

Absence Asymmetry: The Evolution of Monorchid Beetles (Insecta: Coleoptera: Carabidae)

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ABSTRACT Asymmetrical monorchy, or the complete absence of one testis coupled with the presence of its bilateral counterpart, is reported for 174 species of the carabid beetle tribes Abacetini, Harpalini, and Platynini (Insecta: Coleoptera: Carabidae) based on a survey of over 820 species from throughout the family. This condition was not found in examined individuals of any other carabid beetle tribes, or of other adaphagan beetle families. One monorchid taxon within Platynini exhibits symmetrical vasa deferentia at the beginning of the pupal stadium, suggesting that developmental arrest of the underdeveloped vas deferens takes place in pupation. The point at which development of the testis is interrupted is unknown. Complete absence of one organ of a bilateral pair—absence asymmetry—is rarely found in any animal clade and among insects is otherwise only known for testes in the minute-sized beetles of the family Ptiliidae, ovaries in Scarabaeinae dung beetles, and ovaries of some aphids. Based on current phylogenetic hypotheses for Carabidae, testis loss has occurred independently at least three times, and up to five origins are possible, given the variation within Abacetini. Clear phylogenetic evidence for multiple independent origins suggests an adaptive or functional cause for this asymmetry. A previously posited taxon-specific hypothesis wherein herbivory in the tribe Harpalini led to testis loss is rejected. Optimal visceral packing of the beetle abdomen is suggested as a general explanation. Specifically, based on the function of various organ systems, we hypothesize that interaction of internal organs and pressure to optimize organ size and space usage in each system led to the multiple origins and maintenance of the monorchid condition. Testes are the only redundant and symmetrically paired structures not thought to be developmentally linked to other symmetrical structures in the abdomen. Among all possible organs, they are the most likely—although the observed frequency is very small—to bypass constraints that maintain bilateral symmetry, resulting in absence asymmetry. However, based solely on our observations of gross morphology of internal organs, no function conclusively explains the ontogenetic loss of one testis in these taxa. Unlike the analogous absence asymmetry of organs in other animal groups, no dramatic body-form constraint—e.g., snakes and lung loss, ptiliid beetles' small body-size and relatively giant sperm—or adaptive scenario of improved locomotory performance—e.g., birds and ovary loss due to flight constraints—applies to these carabid beetles. We tentatively suggest that testis loss is driven wholly by an

interaction among the internal organs of these beetles, possibly due to selective pressure to maximize the comparatively large accessory glands found in these taxa. However, as the ordering of these evolutionary events of testis loss and accessory gland size increase is not known, large accessory glands might have secondarily evolved to compensate for a decreased testicular output. *J. Morphol.* 000:000–000, 2005. © 2005 Wiley-Liss, Inc.

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Asymmetrical loss of a plesiomorphically paired organ is rare among bilateral metazoan animals. Among vertebrates, such losses are largely restricted to snakes or snake-like animals, where one or the other lung has been reduced during evolution of various lineages (Bellairs, 1970), and to birds, where most taxa are characterized by the loss of one ovary (Kinsky, 1971). Monorchy, or the presence of only one testis, is reported for many nonvertebrate bilaterian groups. At least some monorchid taxa are known in the Entoprocta, Gnathostomulida, Nematoda, Nematomorpha, Rotifera, Platyhelminthes, Gastrotricha, Pterobranchia, Tardigrada, Crustacea, and Chilopoda (Rebecchi et al., 2000; Brusca and Brusca, 2003). In these taxa, the testis is most likely either a medial, symmetrical structure resulting from fusion of paired testes, or an ancestrally bilateral unitary structure.

Among the extremely diverse Insecta, spanning over a million extant species arrayed across 27 ex-

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tant orders, with evolutionary roots in the Devonian (Gullen and Cranston, 2000; Klass et al., 2002), there are only four reported instances of the asymmetrical presence of a primitively paired organ system. Three instances are found in the order Coleoptera and one in Hemiptera. Two cases are ovaries that are asymmetrically lost in some scarab beetles (Halffter and Mathews, 1966; Halffter and Edmonds, 1982) and in aphids (Woodward et al., 1970), both taxa wherein females invest extraordinarily in relatively few offspring. Feather-winged beetles (Coleoptera: Ptiliidae) are a third case, characterized by a single testicular mass—based on a study of eight species representing seven genera from two subfamilies, Ptiliinae and Acrotichinae (de Marzo, 1992). *Nossidium pilosellum* has paired vasa deferentia connected to a single, medial testicular mass; the seven other examined species have the asymmetrical presence of a single testis. Ptiliids are the smallest known beetles, on average about 0.50 mm, ranging from 0.30–2.0 mm. Additionally, they are peculiar in having relatively gigantic sperm, e.g., sperm 1.4 mm long in the 0.7-mm long beetle *Ptinella aptera* (Taylor et al., 1982; Dybas and Dybas, 1987).

