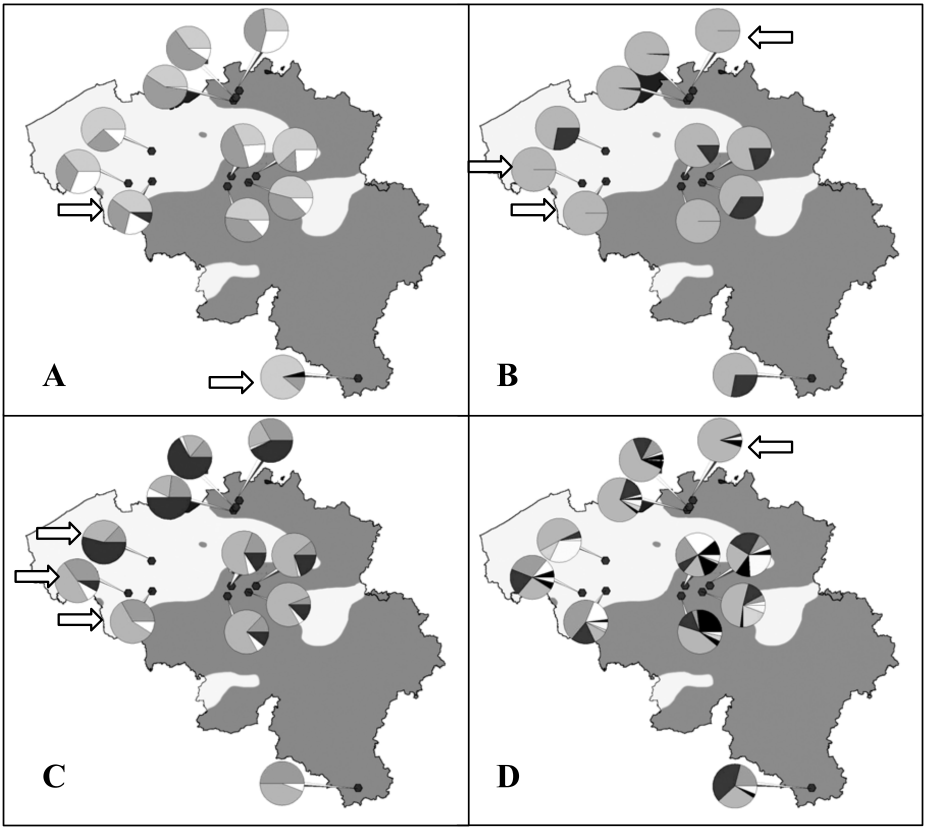

Figure 3. Different patterns of allelic distribution obtained for the polymorphic enzymes (A) PEP-Z, four alleles and (B) IDH2, two alleles and the microsatellites (C) Cpro97, four alleles and (D) Cpro98, with 17 alleles; arrows indicate deviating populations (see text for further explanation); background map of Belgium with schematised distribution area of *Carabus problematicus*.

the distribution area shows a unique allele for Flanders. This rather unexpected result could be an indication for local adaptation and/or the occurrence of relic alleles in this forest.

For IDH2 (Fig. 3B), we observe a number of populations completely fixed for one of the two alleles. This occurs in two of the westernmost populations and could be an indication of genetic drift in smaller isolated populations, whereby the initially rarer allele was lost. The same phenomenon is also observed in the recent forest population from the Campine eco-region. Here, this pattern suggests that recent colonisation took place by a single or a few individuals, carrying only a restricted amount of genetic variability (low genetic diversity because of so-called founder events).

The pattern of allelic distribution obtained for the microsatellite Cpro97 (Fig. 3C) is different from those already described above for enzymes. Based on this marker, isolated ancient forest populations in the westernmost region show higher levels of differentiation between populations as compared to samples in other eco-regions of the more continuous distribution area. This strongly suggests an important influence on genetic structure of isolation (and lack of current gene flow) between these isolated ancient populations in the West and East Flanders eco-region.

The hypervariable microsatellite Cpro98 locus shows two basic patterns (Fig. 3D), indicative of a fast-evolving genetic marker. First, each population shows a more or less unique combination of allele frequencies, indicating a high level of differentiation (rapid evolution and low level of recent gene flow) between all populations even at short distances. Nearly all populations can be characterised by distinct frequency combinations of the numerous alleles in this locus. Second, the single exception, where a much lower genetic diversity (few alleles, dominance of a single allele) is observed, is again the more recent population studied in the Campine region. This last result again suggests the importance of founder events in the colonisation of more recent Campine forests.

Genetic similarity between ancient and recent forests in different eco-regions

The strongly suggested interaction between population genetic structure and eco-regions is further explored in cluster analyses based on genetic distance estimates between *C. problematicus* populations. Fig. 4A shows a genetic distance UPGMA dendrogram (Nei's 78 unbiased distance) based on all investigated enzyme and microsatellite loci for 11 populations of *C. problematicus* from the four regions in Belgium. A similar dendrogram is given in Fig. 4B based on data from 24 populations, studied for genetic variability at the enzyme loci only.

In these dendrograms, populations are mostly grouped within their respective eco-region, with the exception of the isolated populations in the westernmost part of the current distribution area (eco-region 2) of *C. problematicus*. Interestingly, these populations do not cluster together, but rather are scattered between populations of different other eco-regions. The dendrogram based on 24 populations confirms this pattern and moreover shows the special position of populations in more recent forests of the Campine Region, now including two replicate study sites. Populations from Central Belgium (eco-region 3) are clustered, as well as those from Southern Belgium, but in the last-mentioned region there is now a higher observed genetic distance between the additionally studied populations. Microsatellite typing of some of these populations will be performed in the future to study this pattern in further detail, along with data on populations from more distant study sites outside Belgium.

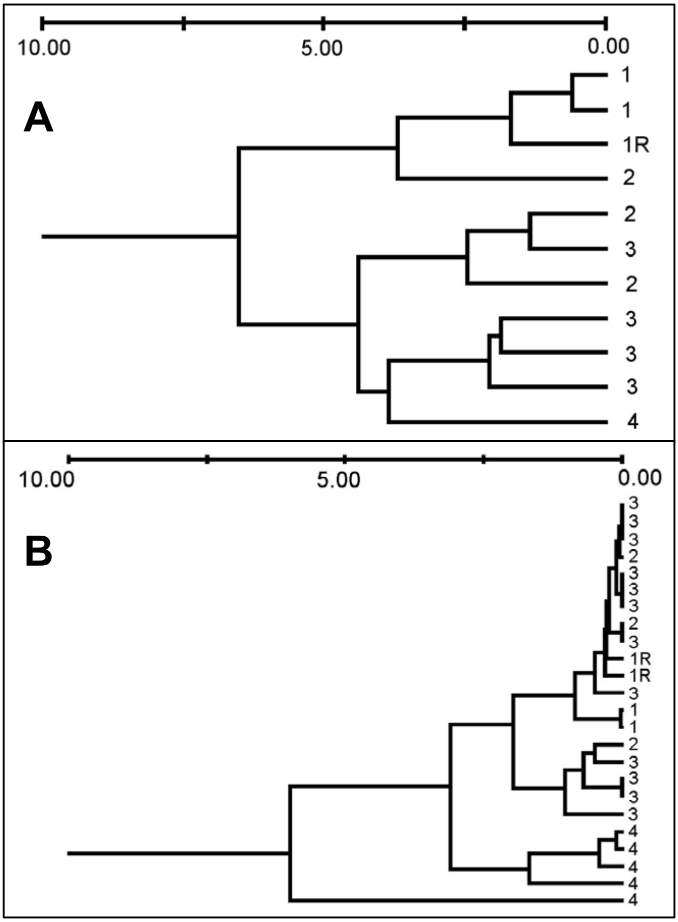

Figure 4. Genetic Distance UPGMA dendrogram (Nei's 78 unbiased distance) based on (A) all investigated enzyme and microsatellite loci for 11 populations, and (B) all investigated enzyme loci for 24 populations of *Carabus problematicus* from four regions in Belgium: 1: Campine region, 1R: population in recent forest, 2: isolated populations in westernmost distribution area, 3: populations in Central Belgium, 4: population from the Gaume region (Fig. 4A) or from different parts of southern Belgium (Fig. 4B). Cf. Fig. 1B for geographic position of the populations.

Discussion

On the relatively small geographic scale of this study, we observe that as much as about 10% of the total genetic variation can be attributed to differences between *C. problematicus* populations. This is a high value in comparison to other organisms studied earlier, certainly when taking the relatively small geographical scale of our study area into account (Ward *et al*., 1992). A comparison with other forest ground beetles shows that the observed genetic structure is moderate to high. The rather eurytopic forest beetle *Abax ater* (Villiers, 1789) showed a significant differentiation between populations (based on allozymes), mainly as a result of reduced gene flow at a relatively large spatial scale (Desender *et al*., 1999). The F_{st} in *A. ater* was estimated at 0.030 only (but statistically highly significant), whereas genetic erosion could not (yet) be observed for this species. Butterweck (1998) obtained a very comparable F_{st} estimate of 0.028 for the same species in a German study area, while also reporting comparable values for the rather eurytopic forest species *Carabus nemoralis* Müller, 1764 ($F_{st} = 0.033$) and *Pterostichus oblongopunctatus* (Fabricius, 1787) ($F_{st} = 0.031$). Brouat *et al.* (2003) performed a genetic study at local scale; they did not mention overall F_{st} values, but obtained estimates between population pairs up to 0.030 for *C. nemoralis* and up to 0.07 for *C. punctatoauratus*. *Pterostichus cristatus* (Dufour, 1820), a typical forest valley carabid species from ancient forests, also showed relatively low (but statistically significant) differentiation estimates, ranging between 0.014 for Belgian populations up to 0.069 when including populations from France and Italy (Desender *et al*., 2002b). Keller & Largiader (2002) studied Swiss populations of *Carabus violaceus violaceus* Linnaeus, 1758 near Bern by means of microsatellites and obtained a relatively low F_{st} of 0.026. *Carabus glabratus* Paykull, 1790, a highly specialized beetle from ancient forests in Germany, showed a much higher F_{st} of 0.168 (Assmann & Günther, 2000). *Carabus auronitens* Fabricius, 1792, to our knowledge the first ground beetle that was studied simultaneously by enzymes and microsatellites (Desender *et al*., 2002c; Drees, 2003), was also investigated in the region of Flanders. This forest carabid showed extreme genetic differentiation with an F_{st}-estimate of 0.490, as far as we know the highest value ever recorded for a ground beetle. That study showed a clear example of isolation by non-distance in relation to forest historical ecology (Desender *et al*., 2002c). Results from both types of genetic markers suggested that genetic drift (loss of genetic variability in small populations), reduced gene flow (as a consequence of increased isolation) as well as local adaptation in conjunction to forest history all could help to explain the observed patterns of genetic variation. Drees (2003) compiled F_{st} estimates for *C. auronitens* in Germany, ranging between 0.040 and 0.304 according to the dataset used. Rasplus *et al*. (2001) obtained a high mean F_{st} value of 0.312 for *Carabus solieri* (including different subspecies) from the French and Italian southern Alps.

To summarise, our estimates of genetic differentiation in *C. problematicus* belong to the higher values obtained for an array of 13 forest ground beetle species studied in the same region (Desender, 2005).

We conclude that different individual markers clearly show divergent patterns of allelic distributions for the same studied populations of *C. problematicus*. The results of these individual markers suggest locus-specific underlying processes, responsible for the observed genetic constitution of the populations. As such, they confirm the need to study different markers in order to elucidate the patterns and processes of genetic variation, a suggestion made a.o. by Arnaud-Haond *et al.* (2003). Our data show that separate interpretation of individual markers also can be helpful, most probably as a consequence of different speed of evolution linked to individual markers. Hypervariable markers are fast-evolving and therefore ideal candidates for showing the effects of recent processes, such as those related to recent habitat fragmentation and deterioration. On the other hand, it is not to be neglected to study at the same time more slowly-evolving markers, such as polymorphic enzymes with a limited number of alleles. Such loci may more easily show the results of other processes, for example loss of alleles through genetic drift in temporarily small populations or patterns of long-term colonisation and differentiation or local adaptation by natural selection. Allozymes also have been related to postglacial recolonisation processes in a number of large scale studies on *Carabus* species (cf. Turin *et al*., 2003).

Our results on *C. problematicus* in many cases differ strongly between eco-regions, as is confirmed by the cluster analyses, but also relate to forest history. Genetic similarities between forest populations from the same eco-region are not always higher as between eco-regions. This is especially true for some isolated forests in West and East Flanders. The special position taken by these ancient forests in the westernmost part of the current distribution area of *C. problematicus* suggests that those populations are of particular conservation genetic interest and are high-priority areas for regional conservation.

The genetic results on *C. problematicus* show a relatively high amount of genetic differentiation, but here, genetic structure as well as diversity appear to depend on or interact with eco-region, forest age as well as forest size (cf. Desender *et al.,* 2004). On the one hand the strong influence of forest history could be a consequence of the extreme degree of forest fragmentation that took place in the major parts of Flanders, on the other hand it could have resulted from the very long history of this fragmentation. These aspects must have important regional conservation genetic implications. In NW and C Europe, *C. problematicus* is rather abundant in many regions and almost nowhere endangered (Assmann, 1999; Turin *et al*., 2003). In many parts of Flanders, however, it is threatened to a high degree. Another conclusion from our study is therefore that conservation (genetic) values can differ dramatically for the same species according to the region and even on a relatively small geographic scale. At least in Flanders, *C. problematicus* therefore can be considered a powerful model species with many possible applications for conservation genetic monitoring at different levels of perception.

Acknowledgements

Regional and national nature conservation and forestry authorities allowed us to sample invertebrates in their forests. B. De Vos (IBW), A. Drumont, V. Choquet, and V. Versteirt (RBINSc), and J.Y. Rasplus (INRA, Montferrier-sur-Lez, France) are acknowledged for their help and support in various ways. This study was supported partly by the RBINSc (Dept.

Entomology) and through financial support by the Flemish Government (AMINAL, Dept. Bos & Groen, projects B&G/15/96 and B&G/29/98; AMINAL/Natuur/ project VLINA 00/015) and the Federal Government (DWTC, project MO 36/006).

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Effect of canopy closure of a young Norway spruce plantation on ground beetles

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Abstract

The effect of canopy closure on carabids was studied by sampling a young Norway spruce plantation for several years, and comparing it to a 15y old stand. We sampled ground beetles in the Bükk Mountains in Northern Hungary by pitfall traps. The first stand, a young Norway spruce (*Picea abies*) plantation was sampled 5, 6 and 8 years after planting. A second stand, a 15 years old forest of the same kind was sampled only in 1998. In the earliest year studied (year 5) of the plantation, there were relatively large open gaps. Shrubs of the native beech forest, grasses and common weeds were present. In years 6 and 8, the canopy started to close, spruce became dominant over shrubs and the herbs almost disappeared. *Bryum* mosses covered the soil surface in the 15-years old plantation. Habitat generalist species had rather constant abundance throughout the three-years of sampling. However, the proportion of the open habitat species decreased in the 8-years old phase, and the proportion of the forest species increased.

Key words: Open gaps, forest species, heterogeneous environment, Norway spruce

Introduction

In Hungary, planting non-native tree species was the preferred way of reforestation in the 1960s. Today, these stands constitute 45% of all Hungarian forests (Mátyás, 1996). The most often planted trees are non-native and include the black locust (*Robinia pseudo-acacia*), Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), black pine (*Pinus nigra*), and different species of poplar (*Populus spp.*). Clear-felled deciduous forests in the hilly regions of Hungary were also replaced with non-native conifer trees, mainly Norway spruce. This species is favoured by foresters because of its high productivity and rapid growth. The reforestation after clear-cutting starts with grubbing (removal of tree trunks and roots) and deep loosening the soil. This practice drastically damages the native fauna and flora, and alters the microclimatic conditions, leading to spatial homogenization of these habitats.

One of the common forest-living arthropod groups affected are ground beetles (Coleoptera: Carabidae). Ground-dwelling carabid beetle larvae develop in the soil (Lövei & Sunderland, 1996) and are consequently sensitive to soil disturbance (Desender *et al.*, 1999; Magura *et al.*, 1997, 2001, 2002; Koivula *et al.*, 2002).

Assemblages of carabids change remarkably after reforestation. In planted, near-monoculture forests, only the habitat-generalist and forest-generalist carabid species are abundant (Szyszko, 1987; Baguette & Gérard, 1993; Niemelä *et al.*, 1993; Elek *et al.*, 2000).

The above studies have concentrated on long time scale and broad patterns, and less on the fine spatio-temporal scale. The mentioned studies concentrated on relatively long time scale, and did not pay attention to short time period (but see Koivula, 2002). We hypothesize that carabid beetles are able to perceive the fine-scale changes in the biotic and abiotic conditions of the area. These small changes may affect carabid species composition and abundance.

To study the carabids of young Norway spruce plantations, we carried out a follow-up study at stand age of 5, 6 and 8 years after clear-cutting and plantation of spruce. As a reference point, a 15-years old plantation was selected to compare the young plantation against an older one. That stand was homogenous (to the human eye), with closed canopy and few herbs or shrubs. Such forests usually have a thick layer of needle-leaf litter due to the acidic soil and slow litter decomposition (Magura *et al.*, 2002, 2003). These conditions can limit the resources, mainly the prey abundance for carabids.

We examined the following parameters of carabid assemblages. (1) The proportions of different carabid-species affinity groups in the different-aged plantations. We expected that the older stand should harbour fewer open habitat specialists and more forest species. (2) The assemblage composition may differ with respect to the overwintering strategy of the species. A high number of species overwintering as larvae would suggest suitable (stable) soil conditions for the larval development. Thus, we expected the share of the larval-overwintering species to be higher in the older stand. (3) We also expected changes in the overall flight ability of the assemblage. The proportion of the winged species should intuitively be higher in the younger stand, as these species are often typical for early successional phases of regenerating stands (e.g. Koivula *et al.,* 2002).

Material and methods

The study was carried out in the Northern Hungarian Mountain Range, at the entrance of the Hor-valley in the Bükk National Park (48° 05' N, 20° 37' E). We selected a Norway spruce (*Picea abies*) plantation and followed the development of its carabid assemblage for three years.

(1) In 1998, the plantation was 5 years old, with an open canopy. Due to the mechanical soil preparation before planting of spruce, grasses, herbs, and other species typical for open habitats still dominated the dense herb layer, while the shrub layer was moderate.
(2) The same plantation was re-sampled in 1999. The canopy had already started to close. The herb and shrub layers did not show marked change.
(3) In 2001, this plantation was 8 years old, the herbs and shrubs had become sparse. The canopy was almost closed, and the herbs and shrubs layers were scarce. The needle litter cover was not remarkable.
(4) For comparison, a 15-years old stand with a similar origin was selected and sampled in 1998. In this stand, the herbs and shrubs were sparse, and a layer of mosses covered the soil. The ground had a thick needle litter cover.

Both plantations were established after the clear-cutting of a beech (*Fagus sylvatica*) forest. Soil preparation, grubbing and deep loosening was applied. Trees were planted in rows. The between-row distance was 2 m, and the average distance between trees was 3 m. Both stands were on a NW slope; 3 km apart from each other. Both stands were ca. 5 ha, and can thus be considered large enough to host self-supporting populations of carabids and not only immigrants from the surrounding habitats (Mader, 1984).

We used plastic pitfall traps (diameter 100 mm, volume 500 ml), partly filled with 75% ethylene glycol and a drop of detergent. The traps were covered with pieces of bark to protect them from litter and rain. In both study stands, 10 pitfall traps, in groups of 5, were placed randomly. Individual traps were 10 m apart from each other, and at least 40 m from the nearest forest edge to avoid edge effect (Murcia, 1995). Trap distance is an important factor in carabid studies. Digweed *et al.* (1995) found that the catch of traps placed less than 10 m from each another were not independent. To test for spatial independence in our trapping, we examined the similarities of the individual traps at different (10 - 90 m) distances from each other. The average similarity showed no trend with increasing distance (data not shown) and we concluded that our traps could be considered independent of each other.

Traps were checked monthly between March and November in 1998, 1999, and 2001. Carabids were transferred to 70% alcohol, and identified to species in the laboratory, using keys in Hůrka (1996). Voucher specimens were deposited in the Department of Ecology, University of Debrecen, Hungary. For the numerical analysis, we pooled samples from the same stand and different months in each year. The seasonal total of a species over the whole growing season gives a good estimate of its abundance (Baars, 1979).

To study the effects of canopy closure on carabids, species were categorized according to their habitat affinity, based on Hůrka (1996). We used three categories: forest, open-habitat and generalist species. Species were also categorized according to their overwintering strategy: adult or larval overwintering according to Hůrka (1996) and Thiele (1977), and flight capacity (flightless, dimorphic or flying species, based on Thiele (1977)). Differences in the number of species in classes of habitat affinity, overwintering and flight ability were tested by Kruskal-Wallis non-parametric ANOVA and Tukey-type multiple comparisons. The analyses were carried out using the SPSS-PC program.

Results

A total of 1548 carabids was trapped, belonging to 38 species (Table 1). Results of the non-parametric ANOVA proved that there was a significant variation among the habitats in the number of carabid species per trap (H= 21.500, *d.f.*= 3, 64, *p*<0.0001; Fig. 1) and the number of individuals per trap (H= 21.709, *d.f.*= 3, 64, *p*<0.0001; Fig. 1). The Tukey-test showed that the number of species as well as the number of individuals (both p<0.001) were significantly higher at year 8 than in other plantation age.

The number of generalist species, forest species, and open habitat species proved to be significantly different among habitats (non-parametric ANOVA, generalists, H= 6.498, *d.f.*= 3, 64, *p*<0.0001 Fig. 2; forest sp. H= 33.805, *d.f.*= 3, 64, *p*<0.0001; Fig. 2; open-habitat sp. H= 25.009, *d.f.*= 3, 64, *p*<0.0001; Fig. 2). The number of generalist species was highest in the 6-years-old plantation (Tukey-test, *p*<0.01), the number of forest species were significantly higher in the 8-years-old plantation (*p*<0.0001), and the number of open habitat species were highest in the 5 and 6-years-old plantations (*p*<0.002).

There was a significant variation in the number of adult - overwintering species (non-parametric ANOVA, H= 16.842, *d.f.*= 3, 64, *p*<0.0001; Fig. 1). The number of adult overwintering species was highest in the 8-years-old plantation (Tukey-test, *p*< 0.015).

Table 1. Selected life history characteristic of the ground beetles captured in different-age. Norway spruce plantations in Northern Hungary. The species are arranged according to their habitat affinity and overwintering stage.

Species	Habitat affinity	Over-wintering stage	Flight capacity	Number of beetles trapped in forest clear-cut			
				5y ago	6y ago	8y ago	15y ago
Abax carinatus	forest	adult		1	0	0	0
Pterostichus anthracinus	forest	adult	macropterous	5	5	8	0
Abax paralellus	forest	adult		11	9	45	8
Aptinus bombarda	forest	adult		1	13	7	0
Carabus convexus	forest	adult		6	2	55	3
Notiphilus biguttatus	forest	adult		0	2	0	0
Pterostichus oblongopunctatus	forest	adult		2	2	14	3
Carabus nemoralis	forest	adult		0	3	30	5
Agonum assimile	forest	adult	macropterous	0	1	0	0
Carabus violacues	forest	larva		14	12	132	5
Carabus coriaceus	forest	larva		10	9	2	1
Carabus glabratus	forest	larva		12	2	103	5
Carabus hortensis	forest	larva		3	4	58	81
Cychrus attenuatus	forest	larva		0	0	0	1
Cychrus caraboides	forest	larva		0	1	26	0
Calosoma inquisitor	forest	larva	macropterous	0	0	1	0
Pterostichus ovoideus	generalist	adult		3	5	0	0
Carabus cancellatus	generalist	adult		0	0	6	0
Stomis pumicatus	generalist	adult		1	1	0	0
Abax ater	generalist	adult		34	26	187	174
Molops piceus	generalist	adult		34	38	17	3
Harpalus marginellus	generalist	larva	macropterous	1	0	0	0
Platyderus rufus	generalist	larva	macropterous	5	1	0	0
Pterostichus niger	generalist	larva	macropterous	37	41	20	23
Harpalus latus	generalist	larva	macropterous	4	15	0	0
Pterostichus melanarius	generalist	larva		13	15	22	46
Metophonus punctatulus	generalist	larva	macropterous	2	1	0	0
Amara aenea	open	adult	macropterous	1	0	0	0
Amara communis	open	adult	macropterous	4	7	0	0
Amara curta	open	adult	macropterous	0	1	0	0
Amar littorea	open	adult	macropterous	1	1	0	0
Amara similata	open	adult	macropterous	0	2	0	0
Panagaeus bipustulatus	open	adult	macropterous	0	2	0	0
Harpalus rubripes	open	larva	macropterous	0	0	1	0
Harpalus rufipes	open	larva	macropterous	20	4	0	1
Semiophonus sigmaticornis	open	larva	macropterous	1	1	0	0
Synuchus nivalis	open	larva	macropterous	1	1	0	0
Harpalus samargdinus	open	larva	macropterous	0	1	0	0
Total number of individuals				270	228	734	359

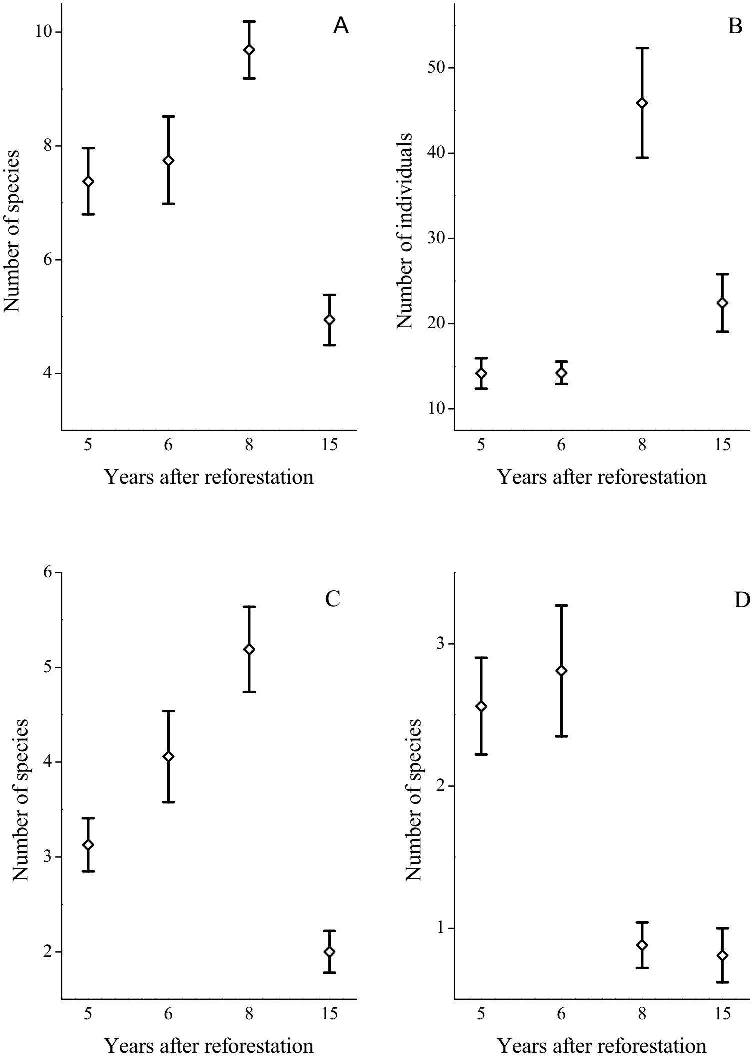

Figure 1. Comparisons of species richness per trap (A), activity density per trap (B), and selected bionomic characteristics (number of adult-overwintering species per trap – C; number of macropterous species per trap – D) among carabid assemblages in different-aged spruce plantations in Northern Hungary. Bars indicate ± one standard error.

Examination of the flight capacity showed a significant difference among habitats (non-parametric ANOVA, H= 23.827, *d.f.*= 3, 64, p<0.0001; Fig. 1). The number of winged species was lowest in the 8 and 15-years-old plantations (Tukey-test, p<0.017).

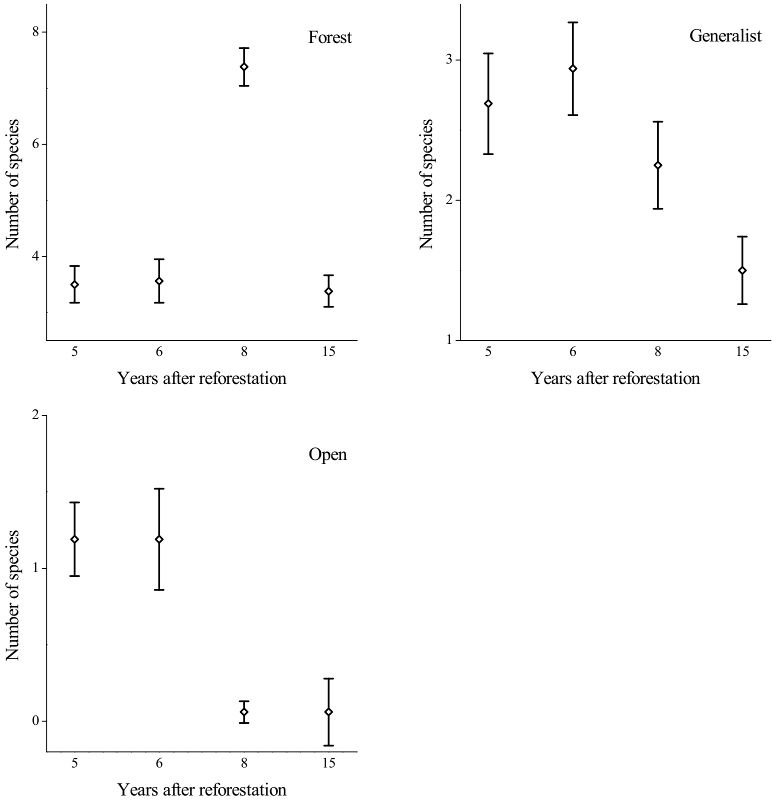

Figure 2. Average number of individuals/trap of forest specialist ("forest"), open-habitat specialists ("open") and generalist ("generalist") species in spruce plantations of different age in Northern Hungary. Bars indicate ± one standard error.

Discussion

The age of the plantation seemed to affect the ground beetle assemblages via habitat structure. Not surprisingly, the 5-y old and 15-y old forests had different assemblages. The degree and nature of such a difference may be influenced by small scale geographic differences, succession, or habitat structure, or a combination of these. Young spruce plantations are patchy, containing open areas with shrubs and a dense herb layer. Open habitat species were abundant in the young plantation. As the developing canopy closes, species that prefer mature forests may appear. This seemed to happen 6-8 years after the establishment of the plantation. This recolonisation is possible because the soil pH is less acidic than under mature spruce trees. The herb and shrub layer is, although becoming more sparse, still present, and provides more food and shelter than the bare ground in mature forest. The thinning of the herbs is the reason that the open habitat species are missing from this stand: they need larger gaps and a more dense herb layer. The deciduous forest species seek out these patches because these conditions are similar to the ones in a (native) deciduous forest, the natural habitat of these species. Due to the lack of proper replication in our study, this cannot be proven, but merits attention.

Theoretically, the fastest colonization is by flying. The high numbers of winged beetles in the 5 and 6 years old plantations, compared to the 15-years old one, can be explained by the colonization of a recently logged stand by winged species that occur in clear-cut boreal (Niemelä *et al*., 1993; Koivula, 2002) and temperate conifer plantations (Baguette & Gérard, 1993; Butterfield, 1997). Beetles that colonize such patches of new habitat need to be good colonizers.

Previous studies have shown that in older plantations generalist species dominate (Baguette & Gérard, 1993; Butterfield, 1997; Elek *et al.,* 2000; Magura *et al*., 2002). We found some evidence that before the complete closing of the canopy, deciduous forest species are dominant, as they were abundant in the 8 years-old plantation. Forest species thus seem to colonize older spruce plantations while herbs and shrubs are still present. The complete closure of the canopy reduces the number of herbs and shrubs, and it may consequently decrease the abundance and species richness of deciduous forest carabids. We found no evidence for larval overwinterers to be less abundant in the young stand compared to the older (15-years old) one, which would possibly have indicated that these carabids suffer from soil alteration but might recover later. However, previous studies (Thiele, 1977; Butterfield, 1997; Magura *et al.,* 2002, 2003) have emphasized that the number of ground beetles that overwinter as adult can be higher in habitats with more variable conditions, compared to stable habitats.

Acknowledgements

The authors would like to express their thanks to the Directorate of the Bukk National Park to give an opportunity to carry out this research work and to Dr. Gabor Lövei for comments which improved the manuscript.

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Food preferences and food value for the carabid beetles *Pterostichus melanarius*, *P. versicolor* and *Carabus nemoralis*

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Abstract

Assuming generalist feeding habits to be the original condition in carabid beetles, we tested two alternative hypotheses about the evolution of food specialisation: that species specialised on prey that 1) were already of high quality to the generalists, or 2) were of low quality to the generalists due to chemical or other defences. We did this by evaluating in laboratory experiments the food quality to three generalist carnivore species of various prey types, selected among those on which carabids are known to have specialised (i.e. insects, earthworms, molluscs, seeds). The carabids used were adults of *Pterostichus melanarius* Illiger, *Pterostichus versicolor* Sturm and *Carabus nemoralis* Mueller. Insects were high-quality food, earthworms were intermediate, and slugs and seeds were low quality food for all species. For *C. nemoralis,* earthworms were the most preferred prey. The results support the second hypothesis, that food specialisation evolved by breaking the defences of low quality but presumably abundant prey.

Key words: Coleoptera, Carabidae, ground beetles, diet, food specialisation

Introduction

Carabid beetles are among the most common predatory invertebrates in agricultural fields and are considered biocontrol agents of different pests. Therefore their habitats, biology, and feeding ecology have been intensively studied. Carabid beetles are mostly polyphagous feeders, which consume a variety of living or dead animal and plant material (Lövei & Sunderland, 1996; Toft & Bilde, 2002). However, feeding specialisations have evolved in several groups of carabid beetles (review in Toft & Bilde, 2002) including mollusc feeders (Hengeveld 1980a,b), earthworm feeders (Symondson *et al.,* 2000), seed feeders (Jørgensen & Toft, 1997a,b), and specialist insect hunters (e.g. Bauer, 1981).

Some generalist carnivore carabids have extremely broad diets, feeding on insects, earthworms and molluscs in varying proportions, but the benefits derived from each of these components are not well known. In spite of this, e.g. some *Carabus* species have been referred to as earthworm and mollusc specialists (Hengeveld, 1980 a,c). Earthworms have been found in high frequencies in the stomach contents of some generalist carabid beetles (Symondson, 1994). Also *Abax parallelepipedus* larvae can be reared on a pure earthworm diet (Symondson, 1994). This might indicate that earthworms are high quality food for some generalist species. Many carabid beetles consume varying amounts of plant material (Johnson & Cameron, 1969; Goldschmidt & Toft, 1997), but the importance of seeds as component in the diet of the generalist species is still unknown. Conflicting evidence exists on the role of slugs for generalist carabid beetles: Symondson *et al*. (1996) and Bohan *et al*. (2000) claim a high preference for slugs in some carabids, while Mair & Port (2001a,b) report the opposite for related species.

The aim of the current study was to evaluate two alternative hypotheses about the evolution of feeding specialisation in carabid beetles raised by Toft and Bilde (2002). The first one suggests that the food types on which some carabids have specialised tend to be high quality food for the generalist feeders. In contrast, the second hypothesis suggests that the specialist feeders have specialised on food types that are generally of low quality to generalists due to chemical or other defences. Our approach assumes that generalist feeding habits are the original condition in carabid beetles (Lindroth, 1992; Lövei & Sunderland, 1996) and that present generalists reflect this situation. Therefore we investigated the preferences and the value of different food types as food for adults of the generalist species *Pterostichus melanarius* Illiger, *Pterostichus versicolor* Sturm and *Carabus nemoralis* Mueller. The food types tested were selected to represent those on which some groups of carabids have specialised, i.e. insects, earthworms, slugs, snails and seeds. Adult Diptera and grasshoppers were selected to represent the insects mainly because they are easily obtained from laboratory cultures, but adult Diptera are also natural prey of several species (Sunderland, 1975; Kromp, 1999). The possibility exists that prey may be insufficient as the only food, but make a positive contribution to predator fitness as part of a mixed diet. We therefore tested the selected prey types both in pure and in mixed diets.

Materials and methods

Study species

Pterostichus versicolor, 8-12.5 mm in length, is a day-active beetle, breeding from April to July. Larvae develop from May to September (Van Dijk, 1994). It is a polyphagous species (Hengeveld, 1980 b; Bruinink, 1990), consuming Diptera, aphids, ants, and larvae of Lepidoptera and Coleoptera (Bruinink, 1990).

Carabus nemoralis, 22-26 mm in length, is a nocturnal species breeding in April. In some studies *C. nemoralis* and other *Carabus* species are referred to as generalist feeders (Digweed, 1994; Turin *et al.,* 2003) while in others they are referred to as specialist feeders preferring molluscs and earthworms (Ayre, 2001; Hengeveld, 1980a,c). Digweed (1994) demonstrated that *C. nemoralis* was able to follow mucus trails of slugs (*Deroceras reticulatum*) and earthworms. This might indicate a preference for such prey.

Pterostichus melanarius, 12-18 mm in length, is a nocturnal species breeding in August-September. It is a highly polyphagous species (Davies, 1953; Pollet & Desender, 1985; Lindroth, 1985/6). Pollet & Desender (1985) reported up to 49 different prey types (at family level) as food for *P. melanarius*, including lumbricids, caterpillars and many other insect groups, as well as plant material. It has been reported to preferentially feed on slugs and to have a significant impact in slug control (Symondson *et al*., 1996; Bohan *et al*., 2000).

Food and prey preparation

The following prey types were used in the experiments: adult houseflies *Musca domestica*, adult fruit flies *Drosophila melanogaster*, grasshoppers *Locusta migratoria*, earthworms, mainly *Lumbricus terrestris*, slugs *Deroceras reticulatum*, snails *Cepaea hortensis*, and seeds of *Taraxacum* sp. Fruit flies or houseflies were used as supposedly high-quality comparison prey in all experiments, as they are highly preferred prey for many generalist predators (Pollet & Desender, 1985; Bilde *et al*., 2000; Toft & Bilde, 2002).

Fruit flies and houseflies were obtained from laboratory cultures. Grasshoppers were bought from a commercial supplier. Earthworms, slugs, snails and seeds were collected from the field. All prey types used were freeze-killed, and earthworms, slugs and snails were cut into small pieces, before being offered.

Standardisation period

The three carabid species were collected in a field at Stjær near Århus, Denmark, between April-July 2002. Beetles were kept in plastic boxes for 1-2 weeks under laboratory conditions before the standardisation period started. Each box contained pieces of wet cotton, leaf litter or wet tissue for shelter, and *ad libitum* dog food (Techni-Cal Maintenance®, Martin Group, Canada). For *P. melanarius,* houseflies were added to minimise cannibalism.

All beetles underwent a 5-days nutritional standardisation procedure before starting the experiments. During these five days beetles were placed individually in Petri dishes (14 cm Ø) with a wet cotton wad. The first 2-3 days the beetles were supplied with food *ad libitum*: *P. versicolor* was offered a diet of dog food and fruit flies; *C. nemoralis* a diet of dog food and houseflies; and *P. melanarius* a mixed diet of all food types used in Experiment 3. Food items were served in small dishes. The remaining 2-3 days the beetles were starved. *P. versicolor* and *C. nemoralis* were kept at room temperature (20- 24ºC) and ambient photoperiod about 16L: 8D. *P. melanarius* was kept in an incubator at 19ºC and a photoperiod of 16L: 8D. Only female *P. versicolor* and *P. melanarius* were used, while for *C. nemoralis* both males and females were used. In all experiments the species were kept under the same temperature and light conditions as during the standardisation period.

Experiment 1: Egg production of *P. versicolor*
Egg production was measured over two weeks (May 2002) on four different diet groups (N = number of replicates): fruit flies (N = 6), earthworms (N = 5), slugs (N = 6), and a mixed diet of the three (N = 6). Prey was renewed every second day to provide *ad libitum* food supply.

During the egg production experiment the females were placed individually in Petri dishes (14 cm Ø). Each dish was divided into two parts by a low barrier of silicone that allowed the beetles to move freely in the dish. One half was filled with moist sand for egg laying. A small glass dish (19 mm Ø) with food was placed in the other half. For egg counting, eggs were sieved every 2-3 days through a 1.2 mm sieve using a gentle stream of tap water (Mols *et al.* 1981). Eggs were counted under the binocular microscope. Fresh, moist sand and food were added to the breeding dishes at the same time before females were returned. Midway in the experiment, males were added to every female, and removed after 6h. Each female was weighed before and after the egg production experiment to determine the body mass change.

Experiment 2: 24-h food consumption measurements with *C. nemoralis*
In May 2002, beetles were divided randomly into four different diet groups: houseflies (N = 10), earthworms (N = 9), slugs (N = 9), and a mixture of the three (N = 6). In the pure diets the amounts offered for each beetle were 25 houseflies, or 1.5 g of slugs or earthworms. The amounts offered in the mixed diet were 10 flies and 0.7 g of both slugs and earthworms.

To determine the consumption during the 24-h (dry mass) prey samples were weighed before being offered (wet mass) and multiplied by a previously established dry mass/wet mass conversion factor to obtain the dry mass of food offered. Food remains were dried in the vacuum oven at 60 ºC for at least 5 days and weighed. The 24-h dry mass consumption was calculated by subtracting the amount of dried food remains from the calculated dry mass of food offered at the beginning of the 24-h measurements. To determine the conversion factor for each prey type, six samples of c. 0.6 g of slugs and earthworms and six samples of 10 houseflies were dried in the vacuum oven at 60 ºC for at least 5 days. Each beetle was weighed before and after the 24-h to determine its mass change. All treatments were run simultaneously.

Experiment 3: 24-h food consumption measurements with *P. melanarius*
In July 2002, females were divided randomly into six different diet groups (N = 16 in all treatments): houseflies, grasshoppers, earthworms, slugs, snails, and seeds. All treatments were run simultaneously. Each female was offered 25 houseflies in the pure housefly diet. Approximately the same wet mass of other food types was offered to other females in the other pure diet groups. In the mixed diet group, each female was supplied with 10 houseflies,

and the other food types were given in approximately the same amounts by weight. The procedure followed was the same as in Experiment 2. For the prey used in Experiment 2, their dry mass/wet mass conversion factors were reused here. For the new food types, conversion factors were established by weighing, drying and re-weighing, using 5 seed samples of c. 0.1 g and 5 samples of c. 0.5 g of snails and grasshoppers.

Statistical analysis

Homogeneity of variances was tested using Bartlett's or Levene's test before the method of analysis was chosen. Data were homogenised with a square-root or a Box-Cox transformation, and one-way ANOVA was applied, followed by post- hoc pair-wise comparisons of treatments using Student's *t*-test. A Welch ANOVA test was used when no transformation was able to homogenise the group variances. Subsequent pair-wise comparisons were made using Welch ANOVA or one-way ANOVA tests. Within each series, the basic α-level of 0.05 was adjusted with the sequential Bonferroni technique (Rice, 1989).

Results

Experiment 1: Egg production of *P. versicolor*

Overall differences in egg production were not fully significant between diets (one-way ANOVA: $F_{3,19}=2.90$, $P=0.062$). Number of eggs produced by beetles fed slugs was significantly lower than by beetles fed other diets (Fig. 1A).

Beetles fed mixed and fruit fly diets had gained mass by the end of the experiment, while beetles fed earthworms or slugs had lost mass (Fig. 1B). The beetle mass change was not significantly different between diets (Welch ANOVA: $F_3=2.42$, $P=0.13$). This was probably due to low sample sizes.

Experiment 2: 24-h food consumption measurements for *C. nemoralis*

There was a significant overall difference in prey consumption between different diets (one-way ANOVA: $F_{3,30}=5.91$, $P=0.0027$). Earthworm consumption was significantly higher than that of houseflies and slugs both in pure diets (Student's *t*-test) and in the mixed diet (Welch ANOVA: $F_2=7.42$, $P=0.013$; Fig. 2A). Housefly consumption in the mixed diet treatment was remarkably low (indiscernible in Fig. 2A). Mixed diet consumption did not differ significantly from earthworm consumption, but was significantly higher than housefly and slug consumption.

Beetle mass change was significantly different between diets (Welch ANOVA: $F_3=18.0$, $P<0.0001$). Mass change of beetles fed slugs was significantly lower than that of beetles fed houseflies, earthworms or mixed diet (Fig. 2B). Mass change of the beetles fed houseflies, earthworms and mixed diet did not differ significantly from each other.

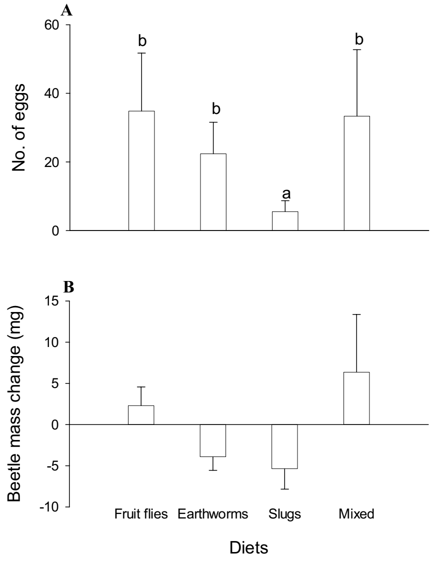

Figure 1. Egg production (total number of eggs per females during two weeks) (A) and mass change (B) in females of *Pterostichus versicolor* kept on different diet regimes under laboratory conditions. Error bars indicate one SE. Different letters indicate significant (p<0.05) difference among treatments.

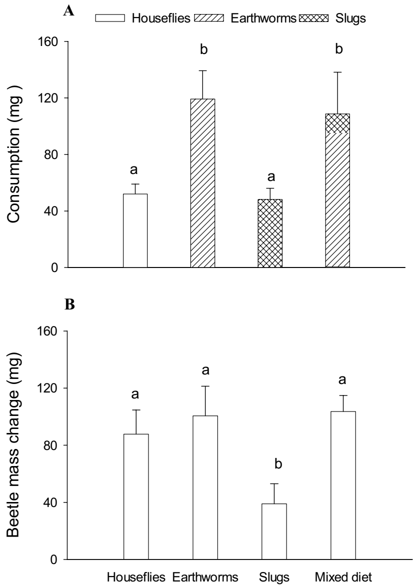

Figure 2. Food consumption (mg dry mass in 24 h) (A) and body mass change (B) in *Carabus nemoralis* adults under different diet regimes. Error bars indicate one SE. Different letters indicate significant difference among treatments.

Experiment 3: 24-h food consumption measurements for *P. melanarius*

Consumption by adult beetles was significantly different between diets (Welch ANOVA: F_5=40.2, *P*<0.0001). Earthworm consumption was significantly higher than the consumption of houseflies, grasshoppers, slugs, snails and seeds (Fig. 3A). Seed consumption was significantly lower than the consumption of the insect diets, but did not differ significantly from slug and snail consumption. Grasshopper consumption was significantly higher than that of slugs, snails and seeds, but did not differ significantly from housefly consumption.

There was a significant overall difference in the beetle mass change between diets (Welch ANOVA: F_5=27.4, *P*<0.0001). Beetles fed insect diets gained more mass than beetles fed slugs, snails and seeds, but not more than beetles fed earthworms. Beetles fed seeds lost mass, and this was significantly different from the mass change in other diet groups.

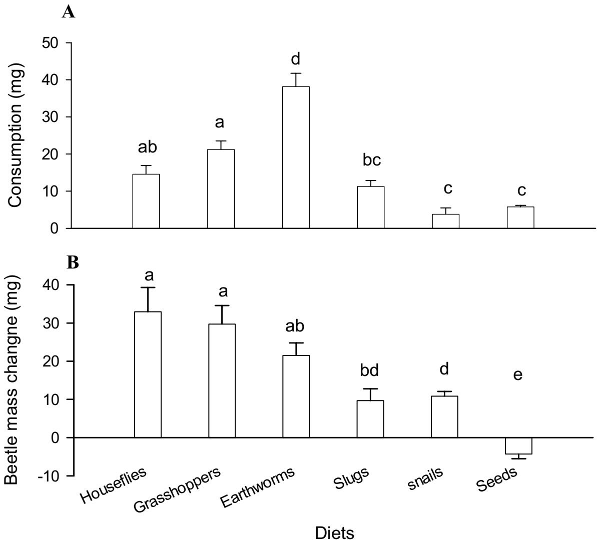

Figure 3. Food consumption (mg dry mass in 24 h) (A) and body mass change (B) in females of *P. melanarius* under different diet regimes. Error bars indicate one SE. Different letters indicate significant difference among treatments.

Discussion

Experiment 1 indicated that fruit flies were of high food quality for *P. versicolor*. Thus, beetles fed fruit flies both gained weight and maintained a high rate of egg production (Fig. 1). This agrees with previous findings on carabid beetles and other generalist predators (Bilde & Toft 1994, 2002; Bilde *et al*., 2000). Slugs were low quality food for *P. versicolor* because the beetles both lost weight and produced very few eggs. Mixing fruit flies with slugs and earthworms did not improve fecundity of *P. versicolor* (Fig. 1A) and increased mass gain only non-significantly (Fig. 1B).

Despite that the beetles fed earthworms lost mass, they produced as many eggs as those in the fruit fly and mixed diet treatments (Fig. 1). This might indicate that *P. versicolor* is more efficient at converting earthworms than slugs into own biomass or eggs and thus have a higher nutritional value than the slugs. As consumption was not measured it cannot be decided whether this was due to pre- or post-digestive effects. Due to the limited duration of the experiment (2 weeks) these results may not fully reflect the possible long-term effects of the dietary restrictions.

In Experiment 2 earthworms were the highly preferred prey for *C. nemoralis*, supporting Hengeveld (1980a) that *C. nemoralis* is to a large part an earthworm consumer. Lukasiewicz (1996) reported that *Carabus* species selectively preyed on large and slowly moving epigean invertebrates such as earthworms. Houseflies were the highest quality food for *C. nemoralis* as the mass gain of the beetles was high in spite of the low consumption (Fig. 2). Slugs were low quality food, as the mass gain of beetles fed slugs was significantly lower than that of other diets (Fig. 2B). These results confirm that *C. nemoralis* is a generalist carnivore consuming a variety of prey types as reported by several authors (Digweed, 1994; Larochelle, 1990; Turin *et al.,* 2003).

Insect diets were also of high quality for *P. melanarius*, since the beetles were able to increase in mass in spite of only intermediate feeding rates. The beetles had a high earthworm consumption capacity (Fig. 3A), supporting Symondson *et al.* (2000) reporting that *P. melanarius* consume many earthworms when other prey types are scarce. But they were not as high quality food as insects, because the beetles gained less mass on a much higher consumption rate. The positive gain in weight of beetles on slug and snail diets in spite of low consumption rates indicate that their low food quality is due to pre- rather than post-digestive effects. Seeds were low-quality food for *P. melanarius* as the low consumption rate was insufficient for maintaining their body mass (Fig. 3B).

Conclusion

Diptera (houseflies or fruit flies) were high quality food for all species, while slugs and snails were low quality and earthworms were intermediate quality. Seeds were low quality and probably useless for *P. melanarius*. Our results regarding molluscs confirm the suggestion of Mair & Port (2001a,b) that generalist carabids have low preference for slugs and may accept only small or dead ones. These authors ascribed this to the deterrent effects of the mucus. However, mucus was not a factor in our experiments, since the beetles were fed small pieces of mollusc meat.

The results refute the hypothesis that food types eaten by specialist feeders are also high quality food for generalists. For example, slugs were low quality food for all beetles and even for *C. nemoralis*, which have been considered a mollusc specialist (Hengeveld, 1980a). The generalist predators seemingly gained nothing from the inclusion of earthworms and slugs in the mixed diets. The results indicate that specialist feeders have adapted to food types that are protected against the generalist feeders. A related question is whether the specialists' performance can be enhanced by a mixed diet that includes prey of high quality for generalists. This is not always the case. Thus, addition of insects to the diet of the seed-eaters *Amara similata* and *Harpalus rufipes* did not improve the performance of the beetles (Jørgensen & Toft, 1997a,b).

Acknowledgements

We are deeply indebted to Else Bomholt Rasmussen for assistance in the laboratory and help with collecting of prey animals; to David Mayntz for statistical assistance; to Gabor Lövei and an anonymous reviewer for many valuable comments; and to Gabor Lövei for taking on the full editorial responsibility for this paper.

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Effects of urbanisation on carabid beetles in old beech forests

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Abstract

In this study, which is part of the international Globenet project, the carabid fauna of 13 woodland sites arranged across a rural-urban gradient in Brussels (Belgium) were sampled over the course of one season (April-September 2002). An increase in species number was found along the gradient from urban to rural. A different habitat preference was observed in the species of the carabid assemblages, with stenotopic forest species almost restricted to rural and suburban forests. Smaller ground beetle species, able to fly, dominated on urban sites, while large flightless species were more abundant in suburban and rural forests.

Key words: Urban-rural gradients, assemblages, diversity, Carabidae

Introduction

Urbanisation modifies the original habitat through different forms of anthropogenic land use (Vitousek *et al.,* 1997). This results in fragmentation and isolation of the original habitat, mostly accompanied by a decrease of local species (Niemelä *et al.*, 2000). Urban ecosystems are characterised by spatially heterogenic and temporally dynamic regions which differ fundamentally from their original environment. The areas are negatively influenced by soil compaction and pavements, are subject to increased pollution, have more exotic species than the original habitat and show changes in the local climate (Pyle *et al*., 1981; Vitousek *et al*., 1997; McIntyre, 2000).

Although urbanisation is considered one of the primary causes for the decline of arthropod populations, a recent review (McIntyre, 2000) shows that surprisingly little attention has been paid to the consequences of urban development on arthropods in general (except for some pest species). Arthropods nevertheless play an important role in the structure, the functioning and the dynamics of urban ecosystems and form the largest part of the total biodiversity. The review of McIntyre (2000) further shows that surprisingly little attention has been paid to the question how urbanisation affects the abundance and distribution patterns of urban arthropods. The way arthropods use the urban landscape needs more detailed investigations to be able to predict changes in their contribution to ecosystem functioning as a consequence of future urban developments.

A better understanding on the functioning of urban ecosystems is also necessary for an improved urban planning with minimal impact on the natural environment (McDonnel & Pickett, 1990; Niemelä, 1999; McIntyre *et al.,* 2001). Preservation of biodiversity, an important indicator for the protection of natural systems, is the key factor (Niëmela *et al*., 2000a), but is not yet sufficiently studied.

Recently, a global network (Globenet: Niemelä *et al.,* 2001) was set up to investigate the effects of urbanisation on ground beetles in forest fragments.

The ultimate aim of the Globenet (http://www.helsinki.fi/science/globenet/) is to assess and compare the effects of urbanisation on biodiversity in several countries around the world. In order to achieve this goal, a unified methodology and one group of organisms have been chosen: carabid assemblages of forests are sampled along urban-rural gradients. Such gradients represent a continuum of increasing human pressure and are subdivided into three classes: rural, suburban and urban. By comparing results from different cities, Globenet seeks to separate general, recurring effects on biodiversity from those that depend on local environments or particular biotic assemblages. This information can be useful in land use planning, depending whether the changes will affect biodiversity in similar ways across the globe or their impact will depend more on unique local conditions.

In this paper, preliminary data, based on a six months sampling of carabid beetles in 13 forests in Brussels (Belgium), are presented. During 2002 and 2003 the same sites have also been sampled by more extensive hierarchical pitfall trapping, but the results of those samplings are not yet available.

Here, we investigated the three main hypotheses proposed in the paper of Niëmela *et al.* (2000a) with our preliminary data, grouped for each of the 13 investigated forests. The first hypothesis is that species richness decreases from rural to urban as a supposed consequence of decreasing habitat quality and increased isolation of more urbanised forests. A second hypothesis is that opportunistic species gain territory in more urban situations due to habitat deterioration. A third hypothesis states that smaller and more mobile species are more abundant in urban situations, possibly due to less stable conditions and/or increased negative influences in urban forests.

Materials and methods

Study area

Figure 1 locates our study area with all investigated forests. We selected a gradient of disturbance within the city of Brussels. In each category (urban, suburban and rural) four study sites were chosen. Division into these three categories was based on GIS-maps with data on the amount of land covered with buildings and pavements in contrast to semi-natural areas such as pastures, gardens or cultivated fields. All forest sites were selected to be as comparable as possible for aspects other than urbanisation based on the presence of mature beech and existence since at least 1775 (ancient forests). In this way we tried to avoid variability between beetle assemblages caused by forest age or forest type differences instead of degree of urbanisation. We also selected a similar, western orientation of sampling sites within each forest. Overall, we selected three different sites. In the southeast of Brussels we studied the large Sonian Forest (U4, U5, S4 & R4) which has the unique situation of finding urban (U4 & U5), suburban (S4) and rural (R4) sites within the same forest, extending into the city of Brussels (Fig. 1). A second site was situated west of the Sonian Forest. Here there are several relic forests which have once been part of the Sonian Forest, but are now separated due to fragmentation and urbanisation. In this region, we selected two rural (R2 & R3), two suburban (S2 & S3) and two urban (U2 & U3) forests. A third site was north of Brussels (U1, S1 & R1). These forests grow on former lime mining areas and have a different history compared to the forests in the south of Brussels.

Pitfall sampling

At each site, six pitfalls (at about 5 m from each other) were installed without fixative in order to obtain living beetles for (future) genetic studies. Results must therefore be interpreted with care, taking into account the possibility of predation, attraction/repulsion and escape of beetles, although this is supposed to be minimal due to the construction of the traps. The top (about 5 cm) of a plastic bottle (diametre = 10 cm) is cut off and put inversely in the lower half of the bottle, functioning as a funnel. A roof is placed above these traps to protect against rain. The traps were all installed at about 100 m from the border of each forest, minimally 5 m from each other. The traps were emptied fortnightly over a period of six months (April until September 2002). All ground beetles captured with these live traps were grouped per site and identified. The species needed for further genetic research (*Abax ater*, *Pterostichus madidus* and *Carabus violaceus*) were frozen in separate tubes and stored at -80°C.

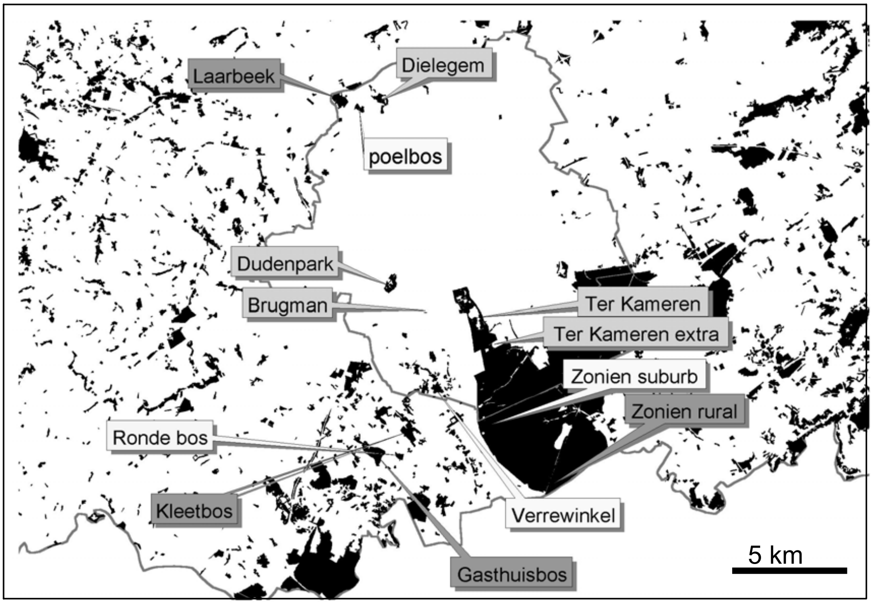

Figure 1. Map of the 13 sampled forest sites in our study. All forests are shown in black. Labels in dark grey = rural forests, in white = suburban forests and in grey = urban forests.

Analyses

We plotted species richness histograms to express carabid diversity. In addition, the expected number of species in each forest for a similar sample size of 250 individuals (minimum number of individuals obtained for all study sites) was estimated using the rarefaction method (Hurlbert, 1971; Heck *et al*., 1975; James & Rathbun, 1981). Habitat preference, dispersal ability and body size were also studied based on data from Desender (1989) and Desender *et al.* (1995). Mean body size was calculated per site for all carabid species. We used detrended correspondence analysis (DCA) (Hill & Gauch, 1980; Hill, 1979a) performed with the program Pcord v.4 to ordinate beetle assemblages along axes according to their species composition. DCA is an eigenanalysis ordination technique based on reciprocal averaging. It is geared to ecological data sets and the terminology is based on samples and species. For this analysis, occasionally trapped species were omitted and we analysed our data using 21 species present in > 10 individuals. The quantitative data of the selected species were transformed to percentages within each species so that each species counts for the same weight. Two Way Indicator Species Analysis or Twinspan (Hill, 1979b; Gauch & Whittaker, 1981) was also applied to the data. Twinspan simultaneously classifies species and samples and is based on dividing a reciprocal averaging ordination space (Gauch, 1982). For this analysis the same 21 species were taken into account, using pseudospecies cut levels of 0, 2, 5, 10 and 20.

Results

Carabid diversity along the urban-rural gradient

During six months we collected a total of 12096 individuals belonging to 49 carabid species (Table 1). Figure 2 shows the species richness (upper) and standardised species number (lower, by rarefaction) for each site. The hypothesis of decreasing species richness from rural to urban was confirmed within the large Sonian Forest (U4, U5, S4, R4), irrespective of whether rarefaction was used or not. In the 6 relics of the Sonian Forest (U2, U3, S2, S3, R2 & R3) the decreasing species richness hypothesis was also confirmed. The most urbanised site of these forests, namely Dudenpark (U3), had the lowest species number. In the three northern forests (U1, S1 & R1) the hypothesis was not confirmed.

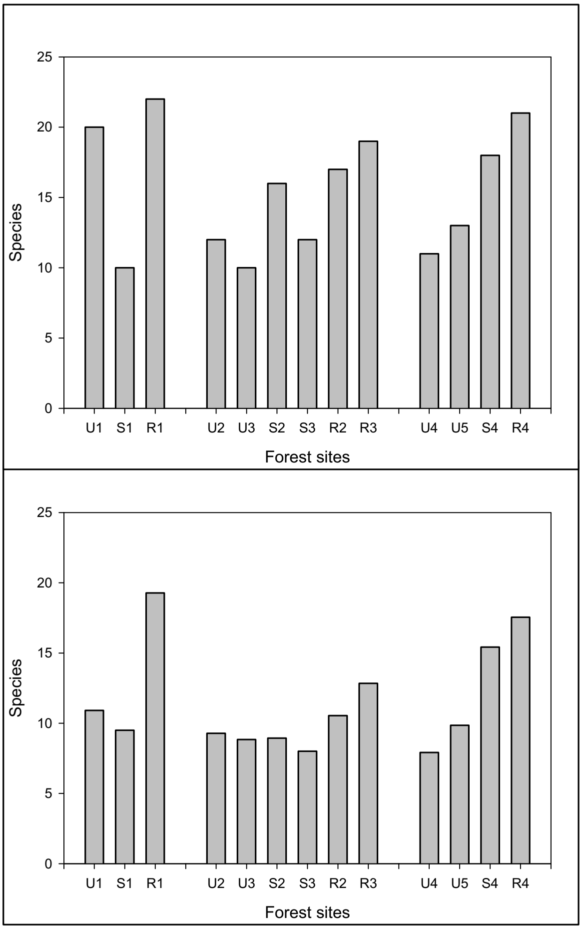

Figure 2. Total species richness (upper graph) and rarefied species number (lower graph, estimated for 250 individuals) for all 13 studied forests. Each graph shows urban-rural gradient for the 3 northern study sites (left), the 6 relic forests of the Sonian forest (middle), and the 4 Sonian forest sites (right); U= urban, S= sub-urban, R= rural.

Table 1. Number of carabids caught from sites U1-5 (urban), S1-4 (suburban) and R1-4 (rural) for each species. The habitat preference is mentioned in the second column (EF= forest eurytopic species, SF= forest stenotopic species, O= open landscape species, E= eurytopic species). The dispersal ability is given in the third column (B= brachypterous, M=macropterous, D= dimorphic or polymorphic).

Species	Habitat preference	Dispersal ability	BRUGMAN U2	DIELEGEM U1	DUDENPARK U3	TER KAMEREN URBAAN U4	TER KAMEREN EXTRA U5	POELBOS S1	RONDEBOS S2	VERREWINKEL S3	ZONIEN SUBURBAAN SS	KLEETBOS R2	LAARBEEK R1	GASTHUISBOS R3	ZONIEN RURAL R4	Sum
Abax ater	EF	B	1	4		163	315	147	67	117	22	148	53	201	307	1545
Abax ovalis	SF	B									253				108	361
Agonum albipes	O	M		30												30
Agonum assimile	EF	M		78						8	2	1	15	10		114
Agonum dorsale	O	M		1												1
Amara aenea	E	M							1							1
Amara lunicollis	E	M		1					2							3
Amara ovata	O	M		1												1
Amara plebeja	E	M						1	1			1				3
Amara similata	O	M								1			1			2
Anisodactylus binotatus	E	M		1					1							2
Asaphidion curtum	EF	M	1	51	38		1		3			7	19	4		124
Asaphidion flavipes	E	M								1						1
Asaphidion stierlini	O	M					1					1				2
Badister bullatus	EF	M	11	3	3	1		3			6	2	3	2	4	38
Badister lacertosus	E	M	1	1												2
Badister sodalis	E	M		2				2								4
Bembidion lampros	E	D									1		8	1		10
Bembidion tetracolum	E	D			12								1			13
Calathus rotundicollis	EF	D	21		3	28		4	1		3	2	27			89
Carabus auronitens	SF	B					29				51				42	122
Carabus monilis	O	B													1	1
Carabus nemoralis	EF	B													5	5
Carabus problematicus	SF	B					1				4			2	22	29
Carabus violaceus purpurascens	SF	B				47	91		40	34	17	41	65	21	56	412
Cychrus attenuatus	SF	B									3			13	18	34
Cychrus caraboides	SF	B											3			3
Harpalus affinis	E	M		1												1
Harpalus latus	E	M								2				1	13	16
Harpalus rufipes	O	M	3													3
Leistus fulvibarbis	EF	M	1		2				2				8			13
Leistus rufomarginatus	EF	M	11	1	4	10	3	2	3	4	5	17	9	33	2	104
Loricera pilicornis	E	M		3									3	4		10
Molops piceus	SF	B									1					1
Nebria brevicollis	E	M	246	202	569	249	33	10	341	140	10	288	149	213	5	2455
Notiophilus biguttatus	EF	D	13	7	14	1	9		18	6	7	19	22	32	10	158
Notiophilus rufipes	EF	M	39	8	27	10	9	1	8	8	7	50	14	29	9	219
Pterostichus cristatus	SF	B					22				19				12	53
Pterostichus cupreus	E	M											2		1	3
Pterostichus madidus	EF	B	306	980	5	472	287	123	854	903	170	711	143	460	41	5455
Pterostichus melanarius	E	D										1	6		3	10
Pterostichus niger	EF	M										1	3		4	8
Pterostichus nigrita	E	M		5									1	2		8
Pterostichus oblongopunctatus	EF	M				1				1	99	17		396	85	599
Pterostichus strenuus	E	D		6		1			1				6			14
Pterostichus versicolor	E	M					1	1								2
Synuchus nivalis	O	M							5			1		1		7
Trechus obtusus	E	D													1	1
Trichotichnus laevicollis	SF	D												4		4
Sum			654	1386	677	983	802	294	1348	1225	680	1308	561	1429	749	12096
Species richness			12	20	10	11	13	10	16	12	18	17	22	19	21	49

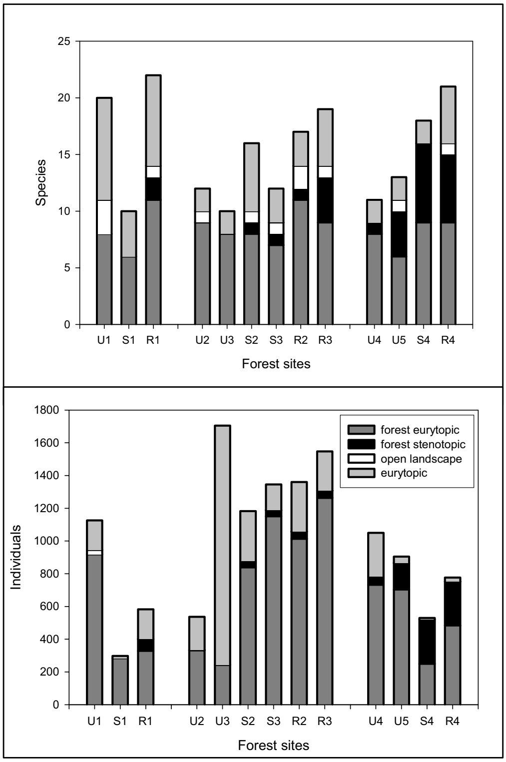

Figure 3. Histograms of the habitat preference of carabids in each sampling site on species and individual level.

Habitat preference and carabid assemblage changes along the gradient

In a second stage, the different species within the gradient were regrouped according to their habitat preference. We distinguished forest stenotopic, forest eurytopic, open landscape and eurytopic species (Table 1, Fig.3). The second hypothesis (opportunistic species gain territory in more urban situations) could not be confirmed with our present data (Fig 3), but stenotopic species disappeared towards more urban sites. Whether this is accompanied by a trend of increasing eurytopic species was not clear from these data and needs to be studied further. In the large Sonian Forest (U4, U5, S4, R4) stenotopic forest species were represented by a large number of individuals, in contrast to the rural sites of the relic forests (R2 & R3) where these species, if present, were represented by low numbers only.

A DCA shows the position of the forests within their respective category (urban, suburban and rural) according to their carabid assemblages (Fig. 4). Sample scores are ordered along the first axis with the urban sites at the right side and the large Sonian Forest at the left side. Urban sites were characterised by the presence of high numbers of *Nebria brevicollis* (highest number in most urbanised site U3) and *Bembidion tetracolum* (only present at the most

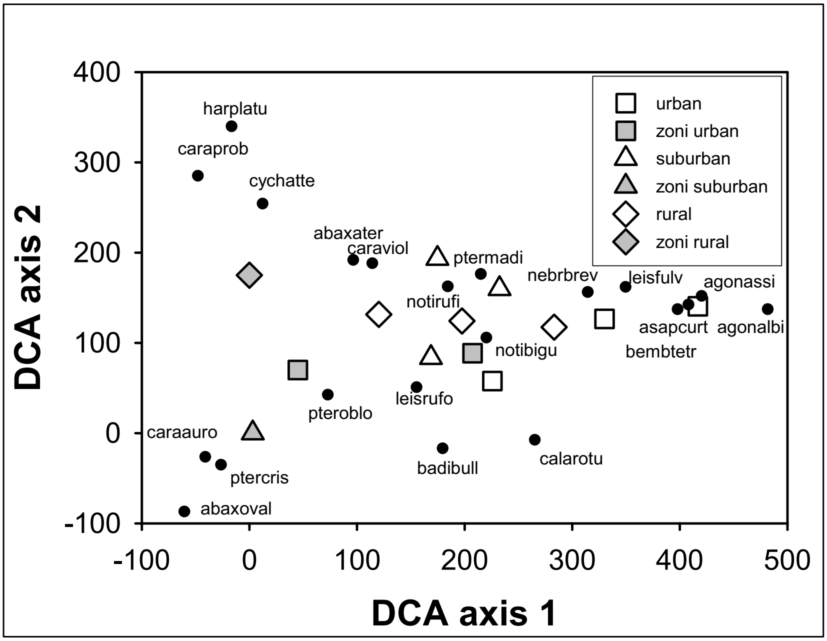

Figure 4. Results of the DCA analysis showing the position of the forests according to their carabid assemblages. The sites in the Sonian Forest (=zoni in legend) are presented separately with grey symbols. The species names are abbreviated and can be found on table 1).

urbanised site U3) and the absence or near-absence of *Abax ater*. The Sonian Forest sites grouped together because of the presence of *Carabus auronitens, Cychrus attenuatus, Abax ovalis, Pterostichus cristatus* and *Carabus problematicus*. These species are present in the Sonian Forest because it is a large, continuous ancient forest. Nevertheless, the most urbanised sites in this forest did not have some of these species: *C. auronitens*, *C. problematicus* & *P. cristatus* are lacking in U4 and *C. attenuatus* does not occur in U4 & U5.

The Twinspan organigram is summarised in figure 5. In the first division three Sonian sites (R4, S4 & U5) and one rural relic site (R3) were distinguished from the other forests. This corresponded to the exclusive presence of C*arabus problematicus* in these sites. In a second division two urban (non-Sonian) sites (U2 & U3) and one rural northern site (R1) were separated where *Leistus fulvibarbis* was more numerous while *Pterostichus madidus* was more abundant in the six remaining forests. A third division separated the urban northern site (U1) based on the presence of *Agonum albipes*. A fourth and last division distinguished the suburban relic sites (S2 & S3) from the other three sites (R2, S1 & U4) were *Badister bullatus* was an indicator species. The results of both assemblage analyses were more or less comparable, i.e. there is a clear separation of the Sonian sites, while the urban sites were not grouped.

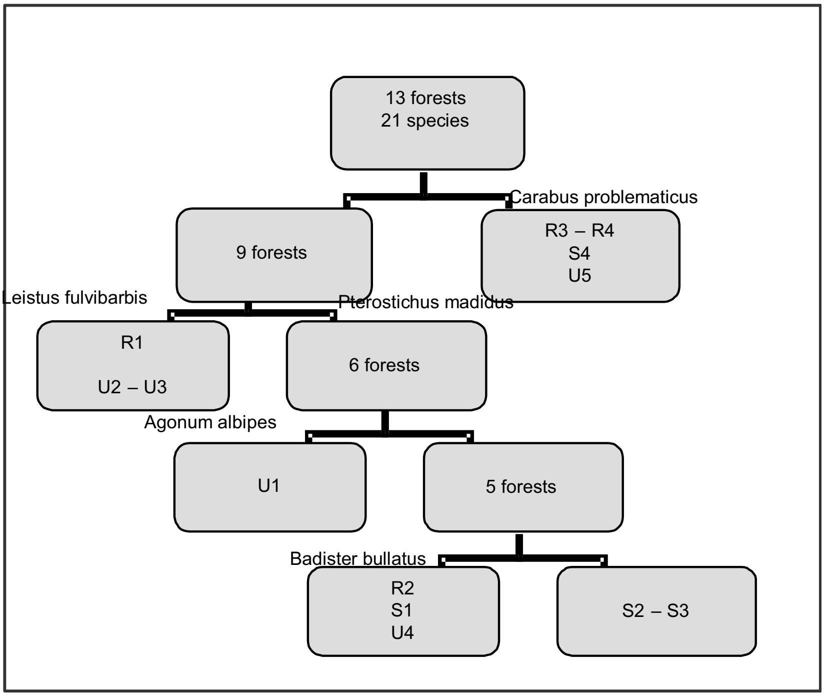

Figure 5. Results of the TWINSPAN analysis of the 13 study sites near Brussels including the 21 carabid species selected.

Dispersal ability and body size along the gradient
The number of brachypterous species obviously declined from urban to rural even within the large ancient Sonian forest (U4, U5, S4 & R4, Fig. 6). The same pattern was found for body size, with a larger mean value in the rural sites and smaller values for the urban sites (Fig. 7). At the level of individuals, there was obviously a large presence of macropterous individuals in Dudenpark (U3) while at species level this was not observed. This was due to the large number of one species only (*Nebria brevicollis*) in this site.

Discussion

Similar studies were done in the scope of the Globenet project in Sofia (Bulgaria), Helsinki (Finland), Edmonton (Canada) (cf. Niemelä *et al.,* 2000) and Hiroshima (Japan) (Ishitani *et al.*, 2003). These initial analyses have clearly shown patterns of response to differ between cities, while common responses to urbanisation are less apparent. A common methodology was set up by different Globenet partners (Niëmela, 2000a), but differences still exist which can make comparison more difficult. For example the altitude of the different cities and the dominant vegetation are completely different in the studies published so far.

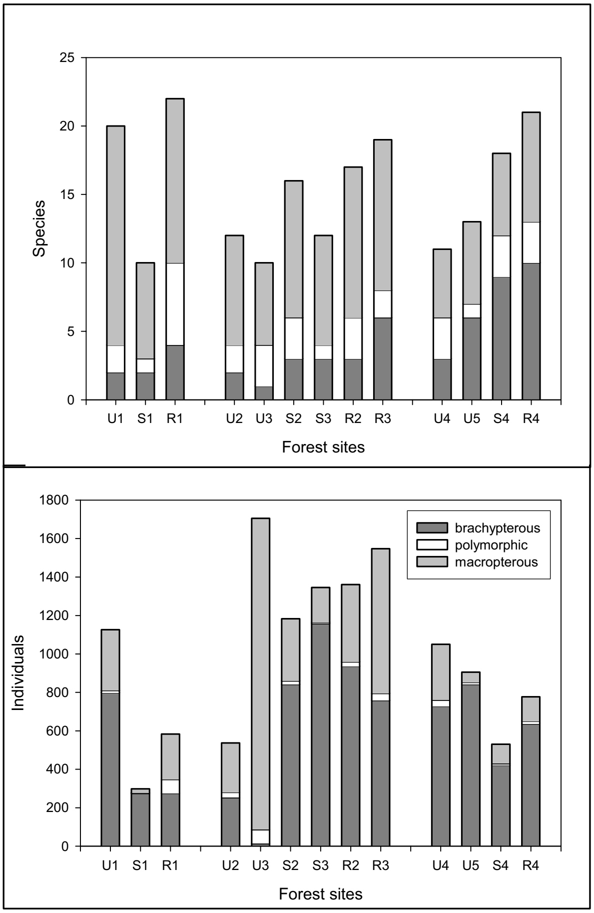

Figure 6. Histograms of the dispersal ability of carabids in the investigated forests on species and individual level.

The first hypothesis that species richness decreases from rural to urban was tested in all of the published studies. In general, the hypothesis was confirmed in Helsinki, Hiroshima and Edmonton (when introduced species were excluded) as well as in our present data from Brussels. In Sofia the hypothesis was neither rejected nor confirmed. The proposed basic idea is that disturbance could cause a homogenisation of the urban forest patches which can eliminate certain micro-habitats. Another possible reason for the decline of some species is that urban sites are in general more fragmented and isolated by a matrix of built-up and inhospitable habitat that make colonisation and dispersal difficult to nearly impossible.

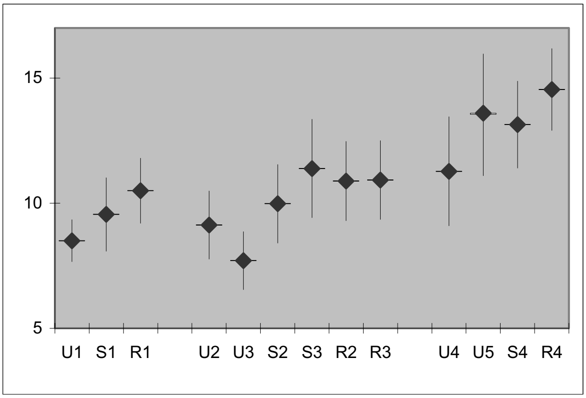

Figure 7. Mean body size (± s.e., mm) of carabid species over the different sites.

In Edmonton and Helsinki the hypothesis that opportunistic species gain territory in more urban situations was confirmed. These sites were even dominated by species of the same genus, *Calathus* (the forest generalists *C. ingrates* and *C. micropterus,* respectively). Remarkably also was that a high dominance by one species was found in the urban sites compared to the suburban and rural sites. In our study this was also expressed by an obvious dominance of *Nebria brevicollis* in U3 (the most urbanised site) and of *Pterostichus madidus* in U1. Also in Hiroshima, more generalists were observed in urban sites and more specialists in suburban and rural sites. The same decline in the presence of stenotopic species from rural to urban was found in our Brussels data. This clearly indicates that stenotopic species are restricted to higher quality forests while eurytopic species are less vulnerable to the effects of urbanisation.

The hypothesis that smaller and more mobile species are more frequent in urban situations was also tested. However, size and mobility are related characters because carabid beetles that are unable to fly, are usually also bigger, while flying carabids are in general smaller (Desender, 1986). The hypothesis was confirmed in Brussels and Sofia. In Helsinki and Edmonton, macropterous (long winged) beetles appeared to be significantly larger than flightless species. This deviating result can possibly be explained by the low number of species, i.e. of larger wingless *Carabus* species, in Helsinki (Finland) as well as the prominence of introduced species in Edmonton (Canada).

In our study, smaller and more mobile species were even also more abundant in urban sites of the large ancient forest. This strongly suggests that the observed effect is not due to isolation or fragmentation, but can presumably be linked to a lower habitat quality (increased disturbance) as a consequence of urbanisation.

In our multivariate analyses we could not find a clear clustering of sites according to their degree of urbanisation. This division is quite unsophisticated and needs a more detailed description. It is important to use additional data, such as the quantitative characterisation of forest surroundings (possible to determine in GIS), forest size, influence of trampling (number and size of paths and compaction degree), light, moisture and temperature measurements, accompanied by a description of the vegetation, litter and soil samples. All these factors can play important roles in structuring the carabid assemblages and can help to explain certain patterns or deviations from expectations. Other imperfections are the possible fluctuations in beetle numbers between years, differences in orientation and the role of edges, which are expected to be relatively larger in smaller forests. Urban forests are usually smaller and edge effects may therefore play an important but hidden role in assemblage composition. All of these aspects, including edge effects, will be studied in more detail in future contributions based on our additional hierarchical sampling campaigns.

Additional data from other countries will be important to further identify common patterns and how far these can be generalized. Finally, we will also investigate possible genetic and population ecological effects of urbanisation on a number of carabid species from our Belgian study sites.

Acknowledgements

We would like to thank A. Drumont, L. Gaublomme and L. Raman for their assistance during field work. We acknowledge the responsible authorities for giving us permission to sample the forests involved in our project. We also thank the Brussels Institute for Management of the Environment (BIM) and the Institute for Forestry and Game Management (IBW) for their cooperation. This research was supported by a grant from the Flemish Institute for the Advancement of Scientific and Technological Research in Industry and Agriculture (IWT) to EG.

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Diversity and habitat preferences of ground beetles (Coleoptera, Carabidae) in a coastal area of North Trøndelag, Central Norway

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Abstract

The study involved carabid beetle diversity patterns in a variety of habitats (forests and open areas), with special reference to anthropogeneous heathland. A line of eight pitfall traps was placed in 34 sites, sampling continuously from 6 May to 11 September 2002. Altogether, 35 species of Carabidae were identified, of which five (14%) were only collected once. Open coniferous and deciduous forests showed the highest species richness, while the lowest species richness was found on a seaweed-influenced dike, in the dense spruce forest and the planted spruce stand. The high species richness in open coniferous and deciduous forests may partly be explained by the Atlantic climate. In general, transition zones were more species rich than the vegetation types. The species accumulation curve and diversity estimators stabilized but did not reach an asymptote, indicating the inventory of the fauna to be incomplete. The diversity estimators indicated true species richness to be three to eight species above the observed total number. Six species occurred in habitats which differed from what is known from literature: *Carabus violaceus* L. and *Cychrus caraboides* L. in heathland; *Trechus obtusus* Erichson in coniferous forest; *Carabus hortensis* L. and *Calathus micropterus* (Duftschmid) occurred eurytopically. *Carabus problematicus* Herbst appeared as a common species in bird fertilized coastal vegetation. Two heathland specialists were found: *Cymindis vaporariorum* (L.) and *C. problematicus*.

Key words: Carabidae, biodiversity, habitat preferences, anthropogeneous heathland

Introduction

In Norway and in Europe, anthropogeneous coastal heathlands are endangered due to changes in agricultural management during the twentieth century (Webb, 1986; Nilsen, 1998; Magura *et al*., 2001). This type of heathland was maintained by clearing, burning, grazing and cutting. Traditionally, fragments of heathland were burnt each year, creating a mosaic of vegetation burnt in different years. The cessation of these practices has lead to a succession towards woodland.

Heathlands are limited to coastal zones with winter mean temperature around 0°C (Nilsen, 1998). Heaths are notably important for carabids, which here have a distinctive and comparatively species-rich fauna (Vermeulen, 1993). Studies in eastern England have stressed the importance of analyzing carabid habitat preferences, in relation to heathland conservation (Telfer & Eversham, 1996).

Native Scottish forests show highest species richness in open regenerating habitats and lowest in dense woodlands (Ings & Hartley, 1999). Species richness was also higher in early than in late stages of grassland succession (Purtauf *et al*., 2003).

There is an abiding interest in patterns of species richness and in explaining its geographical distribution (Rosenzweig, 1995; Longino *et al.,* 2002). Many surveys rely on species lists from unsystematic sampling, although observed species numbers are then often biased samples of the true numbers. Methods for estimating species richness should be based on quantitative sampling in order to get better estimates of the true species richness (Colwell & Coddington, 1994; Longino *et al.,* 2002).

The aim of this study was to establish trends in species richness of carabid beetles in different vegetation types, with special focus on plant communities of anthropogeneous heathland. Secondary aims were to describe the habitat preferences of species from a region of Norway, which is little known with respect to ground beetles.

Materials and methods

Study areas and sampling

This study was carried out in the Nærøy municipal, North Trøndelag County, Central Norway (65.75°N, 11.5°E). In the main study area in Kjeksvika (0-63 meters above sea level) close to Abelvær, altogether 26 sampling sites were established. Botanical surveys and vegetation descriptions of Kjeksvika have been given by Nilsen (1998). A vegetation map with vegetation codes according to Fremstad (1997) and a management plan for approximately 22 ha have been presented by Nilsen (1998). Heathland is the dominant vegetation type in Kjeksvika, making the area a subject for conservation and management. The heathland has a diverse flora, mostly due to calcareous shell-derived sand blowing into the area. Several rare plant species occur, such as the orchid *Ophrys insectifera*, which is a Red List species in Norway (DN, 1992).

Eight additional sites were chosen outside the Kjeksvika area. These were located along the road from Abelvær to Skaga, and consisted of different types of forest.

Altogether, 13 vegetation types and seven transition zones (transitions between vegetation types) were sampled (Table 1). Ten vegetation types were situated in Kjeksvika, of which three were heathlands. The seven transition zones were all in the Kjeksvika area. The sites outside Kjeksvika contained five vegetation types; scrubland; birch forest; open coniferous forest (bilberry woodland); dense spruce forest and deciduous forest (tall fern-downy birch stand). Pitfall traps were used continuously from 6 May to 11 September 2002. This trapping period was divided into two, the first one from 6 May to 15 June and the second from 16 June to 11 September. A trapping line was established in each site, consisting of eight pitfall traps, at 1-2 m intervals. The traps were plastic cups with an upper diameter of 6.5 cm and 9.5 cm deep, half filled with formaldehyde (4%). A metal roof (11x11 cm) was placed approximately 3-4 cm above each trap.

The species were identified according to Lindroth (1961, 1985, and 1986) and checked with specimens in the Andreas Strand beetle collection at Bergen Museum. Voucher specimens have been deposited at the Bergen Museum.

Methods of analysis

Species richness

Species richness based on quantitative sampling was estimated using the software EstimateS (Colwell, 2001). The species accumulation curve and diversity estimator curves were based on summed samples, each sample representing the total catch (eight traps) of a site. A sample-based approach to diversity estimation as opposed to an individual based has been recommended, to account for patchiness in the data (Gotelli & Colwell, 2001). According to the latter authors, it is not always possible to construct a species accumulation curve based on added individuals, i.e. sequence of added specimens during species identification.

In order to smooth the curves, 100 randomizations were performed. The EstimateS software computes a number of different diversity estimators, all based on the rare species in the data set (Colwell & Coddington, 1994). The estimators are the uniques curve (number of species occurring in only one sample); duplicates (number of species appearing in two samples); incidence coverage estimator (ICE, species found in ten or fewer samples); and the Michaelis-Menton mean (MMMean, the estimated MM asymptote, see Colwell and Coddington, 1994). In a recent study on ants, Longino *et al.* (2002) found these estimators to be similar and to outperform estimates based on the lognormal method. Additionally, Coleman’s rarefaction curve was used to look for patchiness in the data set, and the abundance coverage estimator (ACE) was used for diversity estimation based on species with fewer than ten specimens (Colwell & Coddington, 1994). The “cut-point” of ten specimens (Lee & Chao, 1994) is rather arbitrary and was used to discriminate between “rare” species (fewer than ten specimens) and “common” species (ten or more specimens).

Habitat preferences

Carabid species habitat preferences were investigated by applying Hill´s N2 diversity index, which computes “effective number of occurrences”, i.e. species abundance not influenced by sample size (Hill, 1973):

$$N2 = [\sum(Y_i k_i / Y+k)^2]^{-1},$$

Y_i = abundance of a species in sample i, k_i = abundance of all species in sample i, Y = abundance of a species in all samples, k = abundance of all species in all samples.

This was computed by using the CANOCO package (ter Braak & Smilauer, 1998). The index was applied for each vegetation type containing more than one trapping line, in order to find the typical species occurring there. For consideration as typical species, only species with a high relative occurrence (N2 > 1) are presented. A high N2 value indicates the species to be common.

Results

Altogether 2091 specimens were identified, representing 35 species of Carabidae. Of these, five species (14.3%) were singletons and two species (5.7%) were doubletons, while 18 species (51.4%) were represented by ten or more individuals. Most of the common species were large or medium large, and most of the rare species were small to medium large.

Species richness

Open coniferous and deciduous forests contained the highest number of species (Table 1). The species numbers were lowest on the dike, in the planted spruce stand and in the dense spruce forest. The latter one perhaps indicates a loss of species towards climax vegetation in a secondary succession. The transition zones were generally very species rich compared to the vegetation types (Table 1).

Dry heathlands, the dense spruce forest, open coniferous, and deciduous forests possessed a larger fraction of common species compared to the other vegetation types and the transition zones (Table 1). The dense spruce forest and the planted spruce stand contained a high fraction of singletons and doubletons. The dike only possessed singletons and doubletons, and

Table 1. List of the vegetation types and transition zones (transition between two vegetation types) and their respective number of sites, species, singletons and doubletons, rare species, and common species.

Habitat type	No. of sites	Mean no. of species	Mean no. of singletons and doubletons	Mean no. of rare species	Mean no. of common species
Dike	1	2	2	0	0
Meadow	3	7	3	7	1
Wet heathland	3	7	3.33	2	1.67
Dry heathland	1	7	2	1.67	2.5
Dry grass-herb rich heathland	2	6	1	5	0
Bird fertilized coastal vegetation	2	8	4	2	2
Scrubland	3	5.7	2	2.33	1.33
Bog	2	9	4	3.5	1.5
Birch forest	3	5.7	2.33	3	0.33
Open coniferous forest	3	10	3.67	2.67	3.33
Other deciduous forest	2	9.5	4	2	3.5
Dense spruce forest	1	3	3	0	2
Planted spruce stand	1	5	2	1	0
Dike-meadow	1	8	4	3	1
Dry heath-wet heath	1	8	3	4	1
Dry heath-birch forest	1	10	3	7	0
Meadow-birch forest	1	11	6	3	2
Meadow-dry grass and herb rich heath	1	9	3	5	1
Dry grass and herb rich heath-birch forest	1	2	1	0	1
Bog-birch forest	1	11	5	4	2

meadows and birch forests had the lowest fraction of common species. The transition zone between meadow and birch forest, and between bog and birch forest contained the largest proportion of common species among the transition zones. However, in both incidents only two species were common. The transition between dry heathland and birch forest contained no common species. Instead there was a large proportion of rare species.

Species accumulation and diversity estimators

The accumulation and diversity estimator curves stabilized, but did not reach their asymptotes (Fig. 1). The Michaelis-Menton mean and the abundance coverage estimator (ACE) both lie three species above the curve of observed species (Sobs), while the incidence coverage estimator (ICE) was positioned eight species above Sobs. In other words, the estimated species richness was three to eight species higher than the observed number of 35. The uniques curve did not decline but rather stabilized at ten species. Ten species therefore appeared to have an extremely patchy distribution in the study area. The duplicates curve was

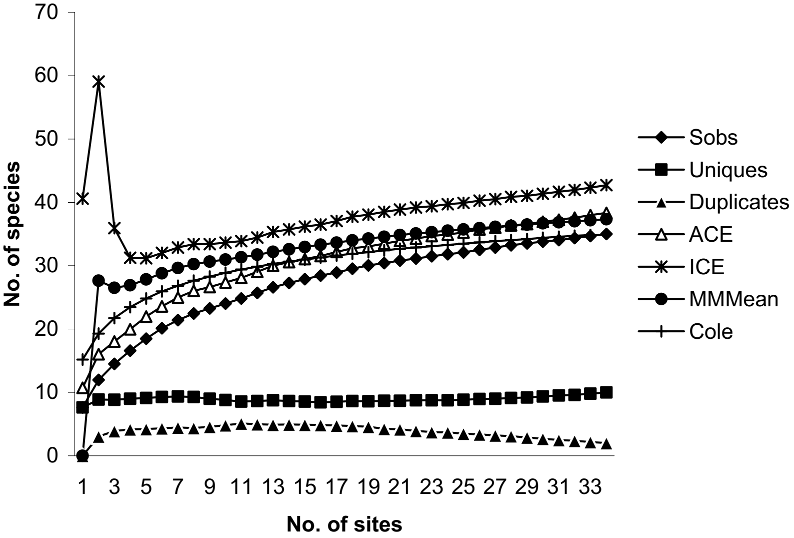

Figure 1. Species accumulation and diversity estimator curves. Sobs = number of species observed; Uniques = number of species occurring in only one sample; Duplicates = number of species occurring in two samples; ACE = abundance coverage estimator (uses species with 10 or fewer individuals in a sample); ICE = incidence coverage estimator (uses species found in 10 or fewer samples); MMMean = Michaelis-Menton mean (estimated MM asymptote; see Colwell and Coddington 1994); Cole = Coleman´s rarefaction curve (expected richness for random sub-samples).

the only one that declined. The Sobs curve reached the Coleman's rarefaction curve (Cole) at the second last added sample, which also indicates a substantial patchiness in the data. The further the species accumulation curve lies below the Coleman's rarefaction curve, the more heterogeneous are the samples (i.e. the more patchily distributed are individuals among samples).

Habitat preferences

Carabus violaceus L., *C. hortensis L., C. problematicus* Herbst*, Trechus obtusus* Erichson*, Cychrus caraboides* L., and *Calathus micropterus* (Duftschmid) differed from habitat preferences previously described for Fennoscandia (Table 2).

Table 2. Hill´s N2 diversity index of the six species which deviated from habitat preferences previously described for Fennoscandia. A high index value indicates a high mean abundance. Car hor = *Carabus hortensis* L.; Car vio = *Carabus violaecus* L.; Car pro = *Carabus problematicus* Herbst; Cyc car = *Cychrus caraboides* L.; Cal mic = *Calathus micropterus* (Duftschmid); and Tre obt = *Trechus obtusus* Erichson.

Species	Meadow	Wet heath land	Dry grass-herb rich heath	Bird fertilized coastal vegetation	Bog	Scrubland	Birch forest	Open coniferous forest	Other deciduous forest
Car hor	0.94	1.88	1.84	0	0.89	3.46	0.93	1.95	0.98
Car vio	1	2.95	1.89	2	0.9	1	0	0.5	0
Car pro	0	0.5	0.5	1	0	0.5	0	0	0
Cyc car	0	2.84	1.87	2	1	0.5	0	0.94	0.5
Cal mic	0.5	1.87	0.5	1.39	1.5	0.5	1.95	1.72	1.47
Tre obt	0	0	0	0	0	0	0	1.47	0

Discussion

Species richness

In contrast to previous studies from other parts of Europe (Ings & Hartley, 1999; Purtauf *et al.*, 2003), open coniferous and deciduous forests had higher species richness than heathlands and other open vegetation types. This may be a coastal phenomenon due to the highly variable environment, causing environmental stress. Consequently the highest peak in species richness seems to be shifted from pioneer vegetation towards climax vegetation of the climatically more unstable coastland. Hence, in this context unstable climate is in terms of winds and precipitation.

It is obvious that transition zones are occupied by species typical of both adjacent vegetation types, which may explain why the transition zones were generally species rich. According to Bommarco & Fagan (2002) carabids probably use such habitat edges during dispersal (e.g. between hibernating and reproducing habitats).

Species accumulation

The species accumulation curve and diversity estimators stabilized, although they did not reach their respective asymptotes (Fig. 1). Our results therefore indicate the inventory of the fauna to be incomplete regarding total species richness of the area examined.

Species appearing rare in data sets may be considered “travelers” caught by chance, rare in the investigated area, or “methodological edge species”. For insect taxonomists, rare species are often thought to be of the latter type (Longino *et al.*, 2002). In the case of ground beetles, pitfall traps have been found to be biased by a multitude of factors (Greenslade, 1964; Adis, 1979; Baars, 1979). Furthermore, pitfall traps seem to overestimate the proportion of large species (e.g. the genus *Carabus*), since they are able to walk for long distances (Spence & Niemelä, 1994; Mommertz *et al.,* 1996). This may partly explain why in the present study most of the common species are of medium to large size, while the rare species are of small to medium size.

Other possible explanations for species occurring rare in the data set may have much to do with the patchy distributions of many species (Vermeulen, 1993; Kinnunen, 1999; Maudsley, 2000; Brose, 2003). *Bembidion lunatum* (Duftschmid) was represented only as a singleton in our study and was the only *Bembidion* found, although this is the most species-rich genus of carabids in Fennoscandia (Lindroth, 1985). Work regarding this genus has been carried out using quadrate sampling (Andersen, 1986) and *Bembidion* species are strongly hygrophilous, living close to water on clay soil (Lindroth, 1985).

The rare species in the data set were either macropterous (hind wings well developed) or dimorphic (having both specimens with reduced and well developed hind wings). Other sampling methods (e.g. window traps, litter washing and D-vac suction) have proven to be better density estimators for such species (Spence & Niemelä, 1994; Mommertz *et al*., 1996), and are sometimes used in addition to pitfall trapping (van Huizen, 1977).

The sites in the Kjeksvika area were situated near the sea, while the sites outside Kjeksvika were situated more inland (8-17 km). This perhaps added more species due to geographical reasons rather than differences in vegetation. In the two birch forest sites in the Kjeksvika area and the birch forest site 9 km more inland, we found four and eleven species respectively. Furthermore, seven species were found in the two scrubland sites in the Kjeksvika area compared to nine species in the scrubland site approximately 10 km more inland. The differences may also be due to age, management or size of the habitats in question.

Habitat preferences

Habitat preferences given for the species in Fennoscandia (Lindroth 1945a, b, 1949, 1985, 1986), are sometimes in accordance and sometimes in contradiction with habitat preferences of the same species in Central Europe (Wachmann *et al.,* 1995). Species are often termed eurytopic or stenotopic. The lingering question is whether habitat occurrences are fixed or flexible. In the present study *C. hortensis*, *C. violaceus*, *C. problematicus*, *C. micropterus*, *T. obtusus*, and *C. caraboides* showed habitat preferences rather different from those given from other parts of Fennoscandia (Table 2).

It seems that *Carabus nemoralis* Müller invades habitats occupied by *C. hortensis* in the southern part of Norway (Lindroth, 1985). However in the northern areas, *C. hortensis* occurs alone, which may widen its habitat range, as the present study suggests. The species clearly seems to be more eurytopic in North Trøndelag than described for Fennoscandia. *C. micropterus* also seems more eurytopic than previously described.

C. violaceus and *C. caraboides* were common species in wet heathlands (Table 2), the former regarded as a eurytopic forest species (Lindroth 1961, 1985, 1986; Wachmann *et al*., 1995) and the latter as a woodland species preferring deciduous forest. They were less common in the dry grass-herb rich heath.

For consideration as a ”heathland species” (de Vries *et al*., 1996), few specimens should be caught outside the six heathland sites (Turin *et al*., 1991). This was not true for *C. violaceus* and *C. caraboides* (see Fig. 6), as both species were common also in the two sites classified as bird fertilized coastal vegetation. However, the bird fertilized coastal vegetation was situated on small fragments surrounded by heathland. *C. problematicus* and *Cymindis vaporariorum* L. are described as heathland species in Fennoscandia (Lindroth 1985, 1986). The former was common in spring in the bird fertilized coastal vegetation sites, suggesting that this species does not distinguish between these two vegetation types. The latter species occurred only as a singleton in heathland.

Similar patterns of habitat preferences as the ones found here have also been found in other studies done in coastal areas of Norway (Waage, 1984; Bruvoll, 1985; Pedersen, 1986). Occurrence of forest species in open country (e.g. *C. hortensis*) has been explained by the damp Atlantic climate, as most of these species prefer wet, shaded environments (Mortensen, 1985).

Acknowledgements

We thank Tom Sømme and Thor Inge Vollan for assisting during the fieldwork, Gaute Velle for statistical advice, and Per Kristian Solevåg for help with the identifications.

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New records of ground beetles (Coleoptera: Carabidae) attracted to light traps in Hungary

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Abstract

Ground beetle species were collected by continuous light trapping operated at 21 different sites in Hungary, between 1982 and 2002. Most light traps were operated with a normal light bulb (100 W). In the carabid material obtained by these traps, there were 22 species that had not before been reported in light trap catches from Hungary. Several species such as *Amara ingenua*, *Badister lacertosus*, *Platynus assimilis*, *Zabrus tenebrioides* are common in Hungary, but there were also several rare species, including *Carterus angustipennis lutschniki*, *Masoreus wetterhalli*, *Parophonus hirsutulus*, *Trechus obtusus*. All species were caught in low numbers only.

Key words: Light trap, ground beetles, new records, Hungary

Introduction

Dispersal by flight is important for many ground beetle species (Den Boer, 1970; Noonan, 1985). Many species have lost or never had the ability to fly and other species fly only during a short period of their life (Thiele, 1977). The flight ability for a given species can be easily detected by special collecting methods used in entomology, for example window traps (Van Huizen, 1980; Basedow *et al*., 1990; Arndt, 2003), flight interception traps (Levesque & Levesque, 1994), suction traps (Lacman, 1986), and light traps (Yahiro & Yano, 1997). This last method is in widespread use to capture carabids (Honek & Pulpán, 1983; Matalin, 1996; Kádár & Szentkirályi, 1997). However, the method can be used only when the nightly air temperature reaches the necessary lower threshold value (Van Huizen, 1979).

In Hungary, there are about 500 ground beetle species, and about 250 species fly to light. So far, we recorded light trap data of 181 species from this country (Kádár & Lövei, 1987; Kádár & Szél, 1989, 1995).

In this paper we report data on further carabid species first caught by light traps in Hungary during a period of 21 years, collecting at 21 locations.

Material and methods

The studied years and localities investigated are given on Table 1. These traps were operated between 1982 and 2002 at different sites in Hungary in different time-periods. Two belonged to the forestry light trap network (Leskó & Szabóky, 2003), 16 to the plant protection light trap network (Szentkirályi, 2002) and three traps were operated as part of a biodiversity monitoring project. Most of the traps were so-called Jermy-type light traps, without baffles, others were Minnesota-type ones, with 3 baffles (Nowinszky, 2003). Most of the light sources were normal 100W bulb with white light, with the exception of five traps. Traps at Sumony, Tompa, Nyékládháza had a 125 W mercury vapour bulb, those at Fülöpháza and Maroslele a compact fluorescent bulb (Philips PL-T 42W/830/4p). The bulbs were placed at 2 m above ground. The habitats of the surroundings of the traps varied, including arable fields, orchards, vineyards, forests, parks, and meadows. The traps were emptied daily. The total trapping effort was 64 trap-years including > 11,000 trap-nights.

Table 1. Localities, their codes of light traps investigated in Hungary, 1982-2002 (see Material and methods).

Code	List of localities Localities	Investigated years
1	Bodrogkisfalud	1998; 2000
2	Csongrád	2001
3	Csopak	1982-1989
4	Fölöpháza	2001-2002
5	Hódmezővásárhely	1990-1992
6	Kenderes	1990-1992
7	Kunszentmiklós	1990-1992
8	Maroslele	2001-2002
9	Mikepércs	1990-1992
10	Nadap	1990-1992
11	Nagytőke	1997-2000
12	Nyársapát	1990-1996
13	Nyékládháza	1990-1992
14	Pacsa	1990-1992
15	Pécs	1990-1992
16	Sumony	1991
17	Székkutas	1998-2000
18	Szekszárd	1991-1993
19	Tanakajd	1990-1992
20	Tata	1990-1992
21	Tompa	1991

Results and discussion

Most of the carabid species obtained by the 21 light traps (about 73,000 individuals of 148 species) were common in light trap catches in Hungary. Many *Acupalpus*, *Amara*, *Badister*, *Bembidion*, *Ophonus*, *Pseudoophonus* species were caught in high numbers. The most common species indluced *Acupalpus parvulus*, *Amara apricaria*, *Bembidion minimum* and *Pseudoophonus griseus*. There were 22 species that have not previously been reported in light traps from Hungary (cf. Kádár & Szél, 1989, 1995). These species are listed in Table 2.

Flight is evidently a favourable ability for many carabids, aiding their dispersal, migration, and enlarging their distribution. For example, the Mediterranean species *C. angustipennis lutschniki* has probably reached Hungary using its flight ability and not by human-assisted dispersal. Previously, the nearest occurrence of this species was about 1000 km from

Hungary, in Bulgaria (Merkl, 1998). However, flight activity plays only a minor role in the life cycle of some abundant species such as the polymorphic species, *P. vernalis* (Desender, 1986).

Table 2. List of new records of carabid beetles captured by light traps in Hungary, 1982-2002.

Species	No. of individuals captured.	Codes of localities (Table 1)	Flight recorded by*
Agonum fuliginosum (Panzer,1809)	1	15	12
Amara ingenua (Duftschmid, 1812)	1	14	9
A. lucida (Duftschmid, 1812)	2	7, 12	13
Badister lacertosus Sturm, 1815	55	1, 4, 17	4
Bembidion dalmatinum (Dejean, 1831)	2	16, 21	6
B. decorum (Panzer, 1801)	1	19	this study
Brachinus bipustulatus Quensel, 1806	1	17	this study
Carterus angustipennis lutshniki Zamotajlov, 1988	1	11	10
Harpalus caspius (Steven, 1806) (= *H. roubali*)	1	14	this study
Masoreus wetterhalli (Gyllenhal, 1813)	1	21	this study
Microlestes fissuralis (Reitter, 1901)	1	6	1
Oodes gracilis A. Villa & G. B. Villa, 1833	3	6, 13, 14	6
Ophonus parallelus (Dejean, 1829)	20	3	this study
Parophonus hirsutulus (Dejean, 1829)	4	2, 4, 8	1, 3
Platynus assimilis (Paykull, 1790)	2	14	2, 9
P. longiventris Mannerheim, 1825	6	1, 9, 10, 12, 18, 20	4, 14
Poecilus cupreus (Linnaeus, 1758)	3	4, 14	3, 4, 7, 9, 11
Pterostichus elongatus (Duftschmid, 1812)	1	12	this study
P. minor (Gyllenhal, 1827)	2	1, 3	6, 12
P. vernalis (Panzer, 1796)	6	1, 5, 14, 15, 17	4, 5, 6, 8, 12
Trechus obtusus Erichson, 1837	1	15	12
Zabrus tenebrioides (Goeze, 1777)	2	5, 10	9

*= 1: Angelini (1998), 2: Arndt (2003), 3: Belousov (1986), 4: Bonn (2000), 5: Desender (1986), 6: Karpova & Matalin (1991), 7: Kegel (1994), 8: Lacman (1986), 9: Lindroth (1945), 10: Merkl (1998), 11: Serrano & Aguiar (1998), 12: Van Huizen (1980), 13: Van Huizen & Aukema (1992), 14: Zulka (1994)

Species in the list are either (1) macropterous, or (2) wing dimorphic with macropterous individuals at the trapping locality. An example for case (1) is *Poecilus (Pterostichus) cupreus*. This is a macropterous species and flight has been recorded (Lindroth, 1945). In the laboratory, some cases of spontaneous flight was observed by Kegel (1994). In Azerbaijan, flight was recorded during the day-time in several in fields (Belousov, 1986). However, no specimen was caught in three window traps used by Kegel (1986) in a rye field, and no specimen was collected in light traps by Belousov (1986). Further, no case of fully developed metathoracic flight muscles were found in random samples of dissected individuals (Geipel and Kegel, 1989). On the basis of this information it is probable that the occurrence in the light trap of *P. cupreus* is only accidental and sporadic.

An example for case (2) is *Trechus obtusus*. This species is recorded in Lindroth's (1945) list as brachypterous. Later it was recorded as a dimorphic species with the ability of flight (Den Boer *et al*., 1980), and individuals were collected in window traps in the Netherlands (Van Huizen, 1980). The proportion of macropterous specimens of *T. obtusus* varies between populations. In Belgium, Desender *et al*. (1980) found very low numbers of macropterous individuals. In contrast, only macropterous individuals were recorded in a Hawaiian population investigated by Liebherr and Takumi (2000). There was a high ratio of macropterous individuals in Canadian (32/54) and North American (141/185) material examined by Kavanaugh and Erwin (1985). The locality (code 1, see Table 1) where *T. obtusus* was caught by light trap was within its distribution area in Hungary as indicated by Horvatovich (1989).

Several species such as *Amara ingenua*, *Badister lacertosus*, *Platynus assimilis*, *Zabrus tenebrioides* are common species in Hungary, but the list includes several rare species, such as *Carterus angustipennis lutschniki*, *Masoreus wetterhalli*, *Parophonus hirsutulus*, and *Trechus obtusus*.

All species were caught in low numbers. *B. lacertosus* was the only species collected in relatively high numbers. In Poland, this species has not been observed flying (Makolski, 1952) but an indication of its flight ability was found recently in Germany (Bonn 2000).

The current paper raises the number of species actively flying to light traps in Hungary to 203.

Acknowledgements

The authors are grateful to G. L. Lövei for critical comments and improving the English of the manuscript and to S. Toft for helpful comments on earlier drafts of this manuscript. This work was supported partly by the Hungarian National Scientific Research Foundation (No. OTKA T023284) and by the Hungarian National Research and Development Project (No. NKFP-3B/0008/2002).

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Carabids of salt meadows at the Baltic Sea coast in Mecklenburg-West Pomerania (Germany) and their variability in mitochondrial genes

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Abstract

The interconnected Project BIOSALT, part of the programme BIOLOG, is concerned with the development of the biodiversity of salt meadows at the Baltic Sea in Mecklenburg-West Pomerania, Germany. Our focus is on the ecogical aspects in carabid beetles at the community and the species level as well as on the genetic differentiation within and between populations of the same species. First results on the level of mitochondrial DNA sequences are presented for some salt marsh species and on *Carabus clatratus* as a representative of species with low dispersal power. The halobionts have a lower subsitution rate than *C. clatratus* as expected because they can fly. Four haplotype groups in Europe were found for *C. clatratus* whose distribution may be explained by postglacial recolonisation. The high diversity of the population from Austria can be explained by its vicinity to putative refuges. The high diversity from Karkensee on Hiddensee was unexpected. There is some evidence for anthropogenic transport but other explanations cannot be excluded.

Key words: Biodiversity, *Carabus clatratus,* dispersal ability, ND5 gene, CO2 gene

Introduction

Following the 1992 Convention on Biological Diversity, the Federal Ministry of Education and Research (BMBF) launched the research programme "Biodiversity and Global Change" (BIOLOG). This programme comprises many projects. Our project BIOSALT was established by the Universities of Greifswald, Berlin and Bremen; it is concerned with the development of the biodiversity of salt meadows at the Bodden coast of the Baltic Sea in Mecklenburg-West Pomerania, Northeastern Germany.

The focus of our group is on the ecological aspects in carabid beetles at the community and the species level as well as on the genetic differentiation within and between species. Genetic diversity is more likely in species living in fragmented habitats such as salt meadows and in species which are restricted in their dispersal ability like small wingless carabids. Carabids are suitable objects for the assessment of change in biodiversity of saline habitats because many species are more or less specific to these habitats. For the examination of genetic variability, molecular data have the advantage of offering different markers with plenty of characters for the examination of genetic differentiation at different time scales.

Questions and hypotheses

Halophilic and halobiont species were the focus of the study. Additionally, *Carabus clatratus* was studied, a halotolerant species which was very common in the salt meadows of our research area. As a usually wingless species, it is in contrast to the more mobile salt specialists. Because of a large pool of material *C. clatratus* was the best candidate to prove the influence of historical processes on actual biodiversity. This was done by reconstruction of putative pathways of postglacial colonization from its refuge areas. Table 1 gives a characterization of the investigated species and Table 2 shows the hypotheses in detail.

Table 1. Characterisation of the investigated species by distribution and bionomic categories.

Species	Distribution	Distribution in Germany*			Habitat salinity type**	Wing type
		North Sea coast	Baltic Sea coast	In-land		
Anisodactylus poeciloides	Euro-Mediter-ranean	0	1	1	3.3	macropterous
Bembidion fumigatum	Euro-Siberian	1	3	1	2	macropterous
Bembidion minimum	Euro-Siberian	3	2	1	2	macropterous
Bembidion pallidipenne	Atlanto-Baltic	2	2	0	3	macropterous
Bembidion tenellum	Euro-Turanean	0	2	1	3.1-2	macropterous
Dyschirius salinus	Euro-Turanean	3	1	1	3.3	macropterous
Carabus clatratus	Euro-Siberian	1	2	2	1	dimorphic

* 0: no population; 1: local; 2: few populations, 3: many populations

** 1 - halotolerant, 2 - halophil, 3 - eury-halobiont, 3.1-2: oligo-mesohaline halobiont, 3.3 - steno-polyhaline halobiont

Table 2. Hypotheses on the genetic differentiations of the investigated salt marsh carabids, and their causes.

Is there a genetic differentiation at the level of mitochondrial DNA sequences?:	
• within the populations?	no
• between populations of the inner research area?	no or low
• between regions?	yes, some
The amount of differentiation depends on:	
• mobility (capability of flight, dispersal power)	yes
• isolation of habitats (patchiness)	yes
• historical processes	
- post glacial recolonisation,	yes
- spreading by human activities	no

Material and methods

DNA sequences of the mitochondrial gene CO2 were determined for 8 specimens of *Anisodactylus poeciloides* (Stephens, 1828), 5 specimens of *Bembidion fumigatum* (Duftschmid, 1812), 8 specimens of *B. minimum* (Fabricius, 1792), 5 specimens of *B. pallidipenne* (Illiger, 1802), 3 specimens of *B. tenellum* Erichson, 1837 and 5 specimens of *Dyschirius salinus* Schaum, 1843. DNA sequences of parts of the mitochondrial gene ND5 were determined for 51 specimens of *Carabus clatratus* Linnaeus, 1761.

DNA was isolated from dried or frozen specimens or from specimens directly stored in ethanol using the QIAGEN DNeasy Tissue Kit. Amplification of the gene parts was performed by PCR using the QIAGEN Taq Master Mix Kit under several conditions on a DNA Thermal Cycler from PERKIN ELMER. After electrophoresis on an agarose gel the PCR products were purified using the QIAGEN Qiaex II Gel Extraction Kit. Sequencing were performed by commercial suppliers. For more detailed information write an email to the author.

Results and discussion

At the present time, the above mentioned questions can be answered as follows (Table 3).

At the level of the mitochondrial DNA sequences of CO2 we had only few (~0-3 substitutions/750 bp) substitutions for the halobionts *Anisodactylus poeciloides*, *Bembidion* *pallidipenne*, *B. minimum, B. fumigatum B. tenellum*, and *Dyschirius salinus*. No further evaluation has been made, because not all the populations shown in Fig. 1 have been sequenced yet.

Table 3. Answer to the hypotheses.

Is there a genetic differentiation at the sequence level:	
• within populations?	in general low, exception: Karkensee, Austria
• between populations (research area)?	low or high (Karkensee)
• between regions?	different
The amount of differentiation depends on:	
• mobility (dispersal power)	yes (halobionts versus *C. clatratus*)
• isolation of habitats (patchiness)	not yet answered
• historical processes	
- post glacial recolonisation,	yes (*C. clatratus*)
- spreading by human activities	yes (*C. clatratus* Karkensee)

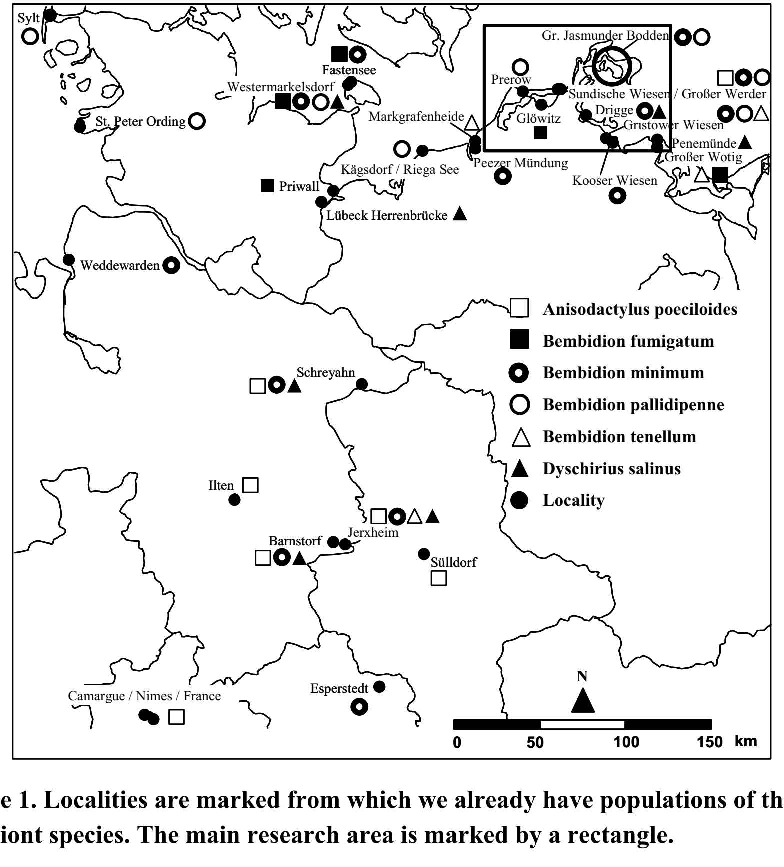

Figure 1. Localities are marked from which we already have populations of the halobiont species. The main research area is marked by a rectangle.

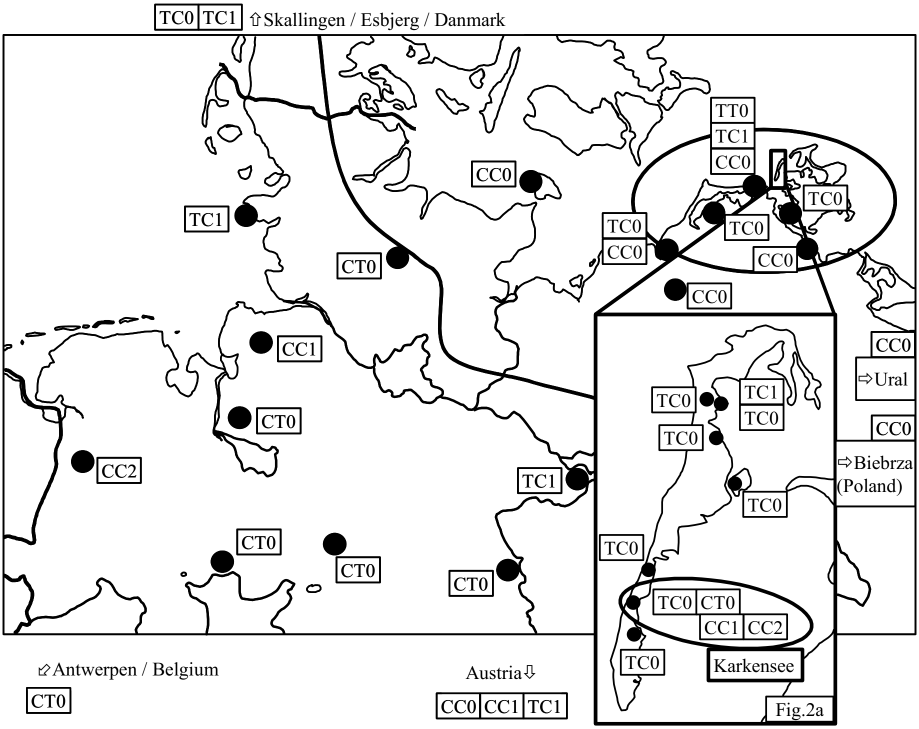

Figure 2. Haplotype distribution of *Carabus clatratus*.
CT1: at first informative position is a C, at the second is a T, additionally, there is one singular substitution. The bended line through Denmark, Schleswig-Holstein and Mecklenburg-West Pomerania marks the edge of the ice-shield of the last ice-age. The different populations are represented by 1 to 3 specimens. Figure 2a: The Island Hiddensee with the population Karkensee.

In *Carabus clatratus* we also found few substitutions (~0-4 subs/1000bp) in the DNA sequences of ND 5, mainly at two positions. Consequently, the 14 haplotypes show a characteristic pattern (Fig. 2): one haplotype group, called Coastal Form with the bases TC, is located at the coasts of the Baltic Sea and North Sea but also at the Elbe River and in Austria. Another haplotype group, called Inland Form, was found from the Ural Mountains across the continent up to Antwerpen in Belgium. The Inland Form can be divided into two subgroups. The Northern Inland Form with CC is distributed in the lowlands of northern Germany, northeast of the region of Masuria and the Ural Mountains. The Southwestern Inland Form with CT is located in the lowlands of Lower Saxony, Schleswig-Holstein and Belgium. There is some spatial overlap of the two inland forms. Additionally, there is support for this pattern from other mitochondrial DNA sequences of some specimens.

The substitutions are transitions and silent. Our interpretation is that convergent substitutions alone are unlikely to explain these patterns. A more likely interpretation is to assume different postglacial recolonisations and further differentiations.

The diversity of the population from Karkensee on Hiddensee (Fig. 2) is remarkable, because the other populations of Hiddensee all belong to the Coastal Form. We first excluded the possibility of anthropogenic transport of *Carabus clatratus* because of the characteristics of its habitat and its hibernation in the soil. However, *Carabus clatratus* was also found hibernating in trunks (pers. com.: T.Aßmann, A.Casale, W.Peil; own records). In former times, there existed a little harbour with a wood-fired lighthouse near Karkensee. In the 30-year War (1618-1648), the woods on Hiddensee were completely cut as a punishment. As a consequence, the wood had to be supplied from outside and this could have brought the Inland Forms to Karkensee. On the other hand, it is remarkable that this population did not spread over Hiddensee, especially with respect to the timescale considered above. Therefore, other explanations have to be taken into account. (i) Dispersal by flight cannot be excluded completely because specimens of *Carabus clatratus* with full wings are described from Sweden, Siberia and Austria (Lindroth, 1945). Additionally, we recently found one winged specimen in the North of Hiddensee. Thus, specimens from Denmark and Sweden are of high interest. (ii) A recent hydrochore drift, e.g. with clods of peat, as we detected for 11 carabid species of reed (unpublished). (iii) Anthropogenic transport, but with reeds.

Outlook

The next step will be to analyse the halobiont species at another level by AFLP (Amplified Fragment Length Polymorphism). We are also trying to get more informative positions of mitochondrial DNA sequences for *Carabus clatratus*.

Acknowledgement

We thank Ragna Misskampf for creating the maps with GIS and for proof-reading.

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Carabid beetles in median strips of three highways around the city of Helsinki, Finland

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Abstract

We trapped carabid beetles in median strips of three main Ring Roads around the city of Helsinki, Finland, in 2002. We collected a total number of 749 individuals and 29 species. As expected, most carabids collected were associated with open habitats, eurytopic and capable of flight. Median strips of the most recently constructed Ring Road II collected slightly more individuals and clearly more species than the two older roads (Ring Roads I and III). According to a Principal Components Analysis, the carabid community of the median strips of Ring Road II was considerably different from the communities at Ring Roads I and III. There appeared to be little difference in community structure at these last two roads. Our catches of the nationally vulnerable *Amara equestris* indicated that the median strips have conservation potential. We propose that verges and median strips should be managed in order to maintain their quality at least as temporary habitat for rare species occurring, for example, in dry meadows.

Key words: Carabidae, conservation, dispersal, grass strips, roads

Introduction

Roads are inevitable consequences of human development. With an increase in human mobility, cities and the surrounding landscape are becoming more connected. The ecological effects of roads are many. Roads fragment and isolate habitats (Forman & Alexander, 1998; Hourdequin, 2000); they act as barriers to dispersal (Mader, 1984); they increase mortality of organisms (Trombulak & Frissell, 2000; Germaine & Wakeling, 2001), and pollute the immediate surroundings (Forman & Alexander, 1998). However, roadsides can also act as valuable dispersal routes and/or (temporary) habitats for open-habitat associated species (Vermeulen, 1995; Niemelä & Spence, 1999; Koivula, 2003), especially those requiring dry meadows, as was shown for an endangered plant species in Finland (Eisto *et al*., 2000).

Although roadside verges have received some attention in ecological research, the median strip has been little studied. Median strips are, usually, artificially created narrow habitats situated between roads, as in the present study. As with roadside verges, the vegetation in these strips can be native or exotic and is disturbed repeatedly by mowing, fertiliser enrichment, and vehicle related factors (noise, chemical pollution, and litter; Forman & Alexander, 1998; Forman *et al*., 2003). Intuitively, these narrow, highly stressed strips appear to be of little functional or conservation value. Functionally, median strips are unlikely to aid in the dispersal of species between 'more' suitable habitat in the roadside verges. Many species avoid roads (Samways, 1994; Charrier *et al*., 1997; Forman *et al*., 2003) and when traffic volume is high, vehicles can cause high mortality. Moreover, in terms of conservation, it is difficult to imagine that these strips will be of use to any species, except hardy generalists.

This primarily exploratory study attempted to evaluate the conservation value of median strips of three highway roads (Ring Roads I, II and III) surrounding the city of Helsinki, Finland. We collected carabid beetles, using pitfall traps, and investigated the following general issues: (1) does the fauna at these three highway strips differ with regard to carabid assemblage structure, abundance and species richness; (2) are highway strips characterised by highly dispersive, open-habitat carabids; and (3) are these strips of any conservation value?

At the time of road construction, the studied median strips consisted of gravel and sand, but some topsoil was added later to promote vegetation growth (Jouni Karjalainen, Finnish Road Administration, pers. comm.). Thus, the beetle fauna we are likely to find in the median strips are probably of two origins: individuals introduced with the addition of soil, and dispersers from habitats adjacent to the roads. The years following road construction are likely to have favoured carabid species that are able to adapt to, or persist in the harsh conditions in the median strips and those species that are good dispersers and colonisers of frequently disturbed, ephemeral habitats. Because Ring Road II was constructed more recently (in 2000) than Ring Roads I and III (constructed from two-lane roads without median strips to four-lane highways with strips in 1989 and 1978, respectively), we expected the beetle community of the median strip of Ring Road II to be different in species richness and abundance, and in community composition. Overall, we expected the median strips to be dominated by highly dispersive, open-habitat generalist species, and that the strips would be of little conservation value.

Material and methods

Carabid beetles were collected in median strips of three main highways (Ring Roads I, II and III) surrounding the city of Helsinki, Finland (Fig. 1). The study sites were 2-4 m wide grassy strips with scarce bushes and trees in some sites (see Table 1). Pitfall traps were placed in four sets of 10 traps per Ring Road (Fig. 1), with sets placed at least 500 m apart. Trapping was continuous from 17 July to 1 October 2002. Carabids were identified using standard keys (Lindroth, 1985, 1986).

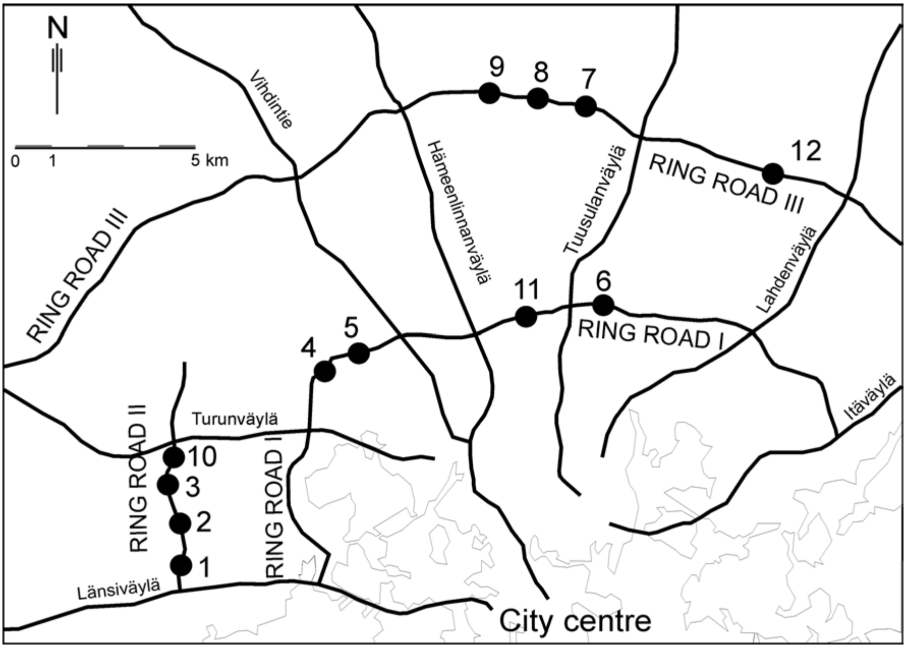

Figure 1. Location of the 12 study sites at Ring Roads I, II and III around the city of Helsinki, Finland.

The carabid beetles collected per 10 traps (i.e. per set) were pooled over the whole sampling period, and analyses were performed at this level. One-way ANOVA tests were performed to test for significant differences in carabid abundance and species richness between the three Ring Roads.

Table 1. Vegetation characteristics of the study sites. ’Site (nr)’ refers to the name of a given part of the city; the numerical code used in Fig. 1 is given in parentheses.

Site (nr)	Field-layer vegetation		Bush-layer vegetation		Trees (h >1.3 m)	
	% cover	Dominant vegetation (>5% cover)	% cover	Dominant species	% cover	Species
Ring Road I						
Leppävaara (4)	40	Grasses, *Taraxacum* sp., *Plantago* sp., *Linaria vulgaris*, *Achillea ptarmica*	-	-	-	-
Lintuvaara (5)	70	Grasses (esp. *Calamagrostis* sp.), *Taraxacum* sp., *Achillea ptarmica*	-	-	-	-
Pakila (11)	10	Grasses	90	*Crataegus* sp.	35	*Ulmus glabra*
Pukinmäki (6)	50	Grasses, *Vicia* sp., *Potentilla anserina*., *Linaria vulgaris*, *Tussilago farfara*	-	-	-	-
Ring road II						
Olari (1)	100	Grasses, *Trifolium* spp., *Taraxacum* sp.	-	-	-	-
Henttaa (2)	80	Grasses, *Trifolium* spp., *Taraxacum*, *Cirsium* sp., *Matricaria* sp.	20	*Lonicera caerulea*	-	-
Taavinkylä (3)	80	Grasses, *Trifolium* spp., *Taraxacum*, *Cirsium* sp., *Matricaria* sp.	25	*Symphoricarpos albus*	-	-
Turunväylä (10)	50	Grasses, *Tussilago farfara* *Artemisia* sp., *Barbarea* sp.	90	*Symphoricarpos albus*	15	*Sorbus intermedia*
Ring road III						
Veromies (7)	100	Grasses, *Potentilla anserina*, *Achillea ptarmica*, *Senecio vulgaris*	-	-	-	-
Ylästö E (8)	90	Grasses, *Potentilla anserina*, *Achillea ptarmica*	40	*Salix fragilis*	-	-
Ylästö W (9)	90	Grasses, *Potentilla anserina*, *Taraxacum* sp., *Tussilago farfara*, *Achillea ptarmica*	-	-	-	-
Tikkurila (12)	10	Grasses, *Trifolium* spp., *Taraxacum* sp., *Achillea ptarmica*	100	*Rosa rugosa*	-	-

In order to test and determine assemblage-level differences among the three roads, we applied Principal Components Analysis (PCA) for the trapping sites and included species with a total catch of > 2 individuals (17 species). The data were ln (x + 1) transformed, and centering by species was applied. We also performed a Redundancy Analysis (RDA) for the same carabid dataset by using percentage field-, bush- and tree-layer vegetation cover as environmental variables, and tested their statistical significance with Monte-Carlo randomisations (199 permutations).

Results

Twenty-nine carabid species and 749 individuals were collected from the Ring Roads (Appendix 1). *Calathus erratus* was the most abundantly collected carabid (167 individuals, 22% of total catch), followed by *Harpalus affinis* (130, 17%), *Pterostichus niger* (106, 14%) and *P. melanarius* (90, 12%). The 17 most abundantly collected species represented 98% of the total carabid catch. The ten largest species (9.1 – 23.9 mm) together formed 78.8% of all the individuals collected. Species with reduced wings (5 species, including *P. niger* with non-functional wings) formed 16.2% of the total catch, while the rest were either wing-dimorphic (10 species, *n* = 274, 36.6% of the total catch) or constantly long-winged (14 species, *n* = 354, 47.3%) (Appendix 1). Moreover, all except one species (*Trechus rivularis* with one individual) are species associated with open habitats or eurytopic in habitat use (see Lindroth 1985, 1986). The median-strip carabid community, therefore, appears to consist mainly of eurytopic species with the capability of flight.

We also captured 11 individuals of *Amara equestris*, a species considered vulnerable in the Finnish Red Data book (Rassi *et al.,* 2000). Moreover, several nationally very rare beetle species other than carabids were captured (M. Koivula, D. J. Kotze and J. Salokannel, unpublished data).

Overall, the highest number of individuals and species was collected from the median strips of Ring Road II. This road also had a slightly higher mean number of individuals than the other two roads, and the highest mean number of species (Table 2, Fig. 2).

The PCA revealed that there were considerable differences in the carabid catches among and within the roads (Fig. 3). Axes 1 and 2 explained 42.5% and 19.1% of the variation in the carabid dataset, respectively. Median strips at Ring Road II were clearly different from the strips at Ring Roads I and III, which in turn also had remarkable within-road variation. As is evident from Appendix 1 and the PCA plot (Fig. 3), some species were associated with certain Ring Road median strips. For example, *Pterostichus melanarius* were mainly found at Ring Road II. According to Appendix 1, this species was collected abundantly in median strips at Ring Road II, and only occasionally at Ring Road I. So too were *P. niger*, *Harpalus rufipes* and *Trechus quadristriatus.* Other species showing an association to a particular Ring

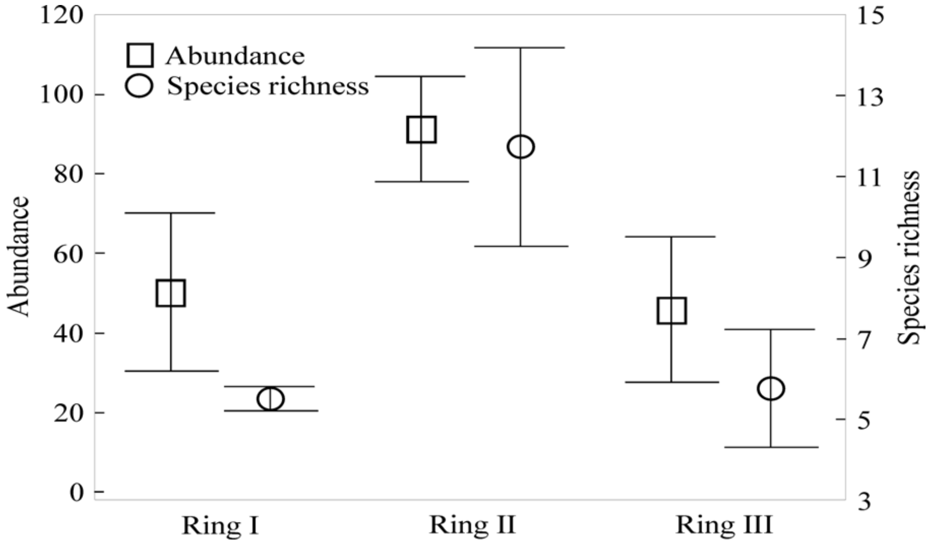

Figure 2. Mean (±1 SE) carabid beetle abundance and species richness in median strips of Ring Roads I, II and III around the city of Helsinki, Finland. *N* = 4 in each.

Table 2. ANOVA tests for differences in carabid beetle abundance and species richness among Ring Roads I, II and III in Helsinki, Finland.

Parameter	*d.f.*	MS	F	*p*
Abundance*				
Ring Roads	2	2514.33	2.08	0.181
Error	9	1207.14		
Species richness				
Ring Roads	2	1.43	4.27	0.050
Error	9	0.33		

*Carabid abundance was not transformed, while species richness was square-root transformed to approach normality.

Road included *Harpalus affinis* (Ring Road I), and *Amara bifrons* and *Calathus erratus* (both Ring Roads I and III). Perhaps surprisingly, in the RDA the vegetation cover did not explain the carabid catches significantly, although field- and bush-layer vegetation together explained 23.6% of the variation in the carabid dataset (not shown).

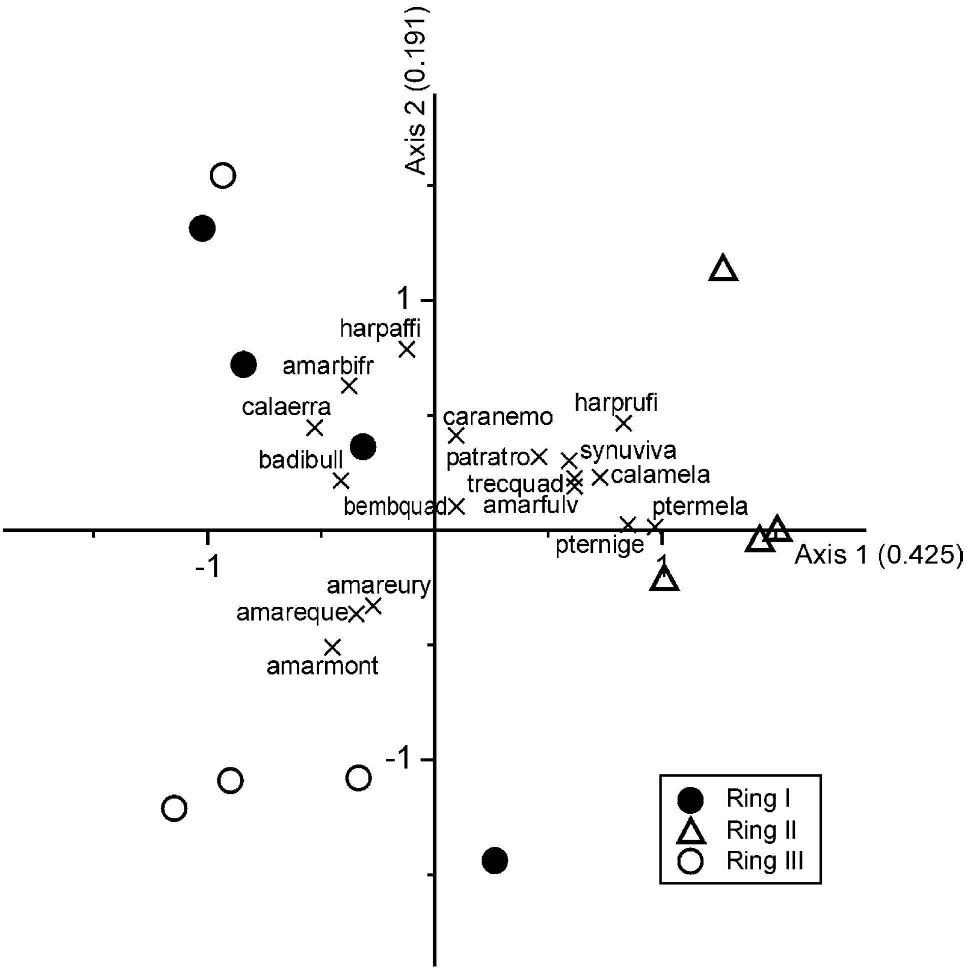

Figure 3. PCA biplot scatter showing the species caught (>2 individuals) and the study sites of Ring Roads I, II and III. The eigenvalues are shown along the axes.

Discussion

This study demonstrated that several carabid species appear to be well adapted to the severe environment of median strips. To our surprise, some of the carabid species were abundantly caught in the median strips, and *Amara equestris*, a species considered vulnerable in Finland (Rassi *et al.*, 2000), was quite abundant at one site. Furthermore, rare beetles other than carabids were also present (data not shown). In the Netherlands, Vermeulen *et al.* (1994) collected several geographically restricted or rare carabid species at road verges. These and the present results indicate that both verges and median strips have potential conservation value. Not surprisingly, the majority of carabid species collected were eurytopic, long-winged (thus probably capable of flight) and associated with open habitats. Furthermore, Lindroth (1985, 1986) classified 20 of the 29 species collected here as either synanthropic or occurring in human-altered habitat such as parks, towns or agricultural land.

As expected from the different ages of the roads, the carabid beetle community in median strips at Ring Road II differed considerably from those of Ring Roads I and III. Significantly more carabid species and more individuals (not statistically significant) were collected from Ring Road II, and the carabid assemblage structure also differed considerably from those of the other Ring Roads. This may be a result of more recent road construction and associated human-caused introduction of carabids. In the older Ring Roads I and III, some species may have disappeared from the median strips because these sites may be hostile habitats (because of frequent disturbances like mowing and traffic-related pollution), the microhabitat has changed along the vegetational succession and/or the patches are too small for them to persist there; consequently, some carabids may not be able to maintain viable populations there. This may not be the only explanation, however, because Ring Road I – constructed in 1989 – had slightly fewer species and individuals than the oldest Ring Road III (constructed in 1978). An additional possibility is a difference in the amount and quality of adjacent habitats along the three roads, thus enabling varying numbers and types of species to colonise the strips. Indeed, Ring Road II had very wide grassy verges and large meadows adjacent to the road, while Ring Roads I and III had narrower verges, and the adjacent habitat was dense deciduous forest, settlements or industrial areas. The species pools of the roadside verges clearly need to be studied in relation to the beetles in the median strips.

But is crossing a big road a likely event? Some carabids occasionally cross paved roads (Mader, 1984), and crossing is obviously easier for those that are able to fly. Crossing by non-fliers can actually be a common phenomenon: during an early summer morning, Ilpo Rutanen (pers. comm.) observed several *Carabus cancellatus* Ill. individuals (trying to) cross a 10-m wide, two-lane paved main highway in southern Finland. Furthermore, several carabid species are active at night (Lindroth, 1985, 1986; Lövei & Sunderland, 1996), when there is less traffic, and beetles trying to cross roads may suffer lower mortality from passing cars. In our study sites, for example, the average traffic volume varies between 40 000 and 80 000 cars/24 hours, but only ca. 11% of the daily traffic is between 22:00 and 7:00 (data from the Finnish Road Administration).

However, even though carabid individuals can travel relatively long distances by walking (Thiele, 1977; Den Boer, 1981) and theoretically can cross these roads, dispersing individuals may avoid crossing inhospitable habitat such as roads, and prefer to disperse along roadside verges or hedgerows (Vermeulen, 1995; Charrier *et al.*, 1997; Petit & Burel, 1998). Mark-recapture sampling at verges and median strips would shed more light on how isolated the median strips actually are.

Although road-effect reviews and studies often emphasise the negative effects of road construction (e.g. Forman & Alexander, 1998), we point out that roadsides and median strips may have some conservation value as well. The carabid (and overall beetle) communities of the median strips around Helsinki were surprisingly species rich, and included rare species. With careful management (mowing, and planting of certain vascular plants) these sites may act at least as stepping-stones and temporary habitat for species associated with habitats that are currently scarce in Finland, for example dry meadows of semi-agricultural landscapes. Currently the strips are managed only by mowing twice a summer and, at sites with recently planted ornamental bushes, by using herbicides with levels lower than farmland use (Arto Kärkkäinen, Finnish Road Administration, pers. comm.). So, should these potentially 'sink' habitats be improved for the benefit of indigenous species? Favouring meadow plants and preventing densely-growing generalist grasses from smothering these plants (i.e. maintaining high vegetational diversity) in the strips and verges will make them more favourable for open-habitat carabids.

Acknowledgements

We thank Matti A. Hämäläinen, Jouni Karjalainen, Arto Kärkkäinen and Jouni Määttä (the Finnish Road Administration, Uusimaa) and Ilpo Rutanen for help and valuable information. Dr. Aulikki Salmia (University of Helsinki) helped with vegetation identification. MK was partly financed by the Izaak Walton Killam Postdoctoral Fellowship, University of Alberta, Canada. We also thank two anonymous referees for their comments.

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Appendix. Carabids collected from Ring Roads I, II and III in 2002 in Helsinki.

Species	Size, mm	Wing form*	Ring Roads		
			I	II	III
Amara apricaria Payk.	7.6	M	-	1	-
Amara aulica Pz.	12.5	M	-	1	-
Amara bifrons Gyll.	6.3	M	31	1	15
Amara equestris Dft.	9.3	M	-	-	11
Amara eurynota Pz.	10.9	M	-	-	6
Amara fulva Müll.	9.1	M	-	3	-
Amara montivaga Sturm	8.5	M	-	-	10
Amara municipalis Dft.	6.5	M	-	1	-
Badister bullatus Schrank	5.6	M	3	-	1
Bembidion bruxellense Wesm.	4.6	M	-	1	-
Bembidion guttula F.	3.1	D	-	2	-
Bembidion lampros Hbst.	3.6	D	-	2	-
Bembidion quadrimaculatum L.	3.1	M	4	1	-
Calathus erratus Sahlbg.	10.0	D	50	16	101
Calathus melanocephalus Dej.	7.3	D	1	3	-
Carabus nemoralis Müll.	23.9	B	2	2	2
Clivina fossor L.	6.0	D	-	2	-
Cymindis angularis Gyll.	7.6	B	-	-	1
Cymindis macularis Fisch. Waldh.	8.8	D	-	-	2
Harpalus affinis Schrank	10.1	M	84	27	19
Harpalus rufipes Deg.	12.9	M	10	59	1
Leistus ferrugineus L.	7.2	B	-	2	-
Patrobus atrorufus Strøm	8.6	B	-	5	1
Pterostichus melanarius Ill.	14.7	D	5	85	-
Pterostichus niger Schall.	17.5	M	6	91	9
Pterostichus vernalis Pz.	6.7	D	-	1	-
Synuchus vivalis Ill.	7.1	D	-	3	-
Trechus quadristriatus Schrank	3.7	M	5	56	3
Trechus rivularis Gyll.	4.6	D	-	-	1
Number of individuals			201	365	183
Number of species			11	22	15

*M= macropterous, D= dimorphic, B= brachypterous

The influence of matrix habitat on ground beetle (Carabidae) species richness patterns in habitat islands

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Abstract

The influence of matrix species on the species - area relationship was tested in a real habitat island situation. Ground beetles (Carabidae) were studied by pitfall trapping in lowland-growing mountain forest fragments in NE Hungary and the Ukraine. There were a total of 56 species, and 48 of these were classified as "matrix" species (not specialists of mountain forests). There was a significant negative correlation between forest fragment size and both the total number of species and the number of matrix species, while a significant positive correlation between the forest fragment size and the number of forest specialist species.

Because of their higher richness, matrix species inverted the slope of the species-area relationship.

Key words: Habitat fragmentation, forest fragments, matrix species, island biogeography theory, carabids

Introduction

The theory of island biogeography (MacArthur & Wilson, 1967), although first suggested to describe species richness patterns on real islands, was soon extended to terrestrial habitat islands such as mountaintops (Diamond, 1981), nature reserves (Diamond & May, 1976), even agricultural fields (Risch, 1979). One major difference between real and habitat islands is the nature of the habitat separating them (Walter, 2004). Real islands are separated by water that cannot sustain dispersers that miss their island target. Also, immigration to the islands by organisms living in the habitat separating them (i.e. water) is not expected. Terrestrial habitat islands are separated by one or more different types of terrestrial habitat. This is termed the "matrix" in which the islands are embedded (Desender, 2005). This matrix is not necessarily hostile to dispersers, and they can survive there for variable periods of time. Species living in the matrix habitat can also colonise the habitat islands and even survive/reproduce there. The importance of the nature of the matrix connecting habitat islands is gradually realised (Desender, 2005). The relationship with habitat island features can be different by the island specialists and the species living in the matrix but also colonising the habitat island (thereafter called matrix species). These species perceive the habitat differently, and their reaction to habitat islands will also be different.