The fourth instance is within the usually predaceous beetle family Carabidae (Coleoptera), the subject of this article. Members of one tribe (Harpalini) have been previously reported to possess a single testis on the right side (Dufour, 1825; Smrž, 1981). We have found asymmetrical monorchy to be more widespread in carabids than previously reported. In this article we describe the phylogenetic distribution of monorchy and discuss its evolution. We show that monorchy has arisen independently several times during diversification of taxa comprising the family. Unlike other reported monorchid conditions in insects or of any other groups, monorchy in Carabidae has proceeded by the asymmetrical loss of either the right or left testis. The absence asymmetry of testes in carabid beetles is therefore a unique type of evolutionary loss among bilateral animals. Whether the testis is absent due to degeneration of gonad primordia in the embryo or larvae, analogous to mandible loss in thrips (Heming, 1980, 1993), or whether the primordial cells never migrate to one side is not known. Herein we present a comparative study of carabid testes and suggest that investigation of the developmental and genetic basis for monorchism in Carabidae would provide an opportunity to study the underlying mechanisms that establish bilateral symmetry in animals and how asymmetries become unilaterally fixed (Palmer, 1996).

Carabidae

Among the families of the coleopteran suborder Adephaga, Carabidae, with nearly 40,000 species (Lorenz, 1998), is the most diverse. Because of the worldwide geographical distribution of the family

and frequent abundance of individuals of some species, more morphological studies have focused on member taxa than on any other coleopteran family. Excellent reviews of the history of classification and phylogenetics (Ball, 1979, 1998), and a comprehensive synopsis noting relationships and distributions of tribes (Bousquet and Laroche, 1993) are available. Recent phylogenetic analyses including exemplars from many carabid taxa have focused on a variety of character systems, e.g., DNA-sequence data (Maddison et al., 1999; Shull et al., 2001; Ober, 2002), female reproductive tract anatomy (Liebherr and Will, 1998), larval morphology (Arndt, 1993, 1998; Beutel, 1993), and cuticular and muscular morphology (Beutel, 1992; Beutel and Haas, 1996). These studies, given their limitations in taxon sampling and character selection, furnish some insights into the evolutionary history of the family. All of these studies agree on several major points; one presently relevant is monophyly of the subfamily Harpalinae (sensu Erwin, 1985). Monorchid taxa presented herein are all members of this subfamilial clade, thereby sharing a significant number of morphological synapomorphies at that taxonomic level. Although relationships within Harpalinae are not understood well enough to allow a mapping of the evolution of monorchy and diorchy on an explicit phylogeny, enough is known about the monophyly of groups that include monorchid taxa to propose minimal numbers of origins of the trait.

Given the number of insect species, relatively few publications present comparative descriptions of the gross morphology of insect testes and male accessory glands (Dufour, 1825; Bordas, 1900; Matsuda, 1976; Suzuki, 1988; Vats and Vasu, 1993; de Marzo, 1992, 1996; Gillott, 2003; Opitz, 2003), although many publications provide isolated descriptions of male structures for individual species. Two forms of coleopteran testes are known: a single, tubular, coiled follicle in the suborders Adephaga and Myxophaga (Reichardt, 1973; Lawrence and Britton, 1994; questionable in Myxophaga, R. Beutel pers. commun.); and multiple follicles in the suborders Polyphaga and Archostemata (Galián and Lawrence, 1993). Within Polyphaga, follicles may be sessile or pedicellate (Lawrence and Britton, 1994).