A detailed, experimental study of species dynamics on habitat islands, mainly concentrating on plants, is reported by Holt *et al.* (1995). In a recent publication, Cook *et al.* (2002) re-analysed this study and showed that the indiscriminate inclusion of matrix species present in habitat islands can distort the species - area relationship. When the matrix species were excluded, the significance of the species-area relationship increased. They conclude that: "further refinements of the paradigm are necessary to adapt and broaden the theory. For island biogeography theory to be applied to terrestrial habitat 'islands' which are heterogeneous and subject to edge effects, methodological allowances need to be made for the likelihood that species can colonise the 'islands' from the sea..." (Cook *et al*., 2002).

In this contribution, we evaluated the effect of matrix species on the species-area relationship to test whether the relationships reported for plants in a manipulated, fine-scale "habitat island experiment" (Cook *et al*., 2002) can also be detected for an arthropod group living in "real", large-scale habitat islands? We found that matrix species made a significant contribution to the ground beetle fauna in the large-scale fragmented forest habitat island setting and their indiscriminate inclusion can seriously distort the species-area relationship.

Material and methods

Study area and sampling

We selected ground beetles (Coleoptera, Carabidae) as test organisms. Carabids form a species-rich beetle family, and are widespread in many types of habitats, including forest fragments (Lövei & Sunderland, 1996). Forest fragments are 'real' habitat islands, formed either naturally, or as a consequence of human activities (Murcia, 1995). We used the carabid material collected over five years (1995-1999) from 19 forest fragments, ranging from 41ha to 3995 ha, located on the Bereg Plain (Magura *et al.,* 2001). The Bereg Plain is at the foot of the Carpathian Mountains, partly in NE Hungary and partly in the Ukraine. This is a relatively undisturbed, forested marginal area of the Great Hungarian Plain. Even in recent times, the area was covered by continuous woodland dominated by deciduous trees, and the species of the closed-canopy deciduous forest of the hills and mountains were able to disperse from the Carpathians to these lowlands. Due to agricultural activities and forest management in the 20th century, this once-continuous woodland became fragmented into patches. These patches of the original mountain forest are now embedded in a matrix of cultivated fields, meadows and other lowland forest patches. The remarkable feature of the carabid fauna is the occurrence of species characteristic of closed canopy deciduous forests of hills and mountains that usually do not occur in lowlands. The original mountain forest patches could be distinguished because they belong to the *Querco roburi-Carpinetum* association; the lowland-forest patches were here considered part of the matrix.

Beetles were collected using unbaited pitfall traps, consisting of plastic cups with 70% ethylene glycol as a killing and preserving solution. There were 9-18 traps/fragment, scattered randomly within individual fragments, and checked monthly from April to October. Further details on sampling and handling are in Magura *et al.* (2001).

Data analyses

The area of the mountain forest islands was measured by the ArcView GIS program package on a digitised 1:25000 map. The 19 studied forest fragments were divided into three area categories (small: <500 ha, medium: 500-1500 ha, large: >1500 ha) and the mean species numbers in the different categories were compared using a one-way Analysis of Variance (ANOVA). Normal distribution of the data was achieved by log(x+1) transformation. When ANOVA revealed a significant difference between the means, an LSD (least significant difference) test was performed for multiple comparison among means (Sokal & Rohlf, 1995).

Linear regression analysis was used to examine the relationships between the area of the forest fragment and the total number of carabid species in the fragment, the number of forest specialist species, and the number of matrix species. The categorisation of species is based on local habitat preference information, including Szél (1996), and our previous studies (Magura *et al.,* 2000, 2001).

Results

The species richness in all fragments combined was 56 species, dominated by the 48 matrix species. Eight species were identified as mountain forest specialists: *Abax parallelus, Carabus arcensis, C. intricatus, Cychrus caraboides, Cymindis cingulata, Leistus piceus, Molops piceus,* and *Pterostichus melas.*

One-way ANOVA on the total species richness and the richness of matrix species did not indicate significant differences by fragment size class (Table 1). However, it detected significantly greater richness of the forest species in large patches than in the small and medium sized fragments (Table 1). There was also a significant difference by fragment size class in the ratio matrix species / total species: small and medium fragments had relatively more matrix species than large fragments.

There was a significant negative (R=0.4846, F(1,17)=5.2161, p=0.0355, n=19) relationship between the size of the forest fragment and the total number of species (log-log scale, Fig. 1a).

Forest fragment size and the number of matrix species (Fig. 1b, log-log scale) showed a significant negative relationship (R=0.5372, F(1,17)=6.8956, p=0.0177, n=19). The forest fragment size and the ratio of matrix species to the total species showed a marginally significant negative relationship (R=0.4504, F(1,17)=4.3275, p=0.0529, n=19). The smaller the fragment, the larger part of the fauna belonged to the matrix species category.

However, there was a significant positive (R=0.4925, F(1,17)=5.4427, p=0.0322, n=19) relationship between the size of the forest fragment and the number of forest specialist species (Fig. 1c, log-log scale).

Table 1. Results of one-way ANOVAs on species numbers in relation to forest fragment size. The 19 studied forest fragments were divided into three groups: (Small: <500 ha, 7 fragments; Medium: 500-1500 ha, 7 fragments; Large: >1500 ha, 5 fragments) and the logarithm of the mean species numbers and the logarithm of matrix species/total species ratio were compared using a one-way ANOVA. If the one-way ANOVA indicated significant differences between samples, then an LSD (least significant difference) test was applied. Post hoc test results indicate which area group differs significantly ($p<0.05$) from the others; for example, S=M<L indicates that species richness was significantly higher in large (L) forests than in the medium (M) and small (S) forests.

	Source	SS	*d.f.*	MS	F	p	Post hoc test
All species	Between Groups	0.036	2	0.018	1.767	0.203	
	Within Groups	0.165	16	0.010			
	Total	0.202	18				
Matrix species	Between Groups	0.080	2	0.040	1.920	0.179	
	Within Groups	0.332	16	0.021			
	Total	0.412	18				
Forest species	Between Groups	0.344	2	0.172	3.825	0.044	S=M<L
	Within Groups	0.719	16	0.045			
	Total	1.063	18				
Matrix species / Total species ratio	Between Groups	0.007	2	0.003	4.340	0.031	S=M>L
	Within Groups	0.012	16	0.001			
	Total	0.019	18				

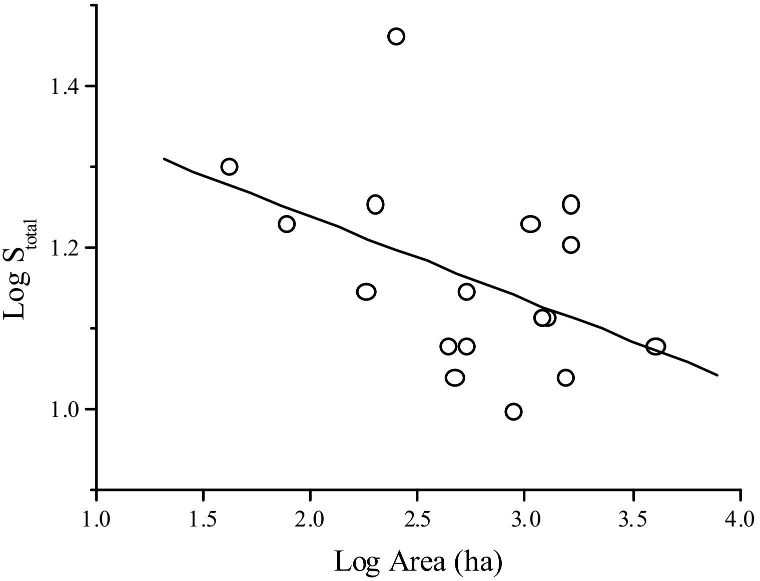

Figure 1a.

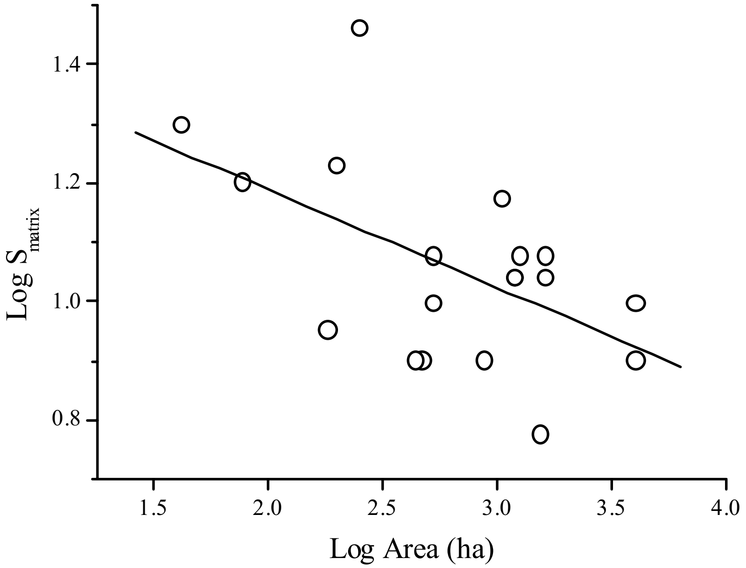

Figure 1b.

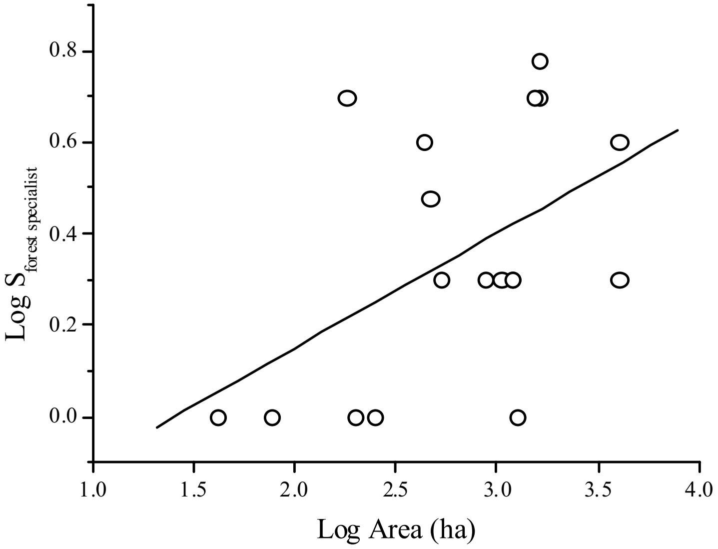

Figure 1c.

Figure 1. Relationship between the area of forest fragment and the number of (a) total ground beetle species; (b) matrix species, (c) forest specialist species on the Bereg Plain in NE Hungary and Ukraine, 1995-1999. All regressions are significant (details see in the text).

Discussion

Several of the relationships found in forest fragments between habitat island area and species numbers in the NE Hungarian Plain ran contrary to the expected positive relationship. Larger fragments had fewer ground beetle species. This pattern was caused by the matrix species that were more common in smaller fragments. The number of forest specialists and habitat area showed a positive relationship, conforming to the conventional prediction of the island biogeography theory. The distinction between matrix and "island" species is warranted but not entirely new. Several authors studying ground beetles (Bauer, 1989; De Vries, 1994; Magura *et al.,* 2001) emphasised that during the study of habitat islands, distinction should be made among species of different habitat affinity. There are species that truly perceive the habitat fragments as islands, and are unable to survive in the surrounding matrix, and those that occur in both the fragment and the matrix. Matrix species did not perceive the fragment as an island.

There can be remarkable differences in the relative importance of the matrix species in comparison to plants. In Holt *et. al*.'s (1995) experiments, the share of matrix species was 23%, while in our material 86% of the species pool consisted of matrix species. This deviation in the share of matrix species could be a general difference between groups of low mobility (plants) vs. high mobility (insects). It may also be due to a large pool of open-area generalist species compared to forest species among carabid beetles. If a similar analysis was made on xylophagous beetles (such as Cerambicidae), we would not expect matrix species to invert the relationship, simply because there would be few matrix species.

The definition of "matrix" species can be based on different considerations. For example, Cook *et al.* (2002) define 'matrix' species as any species occurring outside their experimental islands. This was based on empirical data, yet it is a significant simplification. The mere occurrence in a habitat, especially for mobile organisms, does not indicate ecological links to that habitat. There can be a significant share of ”tourists” in arthropod assemblages (Novotny & Missa, 2000). We defined the habitat affinities (forest specialists, matrix species) based on the literature and our earlier data. We had no parallel sampling in the matrix of this landscape to prove that the matrix species really occurred outside the fragments. However, the habitat affinities of ground beetles are well known and reliably documented (Lövei & Sunderland, 1996) so the allocations could be made with confidence. We believe that a quantitative statistical method such as TWINSPAN (Hill *et al.,* 1975) or the IndVal procedure (Dufrêne & Legendre, 1997) would be able to identify true matrix species more precisely.

We should also emphasise the special nature of the studied forest fragments. These forest fragments represent remnants of the Carpathian high mountain forests growing on the lowland (Magura *et al*., 2001). There were other types of forest fragments in the area, but carabids occurring in these forested fragments were considered matrix species. "Forest specialists" in our context meant species that are restricted to these special types of forests.

In Cook *et al.'s* (2002) experiments, the removal of matrix species increased the strength of the relationship between the species richness and patch size. We note, however, that the relationship remained non-significant. In our study, the relationships were significant, and the impact was even more dramatic: when the matrix species were excluded from the analysis the whole relationship was turned around.

The smallest fragment in our study was still much larger (41 ha) than the assumed minimum necessary for self-sustaining ground beetle populations (about 1 ha, Mader 1984). Including smaller fragments could possibly strengthen the relationship documented.

Acknowledgements

GL was supported by the Domus Hungarica Scholarship, Hungary. We thank E. Varga for assistance, R. Holt for encouragement, S. Toft and an anonymous reviewer for helpful comments on the manuscript. GL thanks S. Toft for taking over the editorial role for this manuscript.

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Composition and diversity of spring-active carabid beetle assemblages in relation to soil management in organic wheat fields in Denmark

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Abstract

Patterns in spring-active carabid beetle assemblages were described in relation to four organic soil management regimes (no soil nutrient addition, undersowing, animal manure, undersowing + manure) in two areas of Denmark by means of pitfall trapping. On the island of Zealand, the Flakkebjerg study area had 22 species, 3-10 species/trap, and the species rank of these was the same for all treatments. The dominant species were *Pterostichus melanarius, Agonum dorsale, Harpalus rufipes* and *Calathus fuscipes*. At Foulum, Jutland, there were 46 species, 12-15 species/ trap, dominated by *P. versicolor, P. melanarius, A. dorsale* and *Nebria brevicollis.* Their rank, however, was not the same for all treatments. There were remarkable differences in the carabid assemblages of the two sites, and manure addition modified the assemblages, more pronouncedly so in the poorer-soil Flakkebjerg site. However, we did not detect clear effects of the treatments on carabid species richness, overall abundance or on *Pterostichus melanarius* alone.

Key words: Soil fertilisation, undersowing, manure, assemblage composition, carabids, diversity scaling

Introduction

Polyphagous predators can survive on many different types of prey and this enables them to persist in habitats that experience fluctuations in prey availability (Symondson *et al.,* 2002). In seasonal climates, however, this fluctuation can result in a complete lack of food, and a subsequent dormancy (not necessarily caused by the lack of food but climatic constraints). A critical period for polyphagous predators is when activity in the spring resumes. At this time, activity can be fuelled by reserves built up before the onset of winter, but this is not usually sufficient. Especially for invertebrate predators, prey availability early in the season is critical, and is often critically low (Toft & Bilde, 2002).

Ground beetles (Coleoptera: Carabidae) are among the dominant soil-surface active arthropods in northern temperate ecosystems (Lövei & Sunderland, 1996). Many species of ground beetles occur in cultivated areas, and many of these are obligate or facultative predators. For this reason, ground beetles are usually considered beneficial in agricultural habitats (Lövei & Sunderland, 1996). Carabids also eat soil-born organisms (Hengeveld, 1980), that can, especially in early spring, be an important food source (Toft & Bilde, 2002).

Increased soil nutrient content can support a more rich soil fauna (Wardle, 2002) and it can also subsidise the above-ground food chain. Several examples of soil subsidies to the above-ground food webs are documented, but the potential effect on above-ground predators remains unexplored (Wardle, 2002).

As carabids are prominent predators active on the ground but spend an unknown part of their activity in the soil (Lövei & Sunderland, 1996), it is plausible to assume that soil food webs can subsidise above-ground food webs, specifically ground-active predators, by providing food for them during times of food shortage. If this occurs, we can expect a change in ground beetle assemblage composition, diversity, density, satiation level, or any combination of the above as a result of different soil management practices. We investigated whether ground beetle assemblages responded to different methods of soil nutrient management in an organic crop rotation. At two different locations in Denmark, we found that soil manipulation influenced the ground beetle assemblage in several ways, but undersowing was less influential than fertilising by animal manure on spring-active ground beetle assemblages.

Study area, material and methods

In order to sample carabids, we used selected plots in a long-term organic crop rotation experiment. This experiment has 10 x 10 m treatment plots as base units in a randomised block design, and is repeated exactly at four Danish locations. We selected two of these: at Flakkebjerg (55°19' N, 11 °23' E), on the island of Zealand and in Foulum (56 °30' N, 9 °34' E), on the peninsula of Jutland, Denmark. The individual plots were separated from each other by grass strips, 5 m within rows and 10 m between rows. We selected plots that had first-year wheat crop in the crop rotation sequence. There were four treatments: unfertilised control, undersowing with legumes, fertilising with animal manure and undersowing + manure. Each treatment had four replicate plots per location, giving us a total of sixteen 10 x 10 m sample plots at each location. Full details of the soil manipulation experiment are in Djurhuus & Olesen (2000).

We sampled ground beetles with pitfall traps, setting one trap near the centre of each of the 32 plots selected. Traps were 10 cm diameter plastic cups, containing ca. 200 ml of 70% ethylene glycol and a drop of detergent. In order to protect the catch from rain and scavenging as well as to prevent accidental killing of frogs and small mammals, we used a 25 cm x 25 cm metal cover, secured ca. 5 cm above the trap. Traps were set at the time of aphid immigration, and were checked fortnightly until aphids emigrated from the crop. In Flakkebjerg, this covered the period of 6 June - 18 July 2002 (3 fortnightly samples), and in Foulum, 17 May - 12 July 2002 (4 samples). The catches were sieved on site, and stored in vials with 70% ethyl alcohol until sorting and identification in the laboratory. For identification, keys by Lindroth (1985, 1986) and Hůrka (1996) were used. Nomenclature follows Lindroth (1985, 1986). Voucher specimens are deposited at the Department of Crop Protection, DIAS Flakkebjerg Research Centre, Denmark.

To evaluate diversity, the generalised Rényi entropy equation was used (Tóthmérész, 1995; Lövei *et al*., 2002). Rényi diversity, $HR(a)$, is calculated as follows:

$$HR(a) = \frac{1}{1-a} \log \sum_{i=1}^{S} p_i^a \quad ,$$

where p_i is the relative abundance of the i-th species, and S is the number of species; a is a so-called scale parameter. The equation is interpreted for the range $a \geq 0$, with the restriction that $\alpha \neq 1$. The results are graphically presented as a 'diversity profile'. The diversity profiles were generated by the DivOrd program package (Tóthmérész, 1993). For multivariate analysis (PCA), the NuCoSa program package (Tóthmérész, 1996) was used. For the PCA, no species were excluded, the Matsusita index was used, and the analysis was centered on samples. A repeated measures ANOVA (Sokal & Rohlf, 1995) was used to test for differences in the number of beetles between study areas, treatments and sampling occasions.

Results

Assemblage composition

In Foulum, 46 species were captured (Table 1) with 12-15 species/trap. The most common species were *Pterostichus versicolor, P. melanarius, Agonum dorsale* and *Nebria brevicollis.* Their rank, however, was not the same for all treatments.

Table 1. The total list of the ground beetles captured in pitfall traps in the different soil fertility treatments. Foulum, Jutland, and Flakkebjerg, Zealand, during 2002.

	Total number of beetles captured in the treatment									
	Foulum					Flakkebjerg				
Species	Control	Under-sowing	Manure	Ma + Us*	Total	Control	Under-sowing	Manure	Ma + Us	Total
Pterostichus versicolor	503	535	185	179	1402	1	6	2	10	19
Pterostichus melanarius	324	399	300	265	1288	1033	965	1380	1363	4741
Agonum dorsale	136	116	126	131	509	130	239	306	434	1109
Nebria brevicollis	134	135	63	137	469	11	10	17	29	67
Bembidion tetracolum	78	43	97	47	265	-	-	-	-	0
Amara plebeja	62	64	39	90	255	-	-	-	-	0
Clivina fossor	44	44	63	39	190	-	-	-	-	0
Harpalus rufipes	66	33	57	33	189	117	86	146	243	592
Harpalus tardus	38	33	38	42	151	-	-	1	-	1
Loricera pilicornis	43	39	38	29	149	1	2	5	3	11
Harpalus affinis	42	36	30	17	125	11	9	12	23	55
Bembidion lampros	44	25	26	18	113	1	1	1	-	3
Amara familiaris	15	11	44	36	106	1	2	2	-	5
Amara aenea	29	9	7	4	49	-	-	-	-	0
Pterostichus niger	7	8	2	17	34	-	-	-	-	0
Synuchus vivalis	5	7	7	6	25	-	-	-	-	0
Trechus obtusus	4	8	4	3	19	-	-	-	-	0
Trechus quadristriatus	9	3	1	2	15	-	-	1	-	1
Calathus cinctus	7	2	2	2	13	-	-	-	-	0
Agonum muelleri	5	2	4	-	11	-	2	-	1	3
Bembidion obtusum	2	4	3	2	11	-	-	-	-	0
Borscus cephalotes	3	3	4	1	11	-	-	-	-	0
Calathus fuscipes	5	5	1	-	11	19	31	27	17	94
Carabus nemoralis	2	2	5	1	10	1	1	1	-	3
Demetrias atricapillus	-	-	-	-	0	-	-	3	7	10
Amara similata	2	1	5	1	9	-	-	-	-	0
Bembidion properans	4	-	3	-	7	-	-	-	-	0

	Total number of beetles captured in the treatment									
	Foulum					Flakkebjerg				
Species	Control	Under-sowing	Manure	Ma + Us*	Total	Control	Under-sowing	Manure	Ma + Us	Total
Calathus melanocephalus	2	4	1	-	7	-	2	1	-	3
Asaphidion flavipes	4	-	-	2	6	-	-	-	-	0
Pterostichus niger	-	-	-	-	0	-	3	2	1	6
Amara bifrons	2	1	1	1	5	-	-	-	-	0
Pterostichus strenuus	-	-	-	5	5	-	-	-	-	0
Stomis pumicatus	1	1	-	2	4	-	-	-	-	0
Amara communis	3	-	-	-	3	-	-	-	-	0
Amara consularis	2	-	1	-	3	-	-	-	-	0
Amara fulva	1	1	1	-	3	-	-	-	-	0
Amara lunicollis	-	2	1	-	3	-	-	-	-	0
Calathus erratus	1	-	1	-	2	-	3	1	1	5
Harpalus rubripes	-	-	2	-	2	-	-	-	-	0
Notiophilus palustris	-	1	1	-	2	-	-	-	-	0
Notiophilus pusillus	2	-	-	-	2	-	-	-	-	0
Pterostichus cupreus		2	-	-	2	-	-	-	-	0
Amara aulica	-	-	-	-	0	-	2	-	-	2
Amara apricaria	1	-	-	-	1	-	-	-	-	0
Anisodactylus binotatus	1	-	-	-	1	-	-	-	-	0
Notiophilus aquaticus	1	-	-	-	1	-	-	-	-	0
Harpalus rufibarbis	-	1	-	-	1	-	-	-	-	0
Trechus micros	1	-	-	-	1	-	-	-	-	0
Amara ovata	-	-	-	-	0	1	-	-	-	1
Notiophilus aquaticus	-	-	-	-	0	1	-	-	-	1
Notiophilus pusillus	-	-	-	-	0	1	-	-	-	1
Total number of individuals	1636	1581	1163	1114	5494	1329	1364	1908	2132	6733
Total numbers of species	40	34	34	28	46	14	16	17	12	22

*** Ma + Us: manure + undersowing**

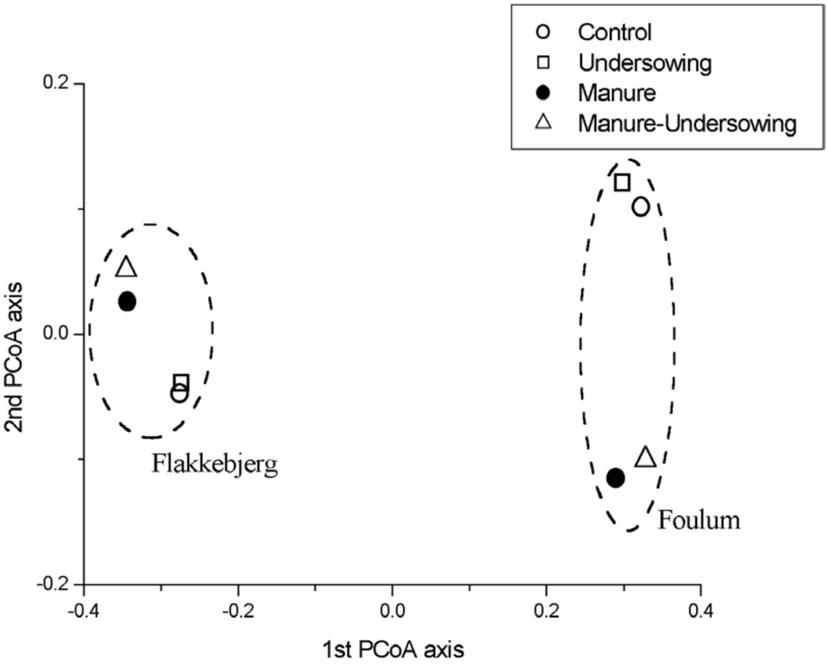

Figure 1. Multivariate analysis (Principal Component Analysis) of the ground beetle assemblages of the soil fertility treatments (4 replicates/treatment combined) at Flakkebjerg (to the left) and Foulum (right). The first two axes explain 93.5% of the variation in the data.

In Flakkebjerg, only 22 species were captured, and the species number / trap was also lower (3-10 species / trap). The most numerous species were *P. melanarius, A. dorsale, Harpalus rufipes* and *Calathus fuscipes*. Their ranks were the same in all treatments (Table 1).

Diversity

The PCA clearly separated the ground beetle assemblages at the two locations (Fig. 1). The first two axes explained 93.5% of the variation in the data. Manure had a larger impact on the assemblage composition than undersowing at both locations.

The Rényi-diversity profiles at Foulum (Fig. 2A) indicated that the combined assemblage of the undersown patches was unequivocally less diverse than the control, and the manure-treated areas. Compared to the manure + undersowing treatment (M+U), it was more diverse only at small values of the scale parameter, sensitive to species richness. From scale parameter α= 0.5 upwards, the undersown plots fell below the profiles of all other treatments (Fig. 2A). The manure or manure + undersowing treatments could not be unequivocally ordered, as the profiles cross each other at different values of the scale parameter. The

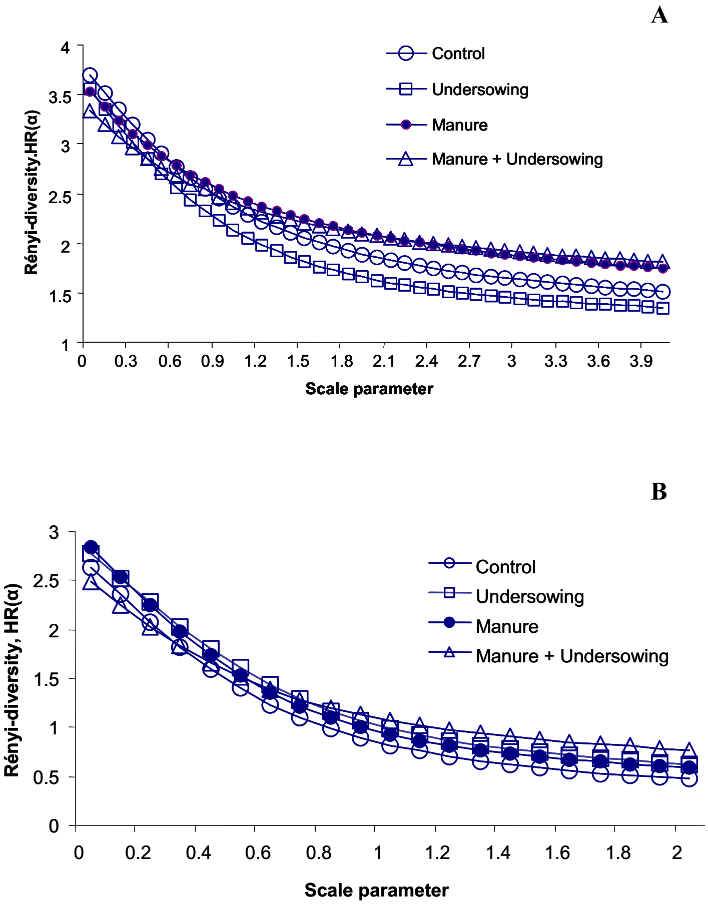

Figure 2. The Rényi diversity profiles of the four ground beetle assemblages studied at Foulum (A) and Flakkebjerg (B), spring 2002. The relative positions of the four profiles did not change at higher values of the scale parameter, so only the intervals α < 4 (a) and α < 2 are shown.

diversity relationship of the assemblages found in the plots that received animal manure and the manure + undersowing combined treatment is complex. The assemblage in manure treatment plots is more diverse when considering rare species (the curve runs above that of the M+U plots), but becomes less diverse (at scale parameters α>2.6, the curves cross, and the diversity of the assemblage in the M+U plots becomes more diverse, being less dominated by the common species) at higher values of the scale parameter (Fig. 2A).

The diversity profiles at Flakkebjerg (Fig. 2B) indicated a partially different situation. The control was unequivocally less diverse than manure or undersowing, but not their combination. However, the only difference was in the part of the curve where species richness has a large influence, at low values of the scale parameter. Near the value $\alpha = 0.3$, the diversity profile of the combination treatment crosses that of the control, and remains consistently above it. At the value of $\alpha \approx 1$, this curve indicates the highest diversity. The relationship between the effect on diversity of manure vs. undersowing is not as different as in Foulum, but the diversity profile for the undersown plots runs mostly above the equivalent curve for the manure-fertilised plots (Fig. 2B).

Carabid response to soil treatments

A repeated measures ANOVA on total number of beetles, and on common species indicated only a few significant relationships. There was a near-significant location * treatment interaction in the total number of beetles, mainly brought about by the significant difference in the number of individuals collected during the four sampling occasions. The only species with a significant treatment * sampling occasion effect on the number of individuals was *Pterostichus melanarius* (Table 2), again, mainly as a result of the different number of individuals collected during the sampling occasions.

Table 2. Analysis of variance on the total numbers of beetles captured, and on one of the common species, *Pterostichus melanarius*.

	numDF	denDF	F-value	Significance, *p*
Total number of beetles				
(Intercept)	1	68	275.44356	<0.0001
Location	1	27	2.47774	0.1271
Treatment	3	27	0.03254	0.9919
Sampling.occasion	3	68	6.24635	0.0008
Treatment*Sampling.occasion	9	68	1.85750	0.0736
Pterostichus melanarius				
(Intercept)	1	68	595.8805	<0.0001
Location	1	27	150.4353	<0.0001
Treatment	3	27	0.3976	0.7558
Sampling.occasion	3	68	40.2885	<0.0001
Treatment*Sampling.occasion	9	68	3.4687	0.0014

Discussion

The strongest impact in our studies was the regional difference in the species richness of the ground beetle assemblages. This probably reflected soil differences. For example, the humus content in the top 25 cm of the soil was nearly two times higher in Foulum than in Flakkebjerg (Djurhuus & Olesen, 2000). Other parameters also indicated a more nutrient-rich soil in Foulum than in Flakkebjerg. This, however, did not result in higher overall activity-density.

The diversity of the assemblages was influenced by the treatments, as well as by regional differences. The soil manipulations seem to have caused a larger effect in Flakkebjerg. This could be a consequence of the poorer soil at this site.

At the activity-density level, however, ground beetles did not show an overwhelming, consistent response to soil treatments. The spring distribution of ground beetles could be influenced by habitat features and the composition of the regional species pool more strongly than soil nutrient status in the local patch. An effect of patch size is also possible. However, this is contradicted by the results of the multivariate analysis: the carabid assemblages in the different treatments separated well from each other, at least in some cases. As the patches were randomly arranged, this seemed to indicate that patch size was not unrealistically small.

Acknowledgements

This work is part of the research program of the Danish Research Centre for Organic Farming. We thank Erling Nielsen and Ilse Rasmussen for allowing us to use the experimental plots, V. Ködöböcz for identifying the species, G. Christiansen, L. Chistensen, Lars Dall, I. W. Nielsen & B.P. Pedersen for technical assistance, Dr. R. Voland for statistical advice and T. Magura and H. Rasmussen for help with the figures. We thank J. Kotze who took over the editorial responsibility for this paper, and to an unknown reviewer for helpful suggestions.

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Biology and ecology of immature stages of ground beetles (Carabidae)

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Abstract

Existing information since 1977 on the biology and ecology of the eggs, larvae and pupae of Carabidae is reviewed. The important biological distinction is between summer- and winter-occurring larvae. For eggs, factors considered include functional morphology, environmental influences and parental care. Larval life-forms are reviewed, but there are still larval morphological features whose functions are unknown. Mortality factors important to newly-hatched larvae include starvation and cannibalism. Larval feeding habits and diets are considered, and related to growth and survival. The potential growth rate of older larvae may be limited by food availability, but will more often be determined by environmental temperatures, especially when diapause is involved. Examples are given of environmental factors, both biotic and abiotic, that have been shown to affect larval growth and mortality. The importance of pre-adult mortality to carabid population dynamics is stressed. Most existing studies have, however, taken place on a limited spectrum of common species, and detailed knowledge of how many environmental factors act on immature stages is still lacking.

Key words: Eggs, larvae, pupae, growth, mortality

Introduction

The purpose of this paper is to provide an overview of our knowledge of some aspects of the functional morphology, biology and ecology of carabid eggs, larvae and pupae. Danish entomologists have had a long history of pioneering work on beetle larvae, since the seminal work of Schiödte (1867). Many of the foundations of our knowledge of beetle life histories and ecology were laid by two Danish coleopterists. August West outlined the life histories and ecologies of all the Danish beetles (West, 1940) and Sven Gisle Larsson paid particular attention to the breeding cycles of carabids (Larsson, 1939) and also published the first keys to species of carabid larvae (Larsson, 1941).

Table 1. Numbers and percentages of papers concerning developmental biology of Carabidae in carabidology conferences since 1976.

Country	Year	Total papers	Developmental papers		Reference
			number	%	
Germany	1978	16	3	18.75	den Boer *et al.* 1979
Germany	1981	22	2	9.09	Brandmayr *et al.* 1983
Poland	1982	14	2	14.29	den Boer *et al.* 1986a
Germany	1984	37	4	10.81	den Boer *et al.* 1986b
Hungary	1986	41	1	2.44	den Boer *et al.* 1987
UK	1989	46	3	6.52	Stork 1990
Belgium	1992	71	10	14.08	Desender *et al.* 1993
Finland	1995	25	1	4.00	Niemelä 1996
Italy	1998	33	5	15.15	Brandmayr *et al.* 2000
Poland	2000	28	1	3.57	Szysko *et al.* 2003
Total		333	32	9.61	

The present review uses as its starting point the information in the excellent book by Thiele (1977) and then considers what we have learned in the ensuing 26 years. Examples are given mainly from European species, unless non-European carabids provide extra information that probably applies also to European species.

Only a small proportion of work on carabids gives substantial information on the biology or ecology of the immature stages. There are active workers on carabid larval taxonomy, but many of their papers give no ecological information. European carabid conferences overall have just under 10% of papers with ecological or biological information on the immature stages (Table 1). Such meetings have sometimes had specific sections on carabid biology and developmental ecology: the proportion of such papers in the literature generally is rather lower in most years.

It is generally considered that every carabid adult has been preceded by developmental stages of egg, three feeding larval instars and pupa. However there are a number of exceptions to this typical life history (Bousquet, 1977). The egg stage is absent in some *Pseudomorpha* species (N. American) which are ovoviviparous. The females lay larvae, probably into or adjacent to the ant nests in which the physogastric larva then develop (Liebherr & Kavanaugh, 1985). This is considered as an adaptation to reduce predation on the eggs by ants. The French cave dwelling *Aphaenops cerberus* lays a single very large egg, and the first instar larvae do not feed before moulting (Deleurance-Glaçon, 1963). It is not clear whether there is any feeding in a later instar, or whether all growth is in the adult stage. Presumably this is a response to the low energy availability in the subterranean environment.

A few carabids have only two larval instars. In some cases this has no apparent biological significance, as in some species of *Amara* (Bílý, 1975) and *Harpalus* (Kirk, 1974). *Lebia* have only two larval instars, but the 2nd is physogastric and adapted as an ectoparasite (Lindroth, 1954). *Thermophilum* (north Africa) also have only two instars, but the second is non-feeding (Paarman 1979a), a possible adaptation to desert conditions and associations with ants (Dinter *et al.*, 2002).

Increased instar numbers are even scarcer. The north American *Eurycolus* has four instars (Erwin, 1975) for no apparent reason. The ectoparasite *Brachinus pallidus* has five larval instars, again physogastric from L2 onwards (Erwin, 1967). However this is not common to all *Brachinus* (Saska & Honek, 2005). But these are the exceptions that prove the rule that indeed 99%+ of carabids do have the conventional developmental stages as outlined above. This begs the question, what is special about the number three? Many other beetle larvae, especially those that are plant feeders, have more developmental instars. It may be, as shown later in this review, that this relates to the maximal growth possible from one instar to another, and that the plant feeding families tend to have relatively smaller eggs.

This paper considers the following aspects of developmental ecology:

- importance of phenology; when in the year do the immature stages occur?
- eggs: their size, environmental interactions and parental care.
- larval morphology in relation to lifestyle and environment.
- larval development, especially the effects of diet, temperature and moisture.
- pupal mortality.
- populations & overall mortality of immature stages, especially in relation to their population dynamics.

Phenology

Between 10% and 80% of the life span of many carabids is in the developmental stages. The classical division of carabids was into spring and autumn breeders (see discussion in Thiele, 1977). Spring breeders have a short larval period, whereas in autumn breeders this can be very extended, as in *Trechus obtusus* (Desender *et al.,* 1981). However these two basic phenologies have been shown to be extremely variable in many species, and other types occur (Paarman, 1979b) as well as there being no clear distinction between them. A better distinction is into when the larvae occur – in summer or in winter. Den Boer and den Boer-Daanje (1990) considered the reproductive cycle and larval period of the 68 most abundant carabid species of Drenthe (Netherlands). The reproductive periods showed a gradual trend, from early spring breeders, to those carabids that reproduced from autumn through into the following spring; no period of the year was without some species' reproductive time. In contrast, consideration of the time of year at which the larvae occurred demonstrated a complete distinction between 40 species with summer larvae, and 28 with winter larvae.

Table 2. Percentages of the developmental period of a range of Carabidae spent as egg, larva or pupa (ordered by % larval period).

Stage			Species, temperature	Reference
egg	larva	pupa		
11.54	47.44	41.03	*Carabus arvensis*	Grüm 1975
12.20	48.78	39.02	*Carabus nemoralis*	Grüm 1975
15.43	50.91	33.66	*Amara eurynota*	Bílý 1972
26.60	53.55	19.86	*Bembidion lampros*, 17°	Boye Jensen 1990
22.69	55.51	21.81	*Bembidion lampros*, 19°	Boye Jensen 1990
22.94	56.21	20.85	*Bembidion lampros*, 12°	Boye Jensen 1990
20.16	58.12	21.73	*Bembidion lampros*, 22°	Boye Jensen 1990
13.47	58.75	27.78	*Amara infima*	Bílý 1975
18.79	60.74	20.47	*Bembidion lampros*, 25°	Boye Jensen 1990
17.11	61.98	20.91	*Amara ingenua*	Bílý 1975
17.72	62.20	20.08	*Bembidion lampros*, 30°	Boye Jensen 1990
14.86	66.22	18.92	*Amara erratica*	Bílý 1971
18.00	72.67	9.33	*Nebria brevicollis*	Nelemans 1987a
7.21	76.58	16.22	*Carabus glabratus*	Grüm 1975
7.10	78.70	14.20	*Carabus hortensis*	Grüm 1975
10.59	81.53	7.88	*Pterostichus madidus* 10°	Luff 1973
9.91	83.08	7.02	*Pterostichus madidus* 15°	Luff 1973
7.41	86.16	6.43	*Pterostichus madidus* 20°	Luff 1973
3.80	91.50	4.70	*Pterostichus melanarius*	Aukema *et al.* 1996

Ribera *et al.* (1999) suggest that winter larvae tend to be found in species with a larger body size.

This is further complicated by species that can have two co-existing cohorts with differing phenologies. *Amara eurynota* (Bílý, 1972) and *Anisodactylus signatus* (Fazekas *et al.*, 1997) have both summer and winter larvae. In others larval development extends over both seasons. e.g. many *Carabus* and other larger species (Ribera *et al.*, 1999), and other species in colder climates (Refseth, 1988), or breeding season switches according to climate or altitude, e.g. *Carabus problematicus* (Butterfield, 1986).

But we should still expect the developmental stages of most species to be pre-adapted to the particular environmental conditions of summer or winter in which development most usually occurs. The larval period is always the longest part of the developmental stages, from >90% to just over 45% (Table 2) and is longest in winter larvae.

Eggs

Both the size and numbers of eggs are important biologically. There is still no detailed information on the morphology, ultrastructure and exact habitat of the eggs of many (possibly most) species of carabid. Carabid eggs are typically pale cream, rather parallel-sided ovoids, devoid of major sculpturing (Luff, 1981). However, detailed photographs show complex ultrastructure, which is sometimes useful taxonomically as in the *Amara communis* complex (Hůrka & Růžičková, 1999), but the functions of these features are not generally known. Eggs of *Poecilus cupreus* have an air layer within the chorion, visible when this is fractured (Kaupp *et al.*, 2000).

Egg size varies between tribes and sub-families: e.g. Luff (1981) showed that Harpalini tended to have relatively fatter eggs that were larger in proportion to body size. But within a closely related group such as *Carabus*, there is an overall positive relationship between a species' adult body size and egg size, despite large inter-specific variations (Turin *et al.*, 2003). In the Hawaiian Platynini, larger egg size has been shown to be correlated with longer larval duration and reduction in adult wings (Liebherr, 2000).

Egg size is also affected by environmental variations. Ernsting & Isaaks (1997, 2000) have shown that egg size in *Notiophilus biguttatus* is inversely related to temperatures experienced by the adults. In *Pterostichus melanarius* and *Poecilus cupreus* diet affected egg size and numbers inversely (Wallin *et al.*, 1992). Larger but fewer eggs were produced on a carbohydrate-rich diet; the resulting larger larvae survived better. But Asteraki (1999) found no effect of different arthropod diets on *P. cupreus* egg size. Within each diet, egg size was only slightly related to resultant larval weight.

Within any one species, egg numbers are generally inversely related to egg size, but this varies from species to species, dependent on both phenology, lifestyle and body weight (e.g. Grüm, 1984). Populations of a species may also differ widely in their fecundity. Thus *Abax parallelepipedus* laid only 12 eggs per female per year in a Belgian forest (Chaabane *et al.*, 1997), but 20-570 (mean 248) eggs per female in laboratory culture in U.K. (Symondson, 1994). Similar variation has been found in *Nebria brevicollis* (Luff, 1976; Nelemans, 1987). Fecundity is low in cave-inhabiting species and in those with brood care. It must be remembered that many data on egg production, mortality and growth obtained from laboratory rearing may differ from real field data, which are so difficult to obtain.

Thiele (1977) summarises existing knowledge on brood care in carabids. This has evolved (probably on several occasions) from simply pushing eggs into the soil (or placing on other substrate) and covering with soil, via covering eggs in a soil capsule to actually watching over the eggs (Brandmayr, 1977; Brandmayr & Zetto Brandmayr, 1974, 1979a; Horne, 1990). These are considered to be responses to ecosystem stability in upland forests and to extreme aridity in Mediterranean regions (Brandmayr, 1985). In *Carterus calydonius* this even extends to parental caring also for larvae and pupae (Brandmayr & Zetto Brandmayr, 1974).

Asteraki (1999) found that egg mortalities of *Poecilus cupreus* and *Nebria brevicollis* were high but not affected by adult diet, which did, however affect fecundity (Table 3). Eggs of *N. brevicollis* also show higher mortality the later they are laid (Nelemans, 1987a). Eggs of *Carabus creutzeri* are very susceptible to desiccation (Brandmayr & Zetto Brandmayr, 1979b), and die above 24°C, even though adults and larvae can survive much higher temperatures. They also suffered more than 64% mortality even at 'favourable' temperatures, possibly due to their removal from the soil. In contrast, egg mortality in *Bembidion lampros* was about 50% at all temperatures from 12-30°C (Boye Jensen, 1990), but their developmental period was highly temperature dependent. Again, the *caveat* mentioned above about the use of laboratory-based data applies.

Table 3. Fecundity and egg mortality (% with s.e.) of two carabid species fed on three diets (from Asteraki, 1999).

Species		**Diet**		
		mealworms	aphids	ant pupae
Poecilus cupreus	fecundity	50.9	12.8	19.9
	egg mortality	58.9	62.0	44.3
	s.e.	7.5	7.2	5.2
Nebria brevicollis	fecundity	210.1	13.5	18.5
	egg mortality	54.4	51.7	37.2
	s.e.	2.3	6.0	5.9

We do not know enough about other environmental relationships of carabid eggs. Exposure of *Pterostichus adstrictus* and *P. pensylvanicus* eggs to –5°C for 24h did not cause any mortality (Goulet, 1974). Eggs have to absorb water to gain weight, but do they survive drowning in very wet conditions? *Carabus clatratus* showed less than 10% egg mortality in both wet and dry peat (Huk & Kühne, 1999), although dry conditions did lead to increased subsequent larval losses.

Larvae

Structure and life-forms

The larvae of most European genera and about 60% of species are now known (Arndt, 1991; Luff, 1993). Functionally, all carabid larvae are essentially an extensible tube (the meso- and meta-thoracic segments plus the abdomen), terminated anteriorly by more sclerotised feeding and locomotory structures (head and pro-thorax), and posteriorly by sensory and sometimes also locomotory or defensive structures (the cerci and anal tube or anal plate).

Carabid larvae are divided (Sharova, 1960, modified by Zetto Brandmayr *et al.,* 1998) into nine main life-forms (details in Table 4). 1. Soil pore explorers; 2. Surface runners; 3. Surface walkers; 4. Sand diggers; 5. Seed feeders; 6. Burrow trappers; 7. C-shaped Harpalines (grub-like subterranean species); 8. Parasitoids and predatory symbionts; 9. Trichome disc larvae (species mostly modified as inquilines). The latter three life-forms are all rather aberrant, and have been combined in Table 4. It is likely that further discoveries of unusual larvae will lead to extension of this scheme in the future (e.g. the crevice-inhabiting larvae of *Siagona*, displayed on video at the present meeting).

Zetto Brandmayr *et al.* (1998) show how life-form relates to the structure of the mandibles. e.g. broadened for seed feeding, long and slender for a diet of Collembola, serrated internally for helicophagy. There are even specific specialisations for seeds of individual plant species (Arndt *et al.,* 1996). However, there are many feeding structures unique to particular taxa, such as the suctorial mandibles of *Graphipterus* (Zetto Brandmayr *et al*., 1994), the maxillary stipes of some *Amara* (possibly for seed crushing) and the labium of *Dromius meridionalis* (Luff, 1993), possibly adapted in some way to a sub-cortical habitat.

At the posterior end of the larvae, there is even greater variability. Surface-active forms have the longest cerci, whereas burrowers have them reduced, and the soil pore dwellers are intermediate. But this does not account for the large cercal variation within closely related taxa such as the Dromiines, whose cerci range from long and segmented, to totally lacking. The Cicindelinae have dorsal hooks near the abdominal apex, and in the Paussinae the whole posterior end of the abdomen is modified to form a terminal disc. In *Pachyteles* this is used to close the hole in which the larva lives, and also as a trapping organ (Di Guilio *et al*., 2000; Di Guilio & Taglianti, 2001). Further examples of adaptations and associated spatial behaviours of some larvae are given by Zetto Brandmayr & Brandmayr (1998).

The basic feeding morphology of the surface-runner *Nebria* is shown by Spence & Sutcliffe (1982). Prey location in *Notiophilus* and *Nebria* has been analysed by Bauer (1982) and Spence & Sutcliffe (1982), respectively. *Notiophilus* detects aggregations of prey by chemical cues, then waits. In *Nebria* there is chance location of actively moving prey by foraging with partly open mandibles, swinging the head from side to side. In both genera, once prey is encountered, the larva turns to face it, and lunges with the opened mandibles, which are then snapped shut. Manipulation of the prey while feeding is by the mouthparts only, so the legs can be used to run. In the surface-active larvae of *Loricera* the maxillary galea has a sticky surface which is used to trap Collembola (Bauer & Kredler, 1988). Not all carabid larvae feed on the ground; some Japanese *Chlaenius* larvae climb cabbage plants to feed on *Plutella* (Lepidoptera) larvae, and indeed have higher consumption rates than adults of the same species (Suenaga & Hamamura, 1988). Other interesting examples of larval behaviour are shown by Zetto Brandmayr & Brandmayr (1988).

Table 4. Simplified table of larval life-forms in Carabidae (after Zetto Brandmayr *et al.*, 1998).

Type(s)	Features	Habitat(s)	Examples
1. Soil pore explorers	small-medium sized, unspecialised	move within soil structure	*Pterostichus, Bembidion, Trechus*
2. Surface runners	small-medium sized, long legs and cerci, often with neck	run on soil surface	*Nebria, Notiophilus*
3. Surface walkers	medium-large sized, often darkened or flattened, cerci short	walk on soil surface	*Carabus, Calosoma*
4. Sand diggers	medium-large sized, cylindrical, cerci short	burrow in loose substrates	*Scarites, Omophron*
5. Seed feeders	medium sized, enlarged head with stout mandibles, cerci short	on soil surface or may live in burrows	*Harpalus, Amara*
6. Burrow trappers	large forebody, cerci reduced but may have abdominal hooks	in vertical burrows in sandy soils	*Cicindela*
7-9. Reduced larvae (3 types)	physogastric, at least in later stages	parasitic, or in ant nests, or family groups	*Lebia, Brachinus, Paussus, Carterus*

Despite these well studied examples, basic behavioural and biological studies on carabid larvae are still clearly needed to understand many aspects of carabid functional morphology, as stressed also by Zetto Brandmayr *et al.* (1998).

Feeding and diet

The first need of any newly-hatched larva is to feed, and therefore its ability to survive until it finds food is paramount. First instar starvation capacities (Luff, 1994; Asteraki, 1999) ranged from 13-45 days at 10°C and constant high humidity, and were affected by temperature and possibly by larval size. One likely source of food is sibling newly hatched larvae, and many carabid larvae are known to be cannibalistic. In the laboratory, artificially raised densities of *Nebria brevicollis* and *Poecilus cupreus* first instar larvae were reduced to single (older) larvae in 66 and 44 days respectively (Asteraki, 1999). However 3rd instar larvae confined together did not eat each other. But Brunsting & Heesen (1983) found that all larval stages of *Pterostichus oblongopunctatus* were cannibalistic. This makes rearing of carabid larvae exceptionally time-consuming and difficult (Goulet, 1976), but cannibalism by *Calosoma sycophanta* in the laboratory can be reduced by design of the rearing conditions and by providing a substrate in which the larvae can shelter (Weseloh, 1996). It was reduced in the more mobile older stages, but even when food was in excess there was 80% mortality of 1st instar larvae, and at most 50% survived to pupate, despite no cannibalism. Weseloh (1998) was also able to rear this species on an artificial diet; development rate and survival did not differ from those on more natural food, but the resulting adults were smaller and less fit.

Effects of larval diet on growth rate result from both the quantity and quality of the food consumed by larvae. Diet in the field has been assessed by dissections (Luff, 1974) or by electrophoresis (Schlevis & Siepel, 1988). Asteraki (1999) found that the effect of feeding frequency of *Poecilus cupreus* larvae interacted with temperature. At 25°C about half (of 10) larvae pupated, the rest died in the first stage, irrespective of frequency of feeding. At 20°C all pupated successfully when fed continuously, but only four with intermittent starvation (4/7 days). At 15°C, they developed further when fed continuously, but none pupated. At both 25°C and 20°C, final weights of the larvae were less on the intermittent regime. Lövei *et al.* (1985) showed in the same species that the rate of consumption and digestion of prey material increased in starved larvae, most digestion taking place within three days of feeding.

Similar results on the effects of food quality and quantity in *Poecilus cupreus, Bembidion tetracolum* and *B. lampros* were shown by Theiss & Heimbach (1993). In *Nebria brevicollis* reduced larval food quantity also decreases the possibility of flight by the adults (Nelemans, 1987b). In *Calathus melanocephalus* both temperature and food availability affect larval growth rate and resultant adult size (van Dijk, 1994). Mortality and growth rate of *Poecilus cupreus* were both affected by type of invertebrate diet when fed on mealworms, ant pupae or aphids (Asteraki, 1999); however on the poorest diet (aphids) none survived to pupation. The caterpillar feeder *Calosoma sycophanta* preferred prey pupae over larvae as 1st or 2nd instars, but 3rd stage preferred larval prey. The younger instars only partly ingested each prey item, so that prey size did not affect their growth rate (Weseloh, 1988).

The type of food eaten by any species will differ primarily according to whether or not the larvae is purely carnivorous, or can grow partly or wholly on plant material. As already mentioned, some larvae, especially in the Harpalini and Zabriini are primarily seed feeders. Jørgensen & Toft (1997a) reared *Amara similata* on seeds, especially *Capsella bursa-pastoris*, although *Amara famelica* had the lowest mortality (33%) and developed fastest on a mixed animal/plant diet (Hůrka 1998). Subsequently, Saska & Jarošík (2001) have shown specific adaptations in common *Amara* larvae. *A. aenea* was found to be omnivorous, whereas *A. similata* and *A. familiaris* are seed feeders, the latter a specialist feeder on *Stellaria media*.

Seed-feeding Harpaline larvae may store seeds in burrows (Alcock, 1976; Luff, 1980; Zetto Brandmayr & Brandmayr, 1975). Foraging for seeds by *Dicheirus* (Alcock, 1976) was non random, the larvae emerging from their burrows and searching near to where the previous seed had been found. Even when the last seed found was 15-19 cm from the burrow, 43% of larvae approached to within 1 cm of this location on their next foraging trip. Seed feeders such as *Ophonus ardosiacus* can only develop on seeds, but may still be cannibalistic (Zetto Brandmayr, 1976). There may be a preference for seeds not only of a host plant species, but also of a particular size: *Harpalus rufipes* ate more biomass of small seeds than large ones (Hartke *et al*., 1998). However Jørgensen & Toft (1997b) showed that the larvae of this species would also eat insect prey; they took longer to develop on a mixed diet than on seeds alone, but their survival rate was not affected.

Growth

The laboratory growth curves of *Poecilus cupreus* (summer larvae) show a more or less continuous exponential increase in weight (Asteraki, 1999). However the time taken to pupate varied widely from 37 to 70 days. The fastest growing larvae were the heaviest at pupation; those that grew slowly were lighter, and took longer to develop to pupation. Because of this, the mean head-width of larvae still in the active population can appear to decrease, as shown in field samples of *Harpalus rufipes* (Luff, 1980). Laboratory reared *Poecilus cupreus* suffered high mortality (45/56 = 80.4%). Those dying had slightly slower growth rate: 1.04 mg/day compared with 1.08 mg/day for those that survived to pupation (Asteraki, 1999). In contrast to *P. cupreus*, the winter larvae of *Nebria brevicollis* have a two-stage growth curve, even at constant temperatures (Asteraki, 1999). After an exponential phase there was a stable phase of similar length, prior to pupation; most (9/10=90%) survived and the larvae dying had a much slower growth rate (0.40 mg/day) compared with those that survived (1.06 mg/day). Final weight was not related to growth rate (unlike in *P. cupreus*). But these conclusions are based on a much smaller dataset than for *P. cupreus*.

These growth curves mask the fact that larval development is not a continuous process, but comprises (usually) three feeding instars with intervening ecdyses. The relative times spent in

Table 5. Percentages of the larval period of a range of Carabidae spent in each instar (ordered by increasing % in 3rd instar).

Instar			Species, conditions	Reference
L1	L2	L3		
11.78	59.43	28.79	*Pterostichus melanarius*, field	Aukema *et al.* 1996
38.75	23.62	37.64	*Poecilus versicolor*, 12°	van Dijk 1994
22.60	37.81	39.60	*Poecilus versicolor*, field	van Dijk 1994
32.57	25.22	42.21	*Poecilus cupreus*, lab.	Asteraki, 1999
20.41	36.73	42.86	*Amara erratica*, field	Bílý 1971
25.81	29.03	45.16	*Bembidion lampros* 12°	Boye Jensen 1990
25.16	28.03	46.82	*Poecilus versicolor* 19°	van Dijk 1994
26.13	27.03	46.85	*Bembidion lampros* 22°	Boye Jensen 1990
25.00	27.78	47.22	*Bembidion lampros* 19°	Boye Jensen 1990
24.50	24.50	50.99	*Bembidion lampros* 17°	Boye Jensen 1990
21.55	27.07	51.38	*Bembidion lampros* 25°	Boye Jensen 1990
11.30	31.33	57.37	*Pterostichus madidus* 20°	Luff 1973
21.52	20.89	57.59	*Bembidion lampros* 30°	Boye Jensen 1990
22.04	20.11	57.85	*Amara eurynota*, field	Bílý 1972
18.16	22.35	59.50	*Amara famelica*, field	Hůrka 1998
15.83	24.29	59.88	*Calathus melanocephalus* 15.5°	van Dijk 1994
18.44	17.73	63.83	*Amara infima*, field	Bílý 1975
15.60	19.27	65.14	*Nebria brevicollis*, lab.	Nelemans 1987a
10.58	20.02	69.40	*Pterostichus madidus* 15°	Luff 1973
13.26	15.79	70.95	*Nebria brevicollis*, lab.	Asteraki, 1999
10.30	15.73	73.98	*Pterostichus madidus* 10°	Luff 1973
12.86	12.19	74.95	*Calathus melanocephalus*, 8.5°	van Dijk 1994
11.97	11.33	76.70	*Calathus melanocephalus*, field	van Dijk 1994

each instar vary widely according to species (Table 5). The third stage is usually the longest, especially in winter larvae that overwinter in this stage.

Under field conditions, growth is temperature dependent. In *Bembidion lampros* all instars have a similar temperature/development rate relationship, so the proportions of development time (and relative growth) in each instar remain more or less constant (Boye Jensen, 1990, and Fig. 1). In *Abax parallepipedus* temperature-retarded slow growth in the early stages is compensated for in the third stage (Chaabane *et al.*, 1997). The second instar of *Pterostichus melanarius* can be extended in winter (thermal parapause) due to low temperatures (Desender *et al.,* 1985) but there was no obligatory diapause (Aukema *et al.*, 1996), in contrast to the results summarised in Thiele (1977), see later. All instars of *Abax parallepipedus* expend most energy on respiration, then growth, then excreted material; respiration costs increase relatively in the oldest larvae (Chaabane *et al.*, 1996). According to Nelemans (1988) each instar of *Nebria brevicollis* needs to increase at least 2.6 times in weight, and can increase up to 3.5 times their initial weight. Similar ratios were shown for *Carabus* species by Huk & Kühne (2000). Such growth ratios set hypothetical upper limits of between about 17 and 42 for the ratio of final pupation weight to hatching egg weight. Any further weight increase must take place in the newly emerged adult stage.

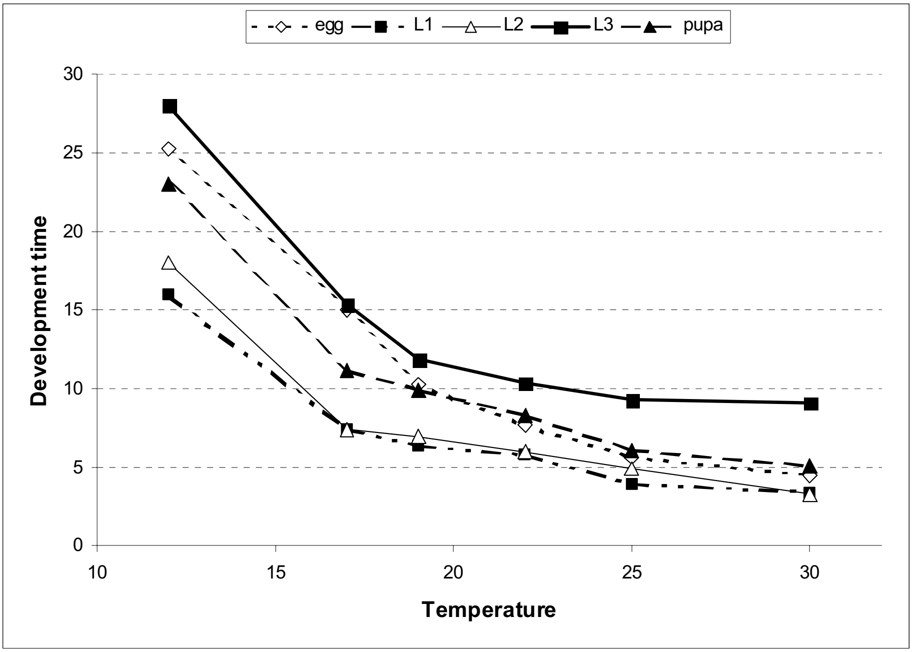

Figure 1. Development times (days) of each stage of *Bembidion lampros* at a range of temperatures (data from Boye Jensen, 1990).

A further complication affecting larval growth of some carabids is diapause. The subject of 'diapause' in its widest sense is a complex one that will not be considered further here. Thiele (1977) gives examples of a thermal hibernation parapause in *Pterostichus melanarius*, which requires low temperature to complete development. It is likely that other winter larvae may have a similar mechanism. Some more recent examples follow. In *Ophonus rotundicollis* a similar obligatory diapause is temperature controlled (Zetto Brandmayr, 1980) and synchronises larvae with their seed food source. The summer larvae of *Pterostichus oblongopunctatus* have a daylength-controlled facultative dormancy in the 3rd instar that synchronises the life cycle; under long-day conditions the 3rd instar duration is extended (van Schaick Zillesen, 1985).

Surface Activity

Most larvae of life-forms 1 (soil pore explorers) and 4 (sand diggers), as well as those living in burrows (life-forms 5, seed-feeders and 6, burrow trappers) are seldom active on the soil surface. Exceptions are foraging seed feeders (see Alcock, 1976, above) and fully grown larvae searching for pupation sites. But those of life-forms 2 and 3 (surface runners and walkers respectively) are active on the surface during their normal foraging. Thus larvae of *Carabus problematicus* were active during winter with a threshold of 3-4°C (Betz, 1992) and could range for 10 m or more (with a mean daily displacement up to about 1 m/day). Corresponding distances moved by *Nebria brevicollis* larvae were a mean of 9.1 m and up to 28 m during the 3rd stage overall (Nelemans, 1988). Older larvae actively left a woodland habitat and moved to open heathland before pupation, and showed both random and directed walk. They are night active, as assessed by time-sort pitfall trapping, which mirrors the activity period of the adults. But *Loricera pilicornis* larvae are diurnal, in contrast to the adults' nocturnal activity pattern (Kegel, 1990 – although the adults can also be diurnal, c.f. Luff, 1978). The Japanese *Carabus insulicola* larvae also showed rhythmical activity, and could be entrained to 24 h 12D/12L conditions, becoming active a few hours after dusk until the middle of the light period (Yamakazi, 1992). This activity rhythm was truly endogenous, persisting in continuous dark, and could also be entrained to a 21h cycle.

Surface activity of larvae can be monitored by pitfall trapping, but may be instar-dependent. Thus only 1st and 2nd instars of *Harpalus rufipes* were trapped, and large soil samples were needed to assess 3rd instar numbers, as these were in burrows feeding on seeds stored by the earlier stages (Luff, 1980). Jensen *et al.* (1989) trapped only 3rd instar larvae of *Agonum dorsale.* Arndt & Arndt (1987) found larvae of 15 out of 28 species in pitfall trapping, but larvae were always fewer than adults in the traps except for *Calosoma inquisitor.* In alfalfa, Barney & Pass (1986) trapped larvae commonly of only six out of 40 species present; in four out of six more 1st instars were trapped than older stages (and in one out of six more 2nd instars). In Russia, Dushenkov & Chernyakhovskaya (1989) trapped larvae of more than 40 species in agricultural land, most in perennial grass, fewest in summer row crops. At high densities, all instars were trapped in pitfalls. In an Austrian potato field, pitfall trapping of larvae, combined with soil sampling, enriched the total species spectrum by 6% (Traugott, 1998). Winter larvae were more likely to be surface-active than summer larvae.

Populations

Estimating actual population densities of subterranean carabid larvae is difficult and time-consuming, requiring intensive soil sampling as many occur at relatively low densities. Thus *Harpalus rufipes* numbers were assessed by large (80 x 80 x 60 cm) hand-sorted samples (Luff, 1980); density ranged from 3.5-20.3/m^2. Larval abundances in Belgian pasture peaked in summer (Desender & Pollet, 1986), with mean yearly values between 30 and 65/m^2. Individual species larval densities varied widely between species and years from 5-28/m^2. It is generally assumed that larvae occur in the same habitat as the adults, as their location is largely determined by where the adult females lay their eggs. Indeed, selection of a suitable oviposition site for the progeny can be seen as the first stage of maternal care. Larvae of *Bembidion femoratum* and *B. punctulatum* showed the same substrate preferences as their respective adults (Meissner, 1984): *B. punctulatum* preferred gravel and sand, whereas *B. femoratum* was less specialist, preferring finer loamy substrates.

Table 6. Carabid larvae found near Newcastle upon Tyne, U.K. in 27 sets of 10 soil samples between April 1985 and October 1986. Total of larvae found = 651. Full details are in preparation to be published elsewhere.

Genus	% of catch	max ensity/m^2
Bembidion	38.25	86.4
Clivina	15.21	22.4
Pterostichus	11.06	14.4
Agonum	8.45	16.0
Amara	8.14	36.8
Trechus	7.99	16.0
Patrobus	5.84	27.2
Nebria	1.54	6.4
Harpalus	0.92	4.8
Abax	0.46	1.6
others	2.15	9.6

Table 6 summarises the total numbers of carabid larvae found by the author in a field near Newcastle upon Tyne, U.K. in 27 sets of 10 smaller (25 x 25 x 10 cm) soil samples between April 1985 and October 1986. Although most of the common genera were found, nearly all the *Pterostichus* were medium or smaller-sized species such as *P. nigrita* and *P. strenuus*. The small summer larvae of *Bembidion* were by far the most numerous, followed by those of *Clivina fossor*. Full details of these larval samples, with statistical analyses, are in preparation for future publication. Larvae of *C. fossor* occur through most of the year (Desender, 1983), but 1st instars only for a short period. The long larval period may relate to the relative stability in the soil for this burrowing species. Although *Amara* larvae made up only 8% of the total larvae found, they had the second highest density, but only occurred for a short period. Only small numbers of the larger *Pterostichus* species, as well as *Harpalus* and *Abax* were found. Densities were greatest under wet soil, lowest in the grass areas. Both winter and summer larval development cycles could be seen. Similar seasonal changes were shown by larvae in the margin of a maize field in Belgium (Desender & Alderweireldt, 1988).

Mortality

It is clear from laboratory rearing of carabids, such as by Asteraki (1999), that there is usually a moderately high ‘background’ mortality of carabid larvae, even under apparently favourable and well-fed conditions. However, Desender (1989) was able to rear *Pogonus chalceus* with less than 10% mortality. In nature also, background mortality may be reduced, but there are a number of other factors known to cause losses. Parasitism by proctotrupid Hymenoptera such as *Phaenoserphus* and *Proctotrupes* species has been recorded by several authors (see illustrations in Basden, 1959 and Sturani, 1962) with rates of parasitism of up to 20% of *Harpalus rufipes* larvae (Luff, 1982), and 25% of *Nebria brevicollis* (Critchley, 1973). Cicindelid larvae are more readily locatable by parasitoids, as they are both readily visible and immobile in their burrows. They are parasitised by the tithiid *Methoca ichneumonoides*, and in America by *Anthrax* species (Diptera, Bombyliidae) with up to 70% parasitism (Knisley, 1987; Arndt & Costa, 2001). Other natural enemies are pathogenic fungi; Steenberg *et al.* (1995) found that 19-50% of overwintering carabid larvae in Danish crops were infected by entonopathogenic fungi, especially *Beauveria bassiana*. Carabid species may also affect one another. In North America *Pterostichus melanarius* and *P. adstrictus* suffered from both interspecific larval predation and cannibalism (Curry *et al*., 1996; Curry & Digweed, 1996).

Data on other mortality effects in the field are sketchy. The amount of rain during the larval development period of *Pterostichus oblongopunctatus* affected the proportion of different supposed genotypes responsible for number of elytral pits (den Boer *et al*., 1993), implying moisture-related larval mortality. In dry summers 'low pitter' larva survived better and vice versa (but body size may also have been inportant). Thiess & Heimbach (1994) found that all larvae of *Poecilus cupreus* died when the soil moisture content was above 60%: their optimum was 15-20% moisture. A detailed study of water relations of *Cicindela* larvae in North America (Hadley e*t al*., 1990) has shown that their water loss increases with temperature, so they adjust the timing of their foraging to avoid excess water loss. In order to maintain a water balance they ingest moist sand and absorb the water through the gut, as water obtained from their prey is insufficient. *Cicindela willistoni* larvae thermoregulate by building turrets which raise them above the very hot soil surface, and provide shade that attracts prey items (Knisley & Pearson, 1981).

Winter larvae may have to withstand periods of low temperatures, and as already mentioned these may be needed to complete diapause development in some species such as *Pterostichus melanarius*. Rossolimo (1997) has shown in Russia that overwintering larval *Pterostichus* have lower supercooling points than those of the adults.

Agricultural management, especially mechanical cultivation, is believed to affect species such as *Carabus* in temperate climates, where their larval development may last over more than one year (Holland & Luff, 2000). Many overwintering *Nebria brevicollis* larvae in fields may be subject to insecticide mortality (Noordhuis *et al*., 2001). Copper contamination caused acute toxic effects to *Poecilus cupreus* (Bayley *et al*., 1995). There was 69% mortality mostly during metamorphosis and pupation. Furthermore, larvae that did not die developed into adults with altered locomotory behaviour.

Pupae

Carabid pupae are all more or less identical, exarate, supported on dorsal pegs, and enclosed by the substrate (usually soil). As with the earlier developmental stages, pupal development time is dependant primarily on temperature (Boye Jensen, 1990; Luff, 1976), but not on the type of substrate (Asteraki, 1999). Even under apparently suitable conditions, many pupae of *Nebria brevicollis* failed to develop into non-deformed adults (Fig. 2). But mortality and deformations were also substrate and temperature dependent; even at outdoor temperatures only 127/200 (63%) produced apparently healthy adults. A similar survival (59.9%) was found on the best (sand) substrate (Asteraki, 1999). However Grüm (1975) considered that pupal mortalities in *Carabus* and *Pterostichus* species were low (under field conditions) compared to those of the larvae. Pupal weight is dependant of feeding conditions for the larvae (Bommarco, 1998), so that small pupae indicate food limitation.

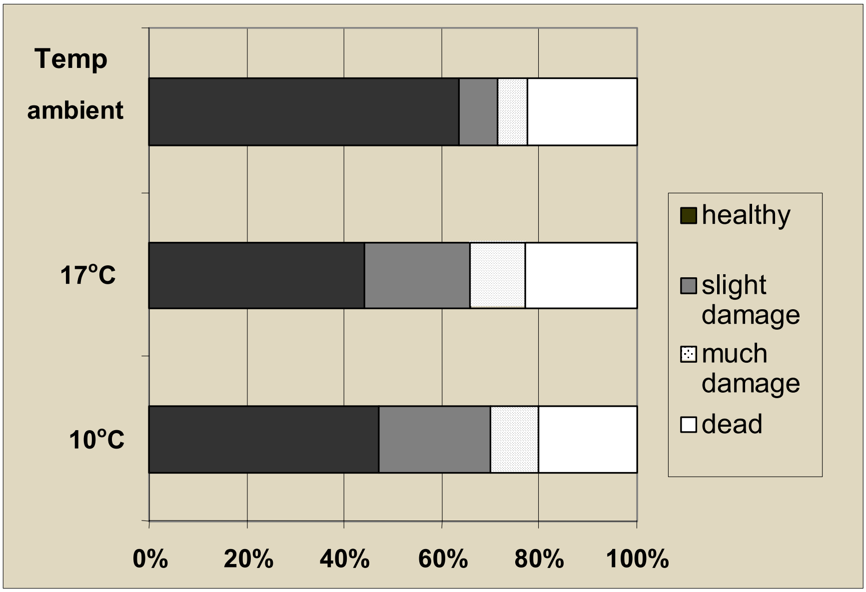

Figure 2. Percentages of pupae of *Nebria brevicollis* that developed into healthy or damaged adult beetles, or which died, at three temperatures (data from Asteraki, 1999). N=159, 153, 155 at 10^0, 17^0 and ambient, respectively.

Overall mortality and conclusions

Table 7 summarises data on overall mortality during the immature stages of several carabids. Species with summer larvae have a similarly high percentage mortality to those with winter larvae, but Grüm (1975) showed that they have higher instantaneous death rates because development takes place over a shorter period. Both *Pterostichus* species had higher mortality rates than any of the *Carabus* species in Grüm's (1975) study. The data on *Nebria brevicollis* from Nelemans *et al.* (1989) show mortalities ranging from 26% to nearly 100% according to food regime. Schlevis & Siepel (1988) give densities for *Pterostichus oblongopunctatus* and *P. rhaeticus*, from which approximate seasonal 'mortalities' of 61% and 71% respectively can also be calculated. It thus seems likely that many ground beetles suffer moderate to very high losses during their developmental stages, even though in most cases the exact causes of this mortality is not clear.

Table 7. Published data on overall mortality during carabid developmental stages.

Overall mortalities	%	author
Summer larvae		
Carabus arvensis	74.4	Grüm 1975
Carabus nemoralis	68.9	Grüm 1975
Pterostichus oblongopunctatus	89.4	Grüm 1975
Pterostichus oblongopunctatus	96.0	Brunsting *et al.* 1986
Bembidion lampros	66.0	Petersen 1998
Winter larvae		
Carabus glabratus	83.7	Grüm 1975
Carabus hortensis	73.2	Grüm 1975
Pterostichus niger	92.3	Grüm 1975
Nebria brevicollis	26.7-97.3	Nelemans *et al.* 1989

Extensive studies of tiger beetles in U.S.A. (Pearson & Knisley, 1985; Knisley & Juliano, 1988) have shown the importance of food availability to these beetles in the larval stage, and suggest that larval food is the main factor limiting the populations. Brunsting *et al.* (1986) stressed that larval mortality plays a key part in carabid dynamics, and this was considered in *P. oblongopunctatus* by Heessen & Brunsting (1981) to be the effects of cannibalism. Population modelling of the same species by Siepel (1988) concluded that cannibalism/mutual predation was indeed the most likely important regulating factor. Similarly, Nelemans *et al.* (1989), based on a 10-year key-factor analysis, concluded that mortality of the pre-imaginal stages was the most influential factor affecting the population fluctuations of *Nebria brevicollis*.

Despite all this existing work, we can conclude:

- We still have large gaps in our detailed knowledge of the developmental ecology of the vast majority of carabid species. Most of the examples cited here have come from a limited spectrum of common and well-studied species. It is not known to what extent these are typical of the family as a whole. One must remember that the larvae of a significant number species are still not yet or only incompletely described.
- The mortality of immature stages, especially from physical environmental factors is largely unknown. There seem to be very few detailed studies of the water or temperature

relationships in terms of the species' survival in particular environments. Such data as exist are often from laboratory studies, and their applicability to the field is uncertain.

- Most carabids eggs never make it from the egg to the adult stage: it is a wonder that so many adult carabids actually exist, and provide the material for the majority of the remaining papers in this volume!

Acknowledgments

I am indebted to the organisers of the 11th European Carabid Meeting for inviting me out of retirement to Aarhus, Denmark in order to present this paper, and for financial assistance. Larval soil sampling at Newcastle upon Tyne was carried out by Alan Craig, and some of the unpublished data presented are from the work of one of my former students, Liz Asteraki.

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Species richness of carabids along a forested urban-rural gradient in eastern Hungary

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Abstract

As part of the international Globenet project, effects of urbanisation on carabid species richness were studied along an urban-suburban-rural gradient representing decreasing intensities of human disturbance in an oak forest in eastern Hungary. Carabid beetles were collected using pitfall traps from four urban, four suburban and four rural sites during the growing season in lowland oak forest patches in 2002. Increased urbanisation had no significant effect on the overall carabid species richness. These results did not support the hypothesis that overall diversity should decrease in disturbed habitats (urban area). They also contradicted the intermediate disturbance hypothesis, as species richness was not the highest in the moderately disturbed suburban sites. However, the habitat specialist decrease hypothesis was supported, as species richness of the forest-specialist species increased from the more disturbed urban area to the less disturbed rural one. Multivariate methods also confirmed that species composition changed remarkably along the urban-rural gradient.

Key words: Increased disturbance hypothesis, intermediate disturbance hypothesis, urbanisation, human disturbance

Introduction

Anthropogenic modification of landscapes linked with a city (i.e. urbanisation) has important direct and indirect influences on the biosphere. Urban ecosystems are characterised by high-density human habitation and only remnants of natural habitats (McIntyre *et al.*, 2001). To ensure that urban areas are managed both for the well-being of city-dwellers and urban nature, knowledge of ecosystem responses to the influence of urbanisation is needed (McDonnell & Pickett, 1990). Central among these considerations is the maintenance of biodiversity, which is an important indicator of the functional state of ecosystems (Naeem *et al.*, 1994). Despite their global ubiquity, relatively little is known about how arthropods respond to urbanisation (Bolger *et al.*, 2000; McIntyre *et al.*, 2001), even though urbanisation is regarded as one of the leading causes of decline in arthropod diversity (Pyle *et al.*, 1981). A way to estimate the anthropogenic effects (urbanisation) on nature is to study ecosystem structure and function along urban-rural gradients (McDonnell & Pickett, 1990).

In 1998, an international research framework to assess and compare the influence of urbanisation on biodiversity was initiated (Niemelä *et al.*, 2000). This project applies the urban-rural gradient approach using a common methodology and a common invertebrate taxon (carabid beetles). Carabids were selected since they were sufficiently varied both taxonomically and ecologically, abundant and sensitive to human disturbances (Lövei & Sunderland, 1996).

Several hypotheses try to explain the effects of disturbance (like urbanisation) on biotic communities. The intermediate disturbance hypothesis (1) predicts an increase in diversity at intermediate levels of disturbance (Connell, 1978). This implicitly involves the increasing disturbance hypothesis (2), that states that species richness should decrease with higher levels of disturbance (Gray, 1989). Disturbance affects primarily the habitat specialists. We hypothesised that in our particular situation, the species richness of forest specialists should decrease from the rural area to the urban area (habitat specialist decrease hypothesis - 3).

In this paper, we tested the following predictions for carabids along a forested urban-rural gradient: (1) diversity should be highest in the suburban area according to the intermediate disturbance hypothesis, (2) diversity should decrease from high in the rural area to low in the urban area (increasing disturbance hypothesis), and (3) the species richness of the forest-specialist species should decrease from the less disturbed rural area to the more disturbed urban area (habitat specialist decrease hypothesis).

Material and methods

Study area and methods

Carabid beetles were studied along an urban-suburban-rural gradient in Debrecen (Eastern-Hungary), the second largest city of the country (Fig. 1). The urban, suburban and rural sampling areas were located in a continuous primeval forest (Nagyerdő Forest Reserve) bordering the city. All areas were situated in continuous patches of old forest (>100 yr) dominated by English oak (*Quercus robur*) and covering an area of at least 6 ha. The typical, native forest association of the sampling sites was *Convallario-Quercetum*. The criteria for distinguishing urban, suburban and rural area was the ratio of the built-up area to the natural habitats. In the urban area the built-up area exceeded 60%, in the suburban area it was approximately 30%, while in the rural area the built-up area was 0%. The area of the built-up environment and the natural habitats was measured by the ArcView GIS program using an aerial photograph. Distance between the sampling areas (urban, suburban, rural) was at least 1 km. In the urban area, several paths with asphalt surfaces had been created and the shrub layer was strongly thinned resulting in a park character, while in the suburban area the fallen trees were removed. The urban-rural gradient covered a distance of approximately 6 km.

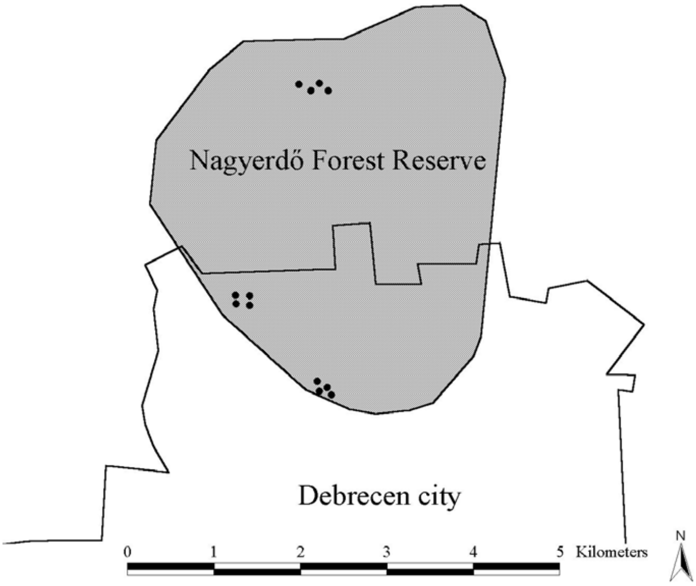

Figure 1. Map of the study area. Filled circles indicate sampling sites.

Four sites, at least 50 m from each other, were selected within each sampling area. Carabid beetles were collected at each of the 4 sites of the 3 sampling areas using pitfall traps. Ten traps were placed randomly at least 10 m apart at each site. This resulted in a total of 120 traps scattered along the urban-rural gradient (3 area × 4 sites × 10 traps). Each pitfall trap was at least 50 m from the nearest forest edge, in order to avoid edge effects (Molnár *et al.*, 2001). The pitfall traps were unbaited, consisting of plastic cups (diameter 65 mm) containing about 200 ml of 75% ethylene glycol as a killing-preserving solution. The traps were covered with bark pieces to protect them from litter and rain. Trapped beetles were collected fortnightly from the end of March to the end of November, 2002. For the numerical analyses we pooled samples from the whole season.

Data analyses

To test differences in overall carabid species richness among the three sampling areas (urban, suburban and rural), and among the 12 sites, nested analyses of variance (ANOVA) were performed using data from the individual traps (sites nested within the sampling areas). Forest specialist species were identified from the literature (Hůrka, 1996). Differences in the species richness of forest specialist carabids were also tested by nested ANOVA. The distribution of data used in the ANOVA model was normal (tested by the Kolmogorov-Smirnov test, Sokal & Rohlf, 1995). When ANOVA revealed a significant difference between the means, an LSD (least significant difference) test was performed for multiple comparison among means.

The composition of the carabid assemblages along the studied urban-rural gradient was compared at site level by cluster analysis, using the Hellinger index of dissimilarity and the Ward fusion algorithm (Gordon, 1981).

Results

The total carabid catch consisted of 2281 individuals representing 46 species. Four hundred and sixty-seven individuals belonging to 38 species were collected in the urban area, 27 species and 569 individuals in the suburban area, and 28 species and 1245 individuals in the rural area. Overall, *Pterostichus oblongopunctatus* (F.) was the most abundant species, making up 45.15% of the total catch, followed by *Carabus violaceus* L. (8.11%).

The overall species richness did not change significantly along the gradient (Table 1), while the species richness of forest specialist carabids increased significantly from the urban to the rural area (Fig. 2).

There was a marked separation among the sites along the urban-rural gradient. The four urban sites separated into a distinct cluster based on the species composition, while the suburban and rural sites formed the other cluster (Fig. 3).

Table 1. Nested ANOVA showing differences in overall species richness and species richness of forest specialists along the urban-suburban-rural gradient and between the twelve sites. ns = not significant.

	Source of variation	d.f.	MS	F	p
Overall species richness	Gradient	2	23.73	3.59	ns
	Sites	9	6.62	1.30	ns
	Error	108	5.08		
Species richness of forest specialists	Gradient	2	23.73	67.79	<0.001
	Sites	9	0.35	0.82	ns
	Error	108	0.43		

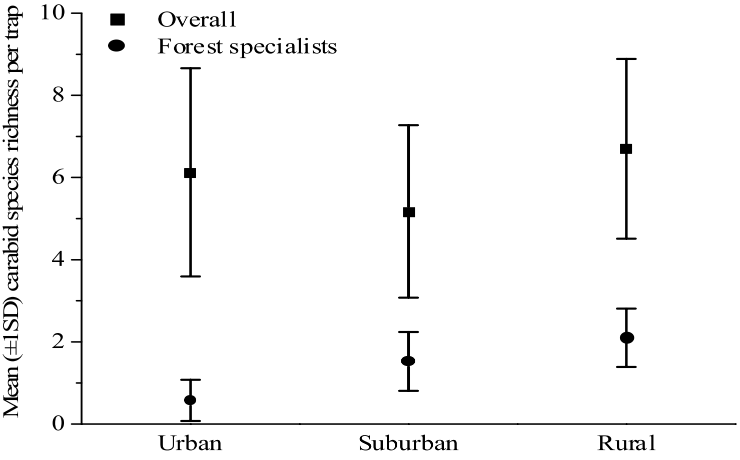

Figure 2. Overall carabid species richness and the species richness of forest specialist carabids along the urban-suburban-rural gradient. The nested ANOVA indicates no significant differences in the overall species richness along the urban-rural gradient. The nested ANOVA indicates significant differences in the species richness of forest specialist carabids along the urban-rural gradient, furthermore subsequent post-hoc comparisons test (LSD test) revealed that the urban area was significantly different from the suburban and rural area and that the suburban area was significantly different from the rural area.

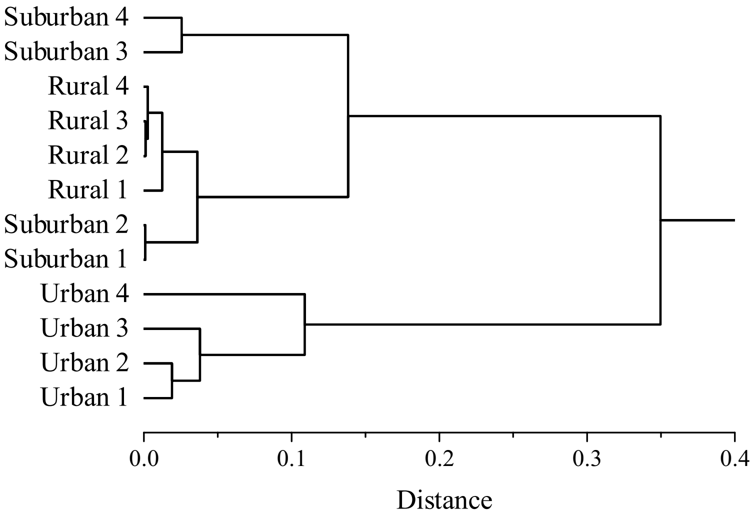

Figure 3. Cluster analysis dendrogram (using the Hellinger index of dissimilarity and the Ward fusion algorithm) showing differences in carabid assemblage structure along the studied urban-rural gradient.

Discussion

Responses of carabids to urbanisation

The findings of earlier studies performed as part of the Globenet project (Alaruikka *et al.,* 2002; Niemelä *et al.*, 2002; Ishitani *et al.*, 2003; Venn *et al.*, 2003; Magura *et al.*, in press) and those of the present study, contradict the Intermediate Disturbance Hypothesis (1; IDH; Connell, 1978). The overall species richness was not the highest in the moderately disturbed suburban areas as IDH predicts. IDH is a general framework considering different kinds of disturbance without precise, verifiable details regarding the ecological mechanisms of the changes in species richness. The increase of species richness may be ecologically important when only the species pool of the local, characteristic habitat type (i.e. native fauna) is involved. However, invasion by species from other habitat types or by generalist, opportunistic species would also increases species richness. The increase resulting from the presence of these species could offset the disappearance of habitat specialists.

Gray (1989) hypothesised that in disturbed habitats, overall diversity should decrease. Our results did not confirm this hypothesis (2). The overall species richness of carabids was almost as high in the urban area as in the rural one. Overall carabid species richness increased significantly with decreasing urbanisation in Canada (Niemelä *et al.*, 2002), in Finland (Niemelä *et al.,* 2002; Venn *et al.*, 2003) and in Japan (Ishitani *et al.*, 2003). The pattern of overall species richness of carabids in Bulgaria was the same as that in our study (Niemelä *et al.,* 2002). Changes of overall species richness along the forested disturbance gradient (urban-rural gradient) can be complex, because individual species, depending on their habitat preferences, may respond differently to disturbance. Species richness of forest specialist species may decrease, while that of generalist and/or open-habitat species may increase as disturbance increases. Basic ecological relationships (such as the effects of urbanisation on carabids in this context) can be masked if one does not take into account the ecological characteristics of the species studied. This complexity may be the reason, why in Helsinki Niemelä *et al.* (2002) and Venn *et al.* (2003) found that overall carabid species richness increased significantly with decreasing urbanisation, while in another year Alaruikka *et al.* (2002) did not find any significant changes.

The habitat specialist decrease hypothesis (3) was confirmed: the number of forest specialist species significantly increased from the urban to the rural area. These results indicate that human impacts caused a pronounced change in the carabid assemblages. Forest species require microsites with a particular kind of environmental heterogeneity, such as favourable microclimate, the presence of dead and decaying trees, significant cover of leaf litter, shrubs and herbs, together forming the undisturbed, forest habitat (Desender *et al*., 1999). Changes caused by urbanisation eliminate such favourable microsites, thus altering the original habitats (Magura *et al*., in press). These disturbances affect the species most closely adapted to the original environment, the forest specialists. The degree of disturbance is higher in the urban area (paved paths, thinned shrub layer), than in the suburban (fallen trees removed), and lowest in the rural area. The changes in species richness of forest carabids closely followed this trend.

Preservation of biodiversity in the urban landscape

Our results showed that urbanisation had an effect on carabid assemblages. Species richness of forest specialist, as well as community composition, changed along the urban-suburban-rural gradient. Urban green areas, including the studied forested areas, improve the quality of urban life and thus should be conserved. But how can the biodiversity preservation function of urban parks be enhanced? We suggest that extensive alteration of habitat structure (e.g. by strong thinning and the removal of decaying wood material) and creating too many asphalt-covered paths should be avoided. Change in habitat structure causes changes to the microclimatic, abiotic and biotic conditions of the area. All these changes affect forest specialist carabids directly. Creating too many paths with asphalt surfaces also influences carabid beetles indirectly by fragmentation. Forest patches divided by asphalt-covered paths can be considered isolated from each other, as carabids usually do not cross such roads (Mader *et al*., 1990). The division of the original forests into smaller, isolated patches results in loss of forest specialist species through reduction in habitat area, an increase in remnant isolation and a decrease in habitat connectivity (Didham *et al*., 1996). Several studies emphasised that the number of carabid species decreased with the decreasing area of the forest patch (Davies & Margules, 1998; Magura *et al*., 2001). The population size of forest specialist carabid species in isolated patches could decrease because the patches are too small to maintain viable populations and because there is too little dispersal between patches. Small populations of forest specialist carabids in isolated patches are at greater risk of local extinction through stochastic population fluctuations than are the larger populations (Den Boer, 1985). Judicious habitat management can both serve the demand of city-dwellers for recreation and the maintenance of biodiversity.

Acknowledgements

We are especially grateful to Gábor Lövei for comments on the manuscript. We thank Zoltán Elek and Viktor Ködöböcz for field and laboratory assistance.

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Phylogenetic relationships among subtribes of Harpalini Bonelli (Coleoptera, Carabidae) inferred from mitochondrial DNA sequences

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Abstract

The tribe Harpalini Bonelli 1810 is included within ‘modern’ lineages of Carabidae and comprises about 2000 species and 238 genera. The systematics of the tribe is currently based on the external morphology of adults and larvae, male genitalia and geographic distribution. The number and limits of the subtribes of Harpalini are controversial and vary between four and six. We have sequenced a fragment of the mitochondrial cytochrome oxydase I gene in 30 species representative of the distinct lineages (subtribes) of Harpalini, and the data have been analysed under different tree-building methods. The main conclusions of the study are that: 1) The current subtribe Harpalina is a polyphyletic taxon and its limits should be much reduced to become monophyletic. 2) The Ditomi is a well-differentiated group that should be ranked as an independent subtribe. 3) The phylogenetic relationships of Amblystomina remain poorly understood but may be related to Stenolophina. 4) The Dapti group is related to Stenolophina instead of Harpalina. 5) The Selenophori (in Noonan’s sense) are a polyphyletic taxon not related to Harpalina, as previously hypothesized; they make up an independent subtribe perhaps related to Anisodactylina. 6) Anisodactylina include two main lineages, Notiobioids and Anisodactyloids. Australian Notiobioids are separated from close relatives that evolved in other regions. The genus *Phorticosomus* (Australian) is not related to Ditomina but to Australian Notiobioids. 7) The Stenolophina are divided into different lineages of which one includes the subtribe Pelmatellina (possibly the Neotropical vicariant of Nearctic Bradycellines); Stenolophi and Acupalpi are closely related taxa.

Key words: Molecular phylogeny, cytochrome oxydase I

Introduction

The tribe Harpalini Bonelli 1810 is included within the Carabidae Conchifera, the group of ground beetles that shows the highest number of derived character states (Erwin, 1985). This Conchifera stock lineage was redefined by Deuve (1988) as the "Harpalidae" on the basis of four autapomorphies. About 2000 species have been described within the tribe, grouped in 238 genera and subgenera (Noonan, 1976) with a worldwide distribution. Open environments including grasslands, semiarid stony places, wet sandy areas, riparian, and salty wetlands are among the preferred habitats of these beetles. Most of the Harpalines are phytophagous and feed on seeds when adults (e.g., Ditomines feed mainly on Umbelliferae seeds; Brandmayr and Brandmayr, 1987). Larvae are phytophagous or carnivorous (Brandmayr *et al.*, 1980), this last habit is hypothesised to be a secondary adaptation (Jeannel, 1942). The harpalines are common members of temperate carabid assemblages that are currently used as ecological indicators in agricultural systems and for conservation purposes (Lövei & Sunderland, 1996; Holland, 2002).

The criteria for establishing the subtribes of Harpalini are based on morphological and geographical data. The number and limits of these subtribes are under discussion, as there are four to six recognised subtribes depending on the author (Csiki, 1932; Jeannel, 1942; van Emden, 1953; Freude *et al.*, 1976; Noonan, 1976; Reichardt, 1977; Lindroth, 1986; Lorenz 1998, etc.). In his checklist of the tribe, Noonan (1976) grouped the genera into four subtribes, Pelmatellina Bates 1882, Stenolophina Kirby 1837, Anisodactylina Lacordaire 1854, and Harpalina Bonelli 1810. This last subtribe includes the Ditomi, Amblystomi, Acinopi, Dapti, Harpali and Selenophori groups. Jeannel (1942) and Freude (1976) considered Ditomina and Amblystomina to be separate taxa from Harpalina, whereas Antoine (1959) proposed the Ophonini (including *Ophonus* and the subtribe Ditomi, among other taxa) as a separate taxon from Harpalina.

Noonan (1973) proposed that Anisodactylina and Pelmatellina might be closely related as they share pubescent ("spongy") male fore tarsi. Arndt (1990) showed that Stenolophina is characterised by primitive larval characters and that the Selenophorines make up a distinctive lineage that is possibly the sister taxon of Anisodactylina + Harpalina. Serrano *et al.* (1994) proposed that Ditomina make up a monophyletic lineage distinct from Harpalina, due to the high number of morphological, karyotypic autapomorphies, and the geographic distribution restricted to the Mediterranean Basin. Martínez-Navarro *et al.* (2003) revised the morphological data used in current taxonomic works to classify the subtribes of Harpalini. They could only select fourteen characters to perform a cladistic analysis by the phylogenetic value of these characters (a karyotypic trait, the diploid chromosome number was lately added to the analysis). Most of the morphological characters turned out to be autapomorphies or homoplasies and only four were useful for phylogenetic inferences. Thus, the relationships between subtribes remained quite unsolved. These relationships are shown in the Fig. 1. Martínez-Navarro *et al.* (2004) noted that karyotypic features (patterns of localisation of ribosomal DNA loci) are conservative within the Harpalines and have little value to establish phylogenetic relationships at most taxonomic ranks.

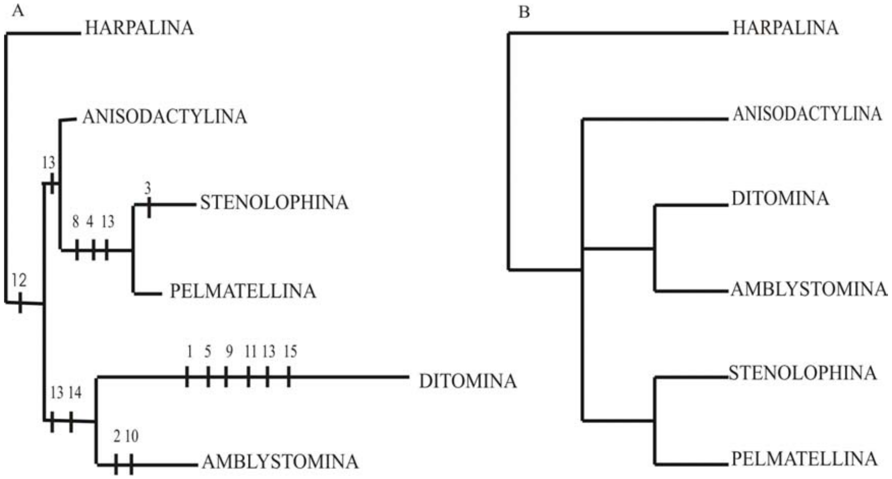

Figure 1. Cladistic analysis of the subtribes of Harpalini based on morphological characters (1-14) and diploid chromosome number (character 15). A) Semistrict consensus tree of the two most parsimonious cladograms. Morphological character state changes are shown above the branches. Branch length is proportional to the number of character state changes. B. Strict consensus tree. Reproduced from Martínez- Navarro *et al*. (2003).

The aim of this study is to analyse the phylogenetic relationships between the subtribes of Harpalini based on molecular data. We initially assume that there are six subtribes (Martínez-Navarro *et al*., 2003), and test their relationships by sequencing a fragment of the mitochondrial cytochrome-oxydase I gene in species representative of the different subtribes.

Material and methods

A fragment of the mitochondrial cytochrome-oxidase I (abbreviated as COI onwards) has been studied in 30 individuals of the tribe Harpalini, that belong to 30 genera and subgenera of all subtribes of Harpalini considered by traditional systematics (Noonan, 1976; Jeannel, 1942). The genus *Zabrus* Clairville, 1806 (tribe Zabrini) has been used as outgroup. Taxa included within the phylogenetic analyses, sampling localities, and EMBL accession numbers are provided in Table 1. Body parts not used for DNA extraction are deposited in the Department of Zoology and Physical Anthropology, University of Murcia.

Table 1. Taxa of the tribe Harpalini sequenced for cytochrome oxydase I, arranged according to the systematics of Noonan (1976).

Species	Country of sample	EMBL accession numbers
Pelmatellina		
Pelmatellus (Thenarellus) leucopus	Costa Rica	AJ583257
Lecanomerus niger (Darlington, 1956)	Australia	AJ583255
Anisodactylina		
A. (Anisodactylus) hispanus Puel, 1931	Spain	AJ583290
Scybalicus oblongiusculus (Dejean, 1829)	Spain	AJ583293
Gnathaphanus sp.	Malaysia	AJ583301
Pseudognathaphanus sp	India	AJ583295
Crasodactylus indicus Andrewes 1933	India	AJ583296
N. (Anisotarsus) politus (MacLeayi 1888)	Australia	AJ583304
Notiobia (Notiobia) sp.	Panama	AJ583297
Hypharpax peronii (Castelnau, 1867)	Australia	AJ583298
Stenolophina		
Stenolophus (Stenolophus) abdominalis (Géné, 1836)	Spain	AJ583267
Egadroma piceus (Guérin-Méneville, 1830)	Australia	AJ583268
Acupalpus elegans Dejean, 1829	Spain	AJ583262
Dicheirotrichus obsoletus (Dejean, 1829)	Spain	AJ583274
Bradycellus lusitanicus (Dejean, 1829)	Spain	AJ583259
Anthracus sp.	Rep. South Africa	AJ583263
Euthenarus promptus (Erichson, 1842)	Australia	AJ583258
Harpalina		
Harpali group		
H. (Harpalus) contemptus (Dejean, 1829)	Spain	AJ583343
Acinopi group		
Acinopus picipes (Olivier, 1795)	Spain	AJ583309
Dapti group		
Daptus vittatus Fischer von Waldheim, 1824	Spain	AJ583306
Bradybaeni group		
Bradybaenus opulentus Boheman, 1848	Kenya	AJ583311
Selenophori group		
Parophonus iberiparcus Zaballos y García-Núñez, 1991	Spain	AJ583324
Stenomorphus sp	U.S.A	AJ583313
Selenophorus sp	U.S.A	AJ583314
Afromizonus sp	Rep. South Africa	AJ583317
Selenophori sp	India	AJ583331
Ditomina		
Dixus clypeatus (Rossi, 1790)	Spain	AJ583275
Odontocarus cephalotes (Dejean, 1826)	Spain	AJ583281
Phorticosomus zabroides (Sloane, 1910)	Australia	AJ583287
Amblystomina		
Amblystomus niger	Spain	AJ583325
Amblystomus sp	India	AJ583326
Zabrini		
Zabrus ambiguus Rambur, 1838	Spain	AJ583328

DNA extraction, amplification and sequencing

Most specimens were kept in 80-100% ethanol (a few of them were kept frozen at -20ºC) until DNA extraction. Only clean legs and thorax muscles of individual ground beetles were used to avoid contamination with gut content or phoretic mites. Total genomic DNA was extracted following Chelex extraction protocol (Walsh *et al*., 1991). The tissues were homogenised in 100 µl Tris-EDTA buffer and protein kinase (0.1 mg/ml) and incubated for 2 h at 56ºC. Ten µl of the homogenates were added to a 100 µl of 5% Chelex resin and incubated for 30 min at 56ºC and 10 min at 95ºC to inactivate protein kinase. After incubation, samples were vortexed for 5 s and centrifuged for 5 s at 12.000xg before use in PCR amplification. Amplification of the fragment was performed using primers C1-J-2138 (Jerry) 5′CAACATTTATTTTGATTTTTTGG 3′, and TL2-N-3014 (Pat) 5′ TCAATTGCACTAATCTGCCATATTA 3′ designed for *Drosophila yakuba* (Clary and Wolstenholme, 1985) using Ready-to-go "PCR Beads" (Amersham Pharmacia Biotech) following the manufacturer's instructions. The amplification protocol was started with denaturation at 95ºC, followed by 35 cycles of 1 min at 95ºC, 1 min at 45ºC and 1 min 72ºC and a subsequent 5 min final extension at 72ºC. PCR products were checked in a 1% agarose gel. The PCR products of the expected length were cleaned with isopropanol and 5 M ammonium acetate and resuspended in 15 µl sterile water. Sequencing of the PCR products was performed on an ABI 377 automated sequencer. Nucleotide sequences are deposited at EMBL under accession numbers shown in Table 1.

Sequence analysis

The COI sequence data were aligned using Clustal W (Thompson *et al.,* 1994). The nucleotide data matrix was formatted with MEGA ver. 1.01 package (Kumar *et al*., 1993). Data matrix was exported into PAUP* 4.0 beta version b10 (Swofford, 1998) in which phylogenetic analyses were carried out.

Data matrix was analysed independently using maximum parsimony (MP), maximum likelihood (ML) and minimum evolution distance (MED) methods. MP analysis was implemented using unweighted parsimony. Heuristic searches were carried out using stepwise addition method, with 10 replicates by random addition of taxa and tree bisection-reconstruction (TBR) branch swapping algorithm. A fifty percent Majority rule consensus tree was calculated from those saved in the MP analysis.

Support at each node in the cladogram was analysed using bootstrap approach with 1000 (TBR algorithm) pseudoreplicates of the data matrix with 10 random sequence addition per replicate. The data set used for phylogenetic analyses was subjected to Modeltest 3.2 (Posada & Crandall, 1998) to find the best-fit model of evolution for use in ML. The data set was then analysed using the maximum likelihood optimality criterion employing the model proposed by Modeltest. Heuristic maximum likelihood searches were performed using random addition sequence and the TBR branch-swapping algorithm. The model of evolution suggested by

Modeltest was also used to estimate the minimum evolution distance tree using Neighbour-Joining as a clustering algorithm.

Results and discussion

Out of 759 bp analysed, a total of 314 bp were variable and 229 bp were parsimony-informative, excluding the outgroup. Thirty-five equal most parsimonious trees (1264 steps) were saved using MP method with unweighted data.

Both methods implemented under Modeltest 3.2, the Likelihood ratio test (hLRTs) and the Akaike information criterion (AIC), selected the same model of evolution for the COI fragment. Likelihood setting from best-fit model selected by AIC was the General Time Reversible, GTR (Yang *et al*., 1994) with an α value of 0.3738 and a proportion of invariant sites I = 0.3811. The optimal tree resulting from the ML optimality criterion had a value of –ln L = 6375. Bootstrap analysis (1000 replicates) resulted in a tree with poor resolution at the basal nodes. Bootstrap support values higher than fifty percent are represented below branches in Fig. 2.

Very similar clade composition and similar topologies of the trees and rearrangements of the taxa were obtained using different inference methods. However, the position of several taxa varied depending on the method carried out. We shall limit the comments to clades that are repeated under the three methods used, ML, MP and MED. The Majority rule consensus tree calculated from those fundamental trees saved under the unweighted parsimony method is shown in Fig. 2 (values above branches).

The mitochondrial COI data only support part of the hypotheses implicitly stated in the current systematics about the phylogenetic relationships of the subtribes of Harpalini (Jeannel, 1942; Noonan, 1976; Freude, 1976; Lindroth, 1986).

The species-groups of the subtribe Harpalina of Noonan (1976), Harpali, Acinopi, Bradybaeni, Amblystomi, Ditomi, Selenophori and Dapti do not make a monophyletic group but show complex relationships and affinities to the other subtribes of Harpalini. Harpali and Acinopi are closely related. Although Bradybaeni is more distantly related and close to the genus *Scybalicus*, appears closely related to the Harpali group in the ML tree. These results suggest that the subtribe Harpalina, as considered by Noonan (1976), is a rather heterogeneous group that deserves a detailed assessment of its limits and the taxa included within.

The two taxa of the Ditomi group make up a monophyletic clade separated from that made up by Harpali + Acinopi. These results support the ranking of Ditomina as an independent subtribe, a proposal (Serrano *et al*., 1994; Martínez-Navarro *et al*., 2003) also based on

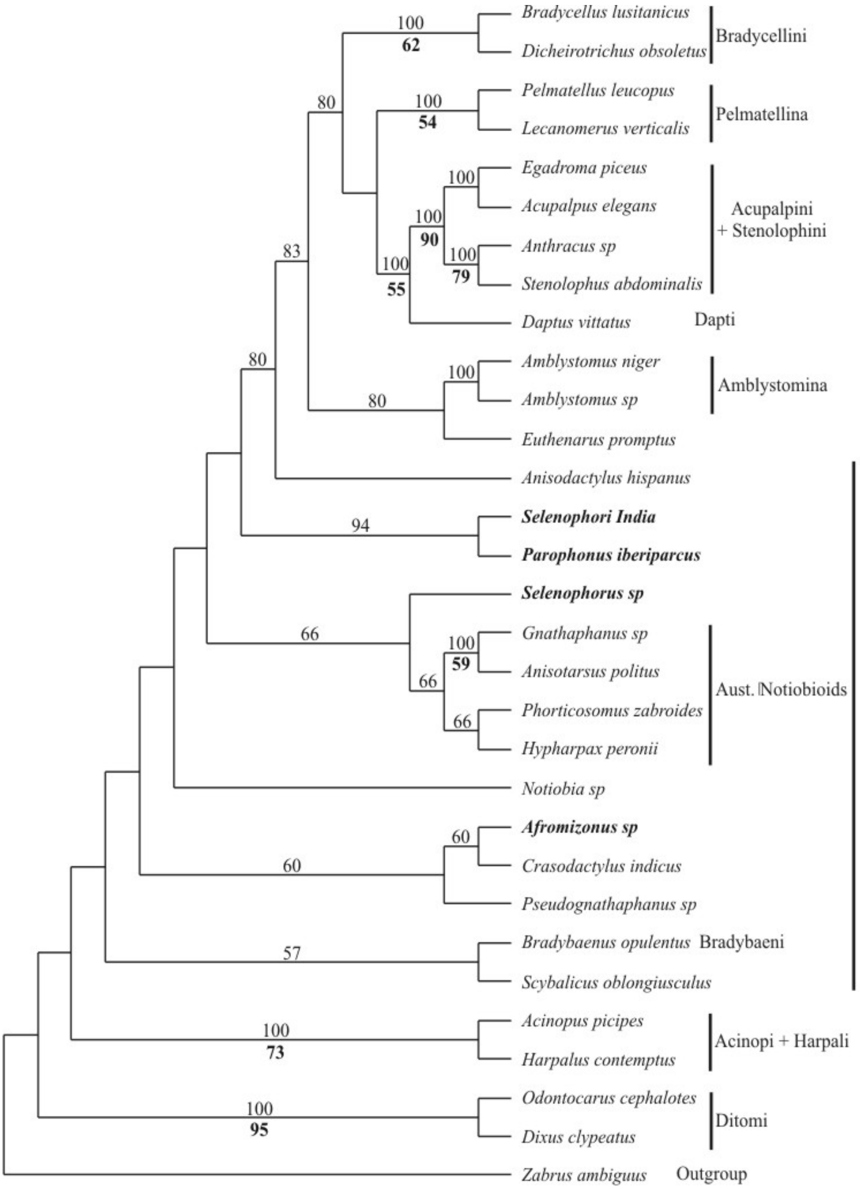

Figure 2. Majority rule consensus tree obtained from the 35 most parsimony trees calculated using unweighted maximum parsimony. 50% Majority rule consensus values above branches and bootstrap support values below them. Bold letters show taxa of the Selenophori lineage.

morphology, chromosomes, true presocial behaviour (Brandmayr and Brandmayr, 1987), and geographic distribution. According to different data sets its sister taxon would be the subtribe Harpalina.

The Amblystomi appears always related to taxa of the subtribe Stenolophina but with low support. The other available data (morphology, karyotypes) are likewise ambiguous and do not help to ascertain a solid relationship of Amblystomi to other subtribes. Freude (1976) placed *Amblystomus* within the Amblystomitae, a separate subfamily from Harpalitae, but did not justify this high ranking. On the other hand, the particular design of the latero-apical surface of the elytron suggests that the Amblystomi are well differentiated from other groups of Harpali. We tentatively suggest that the Amblystomi should be better regarded as an independent subtribe not close to Harpalina.

The close affinity of *Daptus vittatus* (Dapti group) to taxa of Stenolophina poses a note of caution in the interpretation of its morphological characters. It is adapted to a fossorial life in salty places, a specialisation that has possibly occurred independently in different lineages of harpalines (e.g., Acinopi and Bleusei groups), and that causes a convergent evolution with other taxa. Further data are needed for assessing whether *Daptus* represents a fast-evolving lineage of Harpalina or a different lineage superficially resembling some taxa of this last subtribe.

The Selenophori are characterised by the presence of a row of setigerous punctures in the third elytron interneur (also frequent in interneurs two, five, and seven), the first metatarsomere longer than 2 + 3, glabrous paraglossae, and the ostium of the median lobe of the aedeagus not deflected to the left (Noonan, 1985). However, none of these characters are exclusive of the group and there are also some conflictive Selenophori taxa showing different combinations of some (but not all) of the characters indicated above. Noonan (1985, p. 6) considered that the Selenophori are monophyletic on the basis of a character combination that is not found in other taxa of Harpalini. This hypothesis has not been corroborated by the molecular results, as the genera here investigated appear in different clades, clearly suggesting that they make a polyphyletic group. A second conclusion about the Selenophorines is that they are not closely related to other members of the subtribe Harpalina. Instead, Selenophori taxa are interspersed in the molecular analysis with taxa of the subtribe Anisodactylina, a relationship weakly supported that should be further assessed. Arndt (1990) showed that larval characters of *Parophonus maculicornis* (Selenophori) are relatively primitive and not related to those of Harpalina. The Selenophorines should be better considered as the sister taxon of Anisodactylina + Harpalina according to larval characters. The only clear conclusion resulting from these analyses is that most of the Selenophorines should be placed in a subtribe Selenophorina separated from Harpalina.

Taxa of the subtribe Anisodactylina are always found in separate clades. One is well supported and includes the genera from the Australian and Oriental regions (*Gnathaphanus, Anisotarsus, Hypharpax*). These taxa are members of the most plesiomorphic lineages of the Notiobioid branch defined by Noonan (1973). *Scybalicus* is a genus considered to belong to the lineage of modern Notiobioids (Noonan, 1973). Although this hypothesis is poorly supported in Fig. 2, *Scybalicus* and *Notiobia s. str.* make up a monophyletic clade in the ML tree (not shown). Likewise, the two genera representing the Anisodactyloid lineage (mentum and submentum completely or laterally fused, *Anisodactylus* and *Pseudognathaphanus*), are not closely related in Fig. 2, but they make up a monophyletic clade in the bootstrap analysis. The Australian genus *Phorticosomus* was tentatively included within the Ditomi group by Noonan (1976). This decision was perhaps based on the ventral vestiture of male tarsi (not expanded and with irregular to biseriate sparse ventral vestiture), and the presence of numerous short setae on the upper surface of the ligula. However, molecular data clearly suggest that *Phorticosomus* is closely related to the Australian Notiobioids with spongy pubescence in the male fore tarsi. It is worth noting that *Phorticosomus macleayi* (Sloane, 1915) shows ventral dilated and spongy pubescence in male fore tarsi. Likewise, the plurisetose ligula found within *Phorticosomus* is not exclusive of Ditomina, as Noonan (1973) described this same feature in some Anisodactylines (*Progonochaetus* and *Pseudoanisotarsus*). We conclude that *Phorticosomus* should be transferred from the Ditomi to the subtribe Anisodactylina, close to the plesiomorphic Notiobioids of the Australian region.

The subtribe Stenolophina is a monophyletic taxon except for the Australian genus *Euthenarus*, that is more closely related to Amblystomina. Interestingly, the Stenolophina include the two representatives of the subtribe Pelmatellina (*Lecanomerus* and *Pelmatellus*). Martínez-Navarro *et al*. (2003) showed that both subtribes are related by a peculiar chaetotaxy of the second labial palpomere and the prosternal lobe, and it might well be that the Pelmatellina are the Neotropical vicariant of an unidentified Nearctic taxon of Stenolophina. The analysis suggests that this unknown taxon is a member of the bradycellines (the tribe Bradycellini of Jeannel, 1942). This hypothesis implies that the spongy pubescence beneath male tarsi showed by taxa of Pelmatellina and Anisodactylina is convergent.

A close relationship is suggested for the lineages represented by *Stenolophus, Egadroma*, *Acupalpus* and *Anthracus* (the tribes Stenolophini and Acupalpini of Jeannel, 1942). Caution is needed to interpret phylogenetic relationships between the subtribes of Harpalini based on the sequence of COI, because these lineages may have well split off from the basal stock of Harpalines during the Jurassic period. Noonan (1973) estimated that the Anisodactyloid branch evolved independently from the Notiobioids when Laurasia became separated from Gondwana at the beginning of the Cretaceous period (135 my). The Pelmatellina must have a more recent origin (from 100 my onwards) as they are distributed in South America but not in Africa. The same can be said with regard to the Ditomina, a lineage that was possibly originated when the Tethys Sea started its transformation into the present Mediterranean Basin, about 35 my ago. These are periods long enough to permit a likely saturation at the third codon position of the COI gene, what causes an evolutionary "noise" not yet estimated. This "noise" is the most likely explanation for the mixing of taxa belonging to Anisodactylina and the Selenophori lineage depicted in Fig. 2. The increase in length of the sequenced fragment and the addition of the sequence of slow-evolving genes will eventually produce a more solid arrangement of both lineages.

Acknowledgements

We thank the following colleagues for providing beetles or helping in collecting and identifying them: J. L. Lencina, A. Andújar, V. M. Ortuño, C. Andújar, J. F. Sánchez (Iberian Peninsula), B. Guéorguiev (Bulgaria), A. Slipinski, R. Oberprieler, D. Rentz, D. Yeates, L. A. Mound, T.A. Weir, G. Monteith and B. P. Moore (Australia). D. R. Maddison generously gave us alcohol specimens from African, Neotropical, Oriental and Nearctic regions. D. Posada helped us to use the Modeltest program. Two anonymous reviewers improved notably the manuscript with valuable comments. Financial support for this study was provided by the projects PB98-0402 and BOS2002-02870 of the Spanish Ministry of Science and Technology. E. M. Martínez-Navarro was supported by a Ph.D. grant from the University of Murcia.

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Morphological or molecular systematics? A case study of *Carabus*

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Abstract

The genus *Carabus* consists of a large number of species, which are arranged in many subgenera. The phylogenetic relationships of these subgenera and their derived systematics are currently under discussion. Morphological characters, such as larval structure or characters of the endophallus have been classically used in *Carabus* systematics. Numerous DNA sequence data are available, which Imura (2002) used to established a new system. He split the genus *Carabus s. l.* into 29 sections with 137 genera, by following the idea that each well supported clade of the molecular tree should be regarded as a genus. In the present paper the basal phylogeny of *Carabus* is described as more or less unresolved by morphological as well as by molecular data. The DNA data result in a tree in which most of the branches are not sufficiently supported. Therefore, neither the system of Imura is acceptable in most details, nor is a formal step that lifts the subgenera to the genus category.

Key words: *Carabus*, phylogenetic relations, subgenera, morphology, molecular data

Introduction

The genus *Carabus s. l.* includes 476 species according to the classical monograph of Breuning (1932-1937). He reduced the high number of subgenera and other subgroups to 20 subgenera and within his subgenera he established 68 sections. Meanwhile, the number of described species has risen to about 850. Most authors maintained *Carabus s. l.* as a genus (e.g. Deuve 1991, 1997; Brezina, 1999; Turin *et al.*, 2002), by some others it has been split in a moderate number of genera (e.g. Jeannel, 1941).

In this paper, the main attempts to arrange the subgenera of *Carabus* are listed in order to demonstrate the transitions which were based on new morphological characters, the endophallic structures, or on molecular data. The systems of Brezina (1999) and Imura (2002) were established on DNA sequence data. But the phylogenetic methods and the interpretation of these molecular data should come into question as well as the consequence of Imura (2002)

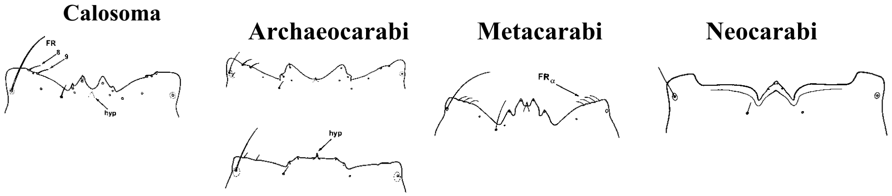


Figure 1. Frontal part of the larval head capsule with nasale and adnasale of *Calosoma* as outgroup and the three larval types of *Carabus*. From ARNDT et al. (2003), modified.

to split the genus *Carabus s. l.* into 29 sections and 137 genera. Two reasons were mentioned by him to establish each well supported branch of the molecular tree as a genus, (i) the interpretation that the major groups evolved by an explosive radiation, which is regarded as an assemblage of distinct lines, and (ii) that his names are more informative and shorter.
There will be discussed two aspects: (i) the phylogenetic relations between the classical subgenera and (ii) the consequences for the categorical ranking of the taxa.

Classical arrangements of the *Carabus* subgenera

The first comprehensive arrangement of the *Carabus* subgenera was published by Reitter (1896). Bengtsson (1927) classified the species into three types according to their larvae (Archeocarabus, Metacarabus, and Neocarabus). The same system was described independently by Lapouge (1929), using other terms, on the basis of his broader knowledge of larvae. To avoid confusion, the terms Archeocarabi, Metacarabi, and Neocarabi are used in the present paper (following Deuve, 1994).

Most authors regard the Archeocarabi as the most primitive type and the Neocarabi as the most progressive forms. But these predicates are only applied correctly in respect to characters. The outgroup comparison shows that some character states of Metacarabi may be plesiomorphic as well (e.g. form of the adnasale). In addition, the character state of the rostral region in Archeocarabi is not uniform. Thus, these arrangements are typological, not all groups are characterised by evolutionary novelties (synapomorphies) except Neocarabi. Additionally, the relationship of the three groups is not yet resolved and only the Neocarabi are based on characters which may be interpreted as synapomorphic: the nasale forms a rostrum and its teeth are reduced; the S-form of the adnasale (Fig. 1), the body shape and the form of the urogomphi are characteristic (Fig. 2).

In Breuning's (1932-1937) system, the Carabi brevimandibulares (merging Archeocarabi + Metacarabi) are based on the form of the mandible of the adults. It is broad and its tip bent sharply. A comparison with *Calosoma*, *Ceroglossus* and *Maoripamborus* as outgroups results in the interpretation that this is the plesiomorphic character state (see also Acorn & Ball,

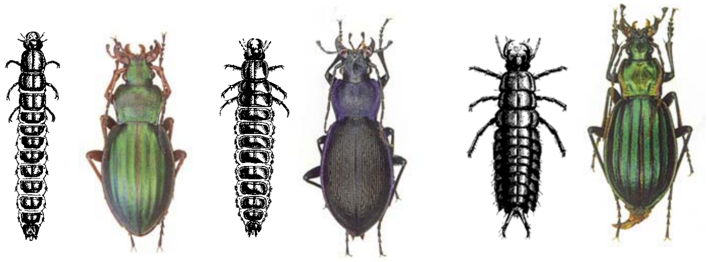


Figure 2. Habitus of larvae and imagines of the three larval types. Larvae from Casale *et al*. (1982), imagines from Forel & Leplat (1995); modified.

1991; Mossakowski, 2002). Therefore, the Brevimandibulares represent a paraphyletic group. The Carabi longimandibulares (Neocarabi plus some other subgenera) with a long, continuously pointed mandible are based on the apomorphic character state. Many, but not all of the old subgenera are well founded by morphological data. Breuning (1932-1937) had already stated that some of his groups were artificial.

Ishikawa (1978) deserves recognition for his systematic research on the internal structures of the aedeagus in *Carabus*. He assembled the subgenera according to their characters regarding the endophallus into 3 groups. This system was expanded by Deuve (1991, 1994), into 5 groups and differentiated later on (Deuve, 1997) (Tab. 1, Fig. 3). Deuve (1994) pointed out that - with some exceptions - the variability of endophallic structures corresponds to that of the larval types.

Studies with the method of phylogenetic systematics

Marciniak (1995) studied the aedeagus of the European *Carabus* species and interpreted his data using the method of phylogenetic systematics (Hennig, 1966). He formed the basic split by facing *C. nitens* towards all the other *Carabus* subgenera. He considered the form of the ligulum in *Carabus (Hemicarabus) nitens* to be plesiomorphic, an interpretation already discussed by Ishikawa (1973). All the other species show the apomorphic state in regard to the ligulum, a character state he assigned also to the form of the aggonoporius of these species. In the remaining groups, *Tachypus* (the former *Autocarabus) auratus* and *cancellatus* split basally. The subgenera *Chaetocarabus* and *Platycarabus* are grouped as sister taxa long isolated from the remaining Neocarabi.

Arndt *et al.* (2003) did not follow his interpretation in respect to *C. nitens*. Therefore, this species was placed in the remainder of the Metacarabi. The authors also found a basic position for *C. (Tachypus) auratus* and *cancellatus* by means of larval and endophallus characters, but their interrelationship was ambiguous: there was a good support for a paraphyletic relation between these species by morphological data (Arndt *et al*., 2003, Fig.

Table 1. System of the *Carabus* subgenera. Compiled from Deuve (1997), Imura (1996) and Imura *et al*. (1998). n: number of subgenera in Imura (1996).

DEUVE 1997	IMURA 1996, IMURA ET AL. 1998	n	subgenera (selection)
1 Spinulati	1.1 Lepidospinulati	2	*Limnocarabus, Euleptocarabus*
	2.1 Spinulati	1	*Apotomopterus*
2 Digitulati	1.2 Digitulati	4	*Carabus,* ^{1}*Eucarabus, Ohomopterus, Isiocarabus*
	2.3 Latitarsi part	2^{x}	*Eurycarabus, Nesaeocarabus*
3 Lipastromorphi	1.3 Lipastromorphi	6	*Morphocarabus, Lipaster, + Rhigoidocarabus*
4 Archicarabomorphi	1.4 Archicarabomorphi	4	*Archicarabus, Ischnocarabus*
5 Lobifera			
basal subgenus ^{x}	2.3 Latitarsi part	1	*Tachypus*
Crenolimbi* ^{x}	2.2 Crenolimbi	2	*Hemicarabus, Homoeocarabus*
Multistriati Reitter			
Tomocaraboides	2.3 Latitarsi part	6	*Tomocarabus, Pachystus, Scambocarabus*
Oreocaraboides	2.3 Latitarsi part	8	*Mesocarabus, Oreocarabus, Meganebrius*
Longimandibulares*			
basal groups	2.5 Procrustimorphi part	1	*Cathoplius*
	2.4 Arcifera part	2	*Hygrocarabus, Heterocarabus*
Neocarabi*			
basal groups* ^{x}	2.4 Arcifera part	2	*Platycarabus, Chaetocarabus*
Procrustimorphi*	2.5 Procrustimorphi	52	*Iniopachys, Tribax, Coptolabrus, Damaster, Megodontus, Procerus, Macrothorax, Chrysocarabus, Procrustes, Lamprostus*

2-3 of Deuve, 1.2-1.4 of Imura = Carabogenici
* hypothetisized to be monophyla (morphology)
^{x} supported by molecular data
2.1-2.5: Multistriata sensu ISHIKAWA nec REITTER ? Lobifera DEUVE.

^{1} Deuve included *Eucarabus* into *Carabus*
+ plus new subgenus in Deuve (1997)

7.16), but a moderate support for a sister group relationship by molecular data (*l. c.*, Fig. 7.17). Therefore, this problem remains unresolved. Archeocarabi and Metacarabi in the old sense could not be confirmed. Many groups referred to in the literature are only based on single characters of the endophallus. Deuve (1994, p. 61) already stated that the Lobifera are likely to be paraphyletic or polyphyletic. The characters of the endophallus are mainly protuberances of its more or less flexible wall. Therefore, similar structures may have evolved in parallel, a phenomenon well known in the morphology of *Carabus* (Fig. 2).

A basic position for *Tachypus* was also concluded by Deuve (1997), who opposed this subgenus to all the other Lobifera in which the Crenolimbi split off first. He used the name Multistriati in the sense of Reitter (Tomocaraboides and Oreocaraboides) and again introduced the Longimandibulares (Table 1). As a consequence of the basal position of *Tachypus* (Arndt *et al*., 2003), and the sister group relationship of *Eurycarabus* and *Nesaeocarabus* which were interpreted as Digitulati (Prüser *et al*., 2000), these taxa could not be included in the Latitarsi of Imura. Imura's Arcifera are at least paraphyletic, because *Chaetocarabus* and *Platycarabus*, a part of the Arcifera, together with his Procrustimorphi are well founded as a monophyletic group (Table 1: Neocarabi). Bengtsson (1927) had already set *Carabus (Chaetocarabus) intricatus* among the Neocarabi. Homologisation of nasale and adnasale of *Platycarabus* species may be determined by the figures of larval characters in Casale *et al*. (1982, p. 393, 394).

A test of the results on *Carabus* phylogeny inferred from molecular data

The discussion about problems of phylogenetic relationships and that on systematics has been reanimated by the increasing use of molecular data for this purpose, in other taxa as well in *Carabus* (first sets of sequences used by Su *et al*., 1996 a, b, c and Prüser, 1996). Studies on morphology were also stimulated by this development. Today, numerous DNA sequences have been published for many species of the genus *Carabus* and many are available in the GenBank. But the majority of them are restricted to only two mitochondrial genes. In consequence, two problems arise: (i) do the resulting gene trees represent the species trees?, and (ii) are mitochondrial genes sufficient to determine the early splits under discussion?

Molecular data (DNA sequences) have many advantages:

- A high number of characters (= positions on the sequence),
- Tests for checking the information content of the data (Ts/Tv; triplet position, etc.)
- Test for checking the validity of the tree or its branches (bootstrap analysis, Decay-Index, etc.)
- They may be evaluated by different methods.

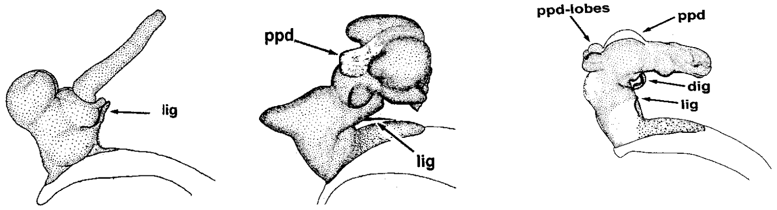
These advantages overcome the disadvantage of the low complexity of a single base substitution.

One of the problems in understanding the evaluation of molecular data seems to be that the output of the programs is always a tree or a number of trees. It is most important to determine the significance of a particular branch or node in the tree. This shall be examined with the results considering *Carabus* subgenera and the mitochondrial subunit 5 of the NADH-Dehydrogenase (ND 5). Arndt *et al.* (2003) presented a comparison of two trees inferred from the same molecular data. The first one shows a well resolved hierarchical pattern, but this structure collapsed in the second tree where the criterion for separating a branch is set to a bootstrap value of at least 70%. The same procedure is performed for the tree given in Imura *et al.* (1998) and the result is represented in Fig. 4.

If we take this gene tree as the species tree, the resolution of the phylogenetic relationships of the *Carabus* subgenera is very poor. In many cases, the 22 branches of the ingroup in Fig. 4 does not fit with the subgenus groups (Table 2). Only 4 are well supported by bootstrap values (taken from the original tree), but among them the Spinulati (in the sense of Imura) contain only one subgenus. Additionally, problems also arise within the same subgenus: *Carabus (Limnocarabus) clatratus* does not cluster with *C. (L.) maacki*, a species regarded as subspecies of *C. clatratus*, e.g., by Deuve (1994).

Table 2. Groups of *Carabus* subgenera and the bootstrap values (%) at their node in the tree of Imura *et al.* (1998). n_1: number of subgenera in the tree; n_2: total number of subgenera in Imura (1996).

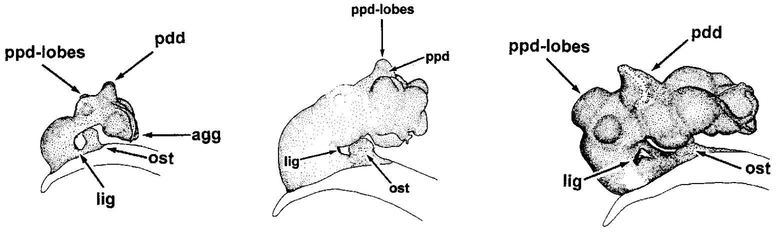
Group of subgenera	n_1	n_2	%
Lepidospinulati	2	3	100
Arcifer	2	4	70
Crenolimbi	2	2	99
Spinulati	1	1	96
Procrustimorphi	5	53	(26)
Digitulati	5	5	(52)
Archicarabomorphi	1	4	-
Lipastrimorphi	1	6	-
Latitarsi	9	17	(26)



Calosoma
outgroup

C. (Limnocarabus) clatratus
Spinulati

C. (Nesaeocarabus) coarctatus
Digitulati



C. (Tachypus) auratus
Lobifera
(Archeocarabi)

C. (Hemicarabus) nitens
Lobifera Crenolimbi
(Metacarabi)

C. (Chaetocarabus) intricatus
Lobifera
(Neocarabi)

Figure 3. Tip of male aedeagus with inflated endophallus. From Marciniak 1995, cf. Arndt *et al.* (2003), modified. agg: aggonoporius, dig: digitulus, lig: ligulum, ost: ostium lobes, ppd: praeputial pad.

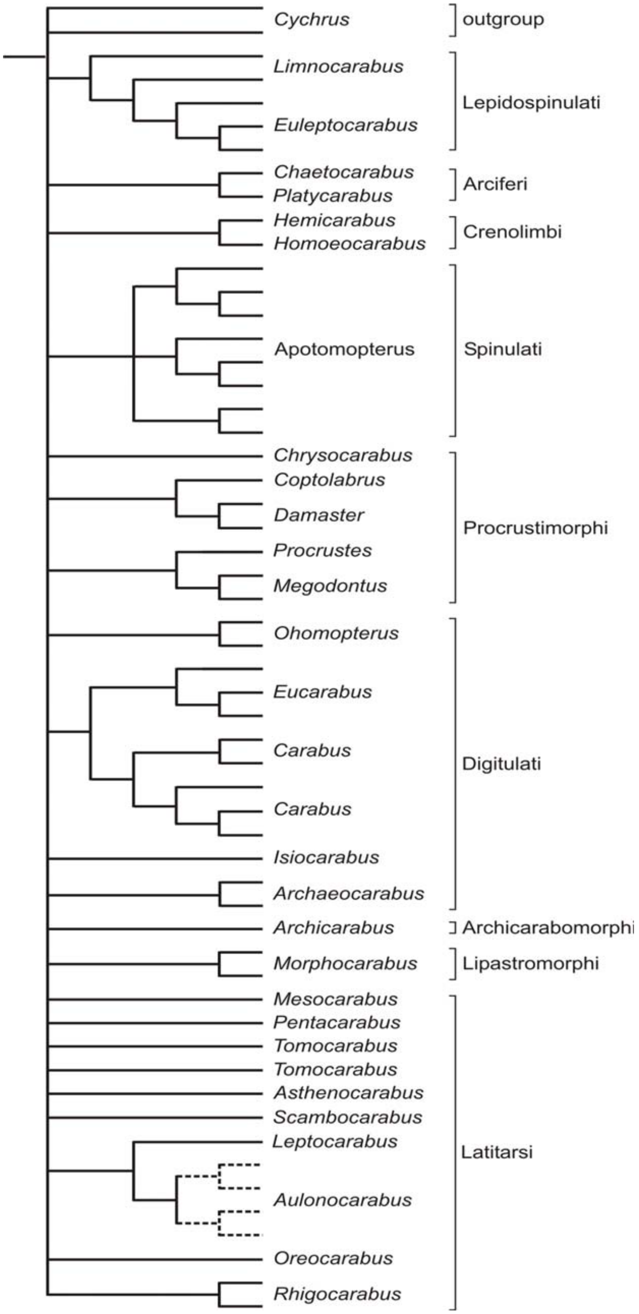


Figure 4. Neighbor Joining tree of *Carabus* based on mitochondrial sequence data (ND5). The original tree of Imura (1996) was redrawn: all the nodes were collapsed with bootstrap values below 70%.

Imura (1996) and Imura *et al.* (1998) deduced their system (compare Table 1) on the basis of results from a molecular tree without using a sufficiently high bootstrap criterion. But in 2002 Imura applied the same criterion (≤ 70%) as did Arndt et al. (2003) and established his 29 sections. Consequently, Imura (2002, p. 2) stated that "…, the first diversification of the Carabina has taken place as an explosive radiation of the major groups. This means that the Carabina should be regarded as an assemblage of distinct lineages rather than simply be unified into a homogeneous group."

Besides these problems, molecular data were helpful in phylogenetic and other respect. E. g., Prüser (1996) questioned the species status of allopatric taxa (*C. famini* & *favieri; C. intricatus* & *lefebvrei*). Prüser *et al.* (2000) reasons for a sister group relationship between *Nesaeocarabus* of the Canary Islands with *Eurycarabus* from North Africa, and Düring *et al.* (2001) found that in *Chrysocarabus* the gene tree of mitochondrial sequence data did not represent the species tree.

Conclusions

- All authors including Imura (2002) interpret the Carabina with the only genus *Carabus* (*s. l.*) as a well supported monophyletic group, but the basic relationships within this taxon are under discussion. Only Ishikawa discussed a sister group relationship between some *Carabus* subgenera with a part of the Calosomina, but this problem arose, because he did not use the argumentation scheme of phylogenetic systematics.

- In contrast to statements of Imura, it is obvious that molecular data do not throw an objective light on the evolutionary history. All kinds of data underlie the same processes of evolution. The similarity of a morphological trait or an identical base in a particular position of the DNA in a group of species may be interpreted as having evolved once (synapomorphy in the sense of Hennig, 1966), as plesiomorphy, or independently evolved multiple times (in parallel or convergent), or as a reversion. Only synapomorphic character states represent the evolutionary signal, the others produce noise (homoplasy).

- Many of the basis near splits in *Carabus s. l.* are unresolved. But there is no real evidence that there was an explosive radiation at the beginning of the *Carabus* evolution. To prove an early radiation, the alternative hypothesis, that the problems to resolve these early splits arise because the mitochondrial genes under use evolved too fast, must be falsified. Therefore, it is necessary to produce data from nuclear genes. Meanwhile, a coarse system like that of Table 1 has yet to be proved.

- Two taxa on a tree may be closely related although they do not cluster like every two of the 22 in Fig. 4. A distinct position only says that the support for a common node is not sufficient.

- The evaluation of molecular data should be done by more advanced methods (e. g., Maximum Parsimony, Maximum Likelihood). Distance methods (Neighbor Joining, UPGMA), as applied for the trees which were the basis for Imura's (2002) scheme, do not use the full information content of the data (e. g., Mossakowski & Prüser, 1999).

- The definition of the category genus of Imura (2002) is open to question. He regards each branch with more than 70% bootstrap support as a genus. But the categorical rank of a taxon is set arbitrarily and should follow practical requirements. Although a category between genus and subgenus is not included in the International Code of Zoological Nomenclature it is not acceptable to change good and established names like Crenolimbi into Hemicarabigenici, etc.

- Taxa are entities of nature, categories are artificial. Taxa above the species level should be established on the basis of a well founded hypothesis on the phylogenetic relationships.

- Although morphological data have disadvantages like an unknown complexity of their genetic basis and a high rate of parallel evolution at this level, they are helpful because they are easy to study for many species, and material of collections can be used almost without restriction. We need more morphological data, more data on genes with an appropriate slow rate of evolution suitable for resolving deeper splits, and an integrated, not a totalised, evaluation of morphological as well molecular data.

Acknowledgements

Many colleagues helped to gather material, and a large number of co-workers produced the many data and results used in respect to *Carabus* phylogeny: Sigrid Kniep (mouth parts), Folkhard Wülfers (elytral fusion), Mathias Marciniak (endophallus), Jana Roihu and Anette Norden (proventriculus); Sabine Braun, Wulf Carius, Dieter Wienrich, Oda Wilkens, Dorothee Just (enzyme data); Frank Prüser, Andreas Düring, Martina Brückner, Marco Zimmermann, Iris Burfeindt, Nordfried Kamer (molecular data, DNA sequences). Hans-Konrad Nettmann was also involved intensely in our discussions. I would like to thank them all, Michael Vicker for correcting my English and two anonymous referees for their stimulating comments.

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Carabid beetles in a Mediterranean Region: biogeographical and ecological features

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Abstract

Calabria is one of the southernmost regions of Italy. The main Calabrian habitats have been sampled with pitfall traps since 1987. Merging field collections with literature data, the known number of species in Calabria is 358 , i.e. 28.5% of the Italian carabid fauna. Owing to the young geological age of the region, only one species (*Pterostichus ruffoi* Sciaky) is endemic to Calabria. Relationships between Italian endemics, Mediterranean species and habitat types are outlined.

Introduction

The faunistical history of the Calabria region is tied mainly to the tectonics of 10-12 My ago, when the Tyrrhenian microplates migrating eastwards from Archeo-Spain gave origin to Corsica, Sardinia and other small islands, and produced the rising of Archeo-Calabria.

For millions of years Archeo-Calabria was a chain of islands, sometimes joined and sometimes separated from each other and from Archeo-Italy (i.e. the earliest emerging mountain ranges of what today are called the Italian Apennines). Archeo-Calabria finally merged with Archeo-Italy 4 My, to form a large peninsula in the middle of the Mediterranean Sea.

Between the end of the Miocene era (6.3 Mya) and the beginning of the Pliocene era (5.3 Mya), there was no connection between the Mediterranean Sea and the Atlantic Ocean (the present Gibraltar Channel absent), and the Mediterranean Sea was reduced to two small basins east and west of Italy (Messinian period). Pleistocenic (2 Mya) glaciations only had an indirect influence in Calabria, because no large ice masses were present, but climate change produced the southward migration of flora and fauna.

These paleogeographic and paleoclimatic phenomena were the main factors affecting the distribution of flora and fauna in the Mediterranean bioregion.

The aim of this paper is to give a zoogeographic picture of the present carabid fauna of Calabria, and to outline the habitat preferences of the most common species.

Study areas

Sample sites were chosen on the basis of vegetation physiognomy, located along altitudinal bioclimatic gradients. In all, 72 sites were sampled, mainly in the northern part of Calabria. They were grouped into habitat types on the basis of vegetation physiognomy as follows:

- beech forests (F): the sites were between 900 and 1600 m above sea level (a.s.l.); winter is cold, with snow lasting from December to May on the central massif of the Sila Grande, but only a few days in the mountains near the coast (15 sample sites)
- oak forests (Q): sites between 500 and 1300 m a.s.l., rainy winter, minimum temperature rarely reaches 0°C (11 sample sites)
- mixed forests (Mx): sites between 500 and 900 m a.s.l.; rainy winter, minimum temperature rarely reaches 0°C. Chestnut forests were included in this category (10 sample sites)
- pine forests (Pi): only mountain pine woods were sampled in the Sila Grande massif; sites between 1200 and 1500 m a.s.l.; in winter the snow lasts from December to May, maximum summer temperatures are around 20-25°C (4 sample sites)
- sclerophyllic forests (Sc): sites between 100 and 600 m a.s.l.; very dry and hot summer, moderately rainy during fall and winter (8 sample sites)
- pastures (Pa): mainly grasslands affected by moderate grazing; sites between 100 and 1400 m a.s.l., (6 sample sites)
- fields (Fi): sites between 40 and 100 m a.s.l. in the sclerophyllic bioclimatic belt; dry and hot summer; olive, orange and wheat fields were sampled (3 sample sites)
- abandoned fields (Fia): abandoned cereal fields at 250 m a.s.l. (4 sample sites)
- azonal habitats (Azf): azonal habitats whose characteristics are tied mainly to topographic features or local factors, rather than altitude and climatic factors, belong to this group. The sites were in a Mediterranean large river bed (“fiumara”), intermittently flowing, and almost completely dry for the largest part of spring and summer. Results apply only for “fiumara” habitats. Sites at 70 m a.s.l. (8 sample sites);
- karstic sites (K): sites at 1400 m a.s.l., strictly tied to karstic phenomena: one was a crevice (2 m deep), two were sinks covered with grassy vegetation (3 sample sites).

Methods

Data on Calabrian carabids come from literature (Magistretti, 1965; Angelini, 1991; Vigna, 1993), and from the authors' field collections with pitfall traps since 1987 (Pizzolotto, 1994; Brandmayr *et al.,* 2001).

The traps were small pots of 9 cm diameter and 11 cm deep, provided with a small hole near the top to allow rainwater to drain away, filled with 200 cl of a mixture of wine vinegar and 5% formalin.

One year-sample was collected at each site with 5-7 traps. Carabids were trapped continuosly between March and November, the traps being emptied every 20-30 days. Thus, approximately 8 trap collections made up a year-sample. In the mountains the carabid activity period is shorter (May to September), while along the coast it lasts 12 months. Tests were made applying a 12 months sampling period in the mountains (where possible) and along the coast. No significant differences in the number of carabids were recorded in comparison with the catches in the March-November and May-September periods (Pizzolotto *et al.*, unpublished).

The number of collected individuals was used to compute the annual activity density of each species, expressed as the number of individuals per trap per 10 trapping days.

The species' geographical distribution was characterized on the basis of the chorotypes of Vigna *et al.* (1993, see also Turin *et al.,* 2003). The chorotypes were grouped into categories (I to V in Table 1) on the basis of the size of the species' geographical range.

Table 1. Chorological features of the Calabrian carabids (358 species).

Chorological categories	Symbol	No. of spp.	%
Calabrian endemics	I	1	0.3
Italian endemics	II	27	7.5
Mediterranean (not exclusively)	IIIm	135	37.7
European	III	73	20.4
Euro-asiatic, Euro-siberian	IV	88	24.6
Palaearctic, Holarctic	V	34	9.5

The IndVal procedure (Dufrene and Legendre, 1997) evaluates the contribution of the fidelity and the specificity of a species for a group of sites, habitats in our case, to produce an index that is maximum 'when the individuals of species *i* are observed in all sites of only one site group' (Dufrene and Legendre, 1997) (habitat in our case). The procedure was applied to evaluate the authors' field collections in order to outline the species' habitat preferences. This index is useful both with qualitative data (presence vs. absence) and with quantitative data, but it is with the latter it gives the best species-specificity measure (cfr. Dufrene and Legendre, 1997).

In a matrix of IndVal values with species in the rows and habitats in the columns (e.g. Table 3), each species may have three types of IndVal index values (cfr., Chemini & Pizzolotto, 1992; Pizzolotto, 1993) as follows:

- central value (a point): where the species shows the maximum IndVal
- nuclear value (an area around a point): where the IndVal is equal or above the average, computed on row total
- orbital values (elements orbiting around a nucleus): IndVal below the average.

Species with wide ecological tolerances might show a central value and several (from 0 to many) nuclear values (see for example *Steropus melas* and *Calathus montivagus* in Table 3). The fewer the nuclear values, the stronger is the preference for the habitat with the central value. It is possible that species strongly linked to a given habitat show central value in that and few or no nuclear values in other habitats. These would be the characteristic species of that habitat.

A problem arises when species are sampled in one habitat only. To define them as central or characteristics species depends on the IndVal value, literature information, and on the researchers' experience.

The same (semi-objective) criterion should be applied to discriminate species seldom caught (travellers) from species caught by chance (tourists) in a habitat.

Results

In Calabria there are 358 species known from literature and from our own field samplings, accounting for 28.5% of the known Italian carabids. In Table 1 the chorological composition is outlined. The Mediterranean chorological category (IIIm) had the highest number of species, acounting for 37.7% (135 species) of Calabrian species. Italian endemics accounted for only 7.5% (27 species) of regional carabids and, except for locally endemic species (*Pterostichus ruffoi*), this is the category with the lowest number of species.

To estimate catching efficiency, the analyses suggested by Colwell (1997) have been performed (Pizzolotto *et al.*, unpublished). They show that not all species have been found.

From the 72 sites sampled by the authors, 159 species were collected. Data on species richness and chorological categories are summarized in Table 2.

It is interesting to see how many species belong to the Italian and Mediterranean (II and IIIm) chorological categories in the sampled habitats (Table 2). Italian endemics are abundant in forest habitats, mainly in mountain pine woods, but also in evergreen sclerophyllic woods and beech woods. Species with a Mediterranean distribution are most abundant in habitats

Table 2. Number of species, and percentage representation among chorological categories of the Calabrian carabids from field collections by the authors (159 species).