Structure of the male testes and accessory glands has been reported for a significant number of taxa in Carabidae and a few other adephagan families (Dufour, 1825; Escherich, 1894, 1898; Bordas, 1900; Holdhaus, 1913; Jeannel, 1942; Ali, 1967; Smrž, 1981, 1985; Witz, 1990; Yahiro, 1996, Yahiro, 1998; Carcupino et al., 2002). Even the earliest studies noted that observed members of Harpalini had no apparent testis on the left side of the body. Dufour (1825: 153) interpreted the single testis on the right side as an agglomeration of two testes, but noted the presence of only one vesicula seminalis. Bordas (1900: 310, and plates XIX fig. 6 and XX fig. 1, wrongly numbered in text, actually plates XVII–

XVIII) claimed that the harpaline taxa he investigated had two equally formed testes. However, both Smrž (1981) and our study confirm that those taxa have only one single follicle testis. In all of these previous studies taxon sampling was limited, regionally circumscribed, and lacked key taxa necessary for understanding the distribution of the condition and its implications for relationships in Carabidae.

Accessory glands are quite various in insects and have been studied in many taxa (Chen, 1984; Gillott, 2003). Paired male accessory glands are probably homologous for all Adephaga. However, gland variation in position and form across Coleoptera and the presence of glands in Polyphaga with apparently distinctly different developmental origins (mesadene and ectadene) (Chapman, 1982) make it difficult to assess homology at deeper levels.

In this study we comparatively survey testes and accessory glands across Carabidae. We describe the gross morphological structure of the reproductive system and present terminology for several basic forms of accessory glands. Given the remarkable asymmetrically monorchid condition in some taxa, we discuss its implications for understanding bilateral symmetry in animals, establish *absence asymmetry* as a subtype of directional asymmetry, and speculate on causes of its origin and maintenance.

MATERIALS AND METHODS

Specimens were collected and either maintained alive, preserved in EtOH (90% or greater), Pampel's or in Kahle's fluid (Barbosa, 1974). Live specimens were anesthetized by placing them in a freezer at approximately -1°C until completely immobilized. Vivisection was done under distilled water. Ethanol-preserved specimens were dissected in 95% and other preserved material under 70% EtOH.

Male reproductive structures were examined in repose in the abdomen and then excised for further examination. Sketches were made using a drawing tube mounted on a microscope, then scanned to create a digital image with drawings completed using standard image editing software on a personal computer. Alternatively, images were taken using a digital camera and drawings made as an overlay using standard image editing software.

Traditionally, insect specimens preserved in high concentration EtOH have not been used for morphological study of internal organs and dried-pinned specimens are generally unsuitable for studying soft internal tissues. For muscle and organ dissection, specimens preserved in high-concentration EtOH for later DNA extraction are inferior to material preserved in FAA or Kahle's solutions (compare Fig. 1C,F). Insects are only rarely preserved in these special fluids, however. Recently, many laboratories and museums worldwide have begun amassing EtOH-preserved material intended for molecular work. This new material contains a wealth of insect taxa otherwise only known from pinned material. In order to study very brittle soft-tissue morphological characters from EtOH material, very slow and careful dissecting technique is needed and frequent images are taken using a microscope-mounted digital camera. Essentially, the dissection of a specimen is documented as layers or structures are removed. Images can then be directly annotated using image-handling software.

No properly preserved specimens of *Amorphomerus* were available, so in this case we determined the state of the testes by examining a pinned specimen that had been killed in EtOH and dried. Lateral structures corresponding in position and structure to testes could be seen in these preparations.

The condition of the early pupal-stage male reproductive tract was investigated by dissecting pupae that had died following incomplete molting from the third larval instar. In all such specimens the pupal head was trapped inside its unmolting larval head capsule. These pupae were preserved in boiling water followed by placement in 70% ethanol within 1 day of the attempted molt. Dissections were made under 70% ethanol, with mercurochrome stain intermittently used to enhance the cuticular structures.

Emphasis was placed on sampling species and genera within the monorchid tribes and in groups thought to be closely related to them. In this study we surveyed or recorded testis condition for over 820 species. This covers exemplars from all but 14 of the nearly 80 tribal level groups typically recognized (Bousquet and Laroche, 1993). Data derive from several sources: 1) >190 taxa were examined exclusively for testes; 2) during routine dissections for two independent chromosome studies, testis condition was noted for 185 species (Serrano and Galián, 1998, their summarized reference numbers 23, 25, 27, 30, 32, and 91), and 167 species (Maddison, 1985); 3) in 185 species testis configurations were summarized from the other primary literature listed above.