	Habitats									
Category	F	Q	Mx	Pi	Sc	Pa	Fi	Fia	Azf	K
No. spp.	41	45	45	18	26	60	68	56	66	31
I	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
II	24.4	20.0	22.2	33.3	30.8	18.3	11.8	12.5	13.6	29.0
IIIm	17.1	20.0	15.6	5.6	19.2	33.3	42.6	33.9	28.8	12.9
III	39.0	33.3	33.3	55.6	23.1	28.3	22.1	35.7	28.8	32.3
IV	9.8	13.3	15.6	5.6	11.5	6.7	10.3	8.9	12.1	9.7
V	7.3	13.3	13.3	0.0	15.4	13.3	13.2	8.9	16.7	16.1

exploited in pre-historical time by man for primeval survival needs (food and shelter), and at present time the original vegetation is still cleared for agriculture and pastures.

The evaluation of IndVal values allowed the species to be separated into a group of species with central and nuclear values in forest habitats (42 species, Table 3), and a group of species with central and nuclear values in open land habitats (117 species, Table 4).

Only three forest species showed strong preference for forest habitats, i.e. they have IndVal higher than 50%. They are *Carabus convexus*, *Calathus piceus* and *Steropus melas* (Table 3).

Several species were caught in one forest habitat only, where they show a high IndVal index. For the beech wood habitat these are: *Calathus fracassi*, *Pterostichus ruffoi* and *Anchus ruficornis* (the latter near forest brooks), with IndVal higher than 20%. It is likely that other species, even with low IndVal, are tied to beech woods (forest specialists in Table 3) because they were caught only there, and previous work (Brandmayr & Zetto Brandmayr 1984; Brandmayr & Pizzolotto 1988, 1990) found similar results. The same argument holds true for the other forest habitats.

Most of the collected species are strictly tied to open land habitats, 23 of them showing IndVal higher than 50% (Table 4).

In Tables 3 and 4 a possible grouping of the species into specialists and generalists is suggested on the basis of the criterion discussed in the Methods section. Tables 3 and 4 only apply to Calabria.

Table 3. Habitat preferences and maximum indicator values (IndVal max) for forest species. X, central; o, nuclear; --, orbital. S, specialist; G, generalist. S*, near brooks. md, more data needed. For definition of habitat abbreviations, see "Study area".

		Habitats										
	Species	F	Qc	Mx	Pi	Sc	Pa	Fi	Fia	Az	K	IndVal max
S	*Calathus fracassii*	X										20.0
S	*Dromius quadrimaculatus*	X										6.7
S	*Pterostichus micans*	X										6.7
S*	*Anchus ruficornis*	X										13.3
S	*Argutor angustatus*	X										6.7
md	*Bembidion geniculatum*	X										6.7
S	*Pterostichus niger*	X										6.7
S	*Nebria andalusiaca*	X										6.7
S	*Pterostichus ruffoi*	X										33.3
S	*Pterostichus (Hapt.) unctulatus*	X		--								33.3
S	*Trichotichnus nitens*	X	--	--								23.1
G	*Notiophilus biguttatus*	X	--		o		--					12.5
G	*Cychrus italicus*	X	--	o	--	--	--				--	43.0
S	*Calathus piceus*	X	--	o	o		--			--		53.8
S	*Notiophilus substriatus*		X									9.1
md	*Amara anthobia*		X	--				--		o	--	19.8
S	*Calosoma inquisitor*		X									18.2
G	*Notiophilus rufipes*	--	X	o		o				--		19.5
md	*Brachinus gr. explodens*			X								10.0
md	*Amara proxima*			X								10.0
S	*Leistus sardous*	o		X								6.0
md	*Harpalus honestus*			X								20.0
G	*Harpalus tardus*	--	o	X			--	--	--	o	--	15.8
G	*Abax ater curtulus*	o	--	X	--	--					--	45.9
G	*Carabus convexus*	--	o	X	--	--				--	--	66.8
G	*Carabus lefebvrei*	o	o	X	--	--				--	--	40.7
G	*Platyderus canaliculatus*	o	o	o	--	--	--	--	--	--	X	21.5
G	*Carabus presli*	o	o	X	o		--			--	--	34.7
md	*Harpalus tenebrosus*			X								10.0
G	*Harpalus rufitarsis*		--	X			--			--	o	35.4
G	*Laemostenus cimmerius*		o	X				--		o		27.9
G	*Pterostichus (Steropus) melas*	--	--	--	X	--	--	--	o	--		56.7
S	*Calathus montivagus*	o	o	o	X	--	--	--	--	--	--	37.2

		Habitats										
	Species	F	Qc	Mx	Pi	Sc	Pa	Fi	Fia	Az	K	IndVal max
S	*Nebria kratteri*	o	--	o	X	--	--	--	--	--		41.7
S	*Synuchus nivalis*	--	--		X							45.1
G	*Calosoma sycophanta*		--	--		X				--		21.3
S	*Percus bilineatus*		--	o		X						14.9
G	*Pseudomasoreus canigoulensis*	--	o	--		X				--		21.1
md	*Masoreus wetterhalli*		--			o	--			--	X	18.1
md	*Harpalus rubripes*		--			X	--					23.7
md	*Laemostenus venustus*		o	o						X		7.6
md	*Trechus obtusus lucanus*	o	--		--				X			40.5

Discussion

Tables 1-4 are only a first attempt to analyse habitat preferences and chorology of the ground beetle assemblages of a Mediterranean region. This kind of information may be of increasing interest if faunas of different continents are compared for evaluating anthropogenic changes (cfr. Kotze & Samways, 1999; Niemelä *et al*., 2000).

Very little is known about the relationships between habitat choice and faunal history in carabids of the Mediterranean landscape. Tables 2, 3 and 4 give a first idea, based on quantitative samples concerning 159 carabid species, of the mode they are distributed in habitat types and the chorotypes involved. On the whole, it is clear that there are more open land species of pastures, cropland and unforested sites than forest species. Another important “ecological group” of ground beetles may exist in riparian habitats (66 species in Azf).

Concerning forest assemblages, Tables 2 and 3 show lower species numbers, but a higher percentage of endemic elements (cat. I, II). In other words, forest carabids of the Mediterranean area are less diverse but probably older in origin. This result fits well with what we know about the history of Mediterranean ecosystem in other areas occupied by this biome (Axelrod, 1973). The “new biome” of the sclerophylls originated probably not before late Pliocene and Quaternary by impoverishment of the tertiary evergreen warm-temperate forest of the Mediterranean Basin. As a consequence of the changes related to the strong climate transition from the first Neogene (subtropical temperatures and summer rains) to the present Mediterranean conditions (strong summer drought and rainy winters), a part of the old forest endemics disappeared or migrated southwards. The most hygrophilic ones (e.g.: *Calathus montivagus*, *Leistus sardous*, *Pterostichus micans*) simply shifted into the “new” mountain forests, the *Fagus-Quercus* belt.

Table 4. Habitat preferences and maximum indicator values (IndVal max) for open land species. X, central; o, nuclear; --, orbital. S, specialist; G, generalist. S*, near brooks. md, more data needed. For definition of habitat abbreviations, see "Study area".

		Habitats										IndVal
	Species	F	Q	Mx	Pi	Sc	Pa	Fi	Fia	Azf	K	max
S	*Microlestes fissuralis*						X					16.7
S	*Syntomus obscuroguttatus*						X	--		--		26.7
S	*Lebia crux-minor*						X					16.7
S	*Lebia marginata*						X					16.7
S	*Cymindis variolosa cyanoptera*				--		X		--			27.7
S	*Ophonus pumilio*						X			--		15.2
S	*Ophonus rotundatus*						X					33.3
S	*Harpalus impressipennis*			--			X					29.8
S	*Harpalus smaragdinus*						X					16.7
S	*Ophonus parallelus*						X					16.7
S	*Syntomus silensis*						X				o	28.1
S	*Cymindis axillaris*						X			o	--	30.4
S	*Notiophilus pusillus*						X				o	27.8
S	*Amara sicula*						X		o		--	19.8
G	*Calathus fuscipes*	--	--	--	o	--	X	--	--	o	o	30.0
S	*Ophonus incisus*						X			o		11.3
S	*Notiophilus geminatus*						X	o		--		17.1
S	*Parophonus suturalis*							X				33.3
S	*Parophonus maculicornis*							X				33.3
S	*Bembidion gudenzii*							X				33.3
S	*Harpalus distinguendus*							X	--	--		64.1
S	*Harpalus oblitus*							X				33.3
S	*Bembidion latinum*							X				33.3
S	*Bembidion lampros*		--	--				X		--		65.5
S	*Ocys quinquestriatus*							X				33.3
S	*Microlestes fulvibasis*							X				33.3
S	*Pseudophonus griseus*							X				33.3
S	*Amblystomus levantinus*							X				33.3
S	*Microlestes corticalis*			--				X				60.6
S	*Microlestes abeillei*						o	X				18.5
G	*Pseudophonus rufipes*			--		--	--	X	--	--		32.1
S	*Bembidion ambiguum*							X				33.3
S	*Tachys bisulcatus*							X				33.3
S	*Amblystomus mauritanicus*							X				33.3
S	*Acinopus megacephalus*							X				33.3
S	*Dinodes decipiens*						o	X	--			18.4
S	*Brachinus immaculicornis*						--	X				33.3
S	*Chlaenius chrysocephalus*							X				33.3
S	*Apotomus rufus*							X				33.3
S	*Bembidion lunulatum*							X	--			32.3

		Habitats										IndVal
	Species	F	Q	Mx	Pi	Sc	Pa	Fi	Fia	Azf	K	max
S	*Graniger cordicollis*							X				33.3
S	*Brachinus psophia*							X				33.3
S	*Asaphidion curtum*							X		--		32.9
G	*Nebria brevicollis*	--	--	o		--		X	o	--		11.1
S	*Brachinus sclopeta*							X	--	--		29.5
S	*Brachinus italicus*					--	o	X	o	--		25.5
S	*Carterus dama*						--	X	--			49.4
S	*Metapedius pantanellii*						--	X	o			28.7
G	*Trechus quadristriatus*	--	--	--		--	--	X		o	--	63.4
S	*Poecilus cupreus*							X	o			22.2
S	*Bradycellus verbasci*							X	o			19.0
G	*Carabus coriaceus mediterraneus*		--	--		--	--	X	o	--		31.5
S	*Microlestes luctuosus*		--			--	o	X		o		21.4
S	*Carterus rotundicollis*						o	X		--		22.2
S	*Anisodactylus binotatus*							X		o		25.5
S	*Agonum dorsale*			--				X	o			83.1
md	*Leistus fulvibarbis*	--	--				--	X	o	--	X	31.6
G	*Pterostichus (Melanius) nigrita*	--							X			42.5
S	*Scybalicus oblongiusculus*							--	X			71.7
S	*Ophonus azureus*			--			--		X			69.9
S	*Siagona europaea*						--	o	X			42.5
G	*Harpalus attenuatus*		--	--		--	--	--	X	--		85.1
S	*Parophonus hispanus*						o	o	X			15.3
S	*Ophonus subquadratus*						--	--	X			61.5
S	*Ophonus sabulicola*						--	--	X			85.7
S	*Bembidion tethys*							o	X	--		35.9
S	*Cicindela campestris*						--		X	--		89.0
S	*Carterus calydonius*						--	o	X			24.2
G	*Brachinus crepitans*		--	--		--	o	o	X			51.9
S	*Ophonus cribricollis*								X			25.0
S	*Ophonus ardosiacus*								X			100.0
S	*Ditomus clypeatus*								X			25.0
S	*Ophonus franzinii*								X			25.0
S	*Ophonus puncticeps*			--					X	--		93.5
S	*Acinopus picipes*								X			25.0
S	*Amara Montana*								X	o		28.0
S	*Brachinus peregrinus*		--			o	--	--	X			45.6
S	*Callistus lunatus*								X			25.0
S	*Diachromus germanus*				--				X			22.7
S	*Harpalus dimidiatus*								X			100.0
S	*Agonum sordidum*								X			25.0
S	*Gynandromorphus etruscus*								X			50.0
S	*Ditomus obscurus*								X			25.0

		Habitats										IndVal
	Species	F	Q	Mx	Pi	Sc	Pa	Fi	Fia	Azf	K	max
S	*Olisthopus glabricollis*						--	--	X	--		60.7
S	*Olisthopus fuscatus*						--	o	X	o		39.1
S	*Licinus silphoides*						--	--	o	X		39.8
S	*Acinopus baudii*			--				o		X		14.8
S	*Amara aenea*		--				--	o	o	X		33.2
S	*Nebria psammodes*							--		X		36.0
S	*Harpalus sulphuripes*		--	--		--	o	o	--	X		12.9
G	*Calathus cinctus*	--	--	--	--	--	o	o	--	X	--	18.8
S	*Zabrus tenebrioides*									X		25.0
S	*Syntomus impressus*									X		12.5
S	*Chlaenius vestitus*									X		12.5
S	*Bradycellus harpalinus*									X		12.5
S	*Asaphidion rossii*	--								X		30.5
S	*Apristus subaeneus*									X		12.5
S	*Amara fusca*									X		62.5
S	*Bembidion inustum*									X		12.5
S	*Bembidion eques*									X		12.5
S	*Asaphidion stierlini*	--								X	o	38.5
S	*Bembidion gr. andreae*									X		12.5
S	*Amara eurynota*		o							X		7.2
S	*Harpalus anxius*									X		12.5
S	*Ocys harpaloides*		--							X		12.3
S	*Microderes scaritides*									X		25.0
S	*Philorhizus crucifer*										X	66.7
S	*Amara ovata*			--							X	60.6
S	*Badister bipustulatus*	--									X	23.8
S	*Philorhizus melanocephalus*										X	33.3
G	*Ophonus jeanneli*	o	--	o							X	17.1
S	*Amara lucida*								--		X	53.3
S	*Harpalus atratus*									--	X	27.9
S	*Cymindis scapularis etrusca*	--					o				X	49.2
md	*Bembidion lucifugum*									--	X	32.1
S	*Leistus spinibarbis fiorii*	--	--	--	--		--				X	80.4
S	*Laemostenus acutangulus*	--	--				--				X	96.9

In this way also the higher species numbers of montane forests could be explained: sclerophylls seem to be a little too dry for most carabid beetles (except for *Percus* and *Pseudomasoreus*). The species assemblages of the upper and foggy forest belts were enriched during cold Pleistocene phases by a second group of species that migrated into the Mediterranean mountains: *Pterostichus unctulatus*, *Calathus piceus*, *Notiophilus biguttatus*. Similar patterns of altitude-related species diversity increase in Mediterranean forests were observed in Sicily (Brandmayr & Pizzolotto, 1990) and in the Karst around Trieste (Brandmayr *et al*., 1983).

In pastures, croplands and abandoned fields the Mediterranean component (IIIm) is particularly rich in species. This means that the Mediterranean open land developed an “own brand”, scarcely overlapping with Central European open land elements, that are mostly Euro-Asian or of steppic origin.

In karstic habitats the Italian species are almost equally or even more abundant than in forests. Calcareous soil cracks and crevices are well known refugia of hypogaean or cave adapted carabids (*Laemostenus acutangulus*, *Bembidion lucifugum*) with low dispersal power.

The chorological features of the Calabrian carabids reflect the young geological age of the region, as the separation from the mainland hampered the colonization by species from Archeo-Italy. It is likely that a more ancient connection with the mainland would have allowed an easier colonization of Calabria where species would have had the (evolutionary) time to generate more endemics (cfr. La Greca, 2002).

As noted by other authors (Casale, 1988; Vigna, 1998), species belonging to the Mediterranean chorotype originate from the phyletic lines dispersed around the Mediterranean basin during Messinian period (e.g. *Percus* genus), or from refugial warm areas during glaciations. Palaearctic (V) and Euro-Asian (IV) species (9.5%, 34 species; 24.6%, 88 species, respectively) originated from Asian or South-East European Pleistocenic refugial areas.

The use of the IndVal index for habitat analysis seems particularly useful for ordering habitat-tied species groups in ecologically poorly known countries or areas, provided that a sufficiently broad spectrum of environments has been sampled. The habitat list presented for Calabria needs completion, e.g. high altitude grass mats and some types of wetlands are severely undersampled or entirely missing. The combined habitat/chorotype approach seems more promising than classic zoogeographical studies if anthropogenic ecosystem changes have to be interpreted.

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Long-term monitoring of ground beetles (Coleoptera, Carabidae) in a Hungarian wetland area

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Abstract

A 10- year study of ground beetles was carried out in the protected area of Kis-Balaton, Western Hungary. Pitfall-traps (15) were placed along a transect and were operating continuously. The traps caught 10,332 individuals, belonging to 127 species. Both the number of species and cumulative number of species increased continuously with no sign of saturation over the 10-year period. The dominant species showed large changes in numbers from year to year. We suggest that fluctuations in abundance, and the year to year changes in the dominant species are caused mainly by the unstable wetland habitat, and only partially by species biology.

Key words: Carabids, wetland, Hungary

Introduction

The study area was formerly the western bay of the Lake Balaton, Hungary. The area was drained in the early 1900s, which led to the eutrophication of the lake and a deterioration of the water quality. In the 1980es, restoration was attempted by using a wetland to clear the most important water supplier of the lake, the River Zala. In 1992, the water level was raised on 16 km^2 of the so-called Kis-Balaton, a protected RAMSAR area. At the instigation of the Hungarian Ministry of the Environment, a monitoring system was established to observe the biological changes during and after the project. Part of this monitoring included ground beetles (Carabidae). This research started in 1993, and aimed to examine the effect of the artificially raised water level on ground living arthropods, particularly ground beetles.

Previous faunistical studies (Kondorosy *et al.,* 1996) found 87 carabid species in the protected area.

Materials and methods

The sampling area was on a natural land bridge (WGS 84 coordinates: 46°41’20” N, 17°16’37” E) in the northern part of the Kis-Balaton area by the Lake Balaton in Western Hungary. This area is protected, and is a wetland of international importance, belonging to the so-called "RAMSAR" network. The pitfall traps were placed along a transect beginning at the edge of the water and continuing 50 m inland. Three vegetation units could be distinguished along the transect: 1) close to the water there was an association of reeds (*Phragmites australis* and sedges *Carex riparia*, *C. acutiformis*); 2) on higher ground, a homogeneous strip of *Solidago gigantea*, giving way to a 3) a *Solidago gigantea* and *Calamagrostis epigeios* association. *S. gigantea,* an invasive weed, causes severe problems in Hungary, since its monoculture spreads aggressively and it almost fully supplants other herbs. The presence of *C. epigeios* indicates disturbance in an area (Mihály & Botta-Dukát, 2004). Fifteen pitfall traps were placed at a distance of 5 m from each other. They were emptied weekly throughout the year, apart from the snowy period. Thus, the pitfall traps were operating continuously for ten years.

To ease the operation of the pitfall traps, an iron pipe (84 mm diameter, length 140 mm) was driven into the ground with its upper rim at 5 mm below the ground level. The reclining rim of the 200 ml plastic cup (88 mm outer and 82 mm inner diameter), serving as the removable part of the trap, rested on the pipe. With the help of three bent aluminium hooks, glass plates were placed over the cups to protect the catch from rain and scavenging. A modified version of the Barber-solution (Barber, 1931) was used as killing and preserving agent, the composition of which was: 1 part alcohol, 5 parts distilled water, ¼ part acetic acid and 1/3 part glycerine. The collected samples were stored in 70% alcohol until processing, when the samples were separated under a microscope and the carabids were identified, using standard classification keys by Freude (1976), Hurka (1996), and Csiki (1905). Distributional data of carabid beetles in Hungary were taken from Kádár & Szél (1989), Kondorosy *et al*. (1996), Kutasi & Szél (2000), Szél (1996). The nomenclature followed Hurka (1996).

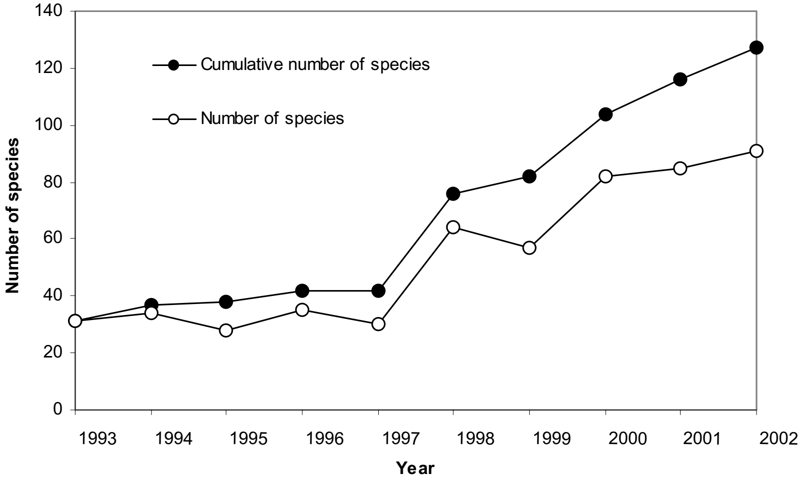

Figure 1. The number of carabid species and the cumulative number of carabid species collected by pitfall traps in the Kis-Balaton, Hungary, between 1993-2002.

Species that were responsible for at least 10% of the samples in at least one year were classified dominant species. The annual cumulative number of species was calculated.

Results

Between 1993-2002, a total of 10,332 individuals were captured, belonging to 127 species (Table 1). This is 25% of the Hungarian carabid fauna, which numbers about 500 species (Horvatovich, 1993). Numerous rare, or very rare carabid species were captured. These include *Trechus austriacus, T. obtusus, Benbidion doris, B. gilvipes, Pterostichus rhaeticus, Amara cursitans, A. municipalis, A. lunicollis,* and *Trichocellus placidus*.

Fig. 1 shows the changes in the number of species each year and the trend in cumulative number of species. Both values increased continuously over the years. The increase was steeper in the second than in the first five-year period (Fig. 1). The number of species caught per year ranged from 28 (1995) to 91 (2002). Fourteen of the 127 species were caught every year, 11 of which were hygrophilous (Table 1).

The total number of individuals collected varied from 416 (1995) to 2009 (2002) with no obvious trend over time.

Table 1. Total yearly catches of ground beetles at the Kis-Balaton Nature Reserve, Western Hungary, in the period 1993-2002. Species sequence follows Hurka (1996). Species captured in every year are in bold.

Species	Year										Total
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	
Leistus ferrugineus (L., 1758)	4		2	6	4	9		26	33	6	90
Nebria brevicollis (Fabricius, 1792)										2	2
Notiophilus palustris (Duftschmid, 1812)						3		14	6	11	34
Carabus cancellatus sopronensis Dejean, 1826	86	50	41	105	150	9	7	4	4	10	466
Carabus clatratus auraniensis Müller, 1902	52	67	62	55	100	21	9	3	14	16	399
Carabus coriaceus coriaceus L., 1758						1				1	2
Carabus ullrichi sokolari Born, 1904								2			2
Carabus granulatus granulatus L., 1758	102	68	91	157	90	129	43	41	172	99	992
Cicindela germanica L., 1758						1		2			3
Cicindela campestris L., 1758							1				1
Elaphrus cupreus Duftschmid, 1812										2	2
Elaphrus uliginosus Fabricius, 1792		1						2	8	10	21
Loricera pilicornis Latreille, 1802							1			1	2
Clivina collaris (Herbst, 1784)									1		1
Clivina fossor (L., 1758)	5	3	1	3	5	4	1	4	5	2	33
Dyschirius aeneus (Dejean, 1825)								2			2
Dyschirius globosus (Herbst, 1784)						26	38	53	91	60	268
Brachinus crepitans (L., 1758)										3	3
Brachinus explodens Duftschmid, 1812							1			2	3
Brachinus ganglbaueri advena Schauberger, 1921										1	1
Epaphius secalis (Paykull, 1790)								5	11	6	22
Trechus austriacus Dejean, 1831									1	3	4
Trechus obtusus Erichson, 1837									1	9	10
Trechus quadristriatus (Schrank, 1781)				29	15	33	58	199	166	56	556
Paratachys bistriatus (Duftschmid, 1812)								1	1		2
Tachita nana (Gyllenhal, 1810)								1			1
Asaphidion flavipes (L., 1761)						1	1	3	4	2	11
Bembidion articulatum (Panzer, 1796)								1			1
Bembidion assimile Gyllenhal,1810	6	2				4	1	11	12	3	39
Bembidion biguttatum (Fabricius, 1779)										3	3
Bembidion fumigatum (Duftschmid, 1812)						1					1
Bembidion inoptatum Schaum, 1857									2		2
Bembidion guttula (Fabricius, 1792)							2	6	20	11	39
Bembidion mannerheimi (Sahlberg,1827)						4	11	20	59	152	246
Bembidion doris (Panzer, 1797)								6	1		7
Bembidion gilvipes Sturm, 1825									1		1
Bembidion lampros (Herbst, 1784)								1			1
Bembidion properans (Stephens, 1828)						8	5	15	3	1	32
Bembidion octomaculatum (Goeze, 1777)								1			1
Bembidion quadrimaculatum (L., 1761)						1		3			4
Bembidion tenellum Erichson, 1837									1		1

Species	**Year**										**Total**
	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	
Stomis pumicatus (Panzer, 1796)						4	6	6	4	24	44
Poecilus cupreus (L., 1758)	77	59	35	93	56	2	20	8	4	11	365
Poecilus versicolor (Sturm, 1824)	8	6		2		17	1		4	12	50
Pterostichus anthracinus (Illiger, 1798)	36	17	5	9	43	35	6	7	10	15	183
Pterostichus aterrimus (Herbst, 1784)	27	16	9	22	7	3	2	26	11	4	127
Pterostichus cursor (Dejean, 1828)	2	1		1					2	2	8
Pterostichus diligens (Sturm, 1824)	8	22	5	28	2	12	8	11	40	21	157
Pterostichus elongatus (Duftschmid, 1812)									1	1	2
Pterostichus gracilis (Dejean, 1828)								1	1		2
Pterostichus melanarius (Illiger, 1798)	83	68	42	84	76	74	2	17	11	70	527
Pterostichus minor (Gyllenhal, 1827)			2	3	6	3		17	27	42	100
Pterostichus niger (Schaller, 1783)	5	38		6		72	22	24	57	33	257
Pterostichus nigrita (Fabricius, 1792)		2	3	19	4	4		10	19	44	105
Pterostichus oblogopunctatus (Fabricius,1787)				1	5						6
Pterostichus rhaeticus Heer, 1837								1			1
Pterostichus strenuus (Panzer, 1797)						25	28	39	25	41	158
Pterostichus vernalis (Panzer, 1796)	51	35	12	37	36	8	7	26	104	151	467
Calathus erratus (Sahlberg, 1827)	1					3					4
Calathus fuscipes (Goeze, 1777)		16	5	11	10	5		2	1	5	55
Calathus melanocephalus (L., 1758)	42	22	9	18	23	14	27	13	23	5	196
Synuchus vivalis (Illiger, 1798)						1	20	28	13	2	64
Oxypselaphus obscurus (HERBST, 1784)							1		2	17	20
Platynus assimilis (Paykull, 1790)	2		1	6	1	1					11
Platynus krynickii (Sperk, 1835)						3	13	7	28	122	173
Agonum lugens (Duftschmid, 1812)	17	9	10	7	31	8		33	9	4	128
Agonum atratum (Duftschmid, 1812)										1	1
Agonum moestum (Duftschmid, 1812)*	*37*	*48*	*7*	*9*	*280*	*75*	*19*	*97*	*176*	*352*	*1100*
Agonum duftschmidi Schmidt, 1994								41	43	47	131
Agonum afrum (Duftschmid, 1812)								17	24	17	58
Agonum permoestum Puel, 1931								39	109	288	436
Agonum sexpunctatum (L., 1758)	1			1	4		2				8
Agonum viduum (Panzer, 1797)		2			5						7
Europhilus fuliginosus (Panzer, 1809)						1	3				4
Europhilus thoreyi (Dejean, 1828)						1		1	8	2	12
Amara aenea (De Geer, 1774)						1	19	8	14	10	52
Amara anthobia A. et G.B. Villa,1833	4	1	1	2	3				3	1	15
Amara bifrons (Gyllenhal, 1810)	3	6	3	3	2	1	3	6		2	29
Amara communis (Panzer, 1797)	62	35	23	56	34	60	53	117	174	45	659
Amara convexior Stephens, 1828						9	7	22	44	23	105
Amara cursitans (Zimmermann, 1831)						1	1	1		1	4
Amara familiaris (Duftschmid, 1812)									10	18	28
Amara fulva (O.F. Müller, 1776)										1	1
Amara lucida (Duftschmid, 1812)										1	1
Amara lunicollis Schiodte, 1837							1				1
Amara municipalis (Duftschmid, 1812)									1	1	2
Amara similata (Gyllenhal, 1810)								2	3	5	10

Species	**Year**										**Total**
	1993	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	
Amara tibialis (Paykull, 1798)						2				1	3
Amara tricuspidata Dejean, 1831								1	1	1	3
Panagaeus cruxmajor (L., 1758)								1			1
Chlaenius tristis (Schaller, 1783)	1	2	1	2				3	4	1	14
Chlaenius nigricornis (Fabricius, 1787)				5			1	3	1	2	12
Chlaenius nitidulus (Schrank, 1781)		1						1	1		3
Oodes gracilis A. et G.B. Villa, 1833	2	3						2	5	1	13
Oodes helopioides (Fabricius, 1792)	71	24	33	45	88	44	25	90	80	189	689
Licinus depressus (Paykull, 1790)	1	1	1			2					5
Badister bullatus (Schrank, 1798)						3	8	3			14
Badister latercosus Sturm, 1815						2		8	3		13
Badister unipustulatus Bonelli, 1813									1		1
Badister meridionalis Puel, 1925	1	4	1	7	1	1	1	4	2		22
Badister dilatatus Chaudoir, 1837								2	4	2	8
Badister peltatus (Panzer, 1797)						4	1		6	3	14
Badister sodalis (Duftschmid, 1812)						2	1	5	5	3	16
Anisodactylus binotatus (Fabricius, 1787)	4	2	2	4		3	6		2	1	24
Anisodactylus signatus (Panzer, 1797)								2		4	6
Stenolophus mixtus (Herbst, 1784)	9	12	6	19	14		2	12	42	57	173
Stenolophus skrimshiranus Stephens,1828						2					2
Trichocellus placidus (Gyllenhal, 1827)								1	2	3	6
Bradycellus collaris (Paykull, 1798)						8	7	14	7	10	46
Bradycellus csikii Laczó, 1912						16	16	81	39	43	195
Acupalpus flavicollis (Sturm, 1825)						1		3	6	1	11
Acupalpus parvulus (Sturm, 1825)										1	1
Parophonus maculicornis (Duftschmid, 1812)						4	2	1	2	1	10
Ophonus diffinis (Dejean, 1829)										1	1
Ophonus puncticeps Stephens, 1828									1	1	2
Pseudophonus griseus (Panzer, 1797)		2					1	8	14	2	27
Pseudophonus rufipes (De Geer, 1774)	16	9	3	9	12	11	7	11	40	42	160
Harpalus distinguendus (Duftschmid, 1812)								1		1	2
Harpalus latus (L., 1758)				4	3	1	4	2	23	18	55
Harpalus luteicornis (Duftschmid, 1812)						2	9	12	6	4	33
Harpalus rubripes (Duftschmid, 1812)							3	2	6	24	35
Harpalus subcylindricus Dejean, 1829									1	1	2
Harpalus tardus (Panzer, 1797)						6	7	2	5	3	23
Drypta dentata (Rossi, 1790)								1			1
Lebia chlorocephala (Hoffmann, 1803)										1	1
Syntomus pallipes (Dejean, 1825)						10	2		6		18
Syntomus truncatellus (L., 1761)						9	9	22	26	26	92
Microlestes maurus (Sturm, 1827)						4	1	2	8		15
Yearly total	**826**	**654**	**416**	**868**	**1110**	**835**	**563**	**1256**	**1795**	**2009**	10332

* *Agonum duftschmidi, A. afrum, A. permoestum* together

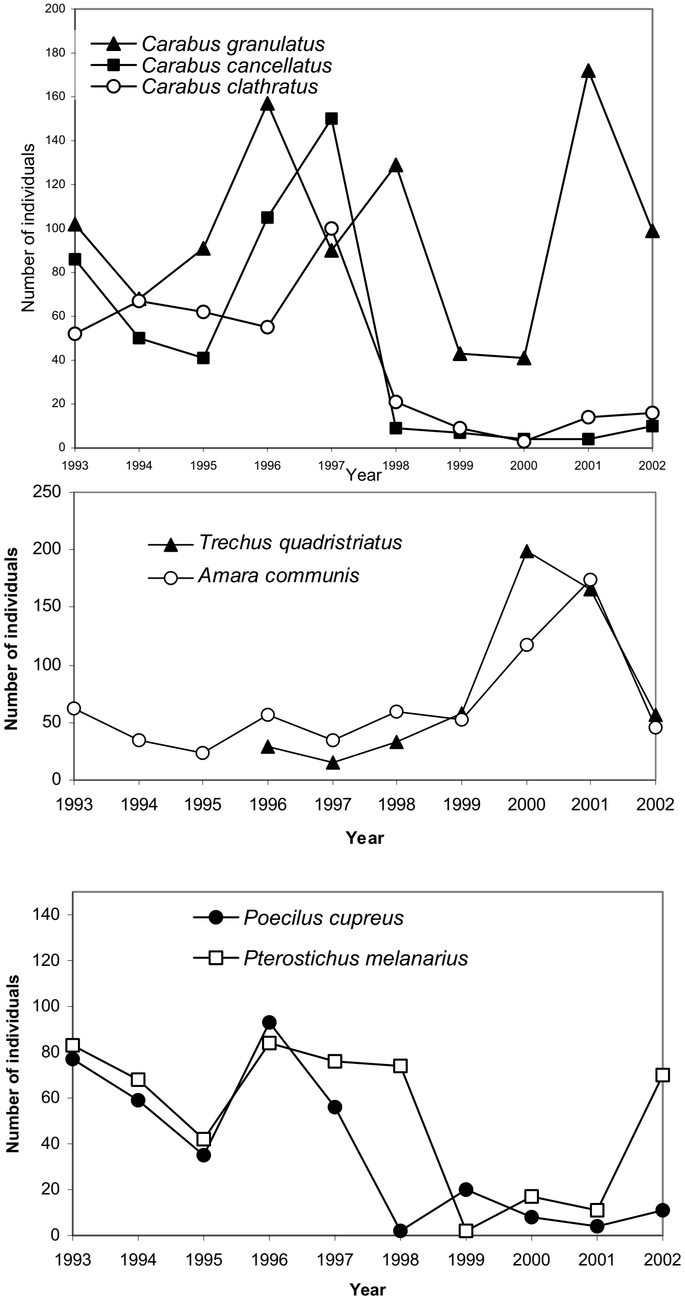

Figure 2A. Year-to-year fluctuations in the yearly total number of individuals captured in the Kis-Balaton, Hungary, between 1993-2002. a) Species common over most of the years: *Carabus granulatus, C. cancellatus, C. clatratus*; b) species common in a short period only: *Trechus quadristriatus, Amara communis;* c) initially common species: *P. cupreus, P. melanarius.*

Between 1993-2002, seven species had relative abundance > 10% in at least one year: these species were considered dominants in the habitat. However, their cumulative relative abundance was < 10%. This list included: *C. granulatus* 8.0%, *Amara communis* 5.4%, *Trechus quadristriatus* 5.4%, *C. cancellatus* 3.2%, *Pterostichus melanarius* 3.2%, *C. clatratus* 2.7% and *Poecilus cupreus* 2.2%. The changes in these populations during the 10 years are shown in Fig. 2. The populations of *C. clatratus* and *C. cancellatus* fluctuated between relatively high values during the first five years, after which (in 1997-1998) their populations drastically fell and stabilised around low values (Fig. 2A). The third species, *C. granulatus*, had relatively high population values. The changes in the population numbers of *A. communis* and *T. quadristriatus* (Fig. 2B) were mostly at low abundances over the whole period, except in 2000 and 2001, when they produced a peak. By 2002, they were back to the former low values. *P. cupreus* and *P. melanarius* (Fig. 2C), fluctuated around high values in the first five years after flooding, and became rare in the second five years.

Conclusions

Common sense would suggest that, with the advance of time, fewer and fewer new species are discovered. Such saturation curves are produced in many species inventories (Magurran, 2003). A small number of new species appears each year because the probability of collecting rare species is small. We witnessed the appearance of new species from year to year and the pace of appearance did not indicate saturation. The results possibly indicated an unstable habitat for carabids, probably due to the year to year changes in the water level. In the case of common species, the population numbers could fall to a fraction of the former value or show a large increase within a few years. In the case of low-population-species the population size can easily slip under the detection threshold and the species would not be detected for years. In the ten years cycle, 28 species (35%) were trapped in just one year. These species could be tourists, or can survive at such low levels that their detection was not possible in most years. Only 14 species (11%) were caught in all years.

Similar investigations were carried out in Germany in an ancient woodland (Gunther & Assmann, 2004). In this research the trapping period was nine years, and the authors found that the catching rates for some species (e.g. *Carabus problematicus* and *Abax parallelepipedus*) fluctuated only slightly, whereas those of other species (e.g. *C. violaceus* and *C. auronitens*) varied as much as ten-fold. Gunther & Assmann (2004) concluded that the amplitude of fluctuations in abundance was a feature of each species rather than a special attribute of their habitats.

In our investigations we found different dominant species and a rising species number each year. Some of the initially dominant species populations eventually stabilised around low values (*C. clatratus*, *C. cancellatus*). We suggest that fluctuations in abundance, and the year-to-year changes in the dominant species were caused mainly by the unstable wetland habitat, and only partly by the species biology, a situation opposite to that in ancient woodlands (Gunther & Assmann, 2004).

Acknowledgements

We wish to express our thanks to Dr. Győző Szél, Imre Retezár and Csaba Kutasi, who helped us with identification.

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Development of the ground-beetle parasitoids, *Brachinus explodens* and *B. crepitans* (Coleoptera: Carabidae): effect of temperature

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Abstract

Establishing the thermal requirements of insects is useful for understanding when they will be present during a season and predicting the period of their maximum abundance. Here the thermal requirements for the development of all stages and certain phases within larval instars of *Brachinus explodens* Duftschmid and *B. crepitans* (Linnaeus) were established at three constant temperatures between 17.7 – 27.4°C. The lower development threshold (LDT) for eggs is 9.4°C for *B. explodens* and 7.2°C for *B. crepitans*, respectively; the sums of effective temperatures (SET) are 154.4 and 180.7 day degrees, respectively. LDT for the total postembryonic development (except the searching phase) is 12.3°C in *B. explodens* and 10.5°C in *B. crepitans*, respectively, and SET are 209.2 and 289.5 day degrees, respectively. Thermal constants for the searching phase of the first instar larva were not calculated because its duration is independent of temperature.

Key words: *Amara*, egg, larva, pupa, thermal constants

Introduction

Precise synchronisation of egg hatching in a parasitoid with the presence of a suitable stage of its host is a prerequisite for the survival of parasitoids with actively searching larvae. There are several ways of synchronising species in time. In the absence of dormancy, this is achieved by the host and parasitoid having similar temperature requirements for development. A useful way of establishing such a relationship is to determine the thermal constants of development, lower development threshold (LDT) and sum of effective temperatures (SET). A linear relationship between development rate and temperature is assumed in order to calculate LDT and SET. Even when there is a positive departure from linearity in the rate/temperature relationship at low temperatures (Charnov & Gillooly, 2003), thermal constants are useful for predicting the duration of development (Jarošík *et al*., 2002). Thermal constants enable the prediction of the duration of development under ecologically relevant conditions. Although thermal constants for a large number of insects are known (reviewed by Honek & Kocourek, 1990; Honek, 1996; Kiritani, 1997) data for Carabidae are scarce (see Saska & Honek, 2003 for review).

In this study the thermal requirements for two ground-beetle ectoparasitoid species, *Brachinus explodens* Duftschmid and *B. crepitans* (Linnaeus) were established. Their ectoparasitoid mode of life was first predicted by Jeannel (1942) based on the fact that North-American species of *Brachinus* are parasitoids of pupae of water beetles (Wickham, 1893). However, the hosts of European species remained unknown until recently, when Saska & Honěk (2004) demonstrated that they develop on the pupae of the carabid genus *Amara* and described their life cycle. There are three larval instars, as in most carabid species, but the first and third instars have two distinct developmental phases, distinguished by behaviour (Saska & Honěk, 2004). Newly hatched first instar larvae immediately search for a host (the first phase). On finding a host pupa a larva crawls over it. When the pupa is young and undamaged the larva starts feeding (the second phase), mostly on the host's antennae or legs. Larvae usually moult to the second instar without moving away from the host pupa. The second instar larva begins to feed immediately. The mode of feeding is as in the first instar: the larva punctures the host cuticle at several places (mostly at intersegmental membranes) and sucks up the exuding haemolymph, and is attached to the host by its ventral surface. Larvae also moult to the third instar on its host. After moulting, the larva adopts a new feeding position: it now attaches itself to the host by its dorsal surface, with the head and thorax bent backwards through 180°. In contrast to the first and second instars, larvae of the third instar chew the tissues of the hosts using their mandibles. Only a few fragments of host cuticle remain on the dorsum of a larva when feeding is finished. Larvae that have consumed a pupa remain and pupate close by. Total larval development lasts 8-12 days at 25°C.

Material and methods

Rearing

The method of rearing *Amara* species is described in Saska & Honek (2003). Adult *Amara aenea* (DeGeer) and *A. similata* (Gyllenhal) were collected in pitfall traps at Praha – Ruzyně (50^o 06' N, 14^o 15' E) in early May 2002 and 2003. They were kept in pairs in plastic Petri dishes (10 cm in diameter, 2 cm high) filled to a depth of 1 cm with a layer of sieved garden soil at a temperature of 18±0.5°C and under a long day (17L:7D) photoperiod. The beetles were fed a mixed diet of pieces of *Tenebrio molitor* (Linnaeus) larvae and seeds of *Capsella bursa-pastoris* (Linnaeus) Medicus twice a week. Their larvae were kept individually in glass Petri dishes (6 cm in diameter, 1.5 cm high, with a 1 cm layer of sieved soil), in the same conditions as the adults. Twice a week the larvae were fed seeds of *C. bursa-pastoris*. Newly pupated individuals (0-48 h old) were used as hosts for the *Brachinus* larvae.

The adults of both *Brachinus* species were collected (May 20 - June 20, 2002 and 2003) and kept under the same conditions in the laboratory as the *Amara* adults, but in groups of 10 per Petri dish. Pieces of *T. molitor* larvae were provided once a week as food. Petri dishes were inspected twice a week until the start of oviposition, and thereafter daily.

For rearing eggs and larvae small glass Petri dishes (5 cm diameter, 1 cm high), filled to a depth of 0.4 cm with plaster of Paris mixed with charcoal, were used. The layer of plaster of Paris was moistened with several drops of tap water twice a week, to keep an optimal humidity.

In 2002, eggs found on the soil surface in the containers in which the parental beetles were reared, were placed singly in Petri dishes. As all of these eggs died (probably because of the damage caused by handling), they were left in the soil until they hatched. The larvae were then removed from the soil within 0-24 h by means of a fine brush. One first instar larva was placed in each Petri dish and provided with a host pupa: *A. aenea* for *B. explodens* and *A. similata* for *B. crepitans* (Saska & Honek, 2004). In 2003, egg-laying females were placed individually in glass Petri dishes (8 cm diameter, 1 cm high) with a layer of plaster of Paris mixed with charcoal in order to determine the egg development. They were removed and placed in a new dish twice a day, and the eggs they laid on the plaster surface during this period were used for temperature experiments.

Thermal constants

The Petri dishes with eggs or the first instar larvae of *Brachinus* and host pupae were placed in light and temperature controlled cabinets kept at temperatures of 18 (2002), 20 (2003), 25 or 28°C (both years), oscillating ± 0.5°C around the mean, and a long-day photoperiod (17L:7D). During the experiments the existing temperature inside the cabinets was recorded at hourly intervals by dataloggers Tinytalk®. All dishes were inspected twice a day (at 08:00 and 20:00) until the larvae died or matured. At each inspection their stage of development was recorded and whether the larvae had moulted.

In calculating the thermal constants a linear relationship between temperature and development rate over the range of the experimental temperatures was assumed. The thermal constants were calculated using development rate (R), calculated as the reciprocal of the mean duration of development of a particular stage (R=1/D) for each temperature (T). Mean values of the temperatures recorded during the experiment were used for calculations: 17.8, 24.7 and 27.4°C in 2002 (larvae and pupae) and 20.0, 24.2 and 27.1°C in 2003 (eggs). For each stage a regression R=aT+b was calculated. From this, the lower development threshold LDT (the temperature below which development ceases) was calculated as LDT [°C]=-b/a, and the sum of effective temperatures SET (number of heat units, called day degrees) as SET [dd]=1/a. Standard errors of LDT were calculated according to J. Janacek (in litt.). Thermal constants were calculated for all stages of development (egg, first, second and third larval instars and pupa) and also for development phases within a stage, distinguished by feeding and locomotory behaviour (Saska & Honek, 2004).

The values for the LDTs and SETs were compared between species and stages. Data for egg, feeding phase of the first instar, the second instar, both phases of the third instar and pupa were used. The calculations were made with commercial statistical software GLIM® 4.09 (Crawley, 1993). LDT or SET were the response variables and species or stages the factors. As only two replicates (species) were available for each development phase, the interaction term between species and stage could not be tested (insufficient degrees of freedom). The differences in the thermal constants were therefore tested by two separate one-way ANOVA's (for species with stages as replicates, and for stages with species as replicates) for both thermal constants. If significant, the differences between factor levels were tested by LSD test (Sokal & Rohlf, 1982). The significance of differences in LDTs was also compared between stages within a species and between species within a stage (or developmental phase) using t-tests (Sokal & Rohlf, 1982).

Results

Thermal constants could not be established for the searching phase of the first instar larva (and consequently for the whole first instar) because of the large variation in the duration of this developmental phase within temperatures (Tables 1, 2). Development rates of the other phases and stages of preimaginal development increased proportionally with temperature (Tables 1, 2) and were well represented by linear relationships.

Table 1. Duration (days) of the different development phases of *B. explodens,* reared on pupae of *Amara aenea* at three temperatures.

Development phase	Temperature					
	17.7°C		24.7°C		27.4°C	
	mean±s.e.	n	mean±s.e.	n	mean±s.e.	n
Egg [a]	15.6±0.8	3	9.8±0.1	2	9.0±0.7	5
Searching phase of L1	2.4±0.5	9	0.9±0.2	4	1.6±0.4	5
Feeding phase of L1	4.7±0.3	8	2.4±0.2	4	2.0±0.1	5
Total L1	7.2±1.2	8	3.3±0.4	4	3.5±0.9	5
Total L2	4.6±0.3	7	2.4±0.2	4	1.3±0.1	4
Feeding phase of L3	3.0±0.4	4	1.4±0.2	4	1.2±0.3	4
Prepupal phase of L3	8.4±0.9	3	3.5±0.1	4	3.0±0.2	4
Total L3	11.2±0.8	3	4.9±0.2	4	4.1±0.1	4
Total duration of larval feeding	12.6±1.1	3	6.2±0.2	4	4.5±0.4	4
Duration of larval stage after attachment to host	20.9±1.0	3	9.7±0.2	4	7.4±0.2	4
Pupa	18.1±1.0	3	9.4±0.3	4	6.1±0.1	4
Total postembryonic development (searching of L1 excl.)	40.0±3.0	3	18.8±1.1	4	13.5±0.4	4

Notes: L1-L3 – larval instars; [a] mean temperature during development: 20.0, 24.2 and 27.1°C.

Table 2. Duration (days) of the different development phases of *B. crepitans,* reared on pupae of *Amara similata* at three temperatures.

Development phase	Temperature					
	17.7°C		24.7°C		27.4°C	
	mean±s.e.	n	mean±s.e.	n	mean±s.e.	n
Egg [a]	14.1	1	-		9.1	1
Searching phase of L1	3.4±0.6	5	3.2±0.4	4	2.3±0.4	4
Feeding phase of L1	5.5±0.3	3	2.3±0.2	3	1.7±0.3	3
Total L1	8.4±1.7	3	5.4±1.3	3	3.9±0.9	3
Total L2	4.5±0.3	3	2.8±0.1	3	1.8±0.5	2
Feeding phase of L3	2.3±0.3	3	1.5±0.3	3	1.1±0.4	2
Prepupal phase of L3	7.3±0.2	3	4.5±0.3	3	3.7±0.3	2
Total L3	9.3±0.2	3	6.0±0.1	3	4.8±0.2	2
Total duration of larval feeding	12.3±0.5	3	6.6±0.3	3	4.5±0.4	2
Duration of larval stage after attachment to host	19.1±0.4	2	11.1±0.4	3	8.1±0.2	2
Pupa	17.9±0.3	2	9.6±0.2	3	7.8±0.2	2
Total postembryonic development (searching of L1 excl.)	36.9±0.1	2	20.6±0.5	3	17.0±1.9	2

Notes: L1-L3 – larval instars; [a] mean temperature during development: 20.0 and 27.1°C.

The values of LDT did not differ significantly between species (ANOVA: $F_{1,10}$=3.299, *p*=0.099) or between stages when species were pooled (ANOVA: $F_{5,6}$=1.119, *p*=0.440). In *B. explodens*, all LDTs were similar (t-test for egg x second instar: $t_{4,2}$=1.025, *p*=0.363) and varied between 9.4°C (egg) and 13.9°C (second instar), with a mean=11.9°C, s.e.=1.4, *n*=6 (Fig. 1A, Table 3). The variation in LDT in *B. crepitans* was greater (mean=9.5°C, s.e.=2.3, *n*=6; Fig. 1B, Table 3) because the egg and third instar (both phases and total duration) have low LDTs of 7.2-7.8°C (mean=7.4°C, s.e.=0.3, *n*=3). The LDTs for the other development phases were similar to *B. explodens*, 9.6-14.4°C (mean=11.7°C, s.e.=1.8, *n*=3) (Table 3). This resulted in significant differences LDTs for feeding phase of the first instar and feeding phase of the third instar (t-test: $t_{4,2}$=3.760, *p*=0.020), and whole third instar (t-test: $t_{4,2}$=3.160, *p*=0.034). LDTs of species were similar within all instars except the feeding phase of the third instar (t-test: $t_{4,2}$=3.428, *p*=0.027). LDTs for total postembryonic development were similar (t-test: $t_{4,2}$=1.049, *p*=0.353) in both species.

SET for particular development stages and phases (Table 3) did not differ significantly between species (ANOVA: $F_{1,10}$=0.287, *p*=0.506) but the differences between phases were significant (ANOVA: $F_{5,6}$=20.040, *p*=0.001) as expected from the variation in development times (Table 1). However, subsequent LSD tests did not find any significant difference between instars. Although the interaction term between species and stages could not be calculated (see Methods), the percentage of the variability explained by both models (2.8%

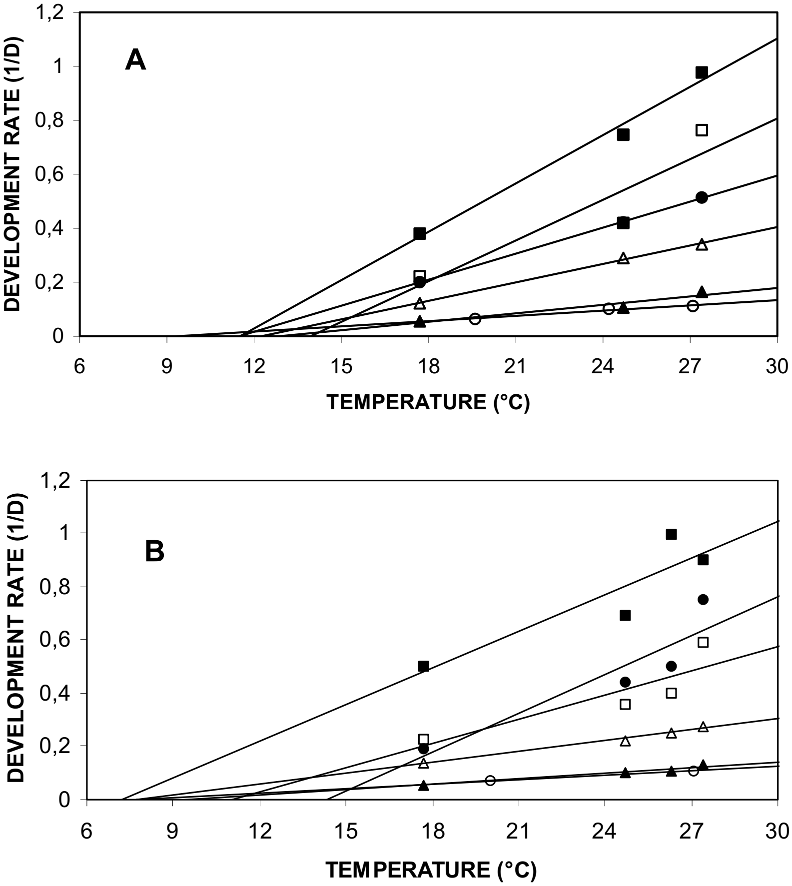

Figure 1. Linear regression of development rate on temperature for all the development phases of A - B. explodens, B - B. crepitans. ○ - egg, ● - feeding phase of L1, □ - L2, ■ - feeding phase of L3, Δ - resting phase of L3, ▲ - pupa.

for between species, and 94.4% for between stages comparison, respectively) clearly show that stage of development accounts for more of the variation than species and thus the interstage variation in SET is greater than the interspecific variation.

Table 3. Lower development threshold LDT (°C) and sum of effective temperatures SET (dd) for *B. explodens* and *B. crepitans*.

Development phase	*B. explodens* [a]		*B. crepitans* [b]	
	LDT	SET	LDT	SET
Egg	9.4	154.4	7.2	180.7
Feeding phase of L1	11.5	31.1	14.4	20.6
L2	13.9	19.9	11.0	33.1
Feeding phase of L3	11.5	16.8	7.2	21.8
Prepupal phase of L3	12.3	43.9	7.8	73.3
Total L3	11.9	63.1	7.5	96.6
Phase of larval feeding	10.1	92.5	11.9	73.1
Total larval stage after attachment to host	12.4	114.0	12.6	102.0
Pupa	12.8	95.9	9.6	147.0
Total postembryonic development (searching of L1 excl.)	12.3	209.2	10.5	289.5

Notes: L1-L3 – larval instars;

[a] reared on pupae of *Amara aenea*;

[b] reared on pupae of *Amara similata*.

Mortality varied between development stages. All eggs removed from the soil in 2002 died, probably because fresh eggs are soft and vulnerable to damage when handled. In 2003, however, many of the eggs that were laid in the dishes in which they were reared also died: 84.1% in *B. explodens* and 94.6% in *B. crepitans*. The average mortality of larvae that accepted a host was 39.0% in *B. explodens* and 46.0% in *B. crepitans*, respectively (Table 4). All pupae of *B. explodens* survived but 14.0% of those of *B. crepitans* died (Table 4).

Table 4. The effect of temperature (T) on survival of *B. explodens* and *B. crepitans*.

Species	T	N	N_{L1}	N_{L2}	N_{L3}	N_P
B. explodens [a]	17.7	9	8	7	3	3
	24.7	4	4	4	4	4
	27.4	5	5	4	4	4
B. crepitans [b]	17.7	5	3	3	2	2
	24.7	4	3	3	3	3
	27.4	4	3	2	2	1

Notes: N – number of individuals that accepted the host;

N_{L1}-N_{L3}, N_P – number of individuals that completed each developmental stage;

[a] reared on pupae of *Amara aenea*;

[b] reared on pupae of *Amara similata*.

Discussion

As expected, the duration of development in both species varied proportionally with temperature, except the searching phase of the first instar. The latter may have two causes: (i) an error in estimating the duration of this phase caused by variation in the age of the larvae at the start of the experiment (0-24 h), which was long relative to the duration of the phase; (ii) the duration of the searching phase is independent of temperature. In fact, larvae only accepted a host after a period of searching even when it was present from the beginning of the experiment. Searching is an obligatory phase in the *Brachinus* life cycle. In another experiment (Saska & Honek, unpubl.), first instar larvae starved for several days accepted a host within a few hours of it being presented. The existence of an obligatory "searching phase" is unique since other beetle parasitoids (*Aleochara* spp.) tend to minimize the time for which their larvae have to search by laying eggs close to the plant damaged by the host (Fournet *et al*., 2001).

In other stages, the development rate increased with temperature. The lower development threshold varied between stages and species but the differences were not significant except for the feeding phase of the third instar in *B. crepitans*. We would expect all stages to have a similar lower development threshold (Jarošík *et al*., 2002) and probably also all related species (Dixon *et al*., 1997). The variance in the lower development threshold was possibly caused by errors in estimating development duration. Some stages or phases of development were completed so quickly that an accurate determination of their length would require more frequent inspections than twice a day. That this explanation may clarify the differences in LDTs, is confirmed by the data for total postembryonic development, which were similar between species despite the differences in the third instar. The sum of effective temperatures varied between stages and species, but the differences were highly significant only for stages. This is expected, as duration of development for stages differ and SET values should reflect this.

It is difficult to compare the thermal constants of *Brachinus* species with those of other Carabidae because *Brachinus* has a complicated life cycle in which only the egg and pupal stages are comparable with those of other species. There are only 16 records for the thermal constants of eggs (Balachowsky, 1962; Paarmann, 1966, 1994; Luff, 1973; Sota, 1986; Jensen, 1990; Saska & Honek, 2003) and 15 for pupae of carabid species (Paarmann 1966, 1994; Luff, 1973, 1975; Hurka, 1975; Sota, 1986; Jensen, 1990; Dijk, 1994; Saska & Honek, 2003). The lower development threshold for the eggs of *Brachinus* species (9.4 and 7.2°C) are similar to that of the eggs of other temperate species, including *A. aenea* and *A. similata* (10.4 and 9.3°C) (Saska & Honek, 2003), i.e. their hosts, but the SET (and development duration) is larger (154.4 and 180.7 dd for *Brachinus* in contrast to 70.9 and 97.2 dd for *Amara*) (Saska & Honek, 2003). We expected the opposite trend (i.e. shorter egg development in the parasitoid), since higher thermal requirements (high SET), disadvantage the parasitoid. The LDTs for pupal development in *Brachinus* (9.6 and 12.8°C) are similar to their *Amara* hosts

(12.6 and 11.0°C) (Saska & Honek, 2003), the SETs are higher (95.9 and 147.7 dd for *Brachinus* in contrast to 70.9 and 97.2 dd for *Amara*) (Saska & Honek, 2003).

Acknowledgements

The work was supported by the project of Ministry of Agriculture of the Czech Republic No. M 01-01-03. We thank Professor A.F.G. Dixon for his critical reading of the MS and improving its English, Ing. Z. Martinková for help with the monitoring of experiments, and Mrs I. Kubečková for excellent technical assistance.

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Land use and ground beetle assemblages in the national park of Cabañeros, Central Spain (Coleoptera: Carabidae)

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Abstract

The carabid assemblages of the National Park of Cabañeros (Central Spain) have been studied in six 1 km^2 squares with varying degrees of agricultural management and diversity of habitats during 2001 and 2002. A natural forest, a planted pine forest, and four sites with mixed forest and open areas but with increasing agricultural use (grasslands, arable land, maize cultivation) were investigated using pitfall traps. Species diversity and abundance increased with the intensity of land use, particularly with the intensive management associated with maize cultivation. Spring breeders of medium to small size, full-winged and marked hygrophilous preferences make up an assemblage of generalist species able to colonise the maize fields. The two forested squares were distinguished from the others by harbouring forest specialist (*Calathus hispanicus, Leistus expansus, Platyderus* sp). Species diversity and abundance resembled those found in other temperate European regions, as a few species made up about 85% of total individuals. Species richness and abundance were higher in spring but diversity (quadratic diversity and Shannon-Wiener indices) increased during autumn. Activity patterns of males and females reflected the diversity in life cycles found in carabid assemblages of Cabañeros.

Key words: Bioassess project, carabid assemblages, disturbance, agricultural ecosystems, biodiversity

Introduction

Carabid beetles are a group of epigaeic arthropods frequently considered as a key group for monitoring changes in biodiversity in response to changes in environmental factors. Many papers on this subject have been published in the proceedings of the European Meetings of Carabidologists (Szyszko *et al*., 2002; Brandmayr *et al*., 2000 and further references therein). Particularly interesting is the role of carabid assemblages in agricultural systems (reviewed in

Holland, 2002) or in characterising urbanisation (Niemelä *et al.*, 2002). Carabid beetles are speciose, make up varying assemblages often abundant in numbers, and are easy to collect by inexpensive pitfall trapping, making them often used ecological indicators at different scales of landscape (Lövei & Sunderland, 1996). Changes in morphology and life history traits can also indicate disturbance and stress (Ribera *et al.*, 2001). These characteristics justified the inclusion of carabid beetles in the "shopping basket" of key groups to be studied in the Bioassess project (http://www.nbu.ac.uk/bioassess/), together with vascular plants, lichens, springtails, soil macrofauna, butterflies and birds. The aims, background and scientific objectives of the project are available on the homepage mentioned above. The project aimed to develop biodiversity indicators or biodiversity assessment tools (these may be structural or functional indicators, including local and landscape level indicators, the latter derived from remote sensing methodology) that can be used to assess the impact of land use change on biodiversity.

The project was pursued in eight European countries. In Spain, the area studied was in the National Park of Cabañeros (Southern Meseta) and its surroundings, which offered a suitable land-use gradient from primeval forest to predominantly agricultural fields. Here we analyse the carabid assemblages in relation to increasing intensity of land use and diversity of habitats during a two-year period (2001-2002). We also aim to characterise the patterns of abundance and activity, and the sex ratio. The hypotheses to be tested were that (i) carabid diversity will be higher in sites with intermediate levels of disturbance (Connell, 1978), and (ii) the increase in diversity is due to opportunistic or generalist species that characterise open temperate habitats.

Material and methods

Areas of study

The project was carried out in the National Park of Cabañeros, located between the provinces of Ciudad Real and Toledo (Central Spain, southern Meseta; Fig. 1). The National Park is part of the Montes de Toledo, an area with a typical Mediterranean forest in the centre of the Iberian Peninsula, limited by the Estena and Bullaque Rivers. This area (41,000 ha) is representative of the vegetation, fauna and land use of the Southern Meseta. The altitude ranges from 620 m to 1448 m. The study areas included six 1 km^2 land-use units (henceforth LUU), that made up a gradient from forested LUUs through mixed ones (forest and agricultural land) to agriculture-dominated ones (Table 1). LUU 1 was a sclerophyllous Mediterranean forest with holly oak (*Quercus ilex ballota*), cork oak (*Quercus suber*), and deciduous oaks (*Quercus faginea, Quercus pyrenaica*). LUU 2 was an old pine plantation (about 80 years) of *Pinus pinaster* with little disturbance, as canopy was well developed and fire breaks were covered with dense shrub. LUU 3 was a typical Mediterranean shrubland with patches of tall *Arbutus unedo*, and "jaral-brezal" shrubland of *Cistus* spp. and *Erica* spp. Three sample plots of this LUU were outside the boundaries of the National Park, in a cereal

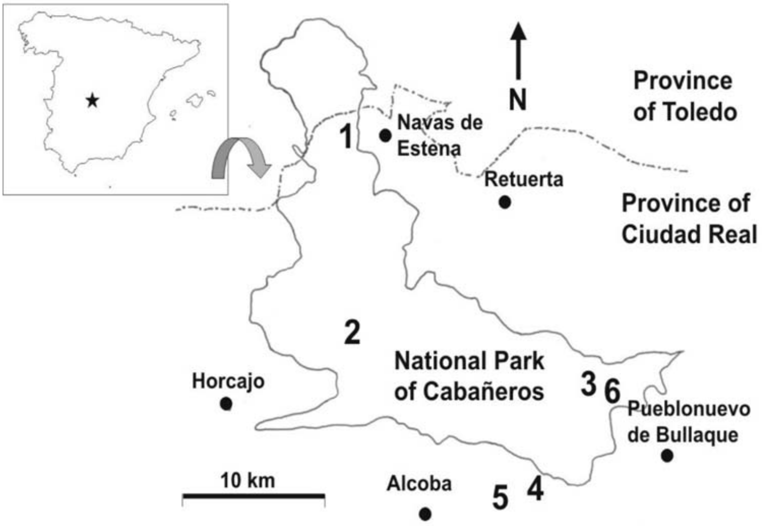

Figure 1. Location of the study areas (the LUUs) in the National Park of Cabañeros, Central Spain. Numbers 1-6 indicate the position of the six 1 km^2 squares sampled: 1 – old forest, 2- managed forest, 3- mixed use dominated by forest-woodland, 4- mixed use not dominated by a single use, 5- mixed use dominated by grasslands, 6- mixed use dominated by crops.

field ploughed and cultivated in both years. Three other plots were located inside the stony “Raña”, where wild animals graze. The “Raña”, at 620 m, is a plain made up of sediments from the neighbouring hills. LUU 4 was on a private property growing maize and wheat, and grazing mostly by sheep. Five sample plots were located within a maize field and three were within the “dehesa”. Dehesa, a typical landscape element of central Spain, is a loose forest-type habitat with isolated trees of *Quercus ilex ballota* and *Q. suber*. The “dehesa” is used for grazing and is often cultivated with a three-year cycle of ploughing, cereal sowing, and fallow. Where possible there is also cork extraction. It represents a moderate to low level of disturbance. LUU 5 was only 1 km away from LUU 4. It differed from the latter in that it included ten sites in the “dehesa” and no maize. The “dehesa” was on fallow during 2001 and ploughed in 2002 (this was done also in LUU 4). LUU 6 was close to LUU 3 and included three sites in the “Raña” and three in the typical Mediterranean shrubland (inside the National Park). Eight sites were in cereal fields with yearly ploughing and sowing, and irregular grazing. This LUU corresponds to a mixed-use landscape dominated by arable crops with moderate to high disturbance.

A list of carabid beetles of the Montes de Toledo, a large area that includes the National Park of Cabañeros, is available (Ruíz-Tapiador & Zaballos, 2001).

Table 1. Proportion (%) of the various habitat types of each land-use unit (LUU) in the National Park of Cabañeros, Spain. Values are derived from measurements of satellite photographs and direct knowledge of the area.

LUU	Forest	Shrub-land	Grass-land	Arable land	Maize field	Water bodies	Paths and others
1-Old forest	60.5	29		5.5		1	4
2- Managed forest	92	5					3
3- Mixed use dominated by forest/shrubland	10.5	46	16	22.5			5
4- Mixed use	30	13	7.5	27	16.5	3	3
5- Mixed use dominated by grasslands	23	6	64	5		1.5	0.5
6- Mixed use dominated by arable lands		15	35	46.5			3.5

Sampling

Sixteen sampling plots 200 m apart were placed in each LUU to give a total of 96 sampling plots. At each sampling plot, four pitfall traps (8 cm in diameter × 10.5 cm in depth) were placed 4-5 m apart from each other in a regular 2 × 2 grid. The traps were partly filled with propylene glycol (20%), and large stones, supported by smaller ones, were placed above them to minimise both flooding of the traps and damage from wild animals. Sampling was carried out with fortnightly controls during 2001 (May 10-June 28, October 11-December 12) and 2002 (April 17- June 6, September 18- November 6). Sampling of LUU 1 during spring 2001 was irregular and catches from many pitfall traps were lost. Further losses included a complete sampling of all LUUs during spring 2002 (except for LUU 1). Wild animals and ploughing caused trap losses in all LUUs of up to 25% of total trap numbers.

The carabid beetles collected were identified to species level using standard keys and are deposited in the collection of our Department. Nomenclature follows the catalogue of Serrano (2003).

Data analysis

Correspondence analysis (CA) was performed to assess how each land-use unit changed through the seasons. A matrix was built consisting of 24 sampling series (6 LUUs in spring and autumn of 2001 and 2002) based on the total number of individuals of each species. The data were analysed using the R package (Ihaka and Gentleman, 1996). The ordination results were subsequently classified with a cluster analysis included in the R package, which classifies the similarities of the LUUs through the four investigated seasons. We also made a correspondence analysis of the changes of carabid assemblages through the seasons within the space defined by the LUUs, by considering the assemblage of each LUU and season as a distinct species set.

The quadratic (or Simpson; in its reciprocal form, 1/D) and the Shannon-Wiener indices of diversity were calculated for the six LUUs.

Results

The species and number of individuals collected in the six LUUs during spring and autumn of 2001 and 2002 are listed in Table 2. LUU 4 showed the highest species richness and abundance during the spring 2001 (34 species and 2829 individuals), particularly in the sampling points 3-4 and 6-8, located in the maize field (23 species and about 1900 individuals). In the following year, 615 individuals of 20 species were collected in this field although maize was not cultivated in that year. Most species of the maize field belonged to the genera *Nebria, Trechus, Bembidion, Poecilus, Agonum, Anchomenus, Anisodactylus, Acupalpus, Stenolophus, Chlaenius,* and *Brachinus.* The species caught are markedly hygrophilous and paludicole or riparian, had a size smaller than 10 mm, and were full-winged. These characteristics correspond to generalist species with high dispersal power. Though we have no data on their life cycle in Cabañeros, they were collected practically only in spring, a fact that suggests that they are spring breeders (see below).

The correspondence analysis of the relationships between the species abundances in the LUUs and the different seasons (Fig. 2) identified two groups of LUUs according to the first axis. The first had only LUU 4 during the springs of 2001 and 2002, and the second one was made up by all the other LUUs in the two seasons of both years, plus LUU 4 in autumn. The second axis divides this second group of LUUs into one including the forested LUUs 1 and 2 and a second with the LUUs 3, 4 (autumn 2001 and 2002), 5 and 6.

The classification of these results in a cluster dendrogram (Fig. 3) shows that carabid assemblages of the forested LUUs 1 and 2 do not cluster with those ones of other units. LUU 4 also has a distinctive assemblage but only in spring, as the species diversity during autumn is similar to that found in the neighbouring LUU 5. LUUs 3 and 6 are related in spring and autumn of both study years and also to LUUs 4 and 5 during autumn.

The results of the second correspondence analysis, change of carabid assemblages through the seasons, are the same as those of the first analysis (data not shown). Assemblages of LUU 4 in spring are separated from the others along the first axis, and assemblages from forested LUUs 1 and 2 are separated from the others along the second axis.

Values of the Simpson's reciprocal index varied within each LUU, both between seasons and years (Table 2). Spring values were typically lower than autumn ones except for LUU 4 in spring 2001 and LUU 6 in autumn 2002. The Shannon-Wiener index of diversity showed the same pattern: lower values in spring than in autumn in all LUUs, except for LUU 4 and LUU 6.

Table 2. List of species and individuals of the family Carabidae collected in six 1 km^2 landscape units (LUUs) in the National Park of Cabañeros (Central Spain) during spring (S) and autumn (A) of 2001 and 2002.

Species	LUU 1				LUU 2				LUU 3				LUU 4				LUU 5				LUU 6				Sum
	2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		
	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	
Cicindela c. campestris												2													2
Cicindela maroccana pseudomaroccana										1							1					1			3
Calosoma maderae													4												4
Carabus rugosus celtibericus		3		10	29	66	14	132			1	4						1	2	11	1	3	9	20	306
Carabus lusitanicus latus	1	100	38	109		14		28	1	123	3	185	1	28	4	58	2	47	5	122	8	167	18	98	1160
Carabus guadarramus			2																						2
Carabus melancholicus costatus		4											26	4	8	3	2	12	9	8					76
Leistusf. fulvibarbis															3										3
Leistus expansus		11		4	11	26	21	4										1							78
Nebria salina		12	1		3	5	1		25	143	1	35	297	440	89	57	333	462	31	87	28	147	3	82	2282
Notiophilus biguttatus					2								1								4				7
Notiophilus marginatus																			1						1
Notiophilus quadripunctatus	1	1				1														1					4
Notiophilus substriatus													1												1
Trechus fulvus										1									1	1		2			5
Trechus obtusus		5					1		1	6	1	3		4	4	4				5	10	2	16	5	67
Trechus quadristriatus					2				3				3							1					9
Asaphidion stierlini																	1								1
Bembidion ambiguum									2				34	2	68	1	3		7		22			1	140
Bembidion antoinei																					1				1
Bembidion vicinum													1		3										4
Bembidion tethys									1								1		8	6	14	1	1	4	36
Bembidion laetum													1												1
Penetretus rufipennis										3				7				3				6			19
Poecilus purpurascens													3	3	18	8	6		1	1					40
Poecilus crenulatus	2				1				3				33		1				1		3				44
Poecilus kugelanni									1				2								3		1		7
Steropus globosus ebenus	19	22	16	54	4	15		21	99	384	235	1081	20	48	38	226	40	143	478	538	283	396	160	788	5108

Species	LUU 1				LUU 2				LUU 3				LUU 4				LUU 5				LUU 6				Sum
	2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		
	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	
Amara metallescens																				2					2
Amara aenea													312		21		2		1		3	1			340
Amara eurynota													1												1
Amara affinis																					1				1
Amara fervida																		1	1						2
Zabrus ignavus														1	28	12									41
Agonum marginatum													7		8		1								16
Agonum muelleri													74		4				9		2				89
Agonum nigrum																			1						1
Agonum viridicupreum													164	1	1				5						171
Anchomenus dorsalis													34	1	11										46
Olisthopus fuscatus																			1		5				6
Olisthopus hispanicus		2		1						1						1		1	1	3	1	2	1		14
Paranchus albipes															1										1
Platyderus sp		4	3		3	10	5	4													1	3			33
Calathus cinctus													1	1	1										3
Calathus circumseptus		2		1																					3
Calathus hispanicus	14	110	151	254																					529
Calathus granatensis	30	130	138	106	324	147	176	240		16		10	54	83	145	74	27	67	47	169	9	57	13	51	2113
Calathus melanocephalus				1																					1
Calathus mollis										20		4	1	11	1	33		3			3	26	2	4	108
Amblystomus escorialensis															1										1
Anisodactylus heros													1												1
Diachromus germanus															1										1
Carterus microcephalus																			1						1
Dixus clypeatus													3		2										5
Cryptophonus tenebrosus												10													10
Harpalus distinguendus													1		7	7									15
Harpalus pygmaeus																			1				1		2
Ophonus subsinuatus												1													1
Pseudoophonus rufipes													1			1									2

Species	LUU 1				LUU 2				LUU 3				LUU 4				LUU 5				LUU 6				Sum
	2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		2001		2002		
	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	
Acupalpus brunnipes													10				1								11
Acupalpus cantabricus													3			1									4
Stenolophus teutonus	1												39		3										43
Licinus punctatulus granulatus																1						1			2
Chlaenius olivieri													7		8				4		1				20
Chlaenius festivus velutinus																	1								1
Chlaenius chrysocephalus													110		17	2									129
Masoreus wetterhallii																1									1
Trymosternus onychinus		1																							1
Pseudomasoreus canigoulensis			2																						2
Metadromius myrmidon			1																						1
Microlestes luctuosus luctuosus															1										1
Paradromius linearis linearis																					5				5
Syntomus fuscomaculatus						1																			1
Syntomus foveatus													22	1	1		2		2		2				30
Drypta dentata															1										1
Brachinus sclopeta													1557	10	528	4	13		2		8				2122
Total no. of individuals	68	407	352	540	379	285	218	429	136	698	241	1335	2829	645	1027	494	436	741	620	955	418	815	225	1053	15346
Total no. of species	7	14	9	9	9	9	6	6	9	10	5	10	34	16	31	18	16	11	24	14	23	15	11	9	
Simpson's diversity	3.16	4.16	2.84	3.22	1.36	2.99	1.5	2.41	1.77	2.65	1.05	1.48	3	2.04	3.34	3.78	1.68	2.28	1.66	2.68	2.13	3.16	1.91	1.73	
Shannon-diversity	1.33	1.69	1.24	1.37	0.63	1.43	0.71	1.50	0.92	1.24	0.14	0.68	1.73	1.19	1.84	1.75	0.98	1.15	1.05	1.32	1.47	1.43	1.12	0.93	

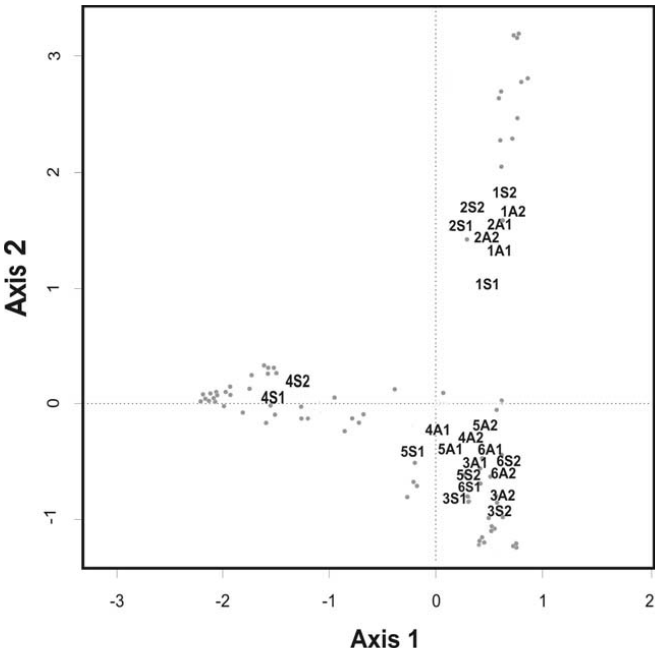

Figure 2. Results of a Correspondence Analysis (CA) of carabid assemblages in six land-use units during spring and autumn of 2001 + 2002, in the National Park of Cabañeros (plot of LUUs through seasons). Eigenvalues are 0.78 (axis 1) and 0.56 (axis 2). Combination of letters and numbers denote the number of the LUU and the season (S spring, A autumn) of 2001 (1) or 2002 (2).

Table 3. Number of males and females of the ten most abundant species of Carabidae captured with pitfall traps in the National Park of Cabañeros (Central Spain).

Species	Spring 2001		Autumn 2001		Spring 2002		Autumn 2002	
	m – f	ratio	m – f	ratio	m – f	ratio	m – f	ratio
Carabus lusitanicus	7 – 6	1.17	286 – 193	1.48	33 – 35	0.94	116 – 61	1.9
Carabus rugosus	9 –10	0.90	56 – 17	3.29	15 – 16	0.94	377 – 233	1.69
Nebria salina	292 – 394	0.74	634 – 575	1.1	48 – 78	0.72	79 – 182	0.43
Bembidion ambiguum	38 – 23	1.65	1 – 2	0.5	58 – 17	3.41	1 – 1	1
Steropus globosus	210 – 255	0.82	517 – 1008	0.51	404 – 523	0.77	1528 – 1180	1.29
Agonum viridicupreum	17 –147	0.12	0 – 0	0	2 – 4	0.5	0 – 0	0
Calathus hispanicus	3 – 11	0.27	24 – 86	0.28	69 – 82	0.84	73 – 181	0.4
Calathus granatensis	193 –251	0.77	44 – 456	0.1	227 – 292	0.78	57 – 593	0.1
Amara aenea	143 –174	0.82	0 – 1	0	14 – 8	1.75	25 – 16	1.56
Brachinus sclopeta	565 – 1013	0.56	5 – 5	1	107 – 423	0.25	2 – 2	1

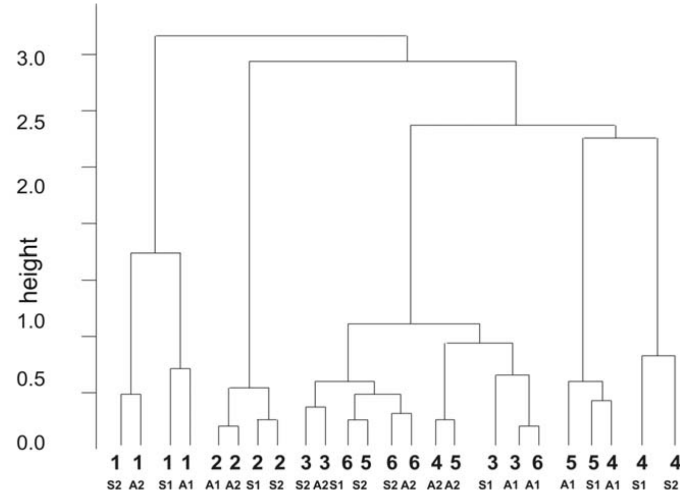

Figure 3. Cluster ordination of LUUs according to the results obtained in the CA indicated in Fig. 2. Abbreviations as in Fig. 2.

Species that showed a clear unimodal pattern of activity in spring included *Bembidion ambiguum, Poecilus crenulatus, Agonum* spp. (four species), *Anchomenus dorsalis, Amara aenea, Stenolophus teutonus, Chlaenius olivieri, C. chrysocephalus* and *Brachinus sclopeta*, while autumn active species were *Leistus expansus, Penetretus rufipennis*, and *Calathus mollis*. Other species showed a bimodal pattern: *Carabus* spp., *Nebria salina, Steropus globosus, Calathus hispanicus* and *C. granatensis*.

Total number of males and females of the ten most abundant species and the sex ratio are shown in Table 3. The ratio varied notably among the most abundant species. The species trapped in both seasons likewise showed either 1) a predominance of males in both seasons, but more marked in the autumn (*Carabus lusitanicus* and *C. rugosus*); 2) a predominance of females in both seasons, more in autumn (*Calathus hispanicus* and *C. granatensis*); 3) a predominance of females in spring and of males in autumn (*Steropus globosus*); 4) an ambiguous pattern (*Nebria salina*).

Discussion

Carabid assemblages in relation to land use and disturbance

Carabid assemblages were clearly affected by the intensive agricultural practices associated with maize cultivation in a patch of LUU 4 that is distinguished by the first CA axis (Fig. 2). The species that caused this separation were mostly those collected in the sampling points of the maize field (data not shown). Irrigation during spring and summer was a significant factor explaining the development of a rich carabid assemblage made up by hygrophilous species with medium to low size and full wings. These species have high dispersal power and most of them are probably spring breeders. They should be considered members of a set of generalist species, able to rapidly colonise habitats associated with irrigated fields that have suitable conditions of soil humidity, prey abundance, and mild temperature.

The species richness of LUU 4 was the highest (34 species in the spring 2001), and this position was maintained during 2002 (32 species) even though maize was not cultivated that year. The soil was still wet and there was much plant debris. The assemblage of this LUU changed substantially during the autumn (16 species in 2001 and 18 in 2002) when it became similar to those found in the surrounding habitats. The species richness of LUU 5 increased from 15 species in spring and 11 in autumn in 2001 to 24 and 14 species, respectively, in 2002. During the spring of 2002 about half of this area (the “dehesa”) was ploughed as a preparation for sowing cereal in the forthcoming year.

The forested nature of the LUUs is probably the factor that distinguishes them along the second axis of Fig. 2, with a clear separation of LUUs 1 (old forest) and 2 (pine forest) from the others. Forested sites in LUUs 3 to 6 are mostly mixed with shrubland, so that the differences in forest cover to LUUs 1 and 2 (Table 1) are possibly not only quantitative but also qualitative.

The correspondence analyses stressed the importance of the intensive agricultural practices associated with maize cultivation in creating a distinctive assemblage of generalist species in LUU 4 during spring. The disturbances in this LUU were possibly over a threshold of intensity, as the other LUUs (together with LUU 4 during autumn) were not well discriminated by the first axis of Fig. 2. Thus, LUU 6 and 5 with a moderate to high disturbance grouped with the forested (and not disturbed) LUUs 1 and 2 and the scarcely cultivated LUU 3.

It seems that disturbance associated with maize cultivation is closer to the optimum of the diversity-disturbance curve than the disturbance of LUUs 5 and 6 (initially postulated to have the higher disturbance). This is indicated by the decrease in diversity observed in the sites of the maize field during autumn and in the neighbouring sites of LUU 4 outside the maize field (all seasons; data not shown).

Effects of habitat disturbance have been found also in the other countries of the Bioassess project (Niemelä *et al*., unpublished). The correspondence between higher disturbance and the increase in small or medium sized species with fully developed wings, and in species diversity seems to be a general pattern also found in other studies (Ribera *et al.,* 2001; Niemelä *et al*., 2002; Luff, 2002).

The second hypothesis has been corroborated to some extent but there are unexpected results. The number of species and individuals increased with increasing agricultural use, as the highest numbers were found in LUU 4 (maize and cereal cultivation), LUU 5 (particularly in 2002 when the "dehesa" was ploughed), and LUU 6 (yearly cultivated for cereal crop). The effect of intensive management is appreciated in the five sites located inside the maize field, where 23 species and 1890 individuals were collected (spring 2001; data not shown). As noted above, this increase was due to the opportunistic or generalist species. These species are in contrast to forest-associated species (*Carabus rugosus, C. guadarramus, Leistus expansus, Platyderus* sp*, Calathus hispanicus, C. granatensis)*, which are wingless, restricted to forests, and with large (*Carabus* spp.) or moderate (*Leistus, Platyderus, Calathus*) body size.
There were two dominant species, *Carabus lusitanicus* and *Steropus globosus* that showed a pattern not clearly related to disturbance derived from agricultural practices. *C. lusitanicus* was found in all habitats but was scarcely collected in LUU 4 (no individuals inside the maize field; data not shown). It was not frequent in the pine forest (LUU 2), where *C. rugosus* is more abundant, but was found in high numbers both in the "Raña" and the cereal fields of LUU 6. *Steropus globosus* showed similar preferences but was even more abundant in cereal fields, possibly taking advantage of agricultural management that does not include irrigation.

Patterns of diversity, abundance and sex ratio

Simpson's reciprocal index suggested that carabid assemblages had a higher equitability in autumn, whereas dominant species had a major role in spring. The opposite was found in LUU 4 during 2001. Here, nine species were represented by few individuals, most of them members of the generalist assemblage that colonised the maize field during spring. Likewise, the high number of individuals (798) of *Steropus globosus* collected in the autumn 2002 in LUU 6 caused a different pattern of evenness. It is evident that increasing agricultural management was directly related to these alterations of the index, as LUUs 4 and 6 were the most disturbed ones.

The number of individuals of the five most abundant species (*Nebria salina, Steropus globosus, Calathus hispanus, C. granatensis,* and *Brachinus sclopeta*), corroborate the empirical observation that a few species often make up about 85% of the total number of captured individuals in many carabid assemblages (Luff, 2002). The genera of the dominant species were the same as those found in temperate agricultural areas of Western Europe and North America: *Nebria*, *Bembidion, Steropus* (formerly ranked as a subgenus of *Pterostichus*), *Agonum, Calathus, Amara* and *Brachinus* (Luff, 2002). This was probably due to the existence of generalist species able to colonise the landscapes changed by agricultural practices. The differences between Central Spain and other western European regions are found at the species level, as most of the abundant species of Cabañeros were endemisms. The only exception was *Nebria salina*, which can be regarded as the Mediterranean vicariant of *N. brevicollis*, a typical abundant species in Europe.

Species that show an unimodal pattern of activity restricted to spring are possibly spring breeders, although this inference should be corroborated by inspection of the reproductive phase through a whole year cycle. Most species showing this pattern were collected in the maize field. *Amara aenea* was collected in a grassland patch not far from the maize field. Three other species are possible autumn breeders, *Leistus expansus* (restricted to forested LUUs), and *Penetretus rufipennis* and *Calathus mollis* (open habitats of different LUUs). These inferences agree with the conclusions obtained by Cárdenas and colleagues on carabid assemblages from various sites of the province of Cordoba, which have environmental conditions similar to those found in Cabañeros (Cárdenas, 1994; Cárdenas & Bach, 1992; Cárdenas & Hidalgo, 1998, 2004).

Most or all species showing a bimodal activity pattern (spring and autumn, *Carabus lusitanicus, C. rugosus, Nebria salina, Steropus globosus, Calathus hispanus,* and *C. granatensis*) are possibly autumn (or winter) breeders, according to the investigations of Cárdenas and colleagues mentioned above. The bimodal activity pattern of *Nebria salina* in Cabañeros was similar to that found in Germany (Dülge, 1994) for *N. brevicollis,* an autumn breeder. The activity pattern of *Steropus globosus* is similar in SW Spain (de los Santos *et al*., 1985). These authors showed that *S. globosus* is a winter breeder as the eggs develop in winter and need a cold shock for hatching.

It seems that disturbance associated with intensive agricultural practices (maize cultivation) is tolerated by spring breeders (i.e., the generalist species), but only by few of the autumn breeders (*S. globosus*, *C. lusitanicus*).

The varying male/female ratios found in carabids from Cabañeros do not follow any clear pattern. In some species, knowledge of their biology can provide a clue. For example, the proportion of males in *Steropus globosus* in SW Spain is higher during autumn, when copulation takes place, whereas the proportion of females is higher from mid December (onset of oviposition) to the beginning of their summer diapause (tenerals actively foraging to reach gonad maturation; de los Santos *et al.,* 1985). In Cabañeros, this species seems to follow the same strategy as in SW Spain. The data on the two most abundant *Carabus* species of Cabañeros suggest that they have a similar pattern. However, it is unknown why males are more abundant than females (or vice versa) in some species of spring breeders, or why there was a constantly higher ratio of females in *Calathus granatensis* and *C. hispanicus*.

Conclusion

Intensive agricultural practices, including irrigation and the use of fertilisers, probably have a profound effect on the carabid assemblages of Central Spain. The relatively low rainfall, mostly in spring and autumn, makes arable land suitable for only a reduced number of carabid species. Irrigation can help the maintenance of a rich epigaeic fauna with a distinctive assemblage of generalist species. Forest development is also a main factor that influences carabid assemblages, as species composition in unmanaged and managed forests is quite different from landscapes with more than 30% open areas.

Acknowledgements

Thanks are due to Spanish colleagues of the Bioassess project for their help with sampling and comments about the development of the research (particularly to the country co-ordinator Pilar Gurrea); to group co-ordinator Jari Niemelä and European colleagues working with carabids; to Allan D. Watt, co-ordinator of the project for his support; to Mr. José Jiménez (Director) and workers of the National Park of Cabañeros for their appreciated collaboration; to Mr. Vicente del Castillo, land-owner of the property of "Las Povedas" (Ciudad Real, Spain) and Daniel Salgado (foreman) for their generous support in sampling LUUs 4 and 5; to José A. Palazón for his help with statistical calculations. Maria Oñate helped with the English. Two reviewers and the editors provided detailed comments and corrections that have much improved the manuscript. This research was founded by the European V Framework project EVK2-CT1999-00041.

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European Carabidology 2003. Proceedings of the 11th European Carabidologist Meeting

Interspecific body size differentiation in *Carabus* assemblages in the Białowieża Primeval Forest, Poland

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Abstract

Nearly 27000 individuals of 10 species belonging to the *Carabus* genus were caught in 12 different habitats in the Białowieża Primeval Forest, Poland. The body length ratios of eight species were analysed. The mean body length ratio of two adjacent species ranged between 1.00 - 1.10, irrespective of the number of *Carabus* species in the assemblage. The mean ratio of the smallest to the largest species increased with the number of species in the assemblage. To study coexistence mechanisms further, automatic traps were used to catch carabid beetles in decaying stumps and tree holes. In tree holes, *C. hortensis* was caught in the greatest numbers. Morphological differences and ecological preferences suggest spatio-temporal niche partitioning as the main coexistence mechanism for *Carabus* species in this forest.

Key words: Body length, Carabidae, Białowieża Primeval Forest, competition avoidance, tree holes, decaying stumps

Introduction

Loreau (1986) suggested that one of the factors organising carabid assemblages in the climax forest was interspecific competition. Interspecific competition occurs whenever two different species attempt to utilise the same resource and when that resource is in limited supply. The importance of competition may also vary among species in the assemblages. The most abundant species may approach their carrying capacity and thus be influenced by competition, whereas the less abundant species may remain well below their carrying capacities and not strongly affected by competition (Loreau, 1992).

However, the coexistence of similar-size carabid species in ruderal and forest environments may be explained without resorting to interspecific competition (Niemelä, 1993). Weber and Heimbach (2001) found no evidence for strong competitive interactions between populations of *Carabus arcensis* and *Carabus nemoralis* in fenced plots. Spatial heterogeneity of habitats may also increase the likelihood of coexistence (Hampton, 2004).

Species belonging to the *Carabus* genus are among the largest Carabidae. In managed European forests, carabid assemblages often contain three – five *Carabus* species, e.g. *C. violaceus, C. glabratus, C. arcensis, C. nemoralis* (Szyszko, 1983; Szujecki *et al*., 1983, Skłodowski, 1995). These species have very similar diets (Turin *et al.,* 2003). In the Białowieża Primeval Forest, carabid assemblages frequently contain five – seven, or even more *Carabus* species (Skłodowski, 2002), providing a good opportunity to study carabid coexistence under natural forest conditions as opposed to managed forests.

The studies of carabid assemblages were part of a larger research program of the Department of Forest Protection and Ecology at the SGGW Warsaw Agricultural University (Szujecki *et al.,* 2001). The objectives of this study were to determine interspecific and intraspecific body size differentiation in assemblages of the *Carabus* genus, and to attempt to clarify the coexistence of closely-related species of the *Carabus* genus.

Study area and methods

The research was performed in the Polish part of the Białowieża Primeval Forest (north-eastern Poland) in two stages. The first phase, in which pitfall traps were used, took place in 1999. I selected three levels of forest soil humidity: damp – wet (70-99% of the soil surface was under water during spring), moderately hygric – moist (humidity of these habitats varied from damp to mesic) and mesic - fresh (no inundation at any time of the year). Further, the traps were set in four different forest stands: coniferous (low fertility habitats), mixed coniferous, mixed deciduous and deciduous, thus providing 12 variants of habitats (Skłodowski, 2002). Two – three coniferous tree species (*Picea abies, Pinus silvestris, Larix decidua*) predominated in the coniferous forest. In mixed coniferous stands, the domination of coniferous species reached 51-90%. In mixed deciduous stands, broad-leaved tree species constituted 51-90% of all trees. Broad-leaved species predominated in the deciduous stands.

Carabids were caught using five pitfall traps per plot. The traps (500 ml glass jars, with plastic funnel, 12 cm diameter, containing 100 ml 70% ethylene glycol) were arranged along transects. The distance between individual traps was 20 m. The traps were inspected every six weeks, giving four collection events between 1 May and 30 October 1999.

In 2000, beetles were studied in decaying stumps, tree stems and tree holes. Three forest habitats were distinguished: mixed coniferous forest, deciduous forest and damp deciduous forests. Six replicates per forest type were selected, giving a total of 18 plots. The following tree species were recorded: *Acer platanoides, Alnus glutinosa, Betula pubescens, Carpinus* *betulus, Fraxinus excelsior, Picea abies, Pinus sylvestris, Quercus robur, Tilia cordata.* Two methods were applied for sampling and collecting carabid beetles dwelling in dead wood. For collecting carabid beetles directly from decaying wood, a special "biocenometer" (tent trap) was used (Szujecki *et al.*, 2001). A decaying stem was enclosed within a tarpaulin tent. The base of the tent was 1 m x 1 m. Inside the tent, a 20 cm diameter plastic dish (330 ml), containing 70% ethylene glycol was suspended beneath its apex. A glass-covered hole was cut above the collecting dish. Beetles emerging from woody stems would climb up inside the tent (towards the light) and fall into the collecting dish. Five decaying stumps were chosen in each plot.

To collect carabids entering tree holes or emerging from them, interception traps were used. The trap consisted of two transparent plastic plates (20 cm x 30 cm) mounted above a 20 cm wide plastic funnel. A plastic bottle containing ethylene glycol was mounted below the funnel, and two small holes halfway up the bottle allowed surplus liquid to flow out during heavy rainfall. Each trap hung directly at the entrance of tree hollows. Tree holes, nine in each plot, located 1.5-2 m above the litter layer were selected for this study.

Evaluation methods

Carabid body length (defined as the length between the tip of the mandibles and the end of elytrae) was measured with the use of a stereo microscope and a graduated ocular. All collected carabids were individually measured (sample sizes see in results).

The species similarities were compared with use of the Ward analysis (Euclidean distances, StatSoft, Inc., 1997). Habitats whose carabid assemblages showed the highest similarity were pooled. This way, the number of habitat types was reduced from the original 12 to a set of six (see details in results).

Prior to statistical analyses, the data were checked for normality of their distribution using Shapiro-Wilk's statistic and for the homogeneity of variances using Levene's test (StatSoft, Inc., 1997). For data with equal variances and normal distribution, a Student's *t*-test for independent variables was applied to assess the statistical significance of differences among mean body lengths. Other data were evaluated using the Mann-Whitney U-test. All calculations were done using the Statistica 5.5A computer program (StatSoft, Inc., 1997)

Results

Nearly 59,000 ground beetles belonging to 105 species were caught during the first part of the study (Skłodowski, 2002). Nearly 37,000 of these belonged to large zoophages (predatory species weighing >100 mg), 27,000 of which belonged to the *Carabus* genus. These were:

Carabus hortensis L. (16,005 individuals), *Carabus arcensis* Hbst. (4116), *Carabus glabratus* Payk. (3204), *Carabus nemoralis* Müll. (1180), *Carabus coriaceus* L. (733), *Carabus violacues* L.(679), *Carabus granulatus* L. (599), *Carabus cancellatus* Ill.(275), *Carabus convexus* F. (181) and *Carabus intricatus* L. (29).

Ward's method of hierarchical cluster analysis was used to determine the similarity between the fauna of the *Carabus* genus occurring in specific forest types. This resulted in the combination of some of the original 12 forest type variants into six types:

- moist deciduous and fresh deciduous habitats, thereafter called deciduous habitat (marked by "D"),
- moist mixed deciduous and moist mixed coniferous habitats, becoming moist mixed (Mm),
- fresh mixed deciduous and fresh mixed coniferous habitats, named fresh mixed (Mf),
- damp deciduous and damp mixed deciduous, named damp deciduous (Dd),
- damp mixed coniferous and damp coniferous, named damp coniferous (Dc),
- moist coniferous and fresh coniferous, named coniferous (C).

The body size ratio of the species of similar lengths was analyzed in the six identified agglomerations (Table 1). Two species, *C. coriaceus* and *C. intricatus,* were excluded from this analysis. *C. coriaceus* had a mean length of 38 mm (the smallest individual was 30 mm, and the largest was 45mm), >1.3 times bigger than the second largest species, *C. violaceus.* In contrast with *C. violaceus* and *C. glabratus*, *C. coriaceus* more frequently preys on big snails. The other species, *C. intricatus,* was excluded because of low occurrence: only 29 individuals were captured.

The analysis showed that the length ratio of species of consecutively smaller sizes in each of identified six agglomerations was smaller than 1.3 (proposed by Hutchinson, 1959).

In the subsequent stage, the body size ratio of the largest to the smallest species (L/S – top line, Fig.1), as well as the mean body length of two adjacent species (TA – lower line, Fig.1), were analysed. The body size ratio of the largest to the smallest species was <1.3 in assemblages "Cd" and "D", (each consisting of two species), as well as in the assemblages "Cd" and "Mf" (with three species each). The mean body length ratio of two adjacent species TA was, in most cases, <1.3, irrespective of the number of *Carabus* species present (Student's *t* -test for all comparisons was $p < 0.05$ to $p < 0.001$). Only in two cases, of the assemblages "C" and "Dd", containing two species each, was this ratio close to 1.3. There was a slight, but statistically not significant, increase in the TA ratio in assemblages consisting five or six species.

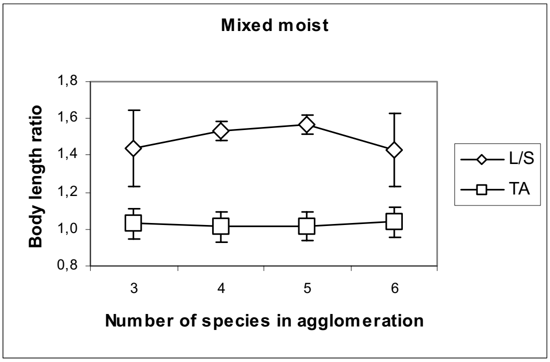
On the basis of current observations, as well as the literature (Burakowski *et al*., 1973, 1974; Szyszko, 1983; Szujecki *et al*., 1983; Skłodowski, 1995), the representatives of the analyzed *Carabus* genus were divided into two groups: species that prefer highly fertile deciduous

Table 1. *Carabus* assemblages and their size ratios in different habitat groups in the Białowieża Primeval Forest, NE Poland, 1999. The habitat groups were identified by Ward's method of hierarchical clustering. Only species with >5% relative abundance were included. The sequence of the assemblages is by size, with the largest species on top.

Coniferous “C"	Relative abundance %	Body length ratio	Damp coniferous “Cd”	Relative abundance %	Body length ratio	Fresh mixed “Mf”	Relative abundance %	Body length ratio
C. convexus		1.01	*C. arcensis*	14	1.08	*C. arcensis*	10	1.05
C. arcensis	40	1.23	*C. convexus*		1.07	*C. granulatus*		1.04
C. granulatus		1.09	*C. granulatus*		1.22	*C. convexus*		1.22
C. cancellatus		1.00	*C. nemoralis*		1.04	*C. nemoralis*	5	1.03
C. nemoralis		1.10	*C. cancellatus*		1.07	*C. cancellatus*		1.07
C. hortensis	34	1.03	*C. hortensis*	43	1.02	*C. hortensis*	68	1.04
C. glabratus	11	1.02	*C. glabratus*	16	1.05	*C. glabratus*	11	1.02
C. violaceus	7		*C. violaceus*			*C. violaceus*		
Moist mixed “Mm”			**Deciduous “D”**			**Damp deciduous “Dd”**		
C. convexus		1.01	*C. arcensis*	22	1.12	*C. arcensis*	9	1.02
C. arcensis	8	1.13	*C. convexus*		1.05	*C. convexus*		1.09
C. granulatus		1.17	*C. granulatus*		1.15	*C. granulatus*	16	1.22
C. cancellatus		1.01	*C. cancellatus*		1.01	*C. nemoralis*	6	1.01
C. nemoralis		1.11	*C. nemoralis*	8	1.12	*C. cancellatus*		1.07
C. hortensis	69	1.03	*C. hortensis*	50	1.01	*C. hortensis*	54	1.03
C. glabratus	14	1.01	*C. violaceus*		1.01	*C. glabratus*	6	1.04
C. violaceus			*C. glabratus*	10		*C. violaceus*		

habitats and those of poorer habitats (low fertility, usually coniferous forests). The group of fertile, deciduous habitat species included *C. hortensis, C. glabratus* and *C. granulatus.* The species *C. violaceus, C. nemoralis, C. convexus, C. arcensis* and *C. cancellatus* were classified as species of poorer, coniferous habitats.

As a rule, the species from the more fertile deciduous habitats attained significantly larger body lengths in fertile deciduous habitats “D” than in poorer coniferous habitats “C”. For example, *C. hortensis* had a mean body length of 26.87 mm (s.d. = 0.54 mm, $n = 2759$) in the former vs. 26.53 mm (s.d. = 0.47 mm, $n = 880$) in the latter. (Student's t test, $p < 0.001$ – Fig. 2). However, the opposite was observed in the case of species from poorer coniferous habitats, where individuals reached a larger body size in poorer, low fertility coniferous habitats “C” rather than in fertile deciduous habitats “D”. A representative of this group, *C. violaceus* had a mean body length of 28.21 mm (s.d. = 0.58 mm, $n = 234$) in the poor habitat vs. 27.16 mm (s.d. = 0.63 mm, $n = 61$) in the more fertile one. This difference was also significant (Student's t-test, $p < 0.001$ – Fig. 2).



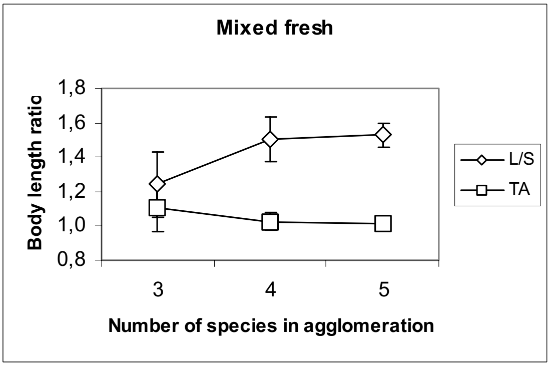


Figure 1. Body length ratio of the largest to the smallest species, L/S (upper line), and the mean body length ratio of two adjacent species, TA (lower line in agglomerations consisting of 2 and more species.

In damp and fresh habitats, the species of both groups seemed to adopt different body size strategies. Species of more fertile (deciduous) habitats were smaller in damp habitats "Dd" than in fresh habitats "D" (Student's *t*-test, $p < 0.001$): *C. glabratus*: 27.34 mm (s.d. = 0.49 mm, $n = 53$) vs. 27.49 mm (s.d. = 0.56 mm, $n = 498$), *C. hortensis*: 26.46 (s.d. = 0.35 mm, $n = 712$) vs. 26.87 (s.d. = 0.54 mm, $n = 2759$), *C. granulatus*: 19.89 (s.d. = 0.50 mm, $n = 175$) vs. 20.68 (s.d. = 0.54 mm, $n = 232$). Conversely, the species with an affinity for poorer coniferous habitats were larger in damp habitats "Dd" than those in fresh environment "D" (Student's t-test, $p < 0.001$) – *C. violaceus*: 28.45 mm (s.d. = 1.04 mm, $n = 20$) vs. 27.16 mm (s.d. = 0.63 mm, $n = 63$), *C.nemoralis*: 24.09 mm (s.d. = 0.61 mm, $n = 95$) vs. 23.97 mm (s.d.=0.43 mm, $n = 441$), *C. cancellatus*: 24.59 mm (s.d. = 2.30 mm, $n = 38$) vs 34.84 mm (s.d. = 0.93 mm, $n = 114$), *C. arcensis*: 17.73 mm (s.d. = 0.73 mm, $n = 101$) vs. 17.48 mm (s.d. = 0.37 mm, $n = 1180$), as well as in damp habitats "Cd", compared to fresh forest "C": *C. violaceus*: 28.75 mm (s.d. = 1.36 mm, $n = 80$) vs. 28.21 mm (s.d. = 0.58 mm, $n = 234$), *C. arcensis*: 17.93 mm (s.d. = 0.10 mm, $n = 53$) vs. 17.80 mm (s.d. = 0.20 mm, $n = 1115$).

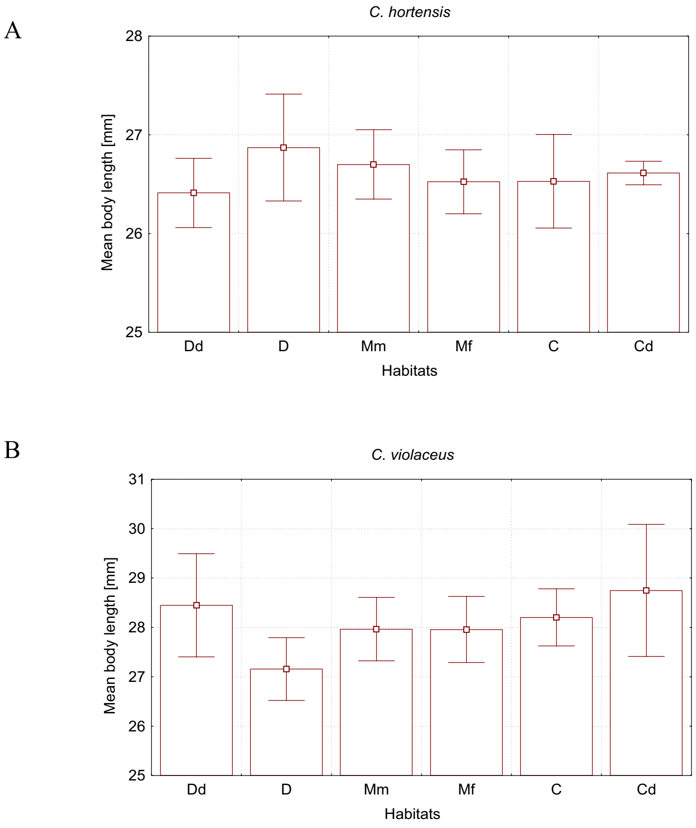


Figure 2. Mean body lengths (mm) and standard deviation (plus-minus) of species in forest habitats. Example of (a) *C. hortensis* a species preferring fertile environments, e.g. (b) *C. violaceus* a species preferring poor habitats.

In the photoeclectors and interception traps, a total of approximately 1000 individuals, belonging to 42 species, were caught. More than 600 individuals, belonging to 34 species, were caught in the tree-stump (photoeclector) samples. Most common were *Pterostichus oblongopunctatus* F. (256), *Pterostichus niger* Schall. (72), *C. hortensis* (69), *Pterostichus aethiops* Panz. (40), *Cychrus caraboides* L. (23), *Agonum viduum* Panz. (21) and *Pterostichus melanarius* Ill. (20). The catchability of remaining species, e.g. *Leistus piceus* Froelich (1) or *C. intricatus* (2), appeared to be fairly low. Despite the fact that the mean number of carabid individuals caught per forest habitat varied from 13.3 in the "Dd" variant to 50.1 in "D" or to 43.4 in "Mc", these differences were not significantly different (data not shown).

Approximately 200 individuals belonging to 23 carabid species were caught in tree hole (interception) samples. Those most commonly caught were: *C. hortensis* (62), *Agonum livens* Gyll. (31), *Agonum assimilis* Payk. (22), *P. niger* (14), *Calosoma inquisitor* L. (12) and *P.*

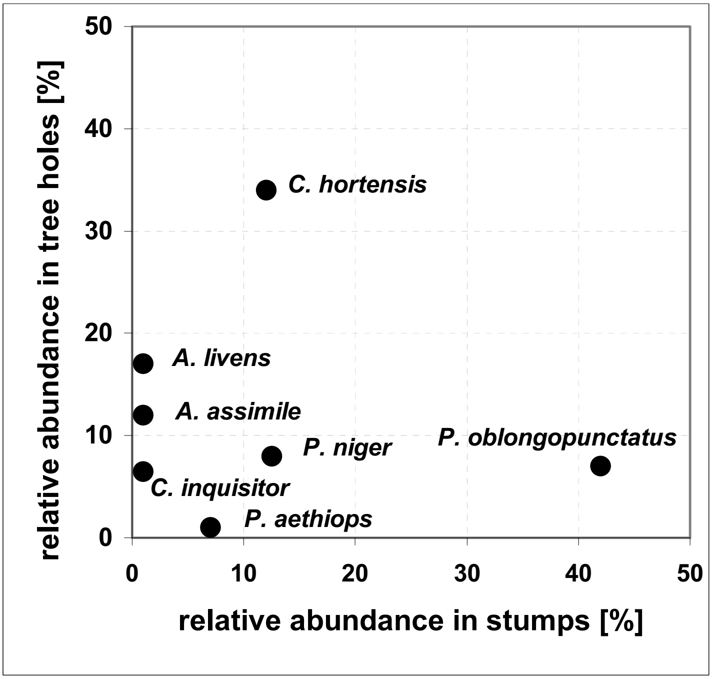


Figure 3. Comparison of relative abundance (%) of the common species observed in decaying stem wood vs. tree holes in the Białowieża Primeval Forest, NE Poland 2000.

oblongopunctatus (12). Typical arboreal species, except *C. inquisitor*, were not caught in large numbers. Other arboreal species were also rare: only three individuals of *Dromius agilis* F. and a single *D. fenestratus* F. were captured. The dominance relations in the two habitat types were graphically compared (Fig. 3). The tree-hole carabid fauna was dominated by the large forest species, such as *C. hortensis*, rather than the smaller ones, whereas the decaying stem wood fauna was clearly dominated by the small forest species *P. oblongopunctatus*.

Discussion

The body size of beetles has been analysed in the context of mating success (Biedermann, 2002), the interrelationship between body size and the size of inhabited islands (Palmer, 2002), or along an urbanisation gradient (Šustek, 1987). The body size of closely related species, e.g. those belonging to members of the *Pterostichus* genus, has been connected to interspecific competition (Brandl & Topp, 1985).

However, my results did not support competition as an explanation for the coexistence of closely related and similar-sized species in the *Carabus* genus. Conditions in the Białowieża Primeval Forest are very probably closer to the original forest habitat than any other forest in Europe. More species of the *Carabus* genus coexist here than in managed forests. Unlike in managed forests, the multi-level old stands continue to grow in a mosaic of fertile habitats. Additional "elements" creating habitat micro-diversity are present in the Białowieża Forest, which are usually missing from managed forests and are rare in others. These include abundant decaying wood, old trees with holes and clumps. The latter occur not just in damp habitats but also in the moist ones.

In Białowieża, there are eight *Carabus* species (including *C. menentiesi* and *C. clatratus*, both collected by hand). In managed forest stands, the number of *Carabus* species is usually only half this number, a consequence of significant structural simplification of those forests, as well as that of the homogeneity of the habitats in which they grow.

The analyzed species were divided into those preferring fertile deciduous habitats (the majority of these represent species of autumnal development type, Thiele, 1977) and those preferring poor, low fertility habitats (here the majority are "spring" species). Species that prefer fertile habitats attained smaller sizes in coniferous forest stands "C", than in deciduous stands "D". However, species typical of poorer forest habitats were larger in coniferous forest stands "C" ("poor habitats") and smaller in deciduous stands "D" (which are fertile habitats). Thus, both types of species attained larger body sizes in their preferred habitats. In less fertile habitats of coniferous stands, non-specialised and less-energy-efficient species appeared to thrive (Skłodowski, 1999a). Among the representatives of the *Carabus* genus, the species of spring development type have lower assimilation and production efficiency than those of autumn development type (Grüm, 1976). Non-specialized spring development type species can be more efficient in poorer habitats and thus achieve larger size than in fertile deciduous stands (Odum, 1971). On the other hand, larvae of autumn species are active in the spring, when deciduous forests are leafless. In Bialowieza, this coincides with the emergence from hibernation of the caterpillars of the forest pest *Dendrolimus pini* L. They, as well as pupae and freshly emerging, still flightless moths provide ample food for the carabid larvae. This is why autumn species can attain larger size in rich, fertile deciduous habitats.

In damp habitats, the species that preferred fertile habitats reached a smaller size than in "fresh" environments ("Dd" vs. "D", "Cd" vs. "C"). Species that prefer poorer habitats, however, exhibited an inverse trend, as their representatives were larger in damp environments than in "fresh" and humid environments. Damp environments of both deciduous forest stands "Dd", and coniferous forest stands "Cd", are difficult to colonize – in the spring the only habitable grass clumps, sedges and moss, as well as tree stumps and trunks, are surrounded by water. However, different species of invertebrates gather in the clumps, providing prey concentrations. Spring carabid species, that prefer poor habitats, overwinter as larvae. In autumn and spring they are active, can feed and reach larger sizes than in other environments. Autumn-active *Carabus* species overwinter as eggs, cannot feed on invertebrates gathered in clumps in spring, so their final size is smaller than in non - damp environments.

The body length ratio of two adjacent species in all habitats was <1.3, the Hutchinson-ratio (Hutchinson, 1959). This ratio was sometimes close to 1.00, as for the dominant *C. hortensis* and the second largest species, *C. violaceus* (habitat “D”) or *C. glabratus* (the remaining habitats, Table 1, Fig. 1).

When the number of species in the assemblage increased, the body length ratio of two adjacent species, TA, stabilized near the value of 1.00 (habitats “C”, “Cd”, “D” or “Dd”, “Mm” and “Mf”, respectively, Fig. 1). A similar stabilization of the body length ratio of two adjacent species, but at higher values – approx. 1.2 – was observed by Sota *et al*. (2000). The stabilization of mean body size ratio at the level close to 1.00 may suggest that the species acquired the ability to avoid competition. Sota (1987) argued that "because of the similar ecology of sympatric species, the body size difference can be a key factor for the coexistence of closely related species". This does not seem to be supported by this study under the arguably "natural" conditions in the Białowieża Primeval Forest.

The species analyzed in this paper are predatory and nocturnally active, but their habitat preferences are different (Burakowski *et al.,* 1973 & 1974; Turin, 2000; Turin *et al*., 2003). *C. granulatus* is a hygrophilous species. This is probably the reason why it ranks second in the dominance structure of assemblages inhabiting damp deciduous forest stands. This species can swim (Turin *et al*., 2003), as well as retire during the long inundation period into crevices with “air bubbles” which may occur within the peat (Fuelhaas, 1997). However, *C. granulatus* is not a forest species in Poland (Burakowski *et al*., 1973, 1974; Szyszko,1983). Its occurrence in damp habitat patches of the Białowieża Primeval Forest is possible not only due to the presence of water, but also due to numerous gaps in stands created by fallen old trees.

Other species occurring at Bialowieza, *C. arcensis* and *C. convexus* are xerophilous. Whereas *C. arcensis* dominated in assemblages inhabiting coniferous forest, *C. convexus* was not common there (Table 1). In Poland, *C. arcensis* prefers poorer forest habitats, as well as ecotones (B. Burakowski, Zoological Museum, Polish Acad. Sci., Warsaw, *personal communication*). This species was ranked second in the dominance structure of the assemblage inhabiting deciduous stands. Being xerophilous, it occurred in wood-stand gap areas.

The remaining species are mesophilous (Burakowski *et al*., 1973 & 1974; Turin, 2000; Turin *et al*., 2003). Among these, *C. hortensis, C. glabratus* and *C. nemoralis* were dominant. These species constituted the core of the assemblage and their body size ratio was close to 1.00. What makes their coexistence possible? As a spring species (Szyszko, 1983), *C. nemoralis* occurs in the first part of the season and thus, at least partially, its activity does not overlap with the other two species. However, the close coexistence of *C. hortensis* and *C. glabratus* remains puzzling.

C. hortensis was dominant in interception traps hung in tree holes 2 m above the ground, meaning that it climbs trees well, which was also observed by Gryuntal & Szyszko (2002). This species avoids entering open spaces (Skłodowski, 1999b). Other carabids (for example, *C. auronitens*) can spend significant time on trees and climb up to 6 m (Weber & Heimbach, 2001), so it is plausible to assume that *C. hortensis* can intensively utilise trees.

Coexistence of these species may be possible by spatial and temporal niche differentiation:

- the main species, *C. hortensis*, climbs trees, thus reducing competition with other, non-climbing species, especially with the similar-sized *C. glabratus,*
- in the remaining habitats, particularly damp ones, the hygrophilous *C. granulatus* inhabits those lower sections of tree stems and stumps that are most subject to inundation, whereas the xerophilous *C. arcensis* inhabits the upper sections. Both species utilise the sunlight-penetrated gaps, created by fallen old trees,
- the occurrence of the "autumn" species *C. glabratus* and the "spring" species *C. nemoralis* (Hurka, 1996) in the same environments seems possible due to different periods of activity,
- the coexistence of the less abundant *C. convexus, C. cancellatus* or *C. violaceus* seems possible due to the heterogenous nature of the environment in the Białowieża Primeval Forest (e.g. clumps of grass, rotting fallen trees).

Spatial and temporal niche partitioning emerges as the main mechanism of coexistence of carabid beetles in the Białowieża Forest. This mechanism seems also the basis of coexistence two or more species of tiger beetles (Schulz & Hadley, 1987), rodents (Morris, 1996) or carnivorous mammals (Fedriani *et al*., 2000).

Finally, I would like to draw attention to the high densities of carabids in this forest. A total of 60,000 carabid individuals were collected in the study, with 27,000 specimens belonging to the *Carabus* genus. The activity density was as high as 5 individuals/day. In managed stands, however, the average acvitity-density rarely reaches 1-2 individuals/day. Trying to explain the coexistence of carabid species on the basis of studies of an impoverished forest may not provide us with the precise picture. Coexistence mechanisms probably developed under drastically different conditions than those that currently prevail in most European forests. Studying habitats such as the Białowieża Forest can thus acquire specific significance.

Acknowledgements

The author thanks two anonymous reviewers and Gabor Lövei for comments and linguistic revisions of the text.

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Influence of land-use intensity on the ground beetle assemblages (Coleoptera: Carabidae) in Central Hungary

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Abstract

Pitfall trapping investigations were carried out in the Csévharaszt area of the Great Hungarian Plain during the years of 2001 and 2002. The aim of our investigations was to find a relationship between the composition of ground-beetle assemblages and the vegetation types and intensity of land-use of the investigated sites. The species richness, total carabid abundance and Shannon-Wiener diversity all increased from forested to agriculture-dominated and open landscapes, while large-sized species and species associated with forests decreased from forested to agriculture-dominated landscapes. The agricultural landscapes were rich in small-sized and disturbance-tolerant species. The highest number of species and individuals were captured in alfalfa crops.

Key words: BioAssess, carabid beetles, land-use intensity, alfalfa, biodiversity.

Introduction

This study is part of the European Union-funded project "Biodiversity Assessment Tools (BIOASSESS)". The project's objective is to develop biodiversity assessment tools for inland terrestrial ecosystems, comprising sets of indicators of biodiversity, and to assess the impact of land-use policies on changes in biodiversity in Europe.

Carabid beetles are species rich and abundant in both natural and arable (or anthropogenic) habitats. Representatives of this group are frequently used as subjects of bioindication investigations all over the world, because they respond sensitively to anthropogenic changes in habitat quality, and they are both ecologically and taxonomically relatively well known (Lövei & Sunderland, 1996).

Landscapes are being fragmented and natural habitats lost throughout the world, while the anthropogenically disturbed area continues to increase (Kromp, 1999). In Hungary, about 70% of the total area is used for agricultural purposes (arable land, gardens, orchards, vineyards, grasslands); of this almost 50% is arable land (Kiss *et al.,* 1994). We studied the effects of land-use intensity on carabid beetle assemblages across a gradient of six sites (1×1 km squares) from forested to agriculture-dominated ones in Csévharaszt in Central Hungary.

Study sites and methods

Our sampling area was in the Csévharaszt Nature Reserve, in Central Hungary, on the Great Hungarian Plain, south of Budapest. It comprised six sites of 1 × 1 km squares each. The sites were situated in a 5 × 7 km area, rather close to each other.Within each site there were 16 plots arranged in a 4 × 4 grid with 200 m between neighbouring plots. At each plot, four traps were placed 4–5 m apart in a regular 2 × 2 grid. The pitfall traps were 8 cm in diameter and 10.5 cm in depth. Every trap was covered with a square aluminium cover to protect the trap content from rain and disturbance by small vertebrates. Traps were emptied every three weeks, and the catches from the four traps in one plot were pooled. Thus we operated a total of 384 traps (6 sites × 16 plots × 4 traps) but had only 96 samples per sampling occasion. The sampling was carried out in the years 2001 and 2002 between April and October. Because of the dry and warm period the catches were interrupted in the second half of July and August.

The typical natural vegetation of the area was lowland oak forest (*Convallario-Ligustro-Quercetum*) on sandy soil in the more humid depressions and juniper-poplar stands (*Junipero-Populetum albae*) on the higher and dry places. On the calcareous sandy patches the most characteristic treeless vegetation of this region, the *Festucetum vaginatae danubiale* grassland occurred, with *Festuca*, *Stipa*, *Koeleria*, *Bromus* species, often interspersed with barren patches. The original vegetation is largely transformed into agricultural land (rye, maize and alfalfa fields) or forest plantations (*Robinia pseudoacacia*, *Pinus sylvestris* and *Populus* spp.).

The sites reflected both the spectrum of land-use intensity and the dominance of forest covering the region. The first site (old growth forest) was dominated by dry forest (white poplar *P. alba* stands with juniper and black locust plantations) without any forestry or agricultural management. The other sites contained increasing proportions of various managed forests (*Populus*, *Quercus robur*, *Pinus sylvestris* stands) or treeless vegetation patches (fallow land, beet-, rye- and alfalfa fields etc.) with increasing influence of agricultural activity.

The second site (mesophilous forest) was dominated by humid natural forest of oak (*Quercus robur*), birch (*Betula pendula*) black locust Scots pine (*Pinus sylvestris*) and poplar plantations under moderate forestry management. About 80% of the site was forested. The third site was also dominated by managed woodland, with about 30% of the area covered with treeless vegetation (agricultural lands, uncultivated areas). The vegetation cover of the fourth site (mixed forest-agriculture site) was about equally managed woodland and various open habitats such as fallow lands and corn (mainly rye) fields. The fifth site was a pasture growing on moderately saline soil, humid in spring and dry in the summer, and mowed twice a year. Treeless vegetation was dominant here with scattered rows of trees and patches of managed poplar plantations. The sixth site was dominated by arable crops with an abandoned orchard and a garbage dump.

The dominance value of individuals of the larger carabid beetles (average body size over 15 mm) was calculated.

Since the carabid fauna of alfalfa crop proved extremely rich, the fauna of this habitat deserve particular attention. This crop type was represented by 1 plot in the 3rd and in the 4th and by 5 plots in the 6th sites.

To calculate the diversity of the carabid assemblages we have used the Shannon-Wiener diversity formula: $H = -\Sigma p_i \ln p_i$ (Shannon & Wiener, 1949). The evenness was estimated by $J = H/\ln S$ formula (where H = Shannon-Wiener diversity, S = richness of species).

Comparison of the carabid assemblages of the six sites was made by metric multidimensional scaling by means of the Horn Index (Krebs, 1989). For calculations we used the Syntax 2000 computer package (Podani, 1997).

Results

During the two years a total of 23619 individuals of 120 species were captured (Table 1). *Calathus fuscipes* Goeze and *Pseudoophonus rufipes* De Geer were the two most common species with 4139 and 3922 (= 8061) specimens, respectively. Other frequent species were *Calathus erratus* Sahlberg, *Calathus ambiguus* Paykull, *Carabus violaceus* L., the relative abundance of each exceeding 5%. These seven species constituted 73% of the total number of individuals. Twenty-five species were singletons (represented by only one specimen).

In the old-growth forest the number of species and of individuals was relatively low (Table 1). The Shannon-diversity of and the evenness value of this assemblage was low, too. The dominant species of this site was *C. erratus* with a high dominance value (Table 2). The second most frequent species was *Harpalus tardus* Panzer. Species with a value higher than 5% were *Carabus convexus* F. and *Calathus melanocephalus*. Ten species were singletons.

Table 1. Number of species and individuals of carabids and Shannon–Weaver diversity values of the carabid assemblages of the six investigated sites in Csévharaszt (Central Hungary).

Sites	1	2	3	4	5	6	Σ
Species number	37	48	62	58	83	70	120
Specimens	1519	4202	4654	3142	2763	7339	23619
Shannon-diversity	1.800	2.230	2.305	2.536	2.502	2.416	
Evenness	0.498	0.576	0.558	0.625	0.566	0.569	

The dominance value of the large-sized ground beetles was 0.091. Characteristic species were *Amara fulva* Müller, *Harpalus autumnalis* Duftschmid and *Harpalus servus* Duftschmid preferring the sandy soil with sparse vegetation.

Table 2. Dominance of the most frequent carabid species in the six sites of Csévharaszt (Central Hungary) (+ = present).

	1.	2.	3.	4.	5.	6.
Calathus ambiguus	+	+	0.084	0.103	+	0.280
Calathus erratus	0.550	+	0.164	0.168	0.098	0.066
Calathus fuscipes	+	0.175	0.296	0.018	0.130	0.140
Calathus melanocephalus	0.072	+	0.084	0.085	+	+
Carabus convexus	0.078	0.059	+	+	+	+
Carabus violaceus	+	0.237	+	+	0.073	+
Harpalus distinguendus	+	+		0.066	+	+
Harpalus tardus	0.114	0.060	+	0.090	+	+
Ophonus rufibarbis	+	0.063		+	+	+
Pseudoophonus rufipes	+	+	0.173	0.144	0.347	0.210
Pterostichus melanarius		+	+	+	0.080	+
Pterostichus niger	+	0.240	+	+	+	+

At the mesophilous forest stands both the number of species and of individuals were higher than at the first locality (Table 1). The Shannon-diversity and the evenness value of this assemblages was moderately high. The two most frequent species were *Pterostichus niger* and *Carabus violaceus* (Table 2). The dominance of *Calathus fuscipes*, *Ophonus rufibarbis* F., *Harpalus tardus* and *Carabus convexus* exceeded 5%. Altogether 17 species were recorded by only one specimen. The number of individuals of the large-sized ground beetles was very high in this site. The dominance value of these beetles reached 0.584. *Zabrus spinipes* F., known as a species of dry sunny steppes, was found here in large numbers in humid forest edges and in depressions with reeds and willows. *Carabus violaceus* and *C.*

convexus were typical inhabitants of the different forest stands, poplar, oak, birch and black locust. The highest number of *Carabus convexus* were captured at this site.

In the mixed forest stands both the number of species and of individuals were high (Table 1). The Shannon-diversity and the evenness value of this assemblages was a little higher than in the case of the previous site. The most dominant species was *Calathus fuscipes* (Table 2) of which we captured the highest number of individuals (1376) at this site. Other species with a dominance value above 5% were *Pseudoophonus rufipes*, *Calathus erratus*, *C. ambiguus* and *C. melanocephalus*. Seventeen species were singletons. The dominance value of the large-sized ground beetles reached 0.043 in this site. More than 52% of the total number of *Amara aenea* De Geer was found in this site. The relatively high individual number of the more sporadic *Licinus depressus* Paykull is equally due to the presence of various habitats (forest edges, alfalfa crops, uncultivated open areas, bank of a canal, etc.).

In the mixed forest stands with large open areas (Site 4) the species number and individual number were similar to those of the previous square (Table 1). The maximum values of the Shannon-diversity and the evenness were reached at this assemblages. The two most frequent species were *Calathus erratus* and *Pseudoophonus rufipes* (Table 2). Further frequent species with more than 5% value were *Calathus ambiguus*, *Harpalus tardus*, *Calathus melanocephalus*, *Harpalus distinguendus* Duftschmid and *Calathus fuscipes*. Eighteen species were found in only one specimen. The dominance value of the large-sized ground beetles reached 0.012 in this site. The presence of *Harpalus flavescens* Piller et Mitterpacher can be explained by the barren sandy patches of this square.

In the saline hay meadow, the number of species was extremely high, while the number of individuals was relatively low (Table 1). The Shannon-diversity and the evenness value of this assemblages was relatively high. The most frequent species was *Pseudoophonus rufipes* (Table 2). Further frequent species with more than 5 % value were *Calathus fuscipes*, *C. erratus*, *Pterostichus melanarius* Illiger and *Carabus violaceus*. The number of species with one individual (30) was also very high. The dominance value of the large-sized ground beetles reached 0.174 in this site. This square proved rather rich in rare species, as *Amara cursitans* Zimmermann, *A. equestris* Duftschmid and *Olisthopus sturmi* Duftschmid.

In the arable land site the species number was high, while the number of individuals was the highest of all sites (Table 1). The Shannon-diversity and the evenness value of this assemblages was relatively high. The two most frequent species were *Calathus ambiguus* and *Pseudoophonus rufipes* (Table 2). Further frequent species with more than 5% dominance value were *Calathus fuscipes* and *C. erratus*. Eighteen species were found in only one specimen. The dominance value of the large-sized ground beetles reached 0.012 in this site. Typical inhabitants of the agricultural land and the disturbed region were *Amara bifrons* Gyllenhal, *A. ingenua* Duftschmid, *A. similata* Gyllenhal, *Dolichus halensis* Schaller and

Harpalus distinguendus. Rare and thermophilous species from this site were *Harpalus albanicus* Reitter, *H. melancholicus* Dejean and *Masoreus wetterhallii* Gyllenhal.

Alfalfa crop was extremly rich in ground beetle species and individuals. The average individual number of the 96 plots over the season was 246 vs. 775 in the alfalfa crop. More than 50% of the recorded species was found in this habitat and 7 species were only encountered here: *Amara apricaria* Paykull, *A. majuscula* Chaudoir, *Harpalus affinis* Schrank, *H. smaragdinus* Duftschmid, *Masoreus wetterhalii* Gyllenhal, *Cicindela germanica* Linnaeus, *Calosoma auropunctatum* Herbst. The three most frequent species, *Calathus ambiguus*, *Pseudoophonus rufipes* and *Calathus fuscipes* constituted 65% of the total number of individuals.

The assemblages of the different sites have been compared with the Horn Index (Fig. 1). According to the hierarchical classification, the 3rd, 4th, 6th and 5th sites show highest similarity, being increasingly separated from the other groups in the order the dry forest (1st site) and mesophilous forest (2nd site)

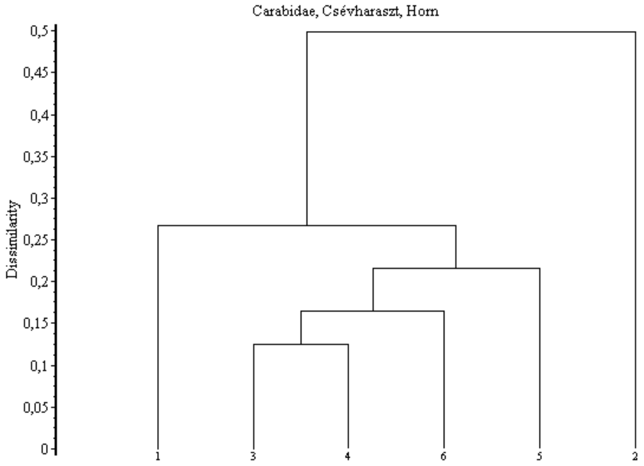

Figure 1. Dendrogram of similarity, based on the Horn Index, of the carabid assemblages of the six different sites in Csévharaszt (Central Hungary).

Conclusions

According to our investigation, the number of species, number of individuals and the Shannon-diversity of carabid beetles show a tendency to increase from forested to agricultural dominated sites. The maximum species richness was reached in the pasture (5th site), while the highest number of individuals was in the agricultural land (6th sites). The lowest number of species and individuals and the lowest diversity values were observed in the dry forest (1st site). The Shannon-diversity reached its maximum at the 4th site (mixed forest).

The average body size of the species pool was large in the humid forest dominated 2nd site, in the rows of trees of the 5th site and smaller in the open vegetation types and in the mixed forest-agriculture sites. The occurrence of larger-bodied species in the 2nd and in the 5th site is in relationship with the humidity and with the richness of nutrition in forested habitats.

The alfalfa crop proved to be a suitable habitat for a lot of species. This interesting phenomenon can be explained by two reasons. On the one hand by the favourable microclimatic conditions produced by this crop type, on the other hand by the partial absence of agricultural treatments. The role of the large quantity of prey was also important.

Acknowledgements

We appreciate the comments and suggestions by Drs. Søren Toft and Gábor Lövei, that improved the former draft of the manuscript.

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Flight of ground beetles towards polarized and unpolarized light sources

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Abstract

The visual system of many insect species is sensitive to polarized light. This ability allows them to use the polarization pattern of skylight, or light reflected by water surfaces for navigation or habitat detection. Many aquatic insects detect their habitats by perceiving water-surface-reflected, horizontally polarized light. Using light trap pairs emitting horizontally polarized or unpolarized light, and operating between April and October, 2001-2002 in central Hungary, we sought for potentially polarotactic flying insect species. Two trap pairs were set up in a wet (near riparian forest on a riverbank) and a dry (ridge of sand dune) habitat. The structural characteristics of carabid assemblages attracted by the two light sources of trap pairs did not differ significantly. The species living in waterside habitats were represented by higher cumulative relative abundance in polarized light traps. From the recorded 115 carabid species, the hygrophilous *Bembidion minimum* and *B. varium* were attracted in significantly greater numbers to polarized than to unpolarized light at both sites. This is the first report about probable polarotaxis of carabid species. Other six ground beetle species seem to be candidates for polarization sensitivity, but this awaits further confirmation.

Key words: Carabidae, light-trapping, polarotaxis, *Bembidion* spp

Introduction

Light can be characterised not just by its colour, but also by its polarization. The visual system of many insect species is sensitive to polarized light due to the arrangement of the photopigments in the receptor cells. Some insects use the polarization pattern of the skylight for navigation during daylight, twilight (Horváth & Varjú, 2004), or moonlight (Dacke *et al.*, 2003). Many aquatic insects can detect water bodies, and use this as cue to their habitat, with the help of the water-surface-reflected, polarized light (Schwind, 1991, 1995; Horváth *et al.*, 1998; Horváth & Varjú, 2004).

Light is a transversal electromagnetic wave with oscillating electric and magnetic vectors perpendicular to the direction of travelling (Fig. 1a). Wavelength is referred to as colour, while the orientation of the vectors gives its polarization. Electric vectors in unpolarized light can be oriented, with equal probability, to any direction in a co-ordinate system perpendicular to the direction of travelling. Light with e-vectors arranged in a given direction is called linearly polarized light and can be characterised by (i) the degree of polarization (0% for unpolarized, while 100% for totally polarized light), and (ii) the direction of polarization. The major natural sources of linearly polarized light are the scattering of sunlight in the atmosphere, and light reflected from smooth surfaces. The reflection-polarization patterns of most horizontal surfaces (natural or artificial) are dominated by horizontally, linearly polarized light (Bernáth *et al.*, 2004).

Polarization sensibility is expected to be widespread among insects, many of them having special dorsal ommatidia that analyse the skylight polarization pattern (Horváth & Varjú, 2004). In some species also ventral polarization-sensitive eye regions were found, that allow them to detect the reflected polarization pattern of water surfaces by polarotaxis. This is the reason why water-seeking aquatic and semiaquatic insects are attracted by any object reflecting linearly polarized light. These are normally water bodies, but also oil spots, car roofs and plastic foils attract these insects (Kriska *et al.*, 1998; Horváth *et al.*, 1998; Bernáth *et al.*, 2001a,b). So far, the ability of polarotactic habitat detection is limited to insects associated with water or moist substrata. They include aquatic Heteroptera (e.g. *Corixa*, *Gerris*, *Notonecta, Sigara* spp.), Odonata, Diptera (Chironomidae, mosquitoes), Trichoptera, Plecoptera, Ephemeroptera, and Coleoptera (Dytiscidae, Hydrophilidae) (Bernáth *et al.*, 2001b; Horváth & Varjú, 2004).

Light trapping provides a convenient method to identify water-associated insect species that can perceive polarized light and are candidates to have polarotaxis. In spite of the frequent use of light traps for insect monitoring (Szentkirályi, 2002), only two publications report the use of polarized light-emitting traps (Kovrov & Monchadskij, 1963; Danthanarayana & Dashper, 1986). In a pilot experiment, we constructed and tested light trap pairs emitting unpolarized and horizontally, linearly polarized light of identical intensity and colour to collect further, potentially polarotactic species from various insect orders. In this paper we analyse catches of ground beetles (Carabidae) only.

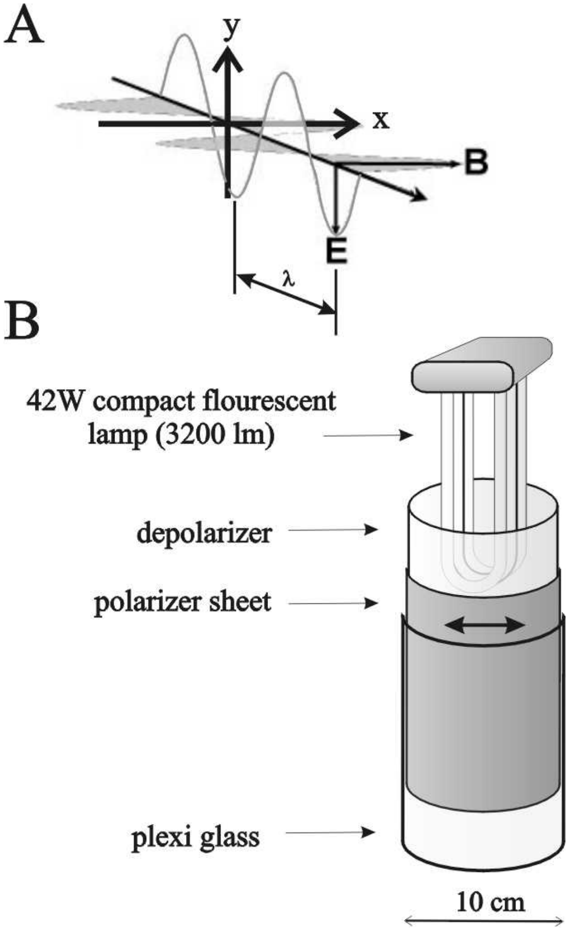

Figure 1. A: Light can be characterised by four parameters, (i) l: wavelength (λ), which is referred to as colour, (ii) I: intensity, (iii) d: degree of polarization, and (iv) a: direction of polarization. In the case of linearly polarized light, the electric (E) and magnetic vectors (B) are more or less oriented in a specific direction (direction of E vectors is referred as direction of polarization) in a (x, y) co-ordinate system defined in the sheet perpendicular to the direction of travel.
B: The structure of the light source emitting polarized light used in the light traps. Horizontal arrow shows the transmission direction of the polarizer sheet. To generate unpolarized light, a depolarizer sheet was placed between polarizer sheet and the plexi-glass cylinder.

In the Hungarian light trap network, hundreds of phototactic ground-beetle species supplied many data sets for studies of seasonality, long-term population dynamics, and biodiversity changes (Kádár & Lövei, 1987, 1992; Kádár & Szél, 1995; Kádár & Szentkirályi, 1997, 1998). Numerous carabid species are hygrophilous, and they are usually more numerous in light trap catches than other species because of their good flight ability. These species emigrate from the drying habitats in search for more suitable ones (Kádár & Szentkirályi, 1997). Carabid species associated with wet ecotones, between watercourses or standing water bodies and their banks are expected to use the water-reflected polarized light for habitat detection. We hypothesised that these ground beetles are polarization-sensitive species, and would appear in greater numbers in polarized than in unpolarized light emitting traps. Such an ability by ground beetles has not been recorded either in catches of polarized light trappings, or in studies on polarotaxis by water-seeking insects (Kovrov & Monchadskij, 1963; Danthanarayana & Dashper, 1986; Kriska *et al*., 1998; Horváth *et al*., 1998; Bernáth *et al*., 2001a,b). The aims of our study were (1) to detect any differences in structural characteristics of assemblages attributed to the greater attraction of horizontally polarized light (treatment) compared with unpolarized light (control); (2) to find species attracted or repelled by polarized light based on comparative analyses of catching rates of trap pairs. This paper reports for the first time about the attraction of certain ground beetle species to horizontally linearly polarized light.

Material and methods

Trapping sites and habitat types

Two trapping sites, one wet, and one dry were chosen on the Great Hungarian Plain, central and southern Hungary. The distance between them was 108 km. A polarized and unpolarized light trap pair was set up in both sites 80 m apart from each other to avoid any interference.

The wet habitat was near Maroslele (46.23˚N, 20.37˚E), in the Körös-Maros National Park: The traps operated in the middle of a 6 m high flood bank at the edge of a riparian forest alongside the River Maros. The bank was covered by natural grassy vegetation, mowed twice yearly. The mixed forest contained old growth poplar (*Populus* spp.), willow (*Salix* spp.), and oak (mainly *Quercus robur*) stands. Several small seasonal ponds were scattered in the understorey. The river was ca. 500 m from the traps. The forest edge was approximately 10 m from the traps, and the light source was at the height of the lower part of the canopy. The power-supply was gained from a nearby house.

The dry habitat was near Fülöpháza (46.87˚N, 19.42˚E), on the ridge of a long sand dune within the Kiskunság National Park. On the top of the sand hill the characteristic vegetation type was open sandy grassland (*Festucetum vaginatae* association), while on the lower parts, and between dunes the surface was covered by closed dry grassland. Scattered individuals of juniper (*Juniperus communis*) and small groups of white poplar (*Populus alba)*, and black locust (*Robinia pseudoacacia*) trees grew on the sand dunes. Other habitats in the vicinity of traps included abandoned gardens with fruit trees, grapes, some arable fields, and abandoned weedy fields. The nearest water body, an alkaline lake, was 1.5 km from the trapping site.

Trap design and trapping characteristics

In both sites we applied the Jermy-type light trap, widely in use by the Hungarian light trap network (Szentkirályi, 2002). In our experiment, the light source was 2 m above the ground. The killing agent was chloroform vaporising from a 12 cm long, 4 cm wide linen-sack filled with cotton, changed daily. Traps were installed with light sources emitting horizontally linearly polarized and unpolarized light, respectively (see Fig. 1b). In both traps the light source was a compact fluorescent lamp of the same type (Philips PL-T 42W/830/4p). A polarizer sheet (KÄSEMAN B+W P-W64) that surrounded the lamp within a plexi-glass cylinder (Fig. 1b) produced the polarized light. To emit light from the unpolarized and polarized light trap with the same intensity and spectral composition, transparent depolarizer sheets were applied to the two traps on the inner and the outer side of the cylindrical depolarizer sheet respectively (Fig. 1b). All light source characteristics of the traps were the same in both sites, except the presence of three metal baffles around the bulb in the sand dune traps.

The traps were set up so that there were no bushes or trees between them. The insects approaching the traps were able to see both light sources. After the first year, the two traps were transposed within habitats. The traps, controlled by twilight switch, were operated every night from 1 April to 31 October 2001 and 2002. Technical problems between mid-July and mid-August, 2002 caused a collection gap at Maroslele. Catches from this site and year were not analysed in this paper.

Data processing and statistical analyses

Due to logistical problems, there was one pair of traps per site, and thus was no real replication within the seasons, limiting the possible statistical evaluation both at assemblage and species levels. For between-trap comparisons of yearly assemblages, we used clustering (UPGMA) with the Bray-Curtis index of similarity (STATISTICA, 2000). Carabid species were selected for comparative statistical analyses if (i) ≥ 40 individuals/trap pair were caught, and (ii) their total yearly catch by trap type differed by a factor of ≥ 1.5. The flight of each individual was considered independent. Yates-corrected χ^2 test was used to detect polarization preferences.

Standard weekly catches were calculated to use for characterisation of the seasonal synchrony and for the binomial test between collections of trap pairs. The degree of synchrony between seasonal activity patterns produced by the polarized and unpolarized light sources was measured with a cross correlation function (CCF) method used in time series analysis. A statistically significant positive CCF indicated highly synchronous flight pattern. In case of very high difference or low captures, the CCF was not applicable (see Table 3). To examine the higher trapping capability of the polarized light emitting trap for a given species we performed binomial tests using the difference between the weekly numbers of individuals caught by the trap pairs over the season (STATISTICA, 2000).

Results

Characteristics of carabid assemblages collected by polarized and unpolarized light

Large number of captured individuals per year were registered at both sites, ranging between 1800 and 4900, except the polarized trap in the dry habitat, which attracted > 26,000 specimens in 2001 (Table 1). This was 14 times greater than the catch of the unpolarized light trap. Nearly equal numbers were captured in 2002. At the riparian forest site (Maroslele) the polarized light-emitting trap collected 1.3 times more individuals than the control trap.

A total of 115 ground beetle species was collected during the two years, 82 species in the wet habitat, and 96 in the dry habitat. The number of species per season was 59-72 species by polarized light and 53-70 species by unpolarized light (Table 1). The majority (62.6%) of the 115 species was hygrophilous, half of them classified as waterside inhabitants. A smaller part of the catch (17.4%) was composed of habitat generalist species. Four more species flew to polarized than to unpolarized light in 2001 in wet and 12 more species in dry habitat. The opposite occurred in 2002: the unpolarized light trap caught 11 species more than the polarized one (Table 1). The Jaccard-similarity index indicated moderate similarities between the assemblages caught with polarized and control light (0.58-0.59). The quantitative Bray-Curtis

similarity index dendrogram separated the assemblages by sites rather than light type. The assemblage sampled with polarized trap during 2001 at dry site was strongly separated from the others because of the extremely high number of individuals in samples. A greater similarity was also shown by the dendrogram between carabid assemblages recorded at Fülöpháza on unpolarized in 2001 and polarized light source in 2002. These finding reflected to a local effect caused by the same trap-position/surrounding inside the dune habitat.

Table 1. Characteristics of ground beetle assemblages captured in light traps with horizontally linearly polarized or unpolarized light sources, near the gallery forest of the river Maros (Maroslele) or on a sand dune (Fülöpháza), Hungary, 2001-2002.

Characteristic	Maroslele, 2001		Fülöpháza, 2001		Fülöpháza, 2002	
	Polarized light	Un-polarized light	Polarized light	Un-polarized light	Polarized light	Un-polarized light
Number of individuals	4829	3610	26107	1887	2916	3153
Number of species	72	68	65	53	59	70
Total number of spp/site*year	82		72		81	
No. of water-side inhabiting species	27	24	27	20	19	23
No. of hygrofrequent species	20	22	19	17	22	23
Number of habitat generalist species	20	16	17	14	14	19
Cumulative relative abundance of						
Water-side inhabitant species, %	49.5	43.0	28.3	17.7	20.1	10.5
Hygrofrequent species, %	30.8	52.3	64.8	66.0	58.0	52.4
Habitat generalist spp, %	19.4	4.2	6.9	16.2	21.8	37.0

The hygrophilous carabids were divided into two categories: typically waterside inhabitants and hygrofrequent species (these are frequently found in wet habitats/soil surfaces but not necessarily close to water edges). At both sites in 2001, the polarized light traps captured more waterside-inhabitant species than the unpolarized ones (Table 1). When the traps were interchanged in 2002, the local difference remained, meaning that in this year four more waterside inhabitant species were captured in non-polarized than in the polarized trap. A similar trend was detected for the number of species of habitat generalist carabids. In the number of hygrofrequent species (range: 17-23 spp.), no characteristic change was found.