RESULTS

General Description of Male Carabid Reproductive Tract

Except as noted below, the carabid male reproductive system is quite typical of insect reproductive organs. The genital opening lies within the endophallus folded within the median lobe of the aedeagus (ml). The ejaculatory duct (ed) emerges from the foramen of the sclerotized median lobe and ramifies anteriorly into two tubular accessory glands (ag). These glands vary from relatively small and simple (Fig. 2C) to large sinuate, convoluted, filling much of the abdominal cavity and even extending into the mesothorax (Figs. 1A, 2B). The accessory glands may be turgid or empty, apparently dependent on breeding condition. We recognize several conformations of accessory glands. Despite size variation, their length, shape, and position in the abdomen are relatively constant within species.

Our survey of male accessory glands is not as extensive as that for testes. The four types of accessory glands we recognize here (Fig. 2) are based only on published illustrations and material dissected by KWW, and do not include all species known for testis condition. As noted above, the accessory glands vary considerably but can be readily recognized as mirror-recurved (Fig. 2A), sinuate (Fig. 2B), simple (Fig. 2C), or elongate tipped (Fig. 2D). The simple form (Fig. 2C), which consists of two paramedial, tomaculate-form glands, is found in many taxa including basally divergent carabids (Nebriini and much more highly derived (Lebiini, Lachnophorini, Zuphiini) members of Harpalinae. The elongate tip form (Fig. 2D) is found only in Rhysodini and Loricerini (Smrž, 1981, 1985; Yahiro, 1996; our data). The tip in *Loricera* is significantly shorter than that found in Rhysodini and is likely an independent derivation of this form. The sinuate form (Fig. 2B) is the most common and widespread type. Although variation exists in the number of curves and where

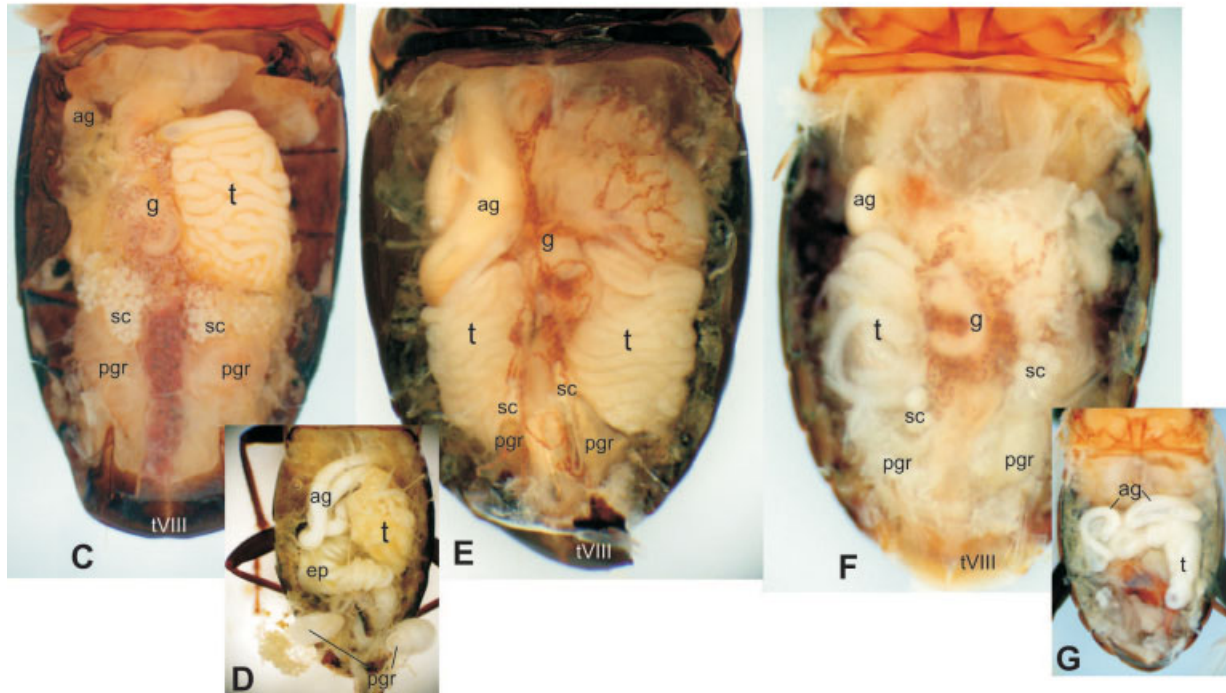
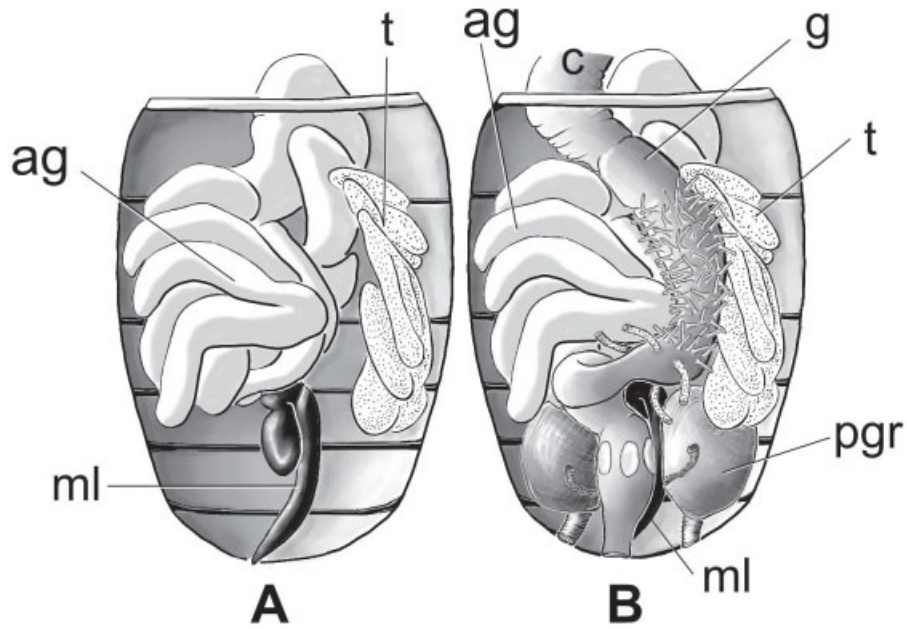