Comparing the percent of total number of individuals (cumulative relative abundance values) of the typical waterside inhabitant carabid group within the collected assemblages, a clear trend appears. Considering the relative abundance of habitat affinity groups, the waterside-inhabitants had a higher share at both sites and seasons in the polarized light-emitting traps (range: 20-50%) than in unpolarized light traps (range: 10-43%) (Table 1). In case of the hygrofrequent or habitat generalist carabids no such relationship appeared.

Table 2. Total yearly numbers of individuals of selected carabid species collected by light traps with linearly polarized and unpolarized light source at two different locations in Hungary, 2001-2002.

Species	Maroslele, 2001		Fülöpháza, 2001		Fülöpháza, 2002	
	Polarized light	Un-polarized light	Polarized light	Un-polarized light	Polarized light	Un-polarized light
Bembidion minimum	305	163	7189	309	360	194
Bembidion varium	673	116	6111	182	349	49
Paratachys bistriatus	496	590	7781	536	804	696
Clivina fossor	103	18	199	25	65	64
Stenolophus discophorus	712	221	32	0	2	1
Ophonus rufibarbis	405	25	40	6	11	5
Pseudoophonus rufipes	148	17	467	62	25	30
P. calceatus	214	26	77	6	67	118

Carabid species that flew in higher numbers to polarized than unpolarized light

Only eight of the 115 species (listed in Table 2) were captured in higher numbers in polarized light traps than in unpolarized ones. Five of them were hygrophilous carabids: *Bembidion minimum*, *B. varium*, *Paratachys bistriatus*, *Clivina fossor*, and *Stenolophus discophorus*. The other three species, *Ophonus rufibarbis*, *Pseudoophonus rufipes*, and *P. calceatus* are common, habitat generalist ground beetles. The differences in their catches were 6-24 fold.

B. minimum, *B. varium*, and *P. bistriatus*, whose catches exceeded 6000 individuals in the polarized trap, had the highest differences. At the wet site (Maroslele) in 2001, the rates of polarized/unpolarized catches varied between 1.9-16.0. The only exception was *P. bistriatus*, where more specimens were collected by the control trap. In 2002 at the dry site, the polarized/unpolarized ratios were found to be higher than 1.5 only for three species: *B.*

minimum (1.8), *B. varium* (7.1), and *O. rufibarbis* (2.2). The other remaining five carabid species were captured either in nearly similar numbers (*C. fossor*, *P. rufipes*, *S. discophorus, P. bistriatus*), or even in higher number in the unpolarized light trap (*P. calceatus*).

In 2001, all eight species at both sites had significant differences in captures by trap type (Table 3). In 2002, at the dry site the value of χ^2 was found significantly ($P<0.001$) higher than expected only for the two *Bembidion* species. Polarized light-emitting traps attracted significantly more individuals of *B. minimum*, and *B. varium* in three trapping cases, and of the other six carabid species (*P. bistriatus*, *C. fossor*, *S. discophorus*, *O. rufibarbis*, *P. rufipes*, and *P. calceatus*) in two cases (in two sites) comparing with catches of unpolarized traps.

Table 3. Comparison of catches of selected carabid species in light traps with polarized vs. unpolarized light source at two locations in Hungary. Results of Chi-square test and the significant cross correlation functions are given.

Species	Maroslele, 2001		Fülöpháza, 2001		Fülöpháza, 2002	
	χ^2	r_0	χ^2	r_0	χ^2	r_0
Bembidion minimum	21.43^{***}	0.91	3995.70^{***}	0.82	24.83^{***}	0.89
B. varium	223.08^{***}	-	3587.30^{***}	0.73	130.03^{***}	-
Paratachys bistriatus	4.00^{*}	0.65	3892.40^{***}	0.89	3.80 NS	0.79
Clivina fossor	31.93^{***}	-	77.80^{***}	0.90	0.00 NS	0.90
Stenolophus discophorus	137.74^{***}	0.78	18.75^{***}	-	-	-
Ophonus rufibarbis	206.45^{***}	-	12.89^{***}	-	-	-
Pseudoophonus rufipes	59.98^{***}	-	179.97^{***}	-	-	-
P. calceatus	85.13^{***}	-	35.23^{***}	-	2.74 NS	-

Trapping sites: Maroslele, Fülöpháza; χ^2: value of Chi-square test (Yates-corrected, df =1); NS: not significant, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$; r_0: significant ($P<0.05$) positive value of cross correlation function without lag.

Where the weekly catches were comparable (see under data processing and statistical analyses) a high degree of synchrony (significant, higher positive r_0 values of CCF) existed between the seasonal activity patterns (Fig. 2, Table 3).

Significantly consistently higher catches in polarized light-emitting traps were found in three cases only for *B. varium*, and in two cases (Fülöpháza, 2001 & 2002) for *B. minimum* (binomial test, $P<0.01$ and $P<0.05$) (Fig. 2). For the remaining six carabid species the binomial test verified significantly higher catches of the polarized light-emitting trap only on the sand dune in 2001 ($P<0.001$: *P. bistriatus*; $P<0.01$: *P. calceatus*, *C. fossor*, *S. discophorus*; $P<0.05$: *O. rufibarbis*, *P. rufipes*). In the other cases the differences were not significant.

Eight other species, even if captured in smaller numbers, seemed to prefer polarized light: *Omophron limbatum* (Fabricius 1776), *Chlaenius spoliatus* (Rossi 1790), *Stenolophus skrimshiranus* Stephens 1825, *Bembidion guttula* (Fabricius 1792), *B. quadripustulatum* (Serville 1821), *B. semipunctatum* (Donovan 1806), *Dyschirius chalybaeus gibbifrons* Apfelbeck 1899, and *D. aeneus* Dejean 1825. These species are hygrophilous, waterside inhabitants, and make likely our hypothesis that several such carabids may be polarization sensitive. However, further investigations are requested to demonstrate their ability to perceive horizontally polarized light.

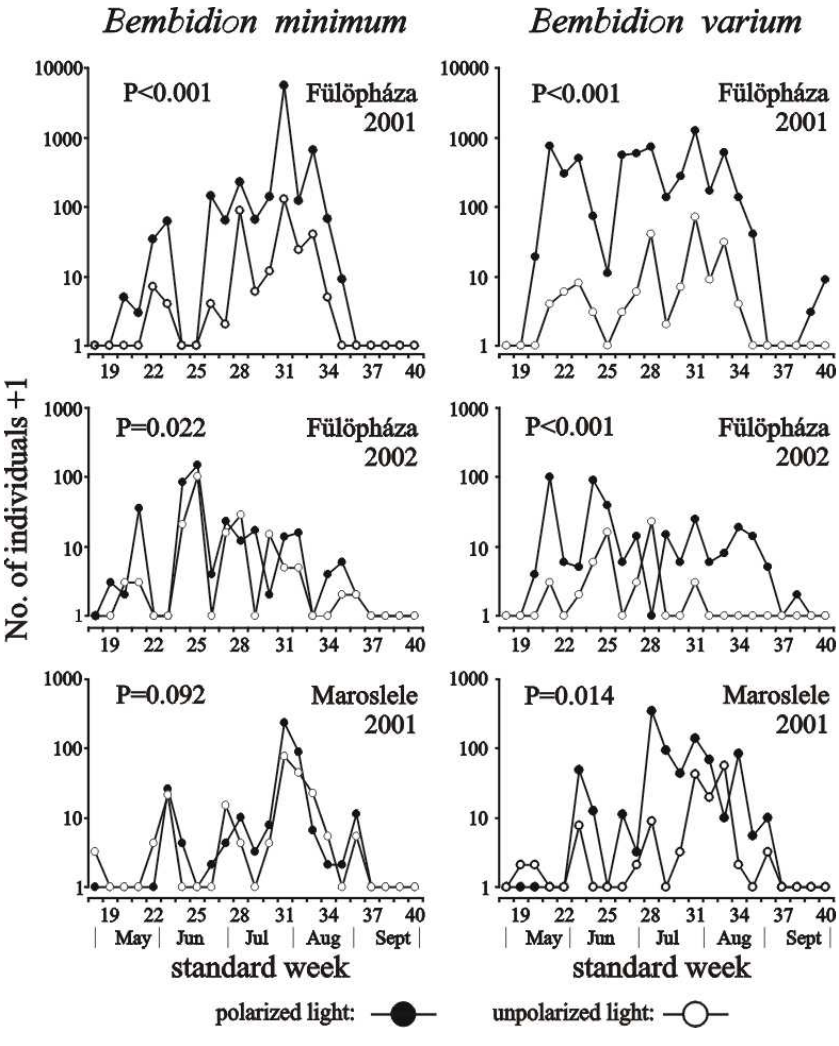

Figure 2. Seasonal flight patterns of the two *Bembidion* species by the weekly catches of polarized and control light-traps in a dry (Fülöpháza) and a wet (Maroslele) habitat in 2001 and 2002. Y-axes are given in logarithmic scale. (P values show the level of significance in binomial test. Time on the x-axis is Julian date (weeks).

Discussion and conclusions

Among the total of 115 species captured, only eight were more common in horizontally polarized light traps. Consistent and significantly higher attraction to polarized light was demonstrated only for *Bembidion varium* and *Bembidion minimum*. These two carabid species are spring breeders (Lindroth, 1985), have long seasonal flying activity (see in Fig. 2), and provide enough information to support the hypothesis that they are polarization sensitive. Both are waterside inhabitant species, so polarotactic behaviour possibly may play a role in their habitat finding. The other six carabid species should remain candidates to have capability for the perception of horizontally polarized light. Stronger synchrony was found between the seasonal dynamics gained from collection by the two light types in case of more species. This fact means on the one hand that the traps in distance of 80 m collected samples from the same local populations, and on the other hand that the polarized light did not cause any shift or modification in seasonal flight activity of ground beetles. Short and sporadic seasonal flight activity may have contributed to the lack of significant results in binomial tests, even though the total yearly catches were many times greater in polarized light traps than in the controls.

Although our results showed clearly that certain species may be more strongly lured by polarized light, it is still questionable that nocturnal flying carabids are able to perceive water-reflected polarized light which have very low intensity under weak nightly illumination. Since the polarization sensitivity of visual system of aquatic insects was discovered in the early 1990es (Schwind, 1991, 1995), several such questions remain open. However, field crickets (*Gryllus campestris*) are able to perceive the polarized light at lower intensity levels than available on a moonless night sky (Herzmann & Labhart, 1989), and a few recent experiments demonstrated unexpected sensitivities in nocturnal insect vision. Kelber *et al.* (2002) pointed out that nocturnal hawkmoths are able to discriminate coloured stimuli even at illumination of extremely low intensity (dim starlight, 0.0001 cd m^{-2}). The photoreceptors in the eye the of a *Scarabaeus* beetle were found to be able to perceive the night-sky polarization pattern produced by very low intensity moonlight (Dacke *et al*., 2003). Polarotaxis can be used by day-flying carabids, frequently captured by window traps (van Huizen, 1979). As far as we know there is no data on the sensitivity of carabid eyes in this respect.

Another question is how carabid beetles can perceive the horizontally polarized light? Detectors for polarized skylight were documented in many insect orders as a specialised ommatidial area at the dorsal rim of compound eye (Labhart & Meyer, 1999). It seems that a diffuse ommatidial structure, a set of specialised photoreceptors at the ventral region of compound eyes is responsible for polarotaxis of aquatic insects (Schwind, 1995; Horváth & Varjú, 2004). Further studies may shed light on the possible presence of such a structure in carabid beetles.

Our results provide the first documentation of possible polarotactic behaviour in ground beetles. In spite of local effect caused by the same trap-position/surrounding inside the dune habitat, higher catching rate was statistically significant for two species, *Bembidion varium*, and *B. minimum*. Six further species should be considered polarization sensitive. To confirm this hypothesis further field investigations are requested using more light traps installed near to each other within the same habitat, to ensure the within-year replications, and to avoid effects of habitat structure on catching rate via trap position.

Acknowledgements

We thank László Kanyó and Dóra Misinszky for the daily operating of our traps, György Rozner and Kriszta Kádár for sorting the ground beetles from the total catch, Béla Kalivoda and Zoltán Vajda from the Directorates of Körös-Maros National Park and Kiskunság National Park, respectively, for their kind permission to operate the light traps. This work was supported by the Hungarian National Research and Development Fund project no. NKFP-3B/0008/2002.

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Carabid conservation within a nature reserve network established for birds

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Abstract

The representation of carabid species on the 182 reserves of the UK's premier bird conservation organisation was assessed. 211 of 356 species (59%) are known to occur on reserves. Some of the missing species are associated with habitats like exposed riverine sediments that are poorly represented on reserves. However, more species certainly remain to be discovered by further survey. Targeted survey work has found 11 of 20 Biodiversity Action Plan species (35%), which are a priority for UK conservation.

Introduction

The Royal Society for the Protection of Birds (RSPB) manages 182 nature reserves spread across the United Kingdom (UK), amounting to 126,846 ha (over 0.5% of the UK land area). This network of nature reserves has been gradually established since 1930 primarily to conserve wild birds but makes an important contribution to the conservation of wider biodiversity and habitats in the UK. The reserves are actively managed to maintain or enhance their key features, which may be birds or other biodiversity. A rigorous management planning process takes account of situations where key features have conflicting management requirements.

The RSPB runs a set of projects to conserve and enhance non-bird biodiversity on reserves, including:

- Biodiversity Survey Programme to establish what species are present on reserves;
- Biodiversity Monitoring Programme to ensure that the RSPB is aware of the status of important species on reserves and that they are being managed appropriately;
- Taxa Teams Project to develop natural history skills across the RSPB staff, leading to more and better survey and monitoring work, and ultimately to better conservation.

How good is the RSPB reserve network at conserving carabids?

A previous assessment of the RSPB reserve network for its contribution to the conservation of carabids and eight other taxonomic groups was made by Hopkinson *et al*. (2000). They looked at the coincidence of reserves with hotspots (areas of high species richness) and complementary areas (sets of sites within which all species are represented). They also looked simply at the total numbers of species recorded from the reserve networks. Their results by all three approaches suggested, rather tentatively, that the RSPB reserve network is comparatively poor for carabid conservation.

Here I use one simple method to evaluate carabid conservation on the RSPB reserve network, namely by looking at the number of carabid species known to be present on at least one UK reserve.

Britain is one of the best-recorded countries for carabid beetles and much of the available information has been collated and computerised by the UK Ground Beetle Recording Scheme (GBRS), organised by Dr. Martin Luff from 1974 to 2000, and by MGT to the present day. The GBRS database contains over 210,000 records, made by 2000 recorders and over 500 determiners. In addition to records provided by recorders, records have also been gleaned from nearly 50 museum collections, and nearly 400 literature sources. Overall validation of the dataset has been provided by Dr. Luff and more recently by MGT. Carabid records from RSPB reserves were collated from the RSPB's own records, from various invertebrate survey reports, and from the GBRS database. This collation of records has been reasonably comprehensive but cannot claim to be complete. I have considered 356 of the total 371 UK carabid species (Luff & Duff, 2002; Telfer, 2003). The nomenclature used here follows the most up-to-date checklist of British carabids (Luff & Duff, 2002). The 15 excluded species have only occurred in the UK as immigrants or short-lived introductions, or have only been recorded as sub-fossils. I have also excluded the Channel Islands which have two or three carabid species that have not been recorded from elsewhere in the UK.

To date, 211 carabid species (59% of the UK list) have been recorded on reserves (Table 1), and this figure will grow with further survey work. All those species with a conservation status are detailed in Table 2. Comparable figures are available for two other groups of invertebrates: dragonflies (Odonata) and spiders (Araneae). Dragonflies are a popular and comparatively well-recorded group in the UK, and 94.5% of the UK's 55 Odonata species have been recorded from RSPB reserves (all except for three species which are extinct in the UK). Spiders are a much less popular group but the RSPB's Spider Taxa Team has been particularly active in recent years and 76% of the UK's 650 spiders have now been recorded from reserves.

The most important role of nature reserves is in conserving rare and restricted range species. Though it is desirable that the reserves should also provide habitat for more common and widespread carabids, these species are reasonably safe in the wider countryside. The RSPB

Table 1. Numbers of carabid species on the RSPB reserve network, including a break-down by conservation status category (see legend for explanation of these).

	Common	Nationally Scarce	Red Data Book species	Biodiversity Action Plan species	New to Britain >1992	Total
Present	146	49	13	11	3	211
Not recorded	31	71	41	20	2	145
% present	82	41	24	35	60	59
Total	177	120	54	31	5	356

Conservation status categories: **Biodiversity Action Plan** species have been selected as priorities for UK government conservation action in response to the Rio convention. Listed in UK Biodiversity Steering Group (1995), and UK Biodiversity Group (1999a, b). **Red Data Book** - Rare (RDB3), Vulnerable (RDB2), Endangered (RDB1), Indeterminate (RDBI), Insufficiently Known (RDBK) or Extinct (RDB EXTINCT) species with ranges of less than fifteen '10-km squares' (10 km × 10 km squares of the UK mapping grid) as listed by Hyman & Parsons (1992). **Nationally Scarce** - range sizes between 16 and 30 (Na) or 31 and100 (Nb) 10-km squares, as listed by Hyman & Parsons (1992). **Common** - all other species.

reserves support 82% of the UK's common carabids (146 of 177 species) (Table 1). For Nationally Scarce species, the proportion is much lower at 41% (49 of 120 species), and lower still for the Red Data Book species at 24% (13 of 54 species). However, it is good to see that the proportion of Biodiversity Action Plan (BAP) species on reserves is higher at 35% (11 of 31 species). BAP species are of the highest conservation priority and the Biodiversity Survey Programme has been successfully targeting this group of species on reserves, as these results show.

Of the five species added to the UK list since Hyman and Parsons (1992), three are recent natural colonists from continental Europe. *Bembidion coeruleum* Serville, 1826 and *Acupalpus maculatus* Schaum, 1860 were first discovered on the Dungeness RSPB reserve (Telfer, 2001; Telfer, 2003). *Microlestes minutulus* (Goeze, 1777) is becoming quite widespread in south-east England and has been recorded from Dungeness and Cliffe Pools RSPB reserves. Dungeness is a unique expanse of coastal sand and gravel in the extreme south-east of England - France is only about 30 km away. The variety of open habitats at the margins of the flooded sand and gravel pits on the RSPB reserve have allowed several carabids and other insects to colonise the UK from across the English Channel. Parts of the reserve contain early-successional bare, wet sediments of fine sand and gravel, supporting *Omophron limbatum* (Fabricius, 1777), *Dyschirius obscurus* (Gyllenhal, 1827), *Bembidion pallidipenne* (Illiger, 1802) and other beetles. After recognition of the value of these habitats for beetles, plans to plant reed *Phragmites australis* to enhance the habitat for birds have been abandoned, and the areas are now managed principally to maintain their beetle interest.

Table 2. All British carabids with a conservation status, or which have been recorded new to Britain since 1992 are listed here in Luff and Duff (1992) sequence with their status from the National Review (Hyman & Parsons 1992) (see legend to Table 1 for brief explanations of status categories). Biodiversity Action Plan Priority species and species recorded from the RSPB reserves are indicated with a 'Yes'.

Species	Priority Species?	National Review	RSPB reserve record?
Omophron limbatum (Fabricius, 1777)		RDB1	Yes
Calosoma inquisitor (L. 1758)		Na	Yes
*Carabus clatratus (*L., 1761)		Na	Yes
Carabus monilis (Fabricius, 1792)		Nb	
*Carabus nitens (*L., 1758)		Nb	
Leistus montanus (Stephens, 1827)		Na	
Nebria complanata (L., 1767)		Na	
Nebria livida (L., 1758)		Na	
Nebria nivalis (Paykull, 1790)		Na	
Pelophila borealis (Paykull, 1790)		RDB3	Yes
Notiophilus aesthuans (Motschulsky, 1864)		Nb	
Notiophilus quadripunctatus (Dejean, 1826)		Nb	
Cicindela hybrida (L., 1758)	Yes	RDB3	
Cicindela maritima (Latreille & Dejean, 1822)	Yes	Nb	Yes
Cicindela sylvatica (L., 1758)	Yes	Na	Yes
Cicindela germanica (L., 1758)	Yes	RDB3	
Elaphrus lapponicus (Gyllenhal, 1810)		Na	
Elaphrus uliginosus (Fabricius, 1792)		Nb	Yes
Blethisa multipunctata (L., 1758)		Nb	Yes
Dyschirius angustatus (Ahrens, 1830)	Yes	RDB3	Yes
Dyschirius obscurus (Gyllenhal, 1827)		RDB2	Yes
Dyschirius extensus (Putzeys, 1845)		RDB1	
Dyschirius impunctipennis (Dawson, 1854)		Nb	
Dyschirius nitidus (Dejean, 1825)		Na	
Miscodera arctica (Paykull, 1798)		Nb	Yes
Perileptus areolatus (Creutzer, 1799)	Yes	Na	
Aepus marinus (Ström, 1783)		Nb	
Aepus robinii (Laboulbène, 1849)		Nb	
Trechus rivularis (Gyllenhal, 1810)		RDB3	
Trechus fulvus (Dejean, 1831)		Nb	
Trechus rubens (Fabricius, 1792)		Nb	Yes
Trechus subnotatus (Dejean, 1831)		RDB1	
Thalassophilus longicornis (Sturm, 1825)		Na	
Lasiotrechus discus (Fabricius, 1792)		Nb	
Asaphidion pallipes (Duftschmid, 1812)		Nb	
Bembidion bipunctatum (L., 1761)		Nb	Yes
Bembidion pallidipenne (Illiger, 1802)		Nb	Yes
Bembidion nigricorne (Gyllenhal, 1827)		Nb	
Bembidion obliquum (Sturm, 1825)		Nb	
Bembidion semipunctatum (Donovan, 1806)		Na	Yes
Bembidion ephippium (Marsham, 1802)		Na	Yes
Bembidion virens (Gyllenhal, 1827)		RDB3	
Bembidion coeruleum (Serville, 1826)		Not Incl.	Yes
Bembidion fluviatile (Dejean, 1831)		Nb	
Bembidion lunatum (Duftschmid, 1812)		Nb	Yes
Bembidion monticola (Sturm, 1825)		Nb	
Bembidion saxatile (Gyllenhal, 1827)		Nb	
Bembidion testaceum (Duftschmid, 1812)	Yes	Nb	
Bembidion stomoides (Dejean, 1831)		Nb	

Species	**Priority Species?**	**National Review**	**RSPB reserve record?**
Bembidion inustum (Jacquelin du Val, 1857)		Not Incl.	
Bembidion nigropiceum (Marsham, 1802)	Yes	Na	
Bembidion gilvipes (Sturm, 1825)		Nb	Yes
Bembidion schuppelii (Dejean, 1831)		Na	
Bembidion clarkii (Dawson, 1849)		Nb	Yes
Bembidion fumigatum (Duftschmid, 1812)		Nb	Yes
Bembidion quadripustulatum (Audinet-Serville, 1821)		Nb	Yes
Cillenus lateralis (Samouelle, 1819)		Nb	
Bracteon argenteolum (Ahrens, 1812)	Yes	RDBK	Yes
Bracteon litorale (Olivier, 1790)		Nb	
Tachys bistriatus (Duftschmid, 1812)		Nb	Yes
Tachys scutellaris (Stephens, 1828)		Na	Yes
Elaphropus parvulus (Dejean, 1831)		Nb	
Pogonus littoralis (Duftschmid, 1812)		Nb	
Pogonus luridipennis (Germar, 1822)		RDB3	
Patrobus septentrionis (Dejean, 1828)		Nb	
Poecilus kugelanni (Panzer, 1797)	Yes	RDB1	Yes
Poecilus lepidus (Leske, 1785)		Nb	
Pterostichus cristatus (Dufour, 1820)		Nb	
Pterostichus aethiops (Panzer, 1796)		Nb	Yes
Pterostichus longicollis (Duftschmid, 1812)		Nb	Yes
Pterostichus oblongopunctatus (Fabricius, 1787		Nb	Yes
Pterostichus quadrifoveolatus (Letzner, 1852)		Nb	
Pterostichus anthracinus (Panzer, 1795)		Nb	Yes
Pterostichus gracilis (Dejean, 1828)		Nb	Yes
Calathus ambiguus (Paykull, 1790)		Nb	Yes
Platyderus ruficolli (Marsham, 1802)		Nb	
Sericoda quadripunctata (De Geer, 1774)		RDB1	
Agonum livens (Gyllenhal, 1810)		Nb	
Agonum scitulum (Dejean, 1828)		Na	Yes
Agonum ericeti (Panzer, 1809)		Nb	Yes
Agonum gracilipes (Duftschmid, 1812)		Na	
Agonum nigrum (Dejean, 1828)		Nb	Yes
Agonum sahlbergii (Chaudoir, 1850)		RDB Ext.	
Agonum sexpunctatum (L., 1758)		Na	
Agonum versutum (Sturm, 1824)		Nb	
Zabrus tenebrioides (Goeze, 1777)		Na	
Amara strenua (Zimmermann, 1832)	Yes	RDB3	Yes
Amara curta (Dejean, 1828)		Nb	
Amara famelica (Zimmermann, 1832)	Yes	RDB3	
Amara lucida (Duftschmid, 1812)		Nb	Yes
Amara nitida (Sturm, 1825)		Na	
Amara spreta (Dejean, 1831)		Nb	
Amara infima (Duftschmid, 1812)		Na	
Amara praetermissa (Sahlberg, C.R., 1827)		Nb	
Amara quenseli (Schönherr, 1806)		Na	
Amara consularis (Duftschmid, 1812)		Nb	Yes
Amara fulva (Müller, O.F., 1776)		Nb	Yes
Amara equestris (Duftschmid, 1812)		Nb	Yes
Curtonotus alpina (Paykull, 1790)		RDB3	
Harpalus froelichii (Sturm, 1818)	Yes	RDB2	Yes
Harpalus cupreus (Dejean, 1829)		RDB1	
Harpalus dimidiatus (Rossi, 1790)	Yes	Na	
Harpalus honestus (Duftschmid, 1812)		RDB1	
Harpalus melancholicus (Dejean, 1829)		RDB1	
Harpalus pumilus (Sturm, 1818)		Na	
Harpalus quadripunctatus (Dejean, 1829)		Na	Yes

Species	**Priority Species?**	**National Review**	**RSPB reserve record?**
Harpalus serripes (Quensel in Schönherr, 1806)		Nb	
Harpalus servus (Duftschmid, 1812)		Nb	
Harpalus smaragdinus (Duftschmid, 1812)		Nb	
Harpalus tenebrosus (Dejean, 1829)		Na	
Ophonus ardosiacus (Lutschnik, 1922)		Nb	Yes
Ophonus azureus (Fabricius, 1775)		Nb	
Ophonus sabulicola (Panzer, 1796)		RDB3	
Ophonus stictus (Stephens, 1828)	Yes	RDB1	
Ophonus cordatus (Duftschmid, 1812)	Yes	RDB3	
Ophonus melletii (Heer, 1837/8)		Na	
Ophonus parallelus (Dejean, 1829)	Yes	RDB3	Yes
Ophonus punctatulus (Duftschmid, 1812)	Yes	Na	Yes
Ophonus puncticollis (Paykull, 1798)		RDB3	
Ophonus rupicola (Sturm, 1818)		Nb	
Ophonus schaubergerianus (Puel, 1937)		Nb	
Anisodactylus nemorivagus (Duftschmid, 1812)	Yes	Na	
Anisodactylus poeciloides (Stephens, 1828)	Yes	RDB3	Yes
Diachromus germanus (L., 1758)		RDB Ext.	
Stenolophus skrimshiranus (Stephens, 1828)		Na	
Stenolophus teutonus (Schrank, 1781)		Nb	
Bradycellus csikii (Lacz¢, 1912)		RDBI	Yes
Bradycellus distinctus (Dejean, 1829)		Na	
Dicheirotrichus obsoletus (Dejean, 1829)		Nb	Yes
Acupalpus brunnipes (Sturm, 1825)		Na	Yes
Acupalpus elegans (Dejean, 1829)		RDB Ext.	
Acupalpus exiguus Dejean, 1829		Nb	Yes
Acupalpus flavicollis (Sturm, 1825)		Na	
Acupalpus maculatus (Schaum, 1860)		Not Incl.	Yes
Anthracus consputus (Duftschmid, 1812)		Nb	Yes
Licinus depressus (Paykull, 1790)		Nb	
Licinus punctatulus (Fabricius, 1792)		Na	Yes
Badister meridionalis (Puel, 1925)		RDBI	Yes
Badister unipustulatus (Bonelli, 1813)		Nb	Yes
Badister collaris (Motschulsky, 1860)	Yes	RDB1	Yes
Badister dilatatus (Chaudoir, 1837)		Nb	Yes
Badister peltatus (Panzer, 1797)	Yes	Na	Yes
Oodes helopioides (Fabricius, 1792)		Nb	
Panagaeus bipustulatus (Fabricius, 1775)		Nb	
Panagaeus cruxmajor (L., 1758)	Yes	RDB1	
Chlaenius nigricornis (Fabricius, 1787)		Nb	Yes
Chlaenius nitidulus (Schrank, 1781)		RDB1	
Chlaenius tristis (Schaller, 1783)		RDB1	
Callistus lunatus (Fabricius, 1775)		RDB1	
Odacantha melanura (L., 1767)		Nb	Yes
Masoreus wetterhallii (Gyllenhal, 1813)		Na	
Lebia chlorocephala (Hoffmann, J., 1803)		Nb	Yes
Lebia cruxminor (L., 1758)		RDB1	
Lebia marginata (Fourcroy, 1785)		RDB Ext.	
Lebia scapularis (Fourcroy, 1785)		RDB Ext.	
Demetrias imperialis (Germar, 1824)		Nb	Yes
Demetrias monostigma (Samouelle, 1819)		Nb	
Dromius longiceps (Dejean, 1826)		Na	Yes
Dromius quadrisignatus (Dejean, 1825)	Yes	RDB1	
Dromius sigma (Rossi, 1790)	Yes	Na	
Dromius vectensis (Rye, 1872)		RDB3	
Microlestes minutulus (Goeze, 1777)		Not Incl.	Yes
Lionychus quadrillum (Duftschmid, 1812)	Yes	RDB3	

Species	Priority Species?	National Review	RSPB reserve record?
Cymindis axillaris (Fabricius, 1794)		Na	
Cymindis vaporariorum (L., 1758)		Nb	
Polistichus connexus (Fourcroy, 1785)		RDB2	
Brachinus sclopeta (Fabricius, 1792)		RDB1	
Brachinus crepitans (L., 1758)		Nb	Yes

Abbreviations (see also text): RDB3 - Rare, RDB2 - Vulnerable, RDB1 - Endangered, RDBI - Indeterminate, RDB Extinct - species with ranges of less than fifteen ‘10-km squares’ (10 km × 10 km squares of the UK mapping grid). Na: nationally scarce - range sizes between 16 and 30 of 10-km squares, Nb: nationally scarce, occurrence on 31-100 10-km squares.

While 59% of UK carabid species are known from RSPB reserves, it is certain that more remain to be discovered. Targeting survey work towards BAP carabids appears to have been successful, as a higher percentage of BAP species has been recorded than would otherwise be expected. Further targeted surveys are the main priority for carabid conservation on RSPB reserves. However, some important carabid habitats are poorly represented within the reserve network, which limits the contribution the RSPB can make to carabid conservation. Monitoring and habitat management work is being carried out for some important carabids on RSPB reserves and this will become an increasing focus of activity in the future.

Acknowledgements

The GBRS is currently organised on a voluntary basis by the author. The database was largely collated by Dr. Martin Luff with the assistance of hundreds of volunteer contributors. Ian Dawson kindly provided records of spiders on RSPB reserves. Malcolm Ausden provided valuable comments on an earlier draft.

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Diversity characterizations in R

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Abstract

Diversity measuring methods are presented in the R program language with special emphasis on the modern techniques that provide scalable diversity comparisons.

Key words: Diversity indices, scalable one-parametric diversity index families, species accumulation plots

Introduction

R is a programming environment for data analysis and graphics (Ihaka & Gentleman, 1996). It is a free-ware version of the program package S-Plus. A wide range of statistical methods are implemented in R, and new applications can easily be developed (Crawley, 2002; Venables and Ripley, 2002). Knowledge of the R and/or S language is useful, although not absolutely necessary to use the procedures in the `'diversity'` package. As an introductory book, Krause and Olsen (2002) may be mentioned.

This paper provides a description and/or explanation of the diversity add-on package for R through examples. It is best used while sitting at a console with R running. This draft of the Guide should be considered a work in progress. It is not a commercial product. Effort is put into maintaining the documentation and make it accessible through the web.

How can R be installed

R can be installed through the web (http://www.r-project.org/) from a CRAN site. It contains binaries for a base distribution and a large number of add-on packages to run on Windows 95, 98, ME, NT4, 2000, and XP on Intel and clones.

Executing commands and saving output to a file

Commands can be executed via the command line of R. Longer sequences of commands or implementations of new functions can be written in a separate file. When the commands are stored in a file, named `'diversity.R'` that has to reside in the working (default) directory, then the command line statement

```
> source("diversity.R")
```

will execute the commands in `'diversity.R'` and load function code contained in `'diversity.R'` file.

If you started the R program package and you choose in the pull down menu the `'File'` and then `'Source R code'` options, you can load the source code of the R procedures and the demo datasets. You can use the usual “`Browse`” button or you make a copy of the `'diversity.R'` file in the default directory of your R version. R is usually installed into the `'C:\Program Files\R'` directory and the working directory is identical with the actual version of the program; at the time of writing the paper it is `'rw2001'`. If you make a copy into the `'C:\Program Files\R\rw2001'` directory you can load the program without browsing the directories.

You can also open `'diversity.R'` in your favourite text editor and use copy/paste to paste commands or sequences of commands into the command line. The output normally will appear on the screen only. You can change this by using the function `sink`. The command:

```
> sink("DivOutput.txt")
```

will divert all subsequent output from the console to an external file, `"DivOutput.txt"`. The command

```
> sink()
```

restores it to the console once again. Alternatively you may copy the results from the R desktop through the clipboard in the usual way. To save the figures, right-click with the mouse button when the pointer is within the figure, and chose a suitable option from the appearing menu.

What is the "diversity" package?

The `"diversity"` package is an add-on package for the R statistical computing system. Several data sets are also included with this package and will be used in examples in this guide. The examples do not include a detailed theoretical and/or technical explanation. See Patil and Taillie (1982), Tóthmérész (1993, 1995, 1997, 1998) and Tóthmérész & Magura (2005) for an introduction to the statistics of diversity.

Sample data sets

There are six sample data frames included in the package. Data frames are matrix-like structures (see e.g. Krause & Olsen, 2002). The simplest data set is named "`trichotom`", originally published by Tóthmérész (2002). It may be used to check and/or demonstrate the calculations. Tóthmérész (1993) used to illustrate the diversity ordering by the "`ABC`" data set (see also Southwood & Henderson, 2000). The three assemblages included in the "`dens`" data frame is to demonstrate the density dependent and density independent representations. This was originally published and discussed in Tóthmérész (1998), as well as the data sets of the "`oAB`" data frame. This latter is a pooled data set, by pooling the 10 separate samples contained in the "`oA`" and "`oB`" data frames.

Methods available in the package

The methods included in the package are presented in the paper of Tóthmérész & Magura (2005). There are a few slightly different methods that are equally useful from a biological point of view. These methods are reviewed by Patial & Tallie (1983), and Tóthmérész (1995, 1997).

Examples of the usage of the procedures

The data set `trichotom` contains the number of individuals of the species in three assemblages. Entering

```
> trichotom
```

displays the entire data set.

```
> trichotom
         nC1 nC2
species1  40  60
species2  30  20
species3  30  10
species4   0  10
```

It contains two assemblages (`nC1` and `nC2`) and 4 species (`species1,...,  species4`). Entering

```
> t(trichotom)
```

displays the data set in the transposed form:

```
    species1 species2 species3 species4
nC1       40       30       30        0
nC2       60       20       10       10
```

Using the

```
> trichotom$nC1
```

commands, only the data vector of the `nC1` assemblage is displayed:

```
[1] 40 30 30  0
```

Traditional diversities are obtained as a special case of the one-parametric Rényi diversity index family. Shannon diversity is obtained for `alpha=1` for the `nC1` and `nC2` assemblages:

```
> div.Renyi(trichotom$nC1, alpha=1)
[1] 1.0889
> div.Renyi(trichotom$nC2, alpha=1)
[1] 1.0889
```

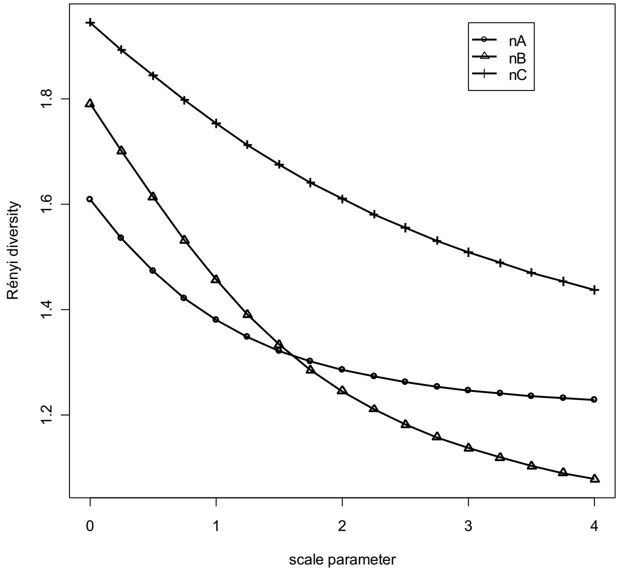

Figure 1. Diversity profiles of the Rényi's one-parametric diversity index family for the `ABC` data set. This resulted from the command line: (`plot.div.Renyi(ABC, from=0, to=4, step=0.25)`).

The default option is the natural logarithm; choosing `base=2` you receive the Shannon diversity with the logarithm of base 2:

```
> div.Renyi(trichotom$nC1, alpha=1, base=2)
[1] 1.570951
```

When `alpha=0`, the logarithm of the number of species is obtained:

```
> div.Renyi(trichotom$nC1, alpha=0)
[1] 1.098612
```

Using the exponential function (`exp`) we get the number of species of the `nC1` assemblage:

```
> exp(div.Renyi(trichotom$nC1, alpha=0))
[1] 3
```

The three variants of the Simpson (or quadratic) diversity is received in the following way:

```
> div.Renyi(trichotom$nC1, alpha=2)
[1] 1.078810
> exp(div.Renyi(trichotom$nC1, alpha=2))
[1] 2.941176
> 1-1/exp(div.Renyi(trichotom$nC1, alpha=2))
 [1] 0.66
```

The first one is $-\log \sum_{i=1}^{S} p_i^2$, the second one is the effective number of species for the quadratic diversity, i.e. $1 \big/ \sum_{i=1}^{S} p_i^2$. The last one is the $1-\sum_{i=1}^{S} p_i^2$ for of the quadratic diversity.

A particular value of the right-tail sum (*RTS)* diversity is received in the following way (*i*=2):

```
> div.RTS(trichotom$nC2, 2)
[1] 0.2
```

If you would like to know each value of the *RTS* diversity for *i*=1,…,*S*, you should enter:

```
> div.RTS.all(trichotom$nC2)
[1] 0.4 0.2 0.1 0.0
```

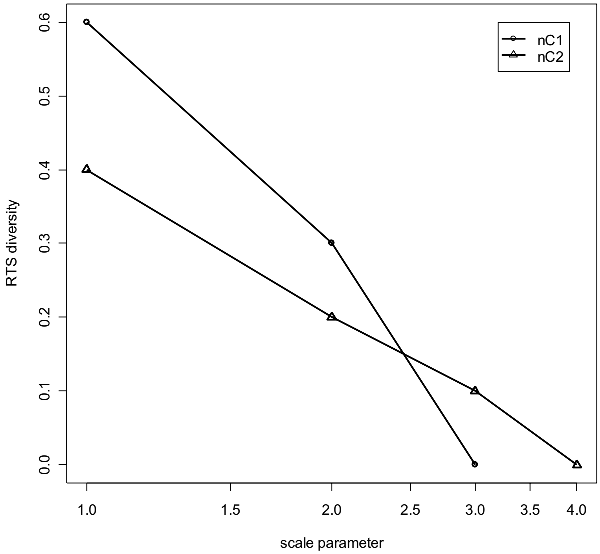

Figure 2. *RTS* diversity profiles for the `trichotom` data set. Output of the command line: (`plot.div.RTS(trichotom, xLabel=0, log="x")`).

The species accumulation plots have more options to choose from than for the other one-parametric diversity index families. For the `dens` data set the expected number of species in a sub-sample containing 25 individuals is the following (for an infinitely large assemblage):

```
> div.ESm.Infinite(dens$nE, 25)
[1] 7.070675
> div.ESm.Infinite(dens$nF, 25)
[1] 6.181578
> div.ESm.Infinite(dens$nG, 25)
[1] 7.070675
```

For the finite case the result is slightly different:

```
> div.ESm.Finite(dens$nE, 25)
[1] 7.082133
> div.ESm.Finite(dens$nF, 25)
[1] 6.192088
> div.ESm.Finite(dens$nG, 25)
[1] 7.099477
```

There are commands to draw the diversity profiles of the assemblages. The simplest way to draw the Rényi diversity profiles of the assemblages of the `ABC` data set is the following (using the default option):

```
> plot.div.Renyi(ABC)
```

You can change the parameters as described in the documentation of the procedures (Appendix):

```
> plot.div.Renyi(ABC, from=0, to=4, step=0.25)
> plot.div.Renyi(ABC, from=0, to=4, step=0.25, main="")
```

RTS diversity profiles of the assemblages in the `trichotom` data set with the default option are produced by the command

```
> plot.div.RTS(trichotom)
```

Using the `log="x"` option, the *x*-axis scale is not logarithmic:

```
> plot.div.RTS(trichotom, log="x")
```

This may be useful for a species-poor assemblage (fewer than 15 species). In the case of species-rich assemblages, the dominance plot using logarithmic scale usually produces a much nicer graph (Tóthmérész, 1997).

To draw a species accumulation plot is slightly more sophisticated than to draw other diversity profiles. The default options still works. On entering

```
> plot.div.ESm(dens)
```

you get a density independent representation for an infinitely large assemblage and default starting and ending values of the number of individuals along the *x*-axis. You can use the options in a longer (and safer) way

```
> plot.div.ESm(dens, type="infinite", representation="individual",
from=2, to=-1, nSteps=10)
```

or just shortly:

```
> plot.div.ESm(dens, "infinite", "individual", from=2, to=-1, nSteps=10)
```

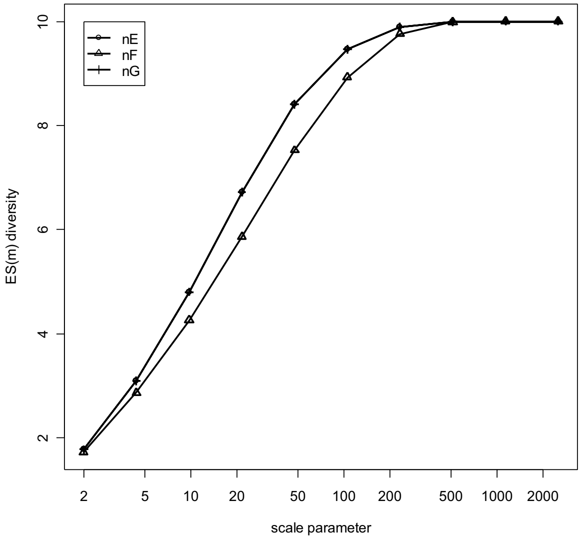

Figure 3. Species accumulation curves for the `dens` data set; density independent representation and infinitely large assemblage is assumed, resulting from: (`plot.div.ESm(dens, "infinite", "individual", from=2, to=-1, nSteps=10)`). Please note that nE≡nG.

Let us assume that you would like to draw the expected number of individuals starting with 5 individuals and increase the sub-sample size up to 400 individuals. You have to type the following command line:

```
> plot.div.ESm(dens, "infinite", "individual", from=5, to=400, nSteps=15)
```

The following two commands produce a figure demonstrating that finite and infinite options may produce really different outcomes. This occurs because the infinite option is based on the relative frequencies of the species:

```
> plot.div.ESm(dens, "finite", "individual", from=60, to=100, nSteps=5)
> plot.div.ESm(dens, "infinite", "individual", from=60, to=100, nSteps=5)
```

There are further commands to help working with the package. You can print the values of the Rényi diversity in the following way:

```
> print.div.Renyi(trichotom, from=0, to=2, step=0.5)
```

```
This will result in the following output:
  scale      nC1       nC2
```

```
1   0.0 1.098612 1.3862944
2   0.5 1.093814 1.2349776
3   1.0 1.088900 1.0889000
4   1.5 1.083891 0.9643264
5   2.0 1.078810 0.8675006
```

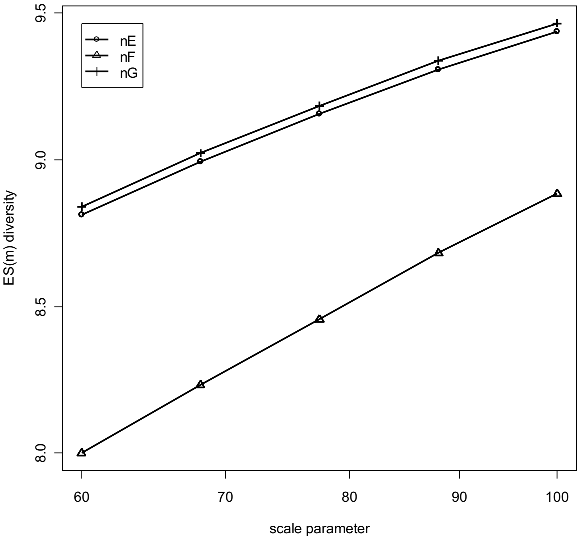

Figure 4. Species accumulatiom curves for the `dens` data set; density independent representation and finite assemblage is assumed. Command line: (`plot.div.ESm(dens, "finite", "individual", from=60, to=100, nSteps=5)`). Please note that nE≠nG, while using the same representation with infinite option they were identical.

If you would like to know the effective number of species, which is defined as `exp(Renyi)`, simply enter:

```
> exp(print.div.Renyi(trichotom, from=0, to=2, step=0.5))
     scale      nC1      nC2
1  1.000000 3.000000 4.000000
2  1.648721 2.985641 3.438302
3  2.718282 2.971004 2.971004
4  4.481689 2.956158 2.623020
5  7.389056 2.941176 2.380952
```

Note that the scale parameter values are also printed as `exp(alpha)` instead of `alpha`.

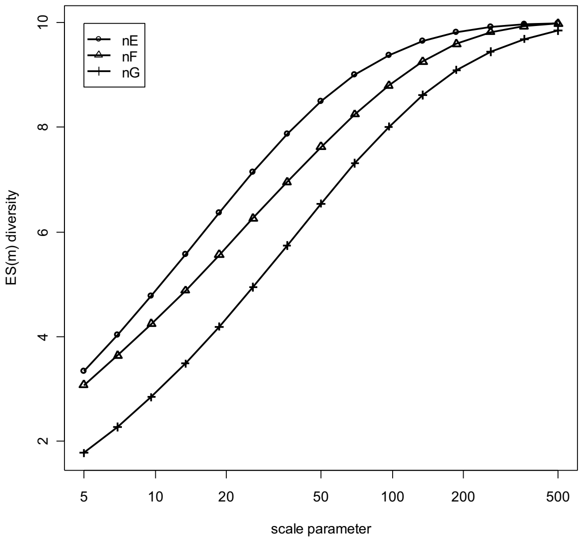

Figure 5. Species accumulation curves for the `dens` data set; density dependent representation and infinitely large assemblage is assumed. Command line: (`plot.div.ESm(dens, type="infinite", representation="area", from=5, to=500, nSteps=15)`).

The total number of species of the `oA` data set, which contains 10 assemblages of 10 samples of an assemblage is produced by

```
> S.total(oA)
[1] 20
```

and similarly, the median of the number of species and the average number of species of the assemblages (or samples) is received as

```
> Si.average(oA)
[1] 5.0 4.7
```

The number of species for each assemblage (samples of an assemblage) is provided by the following command:

```
> Si(oA)
a01 a02 a03 a04 a05 a06 a07 a08 a09 a10
  5   5   4   5   4   5   5   5   4   5
```

The total number of individuals for the 20 species present in the `oA` date set is provided by

```
> ni.total(oA)
   1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
6140 4060 2190 1600  900  752  576   45   15   42   43   12   51  119   16   62
  17   18   19   20
  72   34   97   84
```

The number of individuals for each assemblage (samples of an assemblage) is produced by entering

```
> Ni.total(oA)
 a01  a02  a03  a04  a05  a06  a07  a08  a09  a10
1688 1674 2147 1433 1421 1561 1893 2176 1457 1460
```

Appendix. List of the Procedures

```
 1. div.Renyi(xv, alpha=1, base=exp(1))
 2. div.RTS(xv, parameter=1)
 3. div.ESm.Infinite(xv, subsample=2)
 4. div.ESm.Finite(xv, subsample=2)
 5. div.RTS.all(xv)
 6. plot.div.Renyi(xm, from=0, to=4, step=0.25, xLabel=4,
    xlab="scale parameter", ylab="Rényi diversity", main="Rényi
    diversity profiles", lty=1, lwd=2, ...)
 7. plot.div.RTS(xm, xLabel=0, log="x", xlab="scale parameter",
    ylab="RTS diversity", main="RTS diversity profiles", lty=1,
    lwd=2, ... )
 8. plot.div.ESm(xm, type="infinite", representation="individual",
    from=2, to=-1, nSteps=10, log="x", xlab="scale parameter",
    ylab="ES(m) diversity", main="ES(m) diversity profiles", lty=1,
    lwd=2, ...)
 9. print.div.Renyi (xm, from=0, to=3, step=0.5)
10. S.total(xm)
11. Si.average(xm)
12. Si(xm)
13. ni.total(xm)
14. Ni.total(xm)
```

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Affinity indices for environmental assessment using carabids

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Abstract

Three new habitat-affinity indices were developed, based on the concepts of habitat specificity and fidelity. The usefulness of these indices was assessed using data on ground beetles (Carabidae) from the Hungarian GlobeNet site, in the city of Debrecen (Eastern Hungary), studying a rural-urban gradient. We demonstrated that the carabid assemblage of the rural end of the gradient is characterized by a high forest affinity value that decreased across the gradient towards the urban area. Forest affinity values were attributed to the species based on our earlier research, and literature data. The affinity indices based on fidelity or specificity of the species proved to be useful in comparing different habitat patches. The index based on a combination of specificity and fidelity characteristics produced a good alternative to the otherwise hazy "ecological character" of the studied carabid assemblages.

Key words: Environmental monitoring, ecological indices, forest species, GlobeNet, urban-rural gradient

Introduction

Environmental monitoring to follow and assess the human impact on the environment is of central importance (Loreau *et al.*, 2002). Plants and animals are good indicators of the quality of the environment, so their habitat-affinity and other ecological indices are generally used to assess the natural or "protection" value of an assemblage in a given patch of habitat

(Spellerberg, 1991). Indicator and/or affinity indices are useful tools to characterize carabid assemblages (Pizzolotto 1994); they are also widely used in botany (Ellenberg, 1973), and in water qualification (Ruoppola *et al*., 2003).

Ground-dwelling carabids are especially useful to study environmental impacts, because they are sensitive to environmental changes, abundant and sufficiently variable both taxonomically and ecologically (Lövei & Sunderland, 1996). Carabid beetles are among the most reliable taxa for applied research in environmental evaluation, as they are sensitive to soil disturbance and to other changes in environmental conditions in their habitats (Elek *et al*., 2001; Niemelä, 1999; Desender *et al*., 1999; Magura *et al*., 2000, 2001, 2002, 2003).

This paper deals with some problems related to the use of habitat affinity indices. We evaluated the performance of different indices to assess the natural value of the habitat patches based on the forest-affinity values of the carabid species in habitats across an urbanization gradient. Our hypothesis was that the rural end of the gradient is characterized by an assemblage with a high forest affinity value, and was expected to decrease along the gradient towards the urban area.

Material and methods

Study area and sampling

The study areas were the Hungarian GlobeNet site, in the city of Debrecen (Eastern Hungary) (Magura *et al*., 2004). Three forested sampling areas were selected along an urbanisation gradient; these represented urban, suburban and rural areas, following the GlobeNet protocol (Niemelä *et al*., 2000, 2002). All sampling sites were in continuous patches of forest dominated by English oak (*Quercus robur*), each covering at least 6 ha. Distance between the studied areas was at least 1 km. In the urban park area, there were several asphalt-covered paths and the shrub layer was strongly thinned, while in the suburban area the fallen trees were removed. The urban-rural gradient extended over a distance of approximately 6 km from the city centre through the suburbs to the neighbouring Nagyerdő Forest Reserve. Details of the sampling is discussed in Magura *et al*. (2004).

Data analysis

To test for differences in habitat affinities, nested analysis of variance (ANOVA) were performed on the different habitat affinity index values among the three sampling areas (urban, suburban and rural) and among the 12 sites. The distribution of data used in the ANOVA model was normal (Kolmogorov-Smirnov test, Sokal and Rohlf 1995). When ANOVA revealed a significant difference between the means, an LSD (least significant difference) test was performed for multiple comparisons among means.

Affinity Indices

Although the habitat affinity indices are simple and straightforward from an ecological point of view, the mathematical formulas are technically sophisticated. The base dataset is organized into a table; the entries of the table are x_{ir}, where the species are indexed by *i* and the traps by *r*:

$$\begin{pmatrix} x_{11} & \cdots & x_{1r} & \cdots & x_{1P} \\ \vdots & & \vdots & & \vdots \\ x_{i1} & \cdots & x_{ir} & \cdots & x_{iP} \\ \vdots & & \vdots & & \vdots \\ x_{S1} & \cdots & x_{Sr} & \cdots & x_{SP} \end{pmatrix}, \qquad (1)$$

where x_{ir} is the number of individuals of species *i* captured in the trap *r*. The value of *i* runs from 1 to the total number of species, S; likewise, that of the number of traps, *r* runs from 1 to *P*.

The crucial step of the habitat qualification procedure is how to define the A_i affinity values. We have used the five levels of affinity values, ranging from +1 to –1, suggested by Allegro & Sciaky (2002):

+1:	obligate forest species (forest specialist),
+0.5:	partial forest species (forest generalist),
0:	species indifferent to forest coverage (habitat generalist),
–0.5:	partial open-habitat species (open habitat generalist),
–1:	obligate open-habitat species (open habitat specialist).

The affinity values of the species in our samples were determined from our earlier research in the same area (Magura et al. 2004), and on relevant literature (Hůrka 1996, Thiele 1977). When used the term 'forest affinity' rather than the general term of 'habitat affinity'.

The simplest way to characterize a habitat is to sum up the affinity values of the species (A_i) in the traps; a habitat affinity value, HA_r can be defined as:

$$HA_r = \sum_{i=1}^{S} I_{ir} \cdot A_i \,, \qquad (2)$$

where I_{ir} is the so-called indicator function:

$$I_{ir} = \begin{cases} 1, & \text{when the species } i \text{ is present in the trap } r, \\ 0, & \text{when the species } i \text{ is missing in the trap } r. \end{cases}$$

It is easy to see that the role of I_{ir} is to ensure that species that are not present in a trap do not contribute to the affinity value of the habitat. If species *i* is absent from trap *r*, it will contribute 0 to the sum and not increase the value of *HA*.

HA_r is the simplest way to characterize habitat affinity. A high HA_r value indicates a high number of forest species in the trap, whereas a low value indicates the opposite.

Notice that only presence-absence influences the value of *HA*. The value of the following habitat affinity index, *HAR*, defined by the equation (3), gets the higher values the more forest species are present in the habitat patch. The index is calculated as the weighted average of the affinity values; thus, the affinity values are weighted by the relative frequency of the species:

$$HAR_r = \sum_{i=1}^{S} p_{ir} \cdot A_i \,, \tag{3}$$

where p_{ir} is the relative frequency of the species *i* in the trap *r*. $p_{ir} = 0$, if the species *i* is missing from the trap *r*. Therefore, the I_{ir} indicator function is not necessary in the formula (3). Notice that this is the same as Allegro & Sciaky's (2002) forest affinity index (FAI) but calculated individually for every trap.

The abundances of the species may fluctuate considerably. For this reason, weight other than p_{ir}, based on the consistency of the occurrence of a species in a habitat may be useful (see Dufrêne and Legendre 1997). This kind of weight is defined by the number of traps a given species is present within a habitat patch (fidelity). An alternative approach is when the differences in abundance among habitat patches are used for weighting the affinity of the species (specificity). For these purposes we propose three new habitat-affinity indices (4-6) that include the components of fidelity and specificity.

Habitat-affinity-fidelity index (*HAF*) is defined as:

$$HAF_r = \sum_{i=1}^{S} I_{ir} \cdot \pi_i \cdot A_i \,, \tag{4}$$

where π_i is the relative frequency of traps with species *i* present. A species *i* is present 7 out of 10 traps, then $\pi_i = 7/10 = 0.7$.

Habitat-affinity-specificity index (*HAS*) is defined as:

$$HAS_r = \sum_{i=1}^{S} I_{ir} \cdot e_i \cdot A_i \,, \tag{5}$$

where e_i is the specificity of the species *i*. The specificity is defined as the ratio of the average number of individuals of the species in the habitat patch ($\overline{x}_{ir}$) compared to the average of the individuals across all the studied habitats ($\overline{x}_{i.}$); thus,

$$e_i = \bar{x}_{ir} / \bar{x}_{i.} \ .$$

The combined habitat-affinity index, based on both specificity *and* fidelity (*HAFS*) is defined as:

$$HAFS_r = \sum_{i=1}^{S} I_{ir} \cdot \sqrt{\pi_i \cdot e_i} \cdot A_i \ . \qquad (6)$$

The geometric average of the fidelity and affinity weights, $\sqrt{\pi_i \cdot e_i}$, is used to guarantee a constant unit sum of the weights for all the species.

Results and discussion

The performance of the indices

The ANOVA indicated significant differences in the averages of the habitat affinity values of the compared carabid assemblages (Table 1). All the indices gave similar results regarding the compared habitat patches (Fig. 1).

Table 1. Nested ANOVA for four habitat affinity-index values (*HAR*, *HAF*, *HAS*, *HAFS*). Gradient: urban, suburban, and rural sampling area. There were four sites within each sampling area.

	Source of variation	df	MS	F	p
HAR	Gradient	2	6.8347	44.0097	<0.001
	Sites	9	0.1553	1.4563	0.1736
	Error	108	0.1067		
HAF (fidelity)	Gradient	2	4.6290	56.1772	<0.001
	Sites	9	0.0824	2.0135	<0.05
	Error	108	0.0409		
HAS (specificity)	Gradient	2	28.9290	95.9184	<0.001
	Sites	9	0.3016	0.8501	0.5720
	Error	108	0.3548		
HAFS	Gradient	2	9.9943	87.9006	<0.001
(fidelity and specificity)	Sites	9	0.1137	1.3061	0.2421
	Error	108	0.0870		

The average of the forest affinity values of the carabids was lowest in the urban area with each affinity index, indicating that the number of obligate forest carabids was low (Fig. 1). Moreover, in the urban park there were more open-habitat than forest carabids. In this area, there were several asphalt-covered paths and the shrub layer was strongly thinned, which resulted in higher openness in that area. As soon as the forest affinity is low or negative, the assemblage is characterized by generalist and/or open habitat species.

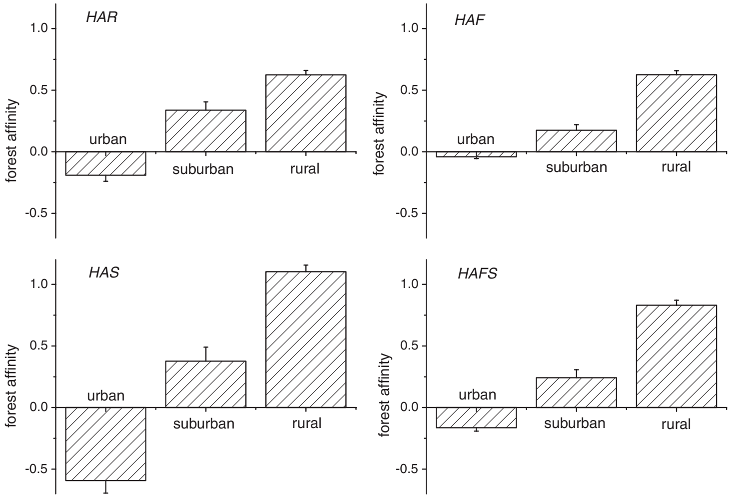

Figure 1. The mean habitat affinity values (± *SE*) for the forest species, weighted by the relative frequency of the species (*HAR*), by fidelity (*HAF*), by specificity (*HAS*), and by the geometric average of fidelity and specificity (*HASF*).

The average forest affinity was positive for the suburban area with each affinity measure, but significantly lower than in the rural area. This is explained by the fact that the species richness of forest carabids increased significantly across the urban-rural gradient (Magura *et al*., 2004).

Every affinity index indicated that the average forest-affinity value was significantly higher in the rural area (Fig. 1, Table 1). The number of individuals belonging to forest-associated species, the number of such species, and the average carabid catch all peaked in the rural area (Magura *et al*., 2004).

The difference between the rural and the suburban areas was pronounced using the fidelity (*HAF*) and the combined habitat affinity (*HAFS*) indices (Fig. 1). The difference was lowest between the suburban and rural areas using the usual habitat affinity index that is based on the relative frequencies of the species (Fig. 1, *HAR*).

The habitat affinity-specificity index produced a relatively large negative forest affinity value for the urban area (Figure 1, *HAS*), while the habitat affinity-fidelity index stressed the high forest affinity value of the rural area (Fig. 1, *HAF*). The combination of these two aspects of the weighting of the carabids affinity values produced a balance between these two aspects (Fig. 1, *HASF*).

There were no remarkable differences between the results provided by the different affinity indices. This is a result of sampling within similar types of habitat. There were no extreme values in our test dataset. The differences and the usefulness of the different indices may be better understood in more extreme comparisons, with more variation among catches.

It is important to stress that fidelity and specificity emphasize two independent aspects of commonness and rarity. For example, a species has high fidelity in a habitat if it is represented by the following catches in five traps: 2, 3, 1, 5, 1.Another species with catches 0, 0, 0, 427, 0 individuals in the same traps may be more abundant overall, but not as "true" to the habitat as as the former one: its occurrence is more sporadic. Thus, its fidelity is low. However, its specificity may be high, if the number of captured individuals in other habitats is low.

The "standard" version of the habitat affinity index, based on the relative frequency of the species in the trap (*HAR*), is sensitive to extreme values of captures. Fidelity quantifies the spatial uniformity of the species, while specificity is based on the variation of the average abundance of the species in the habitats studied. In comparison to indices using the mere occurrence or the relative frequency of species, they capture more aspects influencing their distribution. Therefore this is a fascinating tool for comparisons of carabid communities in ecological and environmental studies. The behaviour of these indices should be studied with more data sets to explore their features and realise their potential.

Acknowledgements

We are especially grateful to Gábor Lövei and Johan Kotze for comments on the manuscript.

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Diversity and scalable diversity characterizations

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Abstract

A short review of recent developments in diversity measuring is presented with a special emphasis on the evolution of these techniques. The importance of scalable diversity characterization through one-parametric diversity index families is stressed. An elementary example is also presented to demonstrate the techniques. A nature management study is discussed to reveal the usefulness of the scalable diversities through one-parametric diversity index families. Density dependent and density independent representation of diversities are also discussed.

Key words: Diversity profiles, one-parametric diversity index families, Rényi diversity, right tail sum diversity, expected number of species diversity, species accumulation plots

Introduction - motivation

The problem of index choice is well-known in the diversity literature. Thirty years ago, Peet (1974) discussed the need for a theory to facilitate choice among diversity indices. One may wish the index to be sensitive to dominant species but relatively indifferent to rare ones. The solution, as proposed by Patil & Taillie (1979), is the use of one-parametric index families that allows the diversity of an assemblage to be characterized by a diversity profile instead of a single numerical value. This is possible with the one-parametric index families, since changing the scale parameter modifies the sensitivity of the diversity index. The change in sensitivity can then be displayed graphically by plotting the calculated diversity value against the scale parameter. The first of these techniques, the generalized entropy, was published by Rényi (1961). Today, there are a number of methods available for scalable diversity characterization (reviewed by Tóthmérész, 1995, 1998).

In the first part of the paper we review the methods stressing the evolution of these techniques; in the second part of the paper the usefulness of the methods is demonstrated by a nature management study.

Material and methods

Some notations

We frequently speak about the number of individuals of a species or about abundance generally. In formal notation, each species is represented by a positive integer. The number of individuals of the first species in the sample is denoted by n_1. Generally, the number of individuals of the *i*-th species is denoted by n_i. The sum of all the individuals in all species is denoted by *N*. The number of species is usually denoted by *S*. So, the total number of individuals, *N* is:

$$N = n_1 + n_2 + \ldots + n_i + \ldots + n_S = \sum_{i=1}^{S} n_i .$$

An assemblage *A* can be described by the abundance vector: $\mathbf{n}(A) = (n_1, n_2, \ldots, n_S)$. For our purposes it is frequently enough to know the relative abundances of species: $\mathbf{p}(A) = (p_1, p_2, \ldots, p_S)$, where $\mathbf{p}(A)$ is the relative abundance vector and $p_i = n_i / N$. These can also be written, more simply as: $\mathbf{n} = (n_1, n_2, \ldots, n_S)$ and $\mathbf{p} = (p_1, p_2, \ldots, p_S)$.

Frequently we would like to know which one is the most frequent species, or the second most frequent, etc. It helps if the species are arranged in descending order, using the following notation:

$$\mathbf{p}^{\downarrow} = \left(p_{[1]}, p_{[2]}, \ldots, p_{[i]}, \ldots, p_{[S]}\right),$$

where $p_{[1]}$ is the relative frequency of the most frequent species, $p_{[2]}$ is the relative frequency of the second most frequent species, ... , and $p_{[S]}$ is the relative frequency of the rarest species. The sign "[ ]" in the subscript means that elements of the vector is arranged in descending order. Therefore:

$$p_{[1]} \geq p_{[2]} \geq \ldots \geq p_{[i]} \geq \ldots \geq p_{[S]} .$$

The notational conventions detailed above will be followed throughout the paper.

Simple artificial data set

We start with an elementary example in order to demonstrate that even in the simplest case we may need sophisticated tools to characterize diversity. Consider two assemblages, denoted by C1 and C2 (Tóthmérész, 2002). There are 3 species in C1 and 4 species in C2; altogether there are 4 species. The abundance vectors (**n**) and the relative abundance vectors (**p**) are as follows:

$\mathbf{n}(\mathrm{C1}) = (40, 30, 30)$, $\mathbf{p}(\mathrm{C1}) = (0.4, 0.3, 0.3)$,
$\mathbf{n}(\mathrm{C2}) = (60, 20, 10, 10)$, $\mathbf{p}(\mathrm{C2}) = (0.6, 0.2, 0.1, 0.1)$

Basic question: Which one is more diverse?
This is a relatively simple question, which is frequently addressed in ecological studies comparing the diversity of animal assemblages.

First, we would like to demonstrate a trichotomy: C1 can be more diverse than C2, they can be equally diverse, or C1 can be less diverse than C2. An easy calculation shows that the Shannon diversity of C1 is:

$$HS(\mathrm{C1}) = 0.4\log 0.4 + 2\cdot(0.3\log 0.3) = 1.0899 \quad \text{and} \quad HS(\mathrm{C2}) = 1.0899\,.$$

The quadratic or Simpson diversity is

$$DQ(\mathrm{C1}) = 1-(0.4^2 + 2\cdot 0.3^2) = 0.66 \quad \text{and} \quad DQ(\mathrm{C2}) = 0.58\,.$$

The numerical results are contrasted in Table 1.

Table 1. The trichtomy of diversity for the assemblages C1 and C2.

	C1		C2
Number of species	3	<	4
Shannon diversity	1.0889	=	1.0889
Quadratic diversity	0.66	>	0.58

Even in such a simple situation we can get all three possible outcomes. Obviously, such ambiguity can emerge in complex situations as well. This is a common problem emerging from the use of traditional diversity indices.

Evolution of the methods of diversity characterizations

The number of species

The number of species is the oldest and the most traditional measure of diversity. However, it depends on the number of individuals in the sample and/or the area to be sampled. This is the basic motivation for the standardizations: the number of species can be divided by the number of individuals or by the area sampled (e.g. plot size). This leads to the diversity ratios.

Diversity ratios

These indices are based on the ratio of the number of species and the number of individuals. The number of species does not increase linearly with the number of individuals, but (usually) with the logarithm of the number of individuals. It is better to use the ratio of linearly related quantities. Therefore, a more correct expression is obtained by dividing the number of species by the logarithm or by the square root of the number of individuals. A few diversity ratios are listed below:

$dsr = S / N$, $dlr = S / \log N$,

$dsqr = S / \sqrt{N}$, $dslr = (S-1) / \log N$.

These simple, richness-type measures of diversity may be useful in many cases, when the abundance is not known for each species, only the total abundance. They do not take into account the abundance-dominance structure of the assemblages. This shortcoming is overcome by the traditional diversity indices, like the Shannon diversity or the quadratic diversity. These methods utilize the information about the relative frequencies of the species of the assemblages. We call them traditional diversity statistics.

Traditional diversity statistics

The most frequently used diversity statistics is the Shannon index of diversity:

$$HS = -\sum_{i=1}^{S} p_i \log p_i \quad .$$

This index was proposed by Claude Shannon as a measure of information (Shannon, 1948; Shannon & Weaver, 1949), and now it is also used as a measure of diversity in ecology. Sometimes it is mentioned as Shannon-Weaver or Shannon-Wiener diversity, even though the publication priority of Shannon is inevitable.

The other frequently used classical diversity statistic is based on a measure of concentration, *C* (Pielou, 1975):

$$C = -\sum_{i=1}^{S} p_i^2 \quad .$$

It measures the un-evenness of the relative abundances. It is evident that the concentration receives its minimum when $p_i = 1/S$ for all $i = 1,\ldots,S$.

Diversity is the opposite of concentration. There are, however, at least three different ways to create an opposite measure. One of them, resulting in the quadratic or Simpson diversity, *DQ*, is the following:

$$DQ = 1 - \sum_{i=1}^{S} p_i^2 \quad .$$

A less frequently used possibility is based on the logarithmic function:

$$HR(2) = -\log \sum_{i=1}^{S} p_i^2 = \log \frac{1}{\sum_{i=1}^{S} p_i^2} \ .$$

It is related to the one-parametric Rényi diversity index family (Tóthmérész, 1998), discussed later on in the paper. The third possibility is producing a measure of effective number of species, discussed in the next section.

The effective number of species

The diversity statistics introduced in the previous section reflect the abundance-dominance structure of an assemblage. These are producing numerical figures without direct ecological meaning. A diversity value, for example 0.58, has no evident meaning. A diversity characterization that has straightforward biological meaning would be advantageous. Such an index is the effective number of species. The number of species has a direct and important ecological message. The effective number of species is defined as the number of species, all with the same number of individuals, that produces the same diversity value as the one under study (Pielou, 1975). This therefore equals the number of species in a hypothetical assemblage of perfect evenness that would have the same diversity as the assemblage whose diversity is to be characterized.

For the Shannon diversity index the effective number of species is defined as

$$SHS = \exp\{HS\} ,$$

where *exp* is the exponential function. Shannon diversity reaches its maximum when all the species are present with the same number of individuals. In this case the diversity is

$$\max\{HS\} = \log S .$$

Therefore, the effective number of species is exactly *S* (i.e, the actual number of species) for an assemblage in which all species are equally abundant, while less than *S* for any other assemblage.

For the quadratic diversity, an opposite of the concentration also can be created in the following way

$$SDQ = \frac{1}{\sum_{i=1}^{S} p_i^2} \ ,$$

which is a measure of the effective number of species. *SDQ* can be used for measuring the effective number of species related to the quadratic diversity. *SDQ* is closely related to *HR*(2), because $SDQ = \exp\{HR(2)\}$. *SHS* and *SDQ* are also strongly related, because they are related to the Rényi diversity index family (Tóthmérész, 1998). Each member of the Rényi diversity index family can be used in the form of an effective number of species, similarly to the diversity indices discussed above.

Scalable diversity by one-parametric diversity index families

In the case of one-parametric diversity index families, a number of diversity values is used to characterize the diversity of an assemblage. The one-parametric diversity indices may be portrayed graphically by plotting diversities against a (scale) parameter (Fig. 1). This curve is frequently mentioned as the diversity profile of the assemblage (Patil & Taillie, 1979, 1982). Members of a one-parametric diversity index family have varying sensitivities to the rare and abundant species as the scale parameter changes. There exists a large family of one-parametric diversity functions (see Tóthmérész, 1993, 1995). The Rényi diversity is a typical member of the generalized entropy functions. Each of the generalized entropies includes the Shannon diversity as a special case.

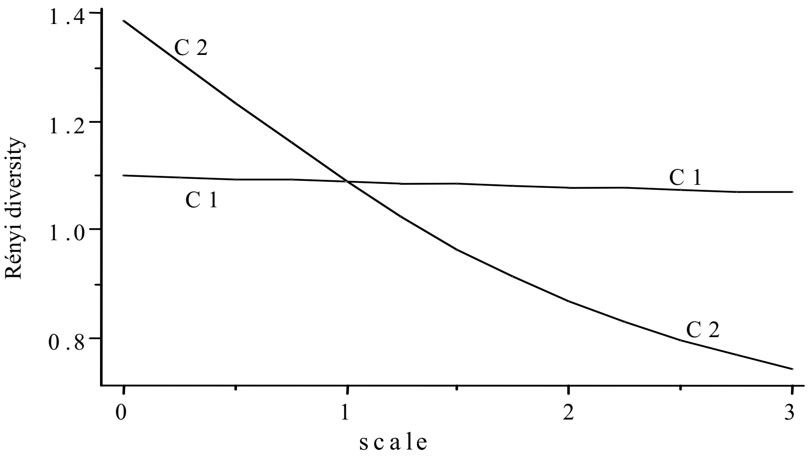

Figure 1. Rényi diversity profiles of the C1 and C2 assemblages.