Fig. 1. Internal organs of the male carabid beetle abdomen. Dorsal view with tergites removed. **A:** Drawing of *Selenophorus* sp from Ecuador (Harpalini) showing size and position of male reproductive organs. Other abdomen contents not shown. **B:** Drawing of *Selenophorus* sp from Ecuador showing size and position of internal organs. Digital images of (C) *Onypterigia tricolor* (Platynini), preserved in Kahle's; (D) *Platynus brunneomarginatus* (Platynini); (E) *Amara* sp from California (Zabrini); (F) *Abacetus* (?*Caricus*) from Malaysia (Abacetini), preserved in 95% EtOH; (G) *Abacetus (Astigis) nitidulus* (Abacetini). ag, accessory gland; c, crop; ep, epididymis; g, gut; pgr, pygidial gland reservoir; ml, median lobe of aedeagus; sc, secretory cells; tVIII, tergite eight; t, testis.

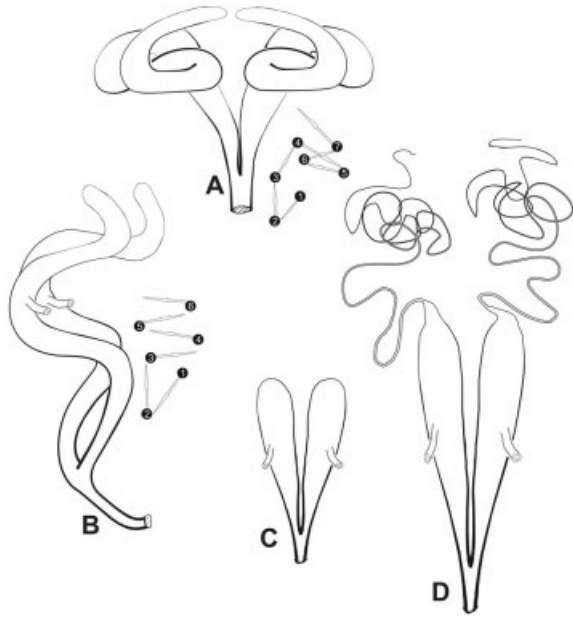


Fig. 2. Generalized drawings of carabid male accessory gland types. **A:** Mirror-recurved. **B:** Sinuate. **C:** Simple. **D:** Elongate tip. Arrow insets of **A** and **B** show the folding path of the gland type.