Diversity profiles are used for scalable diversity comparisons of assemblages. This is also termed diversity ordering. Using diversity profiles, the diversity ordering of assemblages is defined in the following way: assemblage A is more diverse than assemblage B (A>B) when the diversity profile of A is above or equal to the diversity profile of B over the entire range of the scale parameter. It can be shown that diversity ordering is a partial order: if A>B and B>C, then A>C. However, it is not true that for every assemblages A and B, either A>B or B>A; the curves of two diversity profiles may intersect, as illustrated in Fig. 1 for C1 and C2. This situation may reflect important ecological processes and therefore needs to be interpreted carefully. For these two assemblages, the intersection of the diversity profiles means that the assemblage C1 is more diverse than C2 for the rare species, while assemblage C2 is more diverse than C1 for the frequent species.

Generalized entropies

Rényi (1961) extended the concept of Shannon entropy by defining the entropy of order α or Rényi diversity (α ≥ 0, α ≠ 1):

$$HR(\alpha) = \frac{1}{1-\alpha}\left(\log \sum_{i=1}^{S} p_i^{\alpha} \right) .$$

This is the first published family of diversity indices. In the original definition the base of the logarithm was 2; in ecological applications, natural logarithm is more frequently used.

It is important to know some special cases of diversity index families to interpret the result of diversity orderings. For the Rényi index family the following relations are valid:

(i) When the value of the scale parameter is zero (α=0), then the value of the Rényi diversity is the logarithm of the number of species of the assemblage; i.e.

$$HR(0) = \log S .$$

In this case the method is extremely sensitive to the contribution of the rare species to the diversity of the assemblage. At this point, the C2 assemblage is more diverse than the C1.

(ii) When the value of the scale parameter approaches 1 (as there is a division with α - see above- α ≠ 1; but it can take a value infinitely close to 1), then the Rényi diversity is identical to the Shannon diversity:

$$HR(\alpha \to 1) = -\sum_{i=1}^{S} p_i \log p_i \ .$$

In this case the *HR* value is sensitive to the rare species, although less so than at α=0. The diversities of the C1 and C2 assemblages are now identical (Fig. 1).

(iii) When α = 2, the Rényi diversity is related to the quadratic diversity:

$$HR(2) = \log \frac{1}{\sum_{i=1}^{S} p_i^2} \ .$$

In this case the index starts to be more sensitive to the frequent species than to the rare ones, and assemblage C1 is more diverse than assemblage C2 (Fig. 1).

(iv) When the value of the scale parameter is large (i.e. $\alpha \to \infty$) the Rényi diversity is related to the Berger-Parker dominance index that is determined only by the relative abundance of the most common species (Southwood & Henderson, 2000):

$$HR(\alpha \to \infty) = \log \frac{1}{\max\left\{ p_i ; i = 1,\ldots,S \right\}} \ .$$

Cumulative relative abundance plots

RTS diversity (Right-Tail-Sum diversity) also plays a central role in scalable diversity characterizations (Patil & Taillie, 1979; Solomon, 1979). *RTS* diversity is a typical member of the cumulative relative abundance plots, defined as follows (Tóthmérész, 1993, 1998):

$$RTS(i) = p_{[i+1]} + \ldots + p_{[S]},$$

where $p_{[1]}, \ldots, p_{[S]}$ are the relative abundances of the species arranged in descending order. The integer *i*, is the rank of a species, and may be interpreted as a scale parameter. *RTS*(*i*) is the sum of the relative abundances of the rarest (*S*-*i*) species, or the sum of relative abundances remaining after eliminating the *i* most frequent species. Cumulative relative abundance plots are very different from the generalized entropy curves. A diversity profile produced by a generalized entropy function is a continuous curve, usually defined on the [0,∞] or [-1, ∞] range. Cumulative relative abundance plots are discrete functions defined for the integers *i*=1,…,*S*. Traditionally, the discrete values are joined by lines to help in the visual comparison of diversity profiles. Therefore, cumulative relative abundance plots are displayed as a polygon, as demonstrated for the data set of the field study in Fig. 3.

Species accumulation plots

There is a long tradition of species-area and species-counts (number of species – number of individuals) *curves* in biology (Engen, 1978; Fisher *et al*., 1943). We prefer to mention them as *species-accumulation plots*. These curves also can be used for scalable diversity characterization (Patil & Taillie, 1979), and they are defined as follows:

$$ES(m) = S - \sum_{i=1}^{S} (1 - p_i)^m .$$

This is the expected number of species present when *m* individuals are drawn at random from an infinitely large population. Conceptually *m* is an integer, but real values also make mathematical sense. *ES*(*m*) is also referred to as expected species-individual diversity.

An important property of *ES*(*m*) is that here the scale parameter has a direct biological interpretation: it is the number of species in a sub-sample of size *m*. When *m* is small, rare species have a very low probability of appearing in the sub-sample, so *ES*(*m*) is small. When the sub-sample size is increased, the expected number of species also increases. Plotting *ES*(*m*) against *m* produces a diversity profile that is essentially a species-individual curve. It is well known that the number of species depends on the number of individuals in the sample in a non-linear manner. This motivates the proposal of plotting *ES*(*m*) against log *m*, although sometimes it is natural to plot the expected number of species against the sampling units (e.g., number of traps, or the area to be sampled).

In the case of a finite population, where the total number of individuals is *N*, the minimum variance unbiased estimator for *ES(m)* is (Smith and Grassle, 1977):

$$\widehat{ES}(m) = S - \sum_{i=1}^{S} \binom{N-n_i}{m} \Big/ \binom{N}{m},$$

where $\binom{N}{m}$ denotes the binomial coefficients.

Density dependent and density independent representations of species accumulation plots
The expected number of individuals in an area is proportional to the size of the area. We can calculate the *expected species-area curve* using the relationship:

$$m = N \cdot \frac{\mathit{size\ of\ the\ area}}{\mathit{total\ area}} \quad .$$

Specialists of different sub-disciplines of ecology traditionally use different representations. In samples from pitfall traps, the estimation of the species richness is based on the number of individuals in the traps. This may be mentioned as a density-dependent representation, because there are different number of individuals in the traps. In plant ecology, density dependent representation of the species richness is also used since field surveys use plots of the same size, yet they usually contain different number of individuals. In other cases, a density-independent representation of the species richness is used. In algology, species number is often determined by identifying 100 (or 400) individuals. A similar technique is frequently used to determine species richness in samples of soil invertebrates. Both of these are density independent representations of the number of species. Tóthmérész (1993, 1998) stressed the distinction between these two representations of the species accumulation curves, because they may produce strikingly different ecological interpretations.

Which one to use?
Each of the diversity profiles of the one-parametric diversity families shows the same ordering guaranteed by mathematical theorems for the density independent representations (e.g. Patil and Taillie, 1982). The reason to use different kinds of one-parametric diversity index families is that they reveal different aspects of the data set. *RTS* diversity is useful only for species poor assemblages, because it is effective in demonstrating the ordering relations regarding the dominant species (Tóthmérész, 1995). The *RTS* diversity is important from a theoretical rather than practical point of view: it can be used to prove important mathematical theorems (Patil & Taillie, 1982). This may explain why Patil & Taillie (1979) proposed that an assemblage is intrinsically more diverse when its *RTS* diversity is larger for all i (i=1,...,S) than that of another assemblage. The *RTS* diversity profile can conveniently be pictured in the form of a logarithmic dominance plot (Tóthmérész, 1993, 1995). The Rényi diversity is generally useful for most assemblages and can be used very effectively in ecological studies (Tóthmérész, 1995, 1998). Species accumulation plots provide the most sophisticated tools to reveal diversity relationships; the density dependent and density independent representation makes them especially useful (Tóthmérész, 1998, 2002).

Why we speak about scaling?

In the case of generalized entropies and cumulative relative abundance plots, the scale parameter is related to the abundance-dominance structure of the studied assemblage. The interpretation of relative abundance plots is straightforward; for i=1 we eliminate the relative abundance of the most frequent species, for i=2 we eliminate the second most frequent, etc. For generalized entropy plots (especially in the case of Rényi's diversity), the interpretation is indirect because of the sensitivity properties of $HR(0)$, $HR(1)$, $HR(2)$, and $HR(+\infty)$. For the species accumulation plots it is evident that m may be interpreted as a scale parameter; for small m (small sample) the expected number of species is also small and only the frequent species have a real chance to be present. For large m (large sample) the rare species also contribute to the total number of species.

Computing possibilities

Tóthmérész (1993) provided a DOS based computer program to calculate and plot diversity profiles. Recently in R, which is a programming environment for data analysis and graphics (Ihaka and Gentleman, 1996) a package is implemented to calculate one-parametric diversity index families (Tóthmérész, 2005). Oksanen (2004), in his package, called '`vegan`', written in the R program language, also provides a function to calculate the Rényi diversity.

A field study: Management of a non-native spruce plantation

To illustrate the possibilities and approaches by scalable diversity comparisons, a ground beetle study from Hungary is used (Magura *et al.,* 2000). Pitfall catches of carabids from native oak-hornbeam forest were compared with those from managed spruce plantation to examine the effect of management on the diversity of ground beetles.

Study area and sampling

The sampling area was located in the North Hungarian Mountain Range. In this region the typical native forest association was oak-hornbeam, which was the most extensive forest type on this territory. We compared carabids in a native deciduous oak-hornbeam forest and a Norway spruce plantation, where gaps were created as a management practice. The Norway spruce plantation was planted after clear-cutting the native forest stand. The spruce was dominant with 70% cover in the tree layer. The presence of native species in the canopy was due to thinning of the spruce that resulted in a re-invasion of native trees, herbs and shrubs, and produced relatively thick leaf litter patches spreading over the 75% of the soil surface. In the native forest the shrub and herbaceous layer were moderate and the leaf litter layer was thick. Beetles were sampled during the main activity period of the species using unbaited pitfall traps (diameter 100 mm, volume 500 ml) containing ethylene-glycol as a killing-preserving solution (details are in Magura *et al*., 2000).

Result of diversity analysis

There were a combined total of 20 ground beetle species captured in the two habitats; 19 versus 17 species in the native oak-hornbeam forest and in the managed spruce plantations, respectively. There was also a remarkable difference in the number of captured individuals between the managed spruce plantation and the native forest (Table 2). Using traditional diversity statistics, the native forest was more species rich (number of species) and more diverse for the Shannon diversity, while the managed spruce plantation was more diverse using the quadratic or Simpson diversity and the Berger-Parker index of dominance (Table 2).

Table 2. Some statistics for the assemblages of the native oak-hornbeam forest and the spruce plantations.

	native forest		*spruce plantations*
Number of individuals	1199	>	826
Number of species	19	>	17
Shannon diversity	1.75	>	1.72
Quadratic diversity	1.29	<	1.38
Berger-Parker index	0.89	<	1.92

The Rényi diversity profiles of the two carabid assemblages cross each other and the native forest is more diverse considering the rare species, while the plantation is more diverse considering the dominant species (Fig. 2). Using the *RTS*-diversity we can locate the change in the diversity order. The *RTS*-diversity profiles cross each other between the 3-rd and 4-th species (Fig. 3).

Using a density independent representation of the diversity relationships of the native forest and managed plantation by the *ES*(*m*) diversity we receive the diversity profiles shown in Fig. 4. For a small (sub)sample, which includes only a few captured individuals, the managed plantation is more species rich than the native plantation. For a larger (sub)sample including approximately 20 or more captured individuals, the native forest is more species rich.

Using the density dependent representation of the *ES*(*m*) diversity the diversity profiles of the native forest and the managed spruce plantation do not cross each other. The carabid fauna of the native forest is more species rich over the whole range of the scale parameter (Fig. 5). Therefore, they can be unequivocally ordered according to their diversity: the native forest is more diverse than the managed plantation.

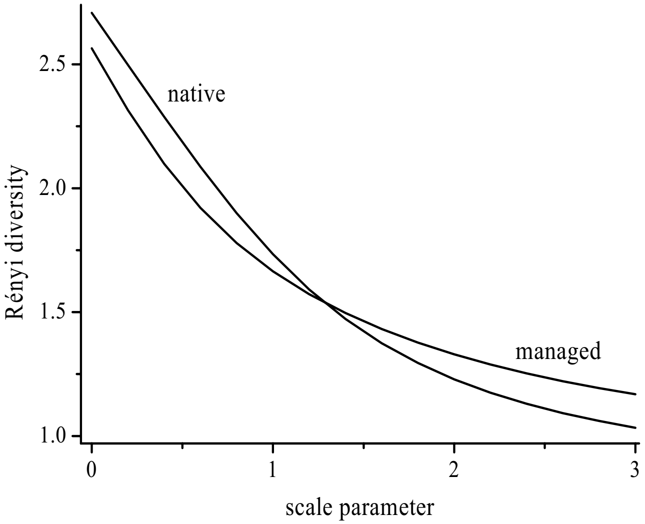

Figure 2. Diversity profiles of the assemblages by the one-parametric Rényi diversity index family.

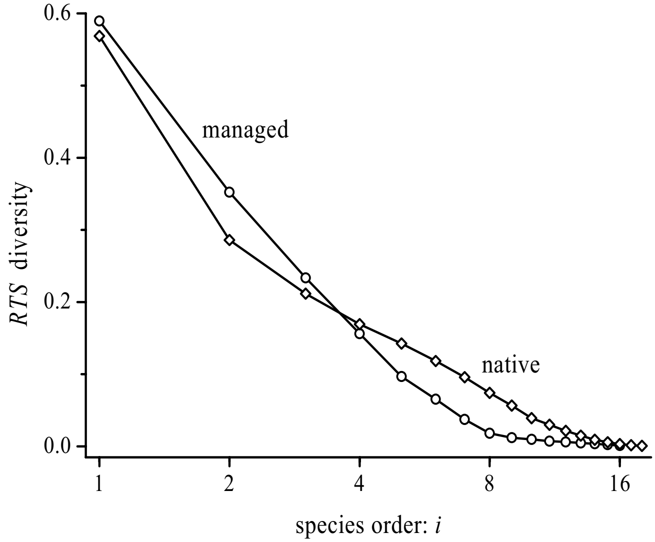

Figure 3. Diversity profiles of the assemblages by the one-parametric *RTS* diversity index family.

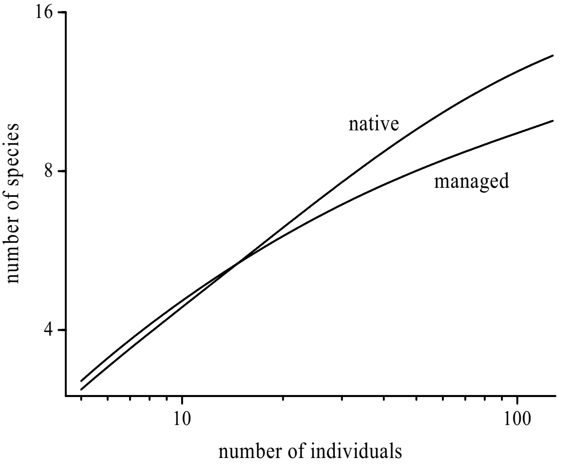

Figure 4. Density independent representation of the expected number of species or *ES*(*m*)-diversity profiles.

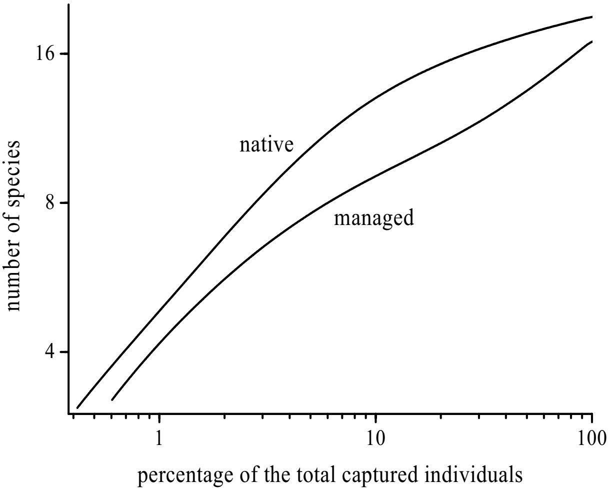

Figure 5. Density dependent representation of the expected number of species diversity or *ES*(*m*)-diversity profiles.

Discussion of diversity comparison of the native forest and managed spruce plantation

Using traditional diversity statistics, the results were confusing (Table 2). This ambiguity is visible on the Rényi diversity profiles (Fig. 2). The profiles cross each other, indicating different relationships at different scale parameter values. Using the *RTS*-diversity profile we can locate the reversal in the diversity ordering: the *RTS*-diversity profiles cross each other between the 3rd and the 4th most frequent species (Fig. 3). Ecologically this phenomena, i.e. the lack of the unequivocal diversity order of the carabid assemblages because of the crossing over of the diversity profiles, occurs because generalist and forest generalist species were more frequent in the managed plantation than in the native forest. There were more rare and/or moderately frequent forest specialist carabids in the native forest causing an increased diversity for this part of the diversity profile.

The usual *ES*(*m*)-diversity profile revealed that using a small (sub)sample (small number of individuals), the managed forest was more diverse: more species occurred in a small (sub)sample than in a (sub)sample of the same size from the native forest. However, with sample sizes larger than 20, the native forest proved more diverse than the managed plantation. This is the usual, density independent representation of the *ES*(*m*)-diversity profile. Each of the diversity profiles produced by the previous methods (Fig. 2 - Fig. 4) resulted in the same diversity relationship.

The number of species in a sample depends on the size of that sample (Gleason, 1922; Fisher *et al.*, 1943). The total number of captured individuals was larger by one-third in the native forest than in the spruce plantation. Therefore, collecting the beetles in a unit area, there were more carabid individuals in the native forest than in the managed plantations. When there are *m* individuals in a unit of the sampled area in the plantation, there are $1199/826 \cdot m \approx 2.9/2 \cdot m \approx 3/2 \cdot m$ individuals in the same area in the native forest. Therefore, a density dependent representation of the *ES*(*m*)-diversity profile is reasonable. Using a density dependent representation of the diversity relationships of the native forest and the managed plantation by the *ES*(*m*)-diversity profile we obtain the diversity profiles shown in Fig. 5. An important difference between the two representations is that in the density dependent representation the curves of the diversity profiles do not cross each other. Therefore, when taking into account differences in density, the two assemblages can be ordered unambiguously according to their diversity. The native forest is more diverse than the managed plantation over the whole range of the scale parameter.

The analysed management practice increased the diversity of the carabid beetles, although there were important and subtle differences that were highlighted by the scalable one-parametric diversity index families. An important difference revealed by the scalable one-parametric diversities that the managed plantation is more diverse for the most frequent or dominant species, which were forest generalist species. The native forest was still more species rich, because of the presence of rare and moderately frequent forest specialist carabid species. In the case of species accumulation plots or *ES*(*m*)-diversity profiles there is an additional benefit besides the scalable comparison. It is the density independent and/or density dependent representation of the diversity profiles. The carrying capacity of the habitat, reflected by density, is frequently an important characteristic of a habitat. In the studied case, the natural forest supported higher density of carabids than the managed plantation. The usual density independent representation of the diversity does not take into account this difference. Using a density dependent representation, we could conclude that the native forest is more diverse than the managed plantation.

We would like to stress that this kind of nature management practice, which encourages the recolonization of herbs, shrubs and trees of the native vegetation by thinning the spruce and especially by creating gaps in the spruce stand is very useful. Advantageous effects of the nature management practice were manifested in the carabid assemblage of the managed plantation. There were subtle differences in the diversity of the native forest and the managed plantation, which were reflected better by the density dependent representation. We do not suggest that the density dependent representation is always automatically more desirable from an ecological point of view than the usual density independent representation. The latter ignores the differences in the densities of the compared assemblages but this is not always important. An appropriate representation should be carefully chosen.

Acknowledgements

We are especially grateful to Gábor Lövei and Søren Toft for comments on the manuscript.

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Investigating isolation - Population biology of *Bembidion monticola*

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Abstract

This paper presents a preliminary study of the only known extant population of *Bembidion monticola* in Finland. The objective of the study was to investigate the isolatedness of populations of *B. monticola* in northern Europe and to reveal sufficient information about the population biology and habitat requirements of the species to facilitate the developement of an appropriate conservation strategy. mtDNA and microsatellite methods were used to investigate the population genetics of the species in Europe.

Key words: Carabid, endangered, mtDNA

Introduction

There were two principle objectives of this study. The first was to gain knowledge of the species *Bembidion monticola* Sturm 1825, by using methods suitable for working with small, sensitive populations. The second objective was to investigate the population biology of species that occur in sparse, highly isolated populations.

The riparian carabid species *Bembidion monticola* is generally distributed over much of Europe (Turin, 2000). Apart from strongholds in the Caucasus and other mountainous regions of central and south-eastern Europe (Lindroth, 1985; Turin, 2000), it appears to be mainly represented by sparsely distributed, small and isolated populations. In Finland, it has been recorded from three sites, though two of these represent only a few old records (Lindroth, 1985) and there is only one known extant population (Helve, 1992) (Fig. 1). All of the Finnish finds originate from ruined water mills, constructed during the 19th century, with footings and workings constructed from stone blocks. Thus this species appears to be synanthropic and lithophilous, at least in this part of its range. Alternatively, it is possible that *B. monticola* was

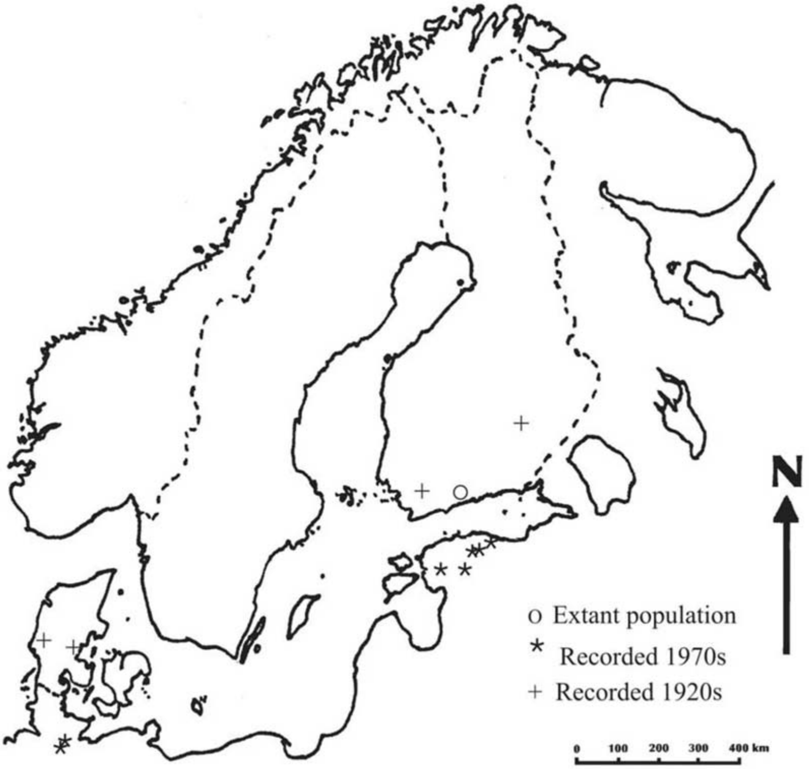

Figure 1. Map of Fennoscandia showing the approximate location of the extant Espoo population, sites of Finnish records from the 1920's and other known records from the region.

better established in this region prior to the construction of water mills, and adopted this synanthropic habitat prior to the species' apparent disappearance from its natural habitat.

Lindroth (1979) has suggested that the species that currently inhabit Scandinavia are either glacial relicts or post-glacial invaders from Europe and Russia. It has been suggested (Lindroth, 1992) that *B. monticola* belongs to the former group, which persisted in high altitude refugia, particularly in the mountains of northern Norway, throughout the last glacial period. After the retreat of the ice sheet, such species expanded from these refugia to become re-established in the region. This would be logical, as the typical habitat of *B. monticola* throughout most of its range (although not in the Baltic states) is mountainous. However, it is difficult to corroborate this, as there are no records of the species from Norway. The existence of extensive populations in the Caucasus and central Europe, old records from the Karelian

Isthmus and a current population in the south of Finland could, however, be considered as suggesting recent expansion into this region from the south-east.

A heterogeneous pool of genetic material is necessary for the persistence of a species (Allendorf & Leary, 1986). Many apparently isolated small populations benefit from dispersal and genetic exchange, for example, as part of a metapopulation (e.g. Hanski, 1999). The genetic analyses in this study will help to resolve the question of whether this population actually has been isolated for a long period or if there is continual dispersal between extant populations, despite their apparent isolation.

Aims

The aims of this study were to:

- Investigate the basic biology of *B. monticola* in Finland, particularly its habitat requirements, so that an appropriate conservation strategy can be developed,
- develop appropriate techniques for investigating small populations of rare carabids,
- compare the status of *B. monticola* populations across northern Europe, particularly their dispersal,
- estimate the size of the population in Espoo, Finland,
- try to detect other populations of *B. monticola* within this region, and in its historical region in Finland.

The closely related (i.e. same sub-species, *Peryphus*) species *Bembidion deletum* Audinet-Serville, 1821 (regionally scarce) and *B. bruxellense* Wesmael, 1835 (regionally common) were used for control purposes. *B. bruxellense* was also used for practising the wing-excision procedure prior to working with *B. monticola.*

Materials and methods

Capture of specimens and mark and recapture study

Dry pitfall traps and hand searching were used to capture arthropods along the banks of the stream from which *B. monticola* has been recorded, in Espoo, Finland (60°14' N, 24°40' E). Six traps were placed at approximately 10 m intervals along the stream bank, throughout the section of the stream that includes the ruined water-mill. A further four traps were also placed at points further away from the stream-bank. The traps were maintained from 25 April until 30 September 2001 and visited twice per week, in order to avoid losses due to the inundation of traps. Subsequently, hand searching alone was used to obtain specimens, as this was found to be more effective. During the season of summer 2002, a mark and recapture study was initiated and flight-wings of specimens were removed for DNA analyses. Site visits were made on a daily basis throughout the season from the 1 May until the 31 August.

All *B. monticola* individuals were counted and marked with a small spot of thinned enamel paint, applied by means of an "000" sized paintbrush and were subsequently re-released. The mark and recapture study was terminated during mid August 2002, when it was observed that the individuals secrete a volatile solvent onto the apex of their elytra, which caused the paint mark to dislodge. It was also observed that *B. deletum* releases a similar solvent, and samples have since been collected for gas-chromatographic analyses.

Individuals of the species *B. deletum* and *B. bruxellense* were obtained from single sites elsewhere in Espoo. Specimens from the same sites were used to enable comparison of within-population genetic variation between the three species in subsequent phases of this study. The extent of genetic variation between these three species is presented in this study.

DNA samples

Flight-wings were used to provide the DNA samples. The live beetles were held firmly but gently by means of foil forceps, as used for handling soft bodied insects. Sharp pointed electron microscopy forceps were then used to prise open the elytra from the apex, taking care not to puncture the elytra or the abdomen of the beetle. These forceps were then used to firmly grip the flight-wing. A second pair of EM forceps secured the wing close to the flight muscle. Subsequently, the flight-wing was excised with a fine micro-surgery scalpel. Such excision technique is necessary when working with sensitive species, as pulling to remove the wing can damage or kill the individual.

Sampling at other sites

Sampling for *B. monticola* was conducted by means of hand-searching on at least two occasions during the season at each of the Finnish sites from which *B. monticola* has been recorded (Fig. 1). Pitfall trapping throughout the duration of one season (20 June-5 September 2001) was also conducted at these sites. These procedures were also used at other potential sites local to the Espoo population, including all known streams with rocky banks and deciduous/mixed forest and water-mills.

DNA extraction

DNA was extracted from the flight-wing using NucleoSpin Tissue kit (Macherey-Nagel), according to the manufacturer's instructions. The only differences were that milliQ-distilled water was used instead of the elution buffer BE, and the final volume was reduced to 30 µl. Concentrations of the DNA extractions were measured using the Gene Quant Pro RNA/DNA calculator (Amersham, Pharmacia Biotech).

DNA sequencing

Universal primers HCO1490 and LCO2198 (Folmer *et al.*, 1994) were used to amplify part of the mitochondrial cytochrome oxidase subunit I (COI) gene. PCR consisted of about 10 ng of DNA, 0.5 µM of each of the forward and reverse primers, 200 µM of each of the dNTPs, 1.5 mM $MgCl_2$, 20 ng of BSA and 0.5 U of Ampli Taq DNA polymerase (PE, Applied Biosystems).

All amplifications were performed in 20 µl volumes using PTC 100 or PTC 200 thermal cyclers (MJ Research, USA). PCR conditions were: denaturation at 95°C for 2 min, followed by 35 cycles of 94°C for 1 min, 49°C for 1 min and 72°C for 1.5 min. Final extension was at 72°C for 10 min. PCR products were purified using the GFX^{TM} purifying kit (Amersham, Pharmacia Biotech) and sequenced in both directions using the BigDye^{TM} terminator cycle sequencing kit (PE, Applied Biosystems) in 10 µl reaction volumes. Sequences were purified using 5% Sephadex^{TM}-solution in Centri-Sep Spin Columns (PE, Applied Biosystems) and resolved on an ABI 377 automated DNA sequencer (PE, Applied Biosystems). Sequences were analysed using ABI PRISM sequencing analysis software version 3.3 (PE, Applied Biosystems) and manually checked and aligned using the SEQUENCHER version 3.0 (Gene Codes Corporation).

Results

In spite of two to four hours of search and 60-67 trap-nights to collect additional *B. monticola,* no individuals were discovered at any of the historic Finnish sites except Espoo. *Nota bene,* the habitat used by this species, i.e. beneath rocks at the water margin, is sufficiently precisely known to permit effective searching in such a short time, during its activity period. A short trapping period using dry pitfall traps at the Espoo site has shown that this capture method is effective for *B. monticola*.

At Espoo, *B. monticola* was the only carabid species found predominantly amongst the rocks at the water margin. The species is a spring breeder (Lindroth, 1985) and was most abundant during the period 20 May – 11 June 2001. It was particularly numerous on 3 June, shortly after the high spring water level had fallen.

Sequencing data

Altogether about 660 bp were sequenced from the mtDNA COI gene from eight *Bembidion monticola* individuals, from one *Bembidion deletum* individual and from one *Bembidion bruxellense* individual. There was no variation between the eight *B. monticola* individuals but the other two species showed a substantial divergence when compared to *B. monticola* or to each other. Pairwise nucleotide divergence between *B. monticola* and *B. deletum* was 11.7%, between *B. monticola* and *B. bruxellense* 12.6% and between *B. deletum* and *B. bruxellense* 13.2%.

Discussion

Sequencing data

The mtDNA analyses revealed that there was no genetic variation at the COI region in the eight individuals tested. As these individuals were collected on different occasions and from a population which appears to be of the order of max. 50 - 100 individuals, it is assumed that they were reasonably representative of the genetic variability present in the population. Further samples have been collected from the population and will also be analyzed. Control material has been collected from *Bembidion deletum*, which is taxonomically closely related to *B. monticola*, morphologically extremely similar and, although not endangered, also appears to be represented by a small number of isolated populations in Finland. Control material has also been collected from *Bembidion bruxellense*, which is in the same sub genus, *Peryphus,* but is common in the region, and their populations are not isolated, and thus dispersal between populations is assumed to be common.

Microsatellites

According to the preliminary results, three of the 12 tested microsatellite loci (originally isolated from *Carabus insulicola* Chaudoir 1869 (Takami & Katada, 2001), appear to function with *B. monticola*. However, further molecular studies are needed in order to develop fully functional microsatellite markers for *B. monticola*. We wish to emphasize that these results are preliminary and considerable work is still required to demonstrate whether or not these microsatellites will function with *B. monticola* and be sufficiently polymorphic for the purposes of this study.

B. monticola appears to occupy a microhabitat at the water margin, as is typical for *Bembidion* species of the *Peryphus* subgenus. Both of the Finnish sites where *B. monticola* has previously been found have been considerably disturbed (one is a mill museum today), so the possibility of the species persisting there was small. However, the reappearance or rediscovery of the species in the 1990's in Espoo suggests at least the possibility that other populations exist in the region. However, it is equally possible that this population is the only one, as surveying of all known potential sites within 10 km failed to find any new populations. An essential element of future work in this study will be continued surveying of potential sites within the region.

The present distribution of *B. monticola* comprises a reasonably stable Euro-Caucasian population (Lindroth, 1985; Bezdek, 2001; Jonaitis, 2001) and a large number of apparently small and isolated populations throughout northern and western Europe. This raises two pertinent questions. Firstly, how did these highly isolated populations arise and secondly, how do they persist?

Regarding the first of these questions, Lindroth (1979) suggests that the carabid fauna of Fennoscandia consists of glacial relict species and species which have colonised from Europe and Russia. The glacial relict species are those which persisted in glacial refugia in the mountains of northern Norway during the last glaciation and subsequently re-colonized the region after the withdrawal of the ice-sheet.

B. monticola may have been previously more abundant in this region, though possibly never having extended onto the Scandinavian peninsula. Subsequent changes in conditions could have resulted in its virtual disappearance from northern Europe, apart from isolated populations at sites where the conditions remained more favourable. It would be difficult to convincingly demonstrate what conditions might be responsible. A strong contender, however, would be climate. The main population strongholds for *B. monticola* are in mountainous regions, where the climate is cool and relatively extreme. Such microclimate could be close to that which was prevalent in northern Europe at some stage subsequent to the Würmsian glacial period, which ended 9-10 000 y B.P.

An alternative potential explanation for the apparent link with water-mills in this region is if they were introduced by a vector linked with the construction or function of these mills. During a visit to one of the Danish sites from which the species has been recorded during July 2003, I found that the only part of the site which possessed the known habitat requirements of the species were also adjacent to a historic century watermill with stone footings. Whilst this hypothesis is intriguing, it is difficult to suggest a means by which this introduction might have occurred.

Regarding the persistence question, there seem to be three plausible explanations. The first of these is that they are not as scarce and isolated as they appear to be. In some parts of northern Europe, such as the United Kingdom (Luff, 1998) and Northern Ireland (Anderson *et al.*, 2002), there are a number of populations with the possibility of dispersal between them. It could be that, besides the few known populations in each country, there are a number of other populations which have not been discovered, with dispersal possible between apparently isolated populations. The apparent isolatedness of these populations could simply be the result of failure to detect additional populations. However, the genetic homogeneity of the individuals so far tested from the Espoo population suggests that the Finnish population is isolated.

A second hypothesis is that macropterous carabid species (*B. monticola* is macropterous) might have greater dispersal ability than has previously been believed. As stated in Thiele (1977), however, it is generally accepted that macropterous carabid beetles do not readily fly and that their use of flight is haphazard. Such behaviour would be unlikely to facilitate the dispersal of a species with such strict habitat requirements. Comparison with genetic material from the Estonian populations would be helpful to investigate this hypothesis. High genetic similarity would support the suggestion that dispersal events between such remote populations are possible. Alternatively, if the Estonian and Finnish (Espoo) populations would be homogenous but substantially different from each other, then that would suggest that there is not dispersal between them.

A third possibility is that the species is on the way to extinction in Finland. Thus the old records for Finland would represent populations which have become extinct and the Espoo population possibly the last population, and likely also to disappear.

In this project, it is the genetic study that is most likely to provide clues to the current distribution of *B. monticola*. The primary objective of this is to assess the homogeneity of genetic material. High homogeneity would suggest that the populations are isolated. This would also support the suggestion that *B. monticola* has been introduced into those regions where it is only represented by such isolated populations. These populations would then simply represent the descendents of the founder individuals, which had managed to breed but not to disperse and colonize beyond these founder populations. This would suggest that *B. monticola* has poor dispersal ability for this region. Alternatively, heterogeneity would indicate that dispersal between different populations had taken place at some stage and would support the possible existence of additional populations. This would suggest that the species is capable of dispersing effectively in this region. These results will thus also allow speculation as to whether the known populations represent genuinely isolated populations or components of metapopulations, and on what scale.

The populations of *Bembidion monticola* in most of northern Europe, not only in Finland, appear to be highly endangered and in need of conservation. In order to formulate a conservation plan, it is necessary to acquire more information about their population dynamics and genetic heterogeneity.

Acknowledgements

The authors wish to thank Professor Ilkka Hanski for advice during the course of this study and Toshka Nyman for performing the laboratory work. We also gratefully acknowledge the financial support provided by the Finnish Entomological Association and Societas Pro Fauna et Flora Fennica.

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The Mantingerveld: effects of fragmentation and defragmentation followed by carabid beetles

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Abstract

In 1958 the reclamation of heathlands and drift sands in the area of Mantingerveld (Netherlands, centre of province Drenthe) came to an end, leaving 300 ha of fragments scattered over this area. In 1992 several areas of arable land between those fragments were restored by removal of the nutrient-rich topsoil layer. The effects of both fragmentation and defragmentation were followed by the Biological Station and compared to a large continuous area (the Dwingelderveld), using ground beetles as indicator species for the soil fauna. Pitfall trapping began in 1959 at the Dwingelderveld and in 1963 at the Mantingerveld and the series have been continued to the present. Although some species were lost from both areas due to overall working factors, more species were lost from the Mantingerveld. After the restoration there was a slight growth in number of individuals in the fragments and some species were caught in the newly restored areas. Also new heathland and drift sand species were caught. As the amount and quality of habitat has increased, it is recommended to reintroduce the non-flying species or to introduce soil transplantates to give the entire poorly dispersing soil fauna a chance to re-establish.

Introduction

By cultivation of the so-called wastelands in the 19th and early 20th centuries, 95% of the Dutch peat bogs, heathlands and poor sandy grasslands were lost (Vermeulen, 1995). Most of the remnant patches were too small for specialised plant or animal populations to survive for long periods. Moreover, the habitat quality in these remnants has decreased because of drying up, acidification and excess fertilisation of the surrounding areas during the second half of the 20th century. The characteristic flora and fauna has decreased and some species have become locally extinct. Because the remnants were scattered in a hostile matrix, populations became isolated from each other, making recolonisation after local extinction nearly impossible. Over the last 10 years attempts have been made in the Netherlands to reverse this process by means of nature restoration, in which the top-soil with its excessive nutrient load was removed. In

DIAS Report, No. 114 (2005),379-389.

this way poor soil conditions were immediately achieved (Klooker *et al.,* 1999, Verhagen *et. al*., 2003), leaving bare substrate for colonisation by characteristic plant species and their corresponding fauna.

One of the largest nature restoration projects in the sandy areas of the Netherlands is the Mantingerveld, Drenthe (Plan Goudplevier, Berris & Gorter, 1991). Up until 1930 there were still several thousand hectares of heathland and drift sand in this area. Cultivation started here relatively recently and stopped at the end of the 1950s. At that time about 300 hectares of the original heathland and drift sand area were left, scattered throughout the area as six fragments of various sizes (Fig. 1), surrounded by arable farmland. From 1990 the nature organisation Natuurmonumenten started to buy the farmland surrounding the heathland fragments. This organisation initiated the first top-soil removal in the area between Hullenzand and Lentsche Veen (number 3 and 4, Fig. 1) in 1992. Today, nutrient poor conditions have been restored in the complex Hullenzand/Lentsche Veen/Martensplek and between Mantinger/Balingerzand and Koolveen. The connection between these two complexes was established in autumn 2003. In the entire area of heathland and drift sand will occupy about 1000 ha. The costs of the project were estimated at about 4 million Euros in 1991.

The hypothesis was made that fragmentation of the Mantingerveld led to a loss of heathland species. Further, it was hypothesised that the decline of heathland species and populations in the fragments could be stopped by restoring the Mantingerveld. The target species were expected to benefit from the habitat improvement of the fragment surroundings and to colonise the restored areas without assistance. For carabid beetles this research aimed to find out whether or not species were lost because of fragmentation; whether or not immigration to the restored areas is taking place, and how quickly.

Methods

In 1963 the Biological Station in Wijster started to sample the carabid fauna in the Hullenzand fragment, which had 23 ha of heathland. Sampling was done with two standard series consisting of two live pitfall traps and one filled with formalin. The traps were emptied weekly up to 1970. In 1986 the sampling was started again with the same trap combination in exactly the same places, and it has continued to the present with only a few years missing.

In 1992, the year of the first topsoil removals, the Mantingerveld was sampled at nine different locations, eight of them repeated in 1993. Of these eight locations several were sampled again in 1996 and 1997 (Fig. 1). The locations in Lentsche Veen and Hullenzand, and those in between, were also sampled in 2002 and 2003.

During the entire period the Dwingelderveld was also sampled; in all but two years since 1959 to the present, traps have been run at two sites at least. The Dwingelderveld is an old

Table 1. The 17 specialist heathland species (A1-species; Turin, 2000, Boeken *et al.*, 2002) that were present before 1970 both at the Dwingelderveld and the Hullenzand, compared to their presence from 1990 up till now. * = species recorded 1990 or later. 'M' = monomorphic macropterous, 'D' = dimorphic in wing-length, and 'B' = monomorphic brachypterous, (fl) : seen in flight.

Species	Dwingelderveld since 1990 (1600 ha)	Hullenzand since 1990 (23 ha)	Dispersal power
Agonum ericeti	*		B
Agonum sexpunctatum	*	*	M (fl)
Amara equestris	*	*	M
Amara infima	*	*	B
Bradycellus ruficollis	*	*	M (fl)
Carabus arvensis	*	*	B
Carabus cancellatus			B
Carabus nitens	*		B
Cicindela campestris	*	*	M (fl)
Cymindis vaporariorum	*	*	B
Harpalus latus	*	*	M (fl)
Harpalus solitaris	*	*	M
Miscodera arctica	*	*	M (fl)
Olisthopus rotundatus	*	*	D
Poecilus lepidus	*	*	D
Pterostichus diligens	*	*	D (fl)
Trichocellus cognatus	*	*	M (fl)
Total	16	14	

heathland area of about 1600 ha, which has never been fragmented and thus can be used as a control site.

The heathland carabid beetles of the Dwingelderveld and Hullenzand

During the 1960s, 22 heathland specialist species (A1-species, Turin, 2000) were found at the Dwingelderveld and 17 at the much smaller Hullenzand (Table 1). These numbers are remarkable considering that no more than 29 heathland specialist species have been found in the North-Netherlands. An additional species, *Acupalpus dubius*, was recently captured by hand at Hullenzand. Though *A. dubius* is a good flyer, it has never been found at Hullenzand before. All the heathland species found at Hullenzand were also present at the Dwingelderveld. Of the species found in the 1960s*, Carabus cancellatus* has disappeared from both. This wingless species is rapidly disappearing in the whole of north-western Europe due to unknown factors (Turin, 2000). Two other brachypterous heathland species, *Carabus nitens* and *Agonum ericeti*, have disappeared from the Hullenzand but not from the Dwingelderveld. These species have also declined elsewhere in north-western Europe. Habitat destruction and fragmentation are thought to play a major role in this decline (de Vries & den Boer, 1990; Turin, 2000).

Table 2. The carabid species of poor sandy and open habitats (B1-species) which have ever been found at the Hullenzand. Their presence is compared with that of the Dwingelderveld. Presence (*) is indicated both for the periods before and after 1990. "M" = monomorphic macropterous, "D" = dimorphic, "B" = monomorphic bachypterous, (fl) = seen in flight.

	Dwingelderveld		Hullenzand		Dispersal power
	1959/ 1989	Since 1990	1963/ 1989	Since 1990	
Bembidion nigricorne	*	*	*	*	B
Calathus ambiguus			*	*	M (fl)
Cicindela hybrida	*	*	*	*	M (fl)
Cicindela sylvatica	*		*		M (fl)
Cymindis macularis	*	*	*	*	D
Harpalus neglectus				*	B
Masoreus wetterhallii			*	*	D
Notiophilus germinyi	*	*	*	*	D
Notiophilus substriatus		*		*	M (fl)
Stenolophus teutonus				*	M (fl)
Total	5	5	7	9	

Originally more heathland specialists were found at the Dwingelderveld. However, it is unknown whether these species never occurred at the Mantingerveld or had already disappeared before the area was first sampled. They might have disappeared during the period before, when habitat destruction and fragmentation took place. Since 1990, 18 heathland specialist species have been found at the Dwingelderveld, while only 14 at the Hullenzand. In addition, the heathland species *Carabus arvensis* was caught for the last time at Hullenzand in 1991 as a single individual. From 2000 to 2003, *C. arvensis* showed a strong recovery at the Dwingelderveld but was still not caught at Hullenzand. This seems to be an important example of the necessity of a large habitat area for the recovery of a wingless species. Since 1977 only 23 heathland specialist species have been found in the North-Netherlands.

Carabid beetles of poor sandy and open soils at Dwingelderveld and Hullenzand

For carabid beetles of poor sandy and open areas (classified as B1-species in Turin (2000)), the Hullenzand is a unique place, even compared to the Dwingelderveld (Table 2). The reason for this probably lies in the history of the two areas. Before 1954 the Hullenzand was part of a very large drift sand and dry heathland area, the Mantingerveld-complex. This kind of habitat is also present at the Dwingelderveld, but only in smaller spots, which are sampled only occasionally (den Boer, 1977; van Essen, 1993).

Cicindela sylvatica has disappeared from both areas. This is a species of old heathland mosaics, which can be found in spots with hard bare sandy substrates. Possibly because such places became overgrown by mosses, a large part of its hunting habitat disappeared. This problem plays a role in many such places in Northwestern Europe (T. Aßmann, pers. comm.).

In the north of the Netherlands, *Masoreus wetterhalli* is only found at the Hullenzand. Another species unique to this area is *Harpalus neglectus*. This flightless species has never previously been found in the north, although sufficient habitat has always been available here (Turin, 2000). According to T. Aßmann (pers. comm.), the species is easily transported with plant material. Indeed, in the beginning of the last decade botanists introduced several plant species here to follow the effect within the restoration areas. Other recently established B1-species at Hullenzand are *Notiophilus substriatus* (also at the Dwingelderveld) and *Stenolophus teutonus*. These two species are excellent flyers and were recorded earlier from neighbouring areas of heathland. Their appearance might be seen as a result of increasing habitat quantity and quality.

The carabid fauna in the fragments of the Mantingerveld

In 1992 Natuurmonumenten started executing the ideas from “Plan Goudplevier”. The first topsoil removals on arable land took place west of Hullenzand, somewhat later north of Hullenzand and between Hullenzand and Lentsche Veen (Fig.1). The forest edge around Hullenzand was also removed. At the present day (2003) connections have been made between Martenplek and Lentsche Veen and Mantingerzand/Hullenzand.

To determine the original state of the carabid fauna, Theo van Dijk started to sample all heathland fragments and the arable land west from Hullenzand. All sample sites are shown in Fig. 1. The results for 1992/1993 for these areas are given in Fig. 2 concerning the heathland specialist beetles and beetles of poor, sandy and open areas (A1- and B1-species of Turin, 2000).

Although not the largest fragment in the Mantingerveld, Hullenzand is the richest, with 13 A1- and B1-species in 1992 and 14 in 1993. Within a small area, Hullenzand shows high heterogeneity in the poor environmental conditions: from peat-like heathland (wet) up to partly vegetated drift sand hills (dry). This variation means that within the poor environmental conditions, most species will be able to find a spot to survive adverse circumstances. Furthermore, in the agricultural period the Hullenzand was surrounded by a row of trees. This probably protected the area from high agricultural influences, so that these species had a higher chance to survive this period. Also the highest number of non-flying stenotopes, indicative of old populations, are found in this fragment. At the other end of this spectrum we find one of the largest fragments, Martensplek. Here only few of the A1 and B1 species are found, and most of those found are good dispersers. This area probably had a high turn-over of disappearing and recolonising species. In 1992/1993 Martensplek was a tree covered area with scattered heathland spots. Even at the much smaller place Koolveen, where birches

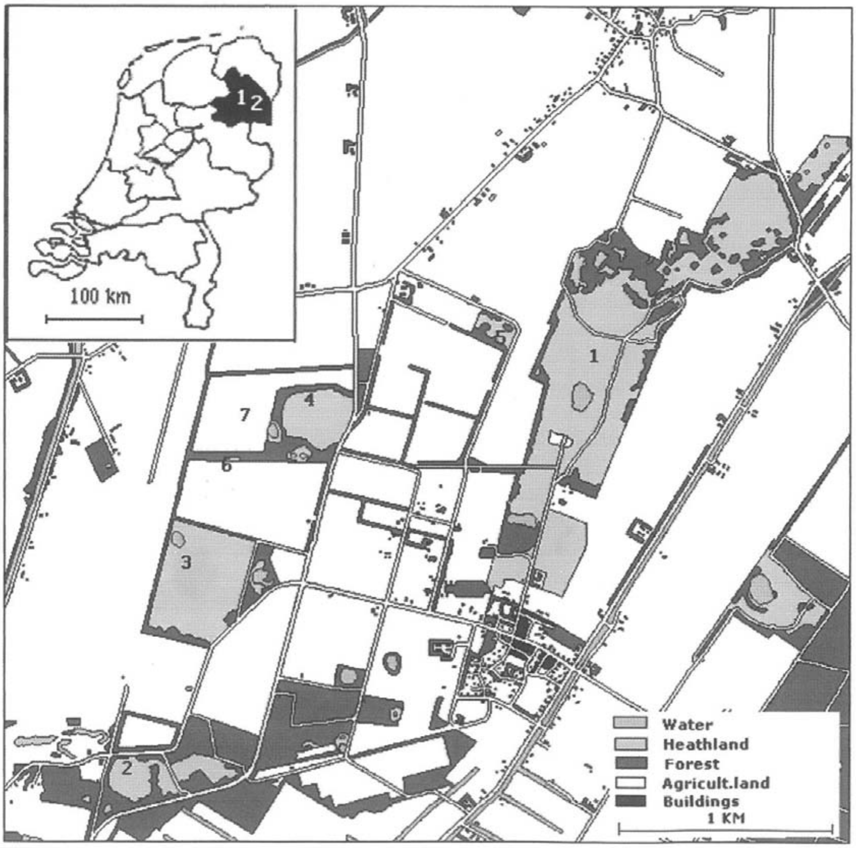

Figure 1. The research areas in Drenthe (Netherlands). 1. Dwingelderveld, 2. Mantingerveld, and enlarged, the Mantingerveld in detail. The situation shown is from between 1959 and 1992. The heath fragments: 1. Mantingerzand (208 ha), 2. Martensplek (56 ha), 3 Lentsche Veen (45 ha), 4. Hullenzand (23 ha), 5. Koolveen (a few ha). Further: 6. Larch-bush, and 7. Arable land from which the topsoil layers were removed in 1992. All locations were sampled in 1992.

invaded the heath, the situation was less adverse. The catches at the large area Mantingerzand were disappointing, but this might be affected by the sampling conditions. Here only one sampling site was used during one year, placed in a field of mainly *Molinia*. In 2004 this area will be sampled more intensively.

The results so far show that Hullenzand and to a lesser extent Lentsche Veen are the backbone of the "Plan Goudplevier", as far as the soil fauna is concerned. This is also shown by the fast colonisation of the adjacent restoration area and the number of species found in a small heath in the nearby larch forest.

Colonisation of the restoration area

Immediately after topsoil removal from the arable land west from Hullenzand the first heathland specialist carabids and carabids of poor sandy and open areas were caught at this site (Figs. 2 & 3). In the first five years only a few individuals were caught, suggesting that colonisation took place, but not settlement. However, from 1997 the numbers also increased and catches of species like *Amara equestris* and *Poecilus lepidus* fluctuated between 10 and 100 individuals per year. This suggests that some heathland species could already find suitable habitat although the area still did not look like a heathland (Verhagen *et al.*, 2003).

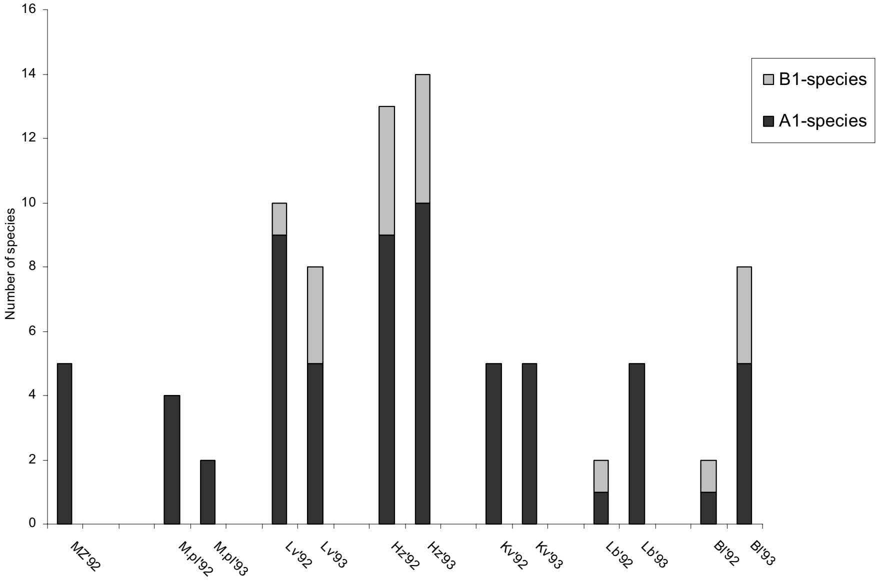

Figure 2. The carabid species of poor soils present on the locations mentioned in Fig 1. A1 = heathland specialists species. B1 = species of drift sand and dry heathland. Since 1977, 23 A1-species and 10 B1-species are known from the northern part of the Netherlands.

Directly after soil removal, the number of eurytopic species and species characteristic of arable land declined dramatically. The yearly catches of species like *Amara plebeja*, *Pseudophonus rufipes* and *Bembidion tetracolum* declined from several hundreds in 1992, to about 10 in 1993, and to hardly any later on. These carabid beetles show rapid responses to changes in soil minerals and are therefore excellent indicators of soil quality. This contrasts with plants, which respond much more slowly (Verhagen *et al.*, 2003). Fig. 3 shows the increase in the numbers of heathland specialist species in the restoration area, compared to the number of species found at the old Hullenzand and Dwingelderveld. In 2002 the number of heathland specialist species found at the restoration area already came close to the number found at Hullenzand. The Hullenzand seems to act as a source for the neighbouring restoration areas, and it looks like these areas will have the same carabid fauna as the

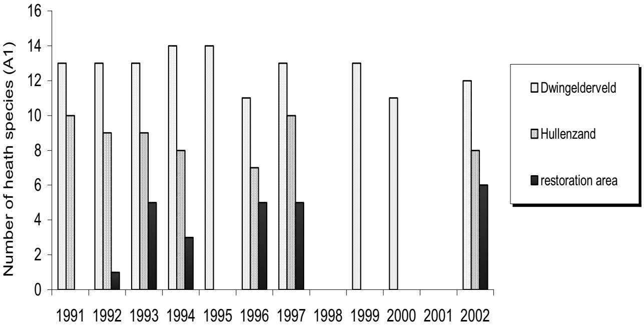

Figure 3. The number of specialist heathland species of carabid beetles caught in successive years on the Dwingelderveld, Hullenzand and the restoration areas adjacent to the Hullenzand (No 7. in Fig 1).

Hullenzand after only a few years. For the vegetation it will probably take some decades more.

New species at the Mantingerveld

Ten new carabid species have been found at Hullenzand and the surrounding areas since the restorations started (Table 3). All these new species are more or less restricted to nutrient-poor sandy areas (Turin, 2000). The species *Laemostenus terricola* must have been there before. This species has still not been caught in the traps of the Biological Station, but was incidentally caught in other research concerning spiders, in traps placed at a site which was previously forest. The non-flying species *H. neglectus* might have been introduced by botanical experiments. *Amara kulti* has never been caught in flight, but it is long-winged and has good flight muscles. There are no flight observations probably because this species is very rare in the Netherlands. This species has also recently been caught near the Dwingelderveld. Thus, eight species have colonised the area spontaneously by flying and can probably find good habitat. This might be due to the exceptional circumstances created by topsoil removal. One might ask whether or not these species will disappear after the area has stabilised as heathland. In that case we have to deal with nomads, species specialised on recent changes in environment. It is worth noting that the eight flying species have their centre of distribution south of the Netherlands; this suggests that recent changes in climate could play a role as well.

Table 3. The 10 new carabid species, found at the Hullenzand and the adjacent restoration areas since 1991/1992. For explanation of dispersal power, see Table 1. A1 = heath/peat moor, B1 = poor sandy and open soils, B2 = extensive arable land on sandy soils. CZ = open sandy soils, but too low number of records (Z = rare) for specific classification, RO = only a few records, habitat definition impossible (in the Netherlands). Codes and distribution according to Turin (2000).

Species	Dispersal power	Habitat	Central point of the distribution
Dromius angustus	M (fl)	CZ	Mid-France/Southern Germany
Harpalus distinguendis	M (fl)	B2	Mid/Southern Europe
Harpalus smaragdinus	M (fl)	B2	Netherlands central
Amara kulti	M	RO	Mid-France/Northern-Italy
Acupalpus dubius	M (fl)	A1	Northern-France
Harpalus melancholicus	M (fl)	CZ	Southern-France/ Northern-Italy (Coast)
Harpalus neglectus	B	B1	Spain/Germany
Notiophilus substriatus	M (fl)	B1	Mid-France/Italy/Balkan
Stenolophus teutonus	M (fl)	BZ	Mid-Europe
Laemostenus terricola	B	B2	Netherlands central

Is nature restoration a success?

In the Netherlands a red list for carabid beetles does not exist. However, some provinces have put carabid beetles on priority lists: lists of species for which the province plays a major role in their distribution. Five carabid species have been placed on the priority list for Drenthe (van Zanten & Dekker, 1995): *C. sylvatica*, *C.nitens*, *A. ericeti*, *Cymindis vaporariorum* and *Harpalus solitaris*. One of these species, *C. sylvatica*, has already disappeared from Drenthe and from the northern Netherlands and has only rarely been found elsewhere in the Netherlands recently. The other four species are still present in Drenthe and, although not widespread, Drenthe is still the province where most catches of these beetles are made.

All five priority species have been recorded at the Hullenzand in the past. At this moment only two of these species are present there and four at the Dwingelderveld (Table 1). In 2003 an introduction experiment with *C. nitens* started. Should this experiment be successful, Mantingerveld and Dwingelderveld will harbour the most of these priority species and must be of importance for the province.

As a result of topsoil removal, *H. solitaris* increased, especially in the restoration sites. Also the increasing numbers of A1- and particularly B1-species suggest that these species benefit from this kind of management, both in the restoration and in the old areas. By topsoil removal agricultural influences are removed so that even in the old areas the situation improves and characteristic beetles can increase. Unfortunately “Plan Goudplevier” probably came too late for *C. arvensis*.

In 5 to 10 years the restoration areas appeared to be colonised by most A1- and B1-species. Especially in 2002 also larger numbers of poor dispersers were found here. The B1- species seem to benefit most from these new areas. At this moment the Hullenzand and surroundings harbour nine of the ten B-1 species that occur in northern Netherlands. Eight of these species have entered the restoration areas as well. Apparently, these larger somewhat bare sandy areas have had a positive effect on these species. However, this means that such areas must be kept partly intact. Extensive grazing with Scottish Highland cattle, as occurs now, might support this.

The fact that “Plan Goudplevier” as far as carabid beetles are concerned can be regarded a success, is mainly determined by the species richness of Hullenzand as a source area. Comparable projects at Eexterveld, Tichelberg and Ennemaborg show more disappointing results so far. In these cases an adjacent source area is missing or the species were already lost in the source area before restoration.

Carabid beetles are often regarded as indicators of the state of the entire soil fauna. Consequently, we should also conclude that “Plan Goudplevier” must be a success for the whole soil fauna that was still present in 1992. Certain species of carabid beetles that have been lost could be reintroduced, giving them a chance to settle again. However, which elements of the soil fauna have been lost during the adverse times is unknown. To give the entire soil fauna a chance for re-settlement, introduction of large and deeply-cut sods might be a solution.

Acknowledgements

We thank Piet den Boer for starting sampling the areas mentioned in 1959 and 1963. Up to the present day, he and his wife Wil are emptying these traps weekly, making these continuous samplings the longest ecological series in the world. We would also like to thank Theo van Dijk, who started the sampling when “Plan Goudplevier” was initiated. Over the last few years, this research has been financed by the Prins Bernhard Cultuur Fonds.

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The phylogeny of African Anthiini beetles (Coleoptera:Carabidae) inferred from mitochondrial NADH dehydrogenase subunit 5 (ND5) DNA sequences

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Abstract

Arid and semi-arid areas of southern Africa are inhabited by predatory ground beetles of remarkable size. Belonging to the tribe Anthiini, beetles of the genera *Anthia* WEBER, 1801 and *Thermophilum* BASILEWSKY, 1950 show a great variability in size, body length and proportions as well as of markings on head, pronotum and elytrae, even within species. The phylogenetic relationships of these ground beetles are inferred on the basis of mitochondrial DNA sequences. Nine species of these two genera- plus one species each from the genera *Cypholoba* CHAUDOIR, 1850 and *Baeoglossa* CHAUDOIR, 1850, - were included in the examination. *Anthia* formed a monophylum which surprisingly contained *Cypholoba. Thermophilum* was paraphyletic in respect to the *Anthia*. The two main groups within *Anthia* contained *A. cinctipennis*.

Key words: Introgression, mtDNA, gene tree

Introduction

The tribe Anthiini comprises 129 species (Lorenz, 1998). Following Schmidt & Gruschwitz (2002) the actual number has to be 127, because 2 species-names have been synonymized in the meantime. In this paper we focus on the genera *Anthia* WEBER, 1801, *Thermophilum* BASILEWSKY, 1950, *Cypholoba* CHAUDOIR, 1850 and *Baeoglossa* CHAUDOIR, 1850. Arid and semi-arid regions of southern Africa are inhabited by 20 species of *Anthia* and *Thermophilum* (Schmidt & Gruschwitz, 2002). The body lengths of these predatory ground beetles range from 21 mm to 58 mm. Within species the body length may vary considerably due to different climatic conditions in their large area of distribution (Schmidt, 2002). Body proportions and markings on head, pronotum and elytrae show an enormous variability as well (Schmidt & Gruschwitz, 2002).

Thermophilum was originally described as a subgenus of *Anthia* named *Thermophila* HOPE, 1938 but Basilewsky (1948) changed the status of *Thermophila* into a genus (cf. Schmidt & Gruschwitz, 2002). He justified this with sexual dimorphism which occurs only in *Anthia* species. In 1950 Basilewsky changed *Thermophila* into *Thermophilum* as the former was already in use for the lepidopteran genus *Thermophila* HÜBNER, 1819 (Schmidt & Gruschwitz, 2002). The mandibles of male individuals of *Anthia* are enlarged on one side and the base of the pronotum is widened characteristically (Fig.1). Much more than in other species of *Anthia*, the size of these features depends on the body length in *Anthia cinctipennis*. Body length itself depends on climatic and nutritional factors during larval development (Schmidt, 2002). Not only *Anthia* and *Thermophilum* are discussed controversially in respect to phylogeny and systematics but all the 127 species of the tribe Anthiini. All previous attempts to infer the phylogeny of the Anthiini have been based on morphological characters (e.g. Arndt & Paarmann, 1999). The use of molecular data offers the possibility of examination of a wider range of characters. The ND5 gene was chosen because it is one of the fastest evolving mitochondrial genes and is therefore especially suited to resolve phylogenetic relationships within genera (Su *et al*., 1996).

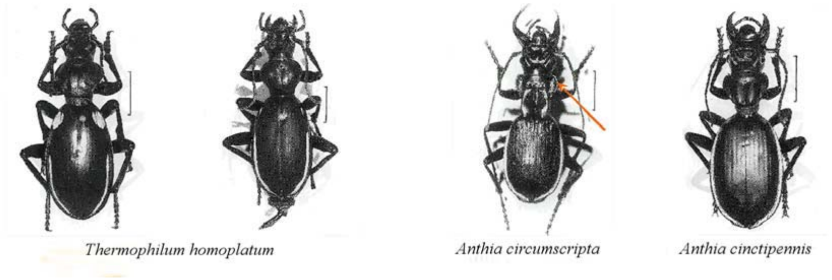

Figure 1. Some Anthiina species. The arrow indicates the hair marking on the pronotum which distinguishes *Anthia circumscripta* from *Anthia cinctipennis*. Note the different marks on the elytrae of *Thermophilum homoplatum*. The bars correspond to 10 mm. Photos © by Almuth Schmidt.

Materials and methods

Twenty-eight specimens of ten Anthiini species (*Anthia, Baeoglossa* and *Thermophilum*) and one specimen of the Anthiini species *Cypholoba* were examined as ingroup (Table 1). The outgroup includes one specimen each of *Carabus nemoralis*, *Abax carinatus* and *Percus strictus*. The sequences of a part of the NADH-dehydrogenase subunit 5 (ND5) comprise a total of 795 bp. DNA was extracted from hind leg muscle of frozen specimens using the Qiagen DNeasy Tissue Kit. DNA was subsequently amplified using the Qiagen PCR Master Mix Kit with ND5 primers as described in Su *et al*. (1996) and Düring & Brückner (2000), respectively. PCR fragments were separated by electrophoresis on an agarose gel (1.5%).

PCR products were purified using the Qiagen QiaEx Gel Extraction Kit. Sequencing was performed on an ABI 737 stretch automatic sequencer using Applied Biosystems´ Dye Terminator Cycle Sequencing Ready Reaction Kit. Obtained sequences were aligned using the multiple sequence alignment computer programme ClustalX 1.81 (Thompson *et al*., 1997). Phylogenetic analysis was carried out using the maximum parsimony method (MP) implemented in the computer programme PAUP 4.b10 (Swofford, 2002). For indication of branch support, bootstrap values at 1000 replicates as well as decay indices (Bremer Support) were calculated.

Table 1. List of examined species.

Species	**Author/year**
Anthia cinctipennis	Lequien 1832
A. circumsripta	Klug 1853
A. thoracica	(Thunberg 1784)
Baeoglossa anthracina	(Guerin-Meneville 1847)
Cypholoba chaudoiri	(Peringuey 1892)
Themophilum œmilianum	C.A. Dohrn 1881
T. burchelli	Hope 1832
T. cephalots	Guerin-Meneville 1845
T. homoplatum heres	Lequien 1832
T. limbatum kolbei	Obst 1901
T. massilicatum	Guerin-Meneville 1845

Table 2. Places where specimens were collected.

Places of origin	**Abbreviation**
Blouberg N.R. RSA	blou, rsa
Botswana (26.26.38 S, 21.08.09 E)	bots
Botswana, Gangwe Pan	gang.bots
KGNP, RSA	kgnp
Khorixa, Namibia	ko, nam
Kutse Game Reserve, Botswana	kuts, bots
Langjan N.R. Nordtransvaal/RSA	lang, rsa
Mountain Zebra N.P., RSA	mtzebra
Ovisten N.S., RSA	OVIS
Leicester, Namibia	leic,nam
Windhoek,Namibia	win, nam

Results and discussion

The NCBI gene bank accession numbers for the outgroup specimens are: *Abax carinatus* – AF190045; *Carabus nemoralis* – AB047265; *Percus strictus* – AF537170. The ingroup specimens have not yet been submitted. The data set contained 795 bp, 273 characters were

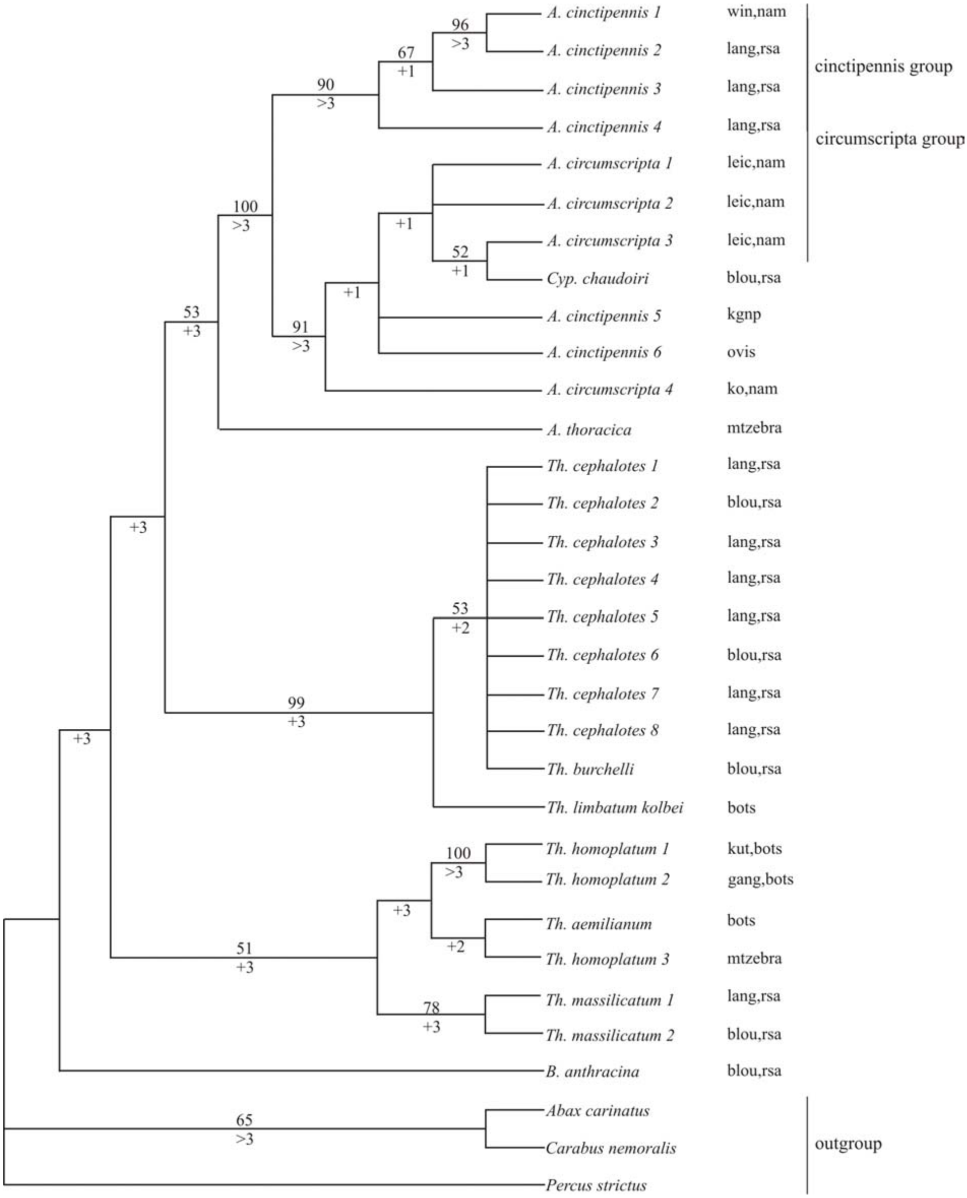

Figure 2. Consensus Tree of 25 equally parsimonious trees based on ND5 sequences (795 bp). Length of the tree: 530 steps; 273 characters variable, 149 characters informative. The numbers above the branches represent bootstrap values (those below 50% are not shown). The numbers below the branches represent decay indices. Abbreviations for species: A.: *Anthia*, B.: *Baeoglossa*, Cyp: *Cypholoba*, Th.: *Thermophilum*. Abbreviations behind the species names: Places of origin.

variable and 149 were informative. The obtained consensus tree (Fig. 2) comprised three major groups. The first *Thermophilum* group included *T. homoplatum, T. massilicatum* and *T. aemilianum.* The second group contained *T. cephalotes, T. burchelli* and *T. limbatum kolbei.* The *Anthia* species formed a group which also included *Cypholoba chaudoiri*. This was remarkable because this species represents a different genus and in Lorenz's classification system (1998) it is allocated to the Anthiini subtribe Cypholobina STROHMEYER, 1928. Therefore, *Cypholoba* should have been an outgroup in relation to the Anthiina (*Anthia* and *Thermophilum*). In contrast, it was located at one of the terminal splits in our tree. Thus, a repetition of the sequencing for *Cypholoba* was performed to exclude the possibility of a fault caused by polluted DNA. As *Cypholoba* does not show sexual dimorphism, this character may have been reduced secondarily according to the position in our tree and therefore led to the systematic classification. Since the molecular investigation included solely mitochondrial gene sequences, it can not be excluded that the obtained tree represents a gene genealogy rather than a species genealogy. Possible explanations for the position of *Cypholoba* in our tree are introgression (see general remark in Ballard 2000; Düring *et al*. 2001 for the carabid taxon *Chrysocarabus*), the inadvertent sequencing of a mitochondrial pseudogene (Bensasson *et al*., 2000, 2001) or a very recent split of the species in question (Li, 1997). However, Arndt & Paarman (1999) also found *Cypholoba bihamata* and *Cypholoba macilenta* to be included within the Anthiina in an examination of larval characters. In an examination of 28S rDNA sequences, *Anthia* groups with *Cypholoba* as well, although *Thermophilum* was not included in that study (Ober, 2002).

The *cinctipennis* specimen # 1 from the population within the *circumscripta* distribution area forms a well supported cluster with the *cinctipennis* specimens of the northern part of South Africa near to the *circumscripta* range. Those specimens coming from remote populations far from the *circumscripta* distribution area form a different cluster together with the *circumscripta* specimens. This pattern looks like a character displacement. Schmidt & Gruschwitz (2002) stated that *A. cinctipennis* and *A. circumscripta* are sibling species. The feature for distinguishing both species is a marking with white hair on the side of the pronotum of *Anthia circumscripta* (Fig. 1) which is absent in *A. cinctipennis*. Both species inhabit a wide area and therefore they show significant variability. This results in the following question: Are *Anthia cinctipennis* and *A. circumscripta* really distinct species or is introgression the reason for this grouping?

Thermophilum was grouped together as well and is furthermore paraphyletic in respect to the *Anthia/Cypholoba* group. The support by bootstrap values and decay indices was rather weak for the paraphyletic state. For this reason, the phylogenetic relationships between the *Anthia* and the two major *Thermophilum* groups remain unresolved. The position of *Cypholoba* as well as of some species like *massilicatum* in our tree does not fit to the phylogenetic relationships inferred from morphological data. Our aim will be the analysis of a fast evolving nuclear gene to answer the question whether an introgression produced the conflict in the monophyly of *Anthia.*

Acknowledgements

We thank Almuth D. Schmidt for providing us with the specimens and letting us use some of her photographs for this paper.

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