in the body cavity the glands terminate, all share a basic plan. The generalized path from the foramen of the median lobe is shown in Figure 2B. In all cases the ejaculatory duct leaves the foramen directed toward the apex of the abdomen. The ejaculatory duct then recurves anteriorly, then bends to the right across the body. At the ramification of the ejaculatory duct into the accessory glands, the right gland lies on top of the left, and as a pair, the accessory glands lie across the body cavity, looping back and forth laterally, normally forming three to four loops. The accessory glands lie against the ventral surface of the abdomen (above the ventral nerve cord) and may extend forward into the metathorax, or be compacted posteriorly. A few taxa are known to have the mirror-recurve type (Galeritini, Helluonini). In this unusual conformation, the glands extend to the margins and then back to the centerline, a total of four times, distal to their connection near the basal bifurcation of the accessory glands (Fig. 2A). These unusual glands lie anterad and partially dorsad of the testis.

Past the point of bifurcation of the ejaculatory duct into the accessory glands two vasa deferentia (vd) intertwine to form an epididymis (Fig. 3, ep). If only a single vas deferens is present it may be loosely or compactly twisted. The vas deferens may have a slightly expanded and/or entwined region identified as the vesicula seminalis (Figs. 4, 5). Normally, the vesicula is only an enlargement of the diameter of the vas deferens. This section is coiled separately from the testis. In Scaritini (Figs. 4, 5) and Rhysodini (Fig. 5) the vesiculae are well-developed evaginations. In fresh specimens of *Scar-*

ites, the vesiculae are filled with a whitish fluid, the same color as the accessory glands. The form of the vesiculae varies in Rhysodini (Smrž, 1981, 1985; Yahiro, 1996), but in vivisections of *Omoglymmius* Ganglbauer and *Clinidium* Kirby the vesiculae are elongate with a globose terminus. The globular end is filled with a yellowish fluid quite unlike the white fluid in the accessory glands.

Taxa may have only one testis located on the right (Figs. 1A–D,G, 3) or left (Fig. 1F) or two testes located laterally (Figs. 1E, 4) in the abdomen. They are generally oval or pyramidal in form (Fig. 5) and their shape may be defined by adjacent organs, e.g., accessory glands or gut. Members of Apotomini are the only carabids observed where testis form is significantly different. In *Apotomus* the monofollicular testes are coiled into a disc of uniform thickness.

Description of Monorchid Taxa

The male reproductive tract of Harpalini (Figs. 1A,B, 3), aside from the asymmetrical loss of a testis, possesses reproductive organs configured similarly to those in taxa with paired testes. When present, the left vas deferens is a simple, blind tube (Fig. 3B).

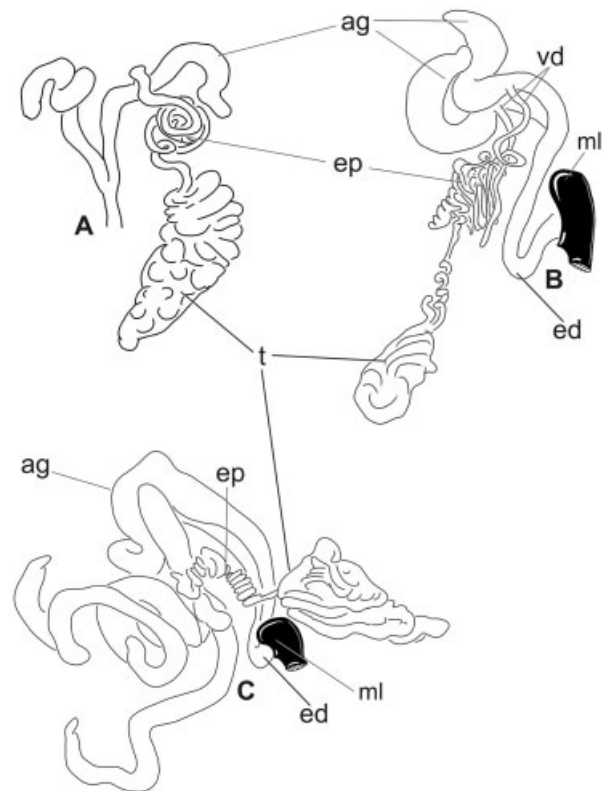


Fig. 3. Male reproductive structures in Harpalini taxa. **A:** *Bradycellus rufipennis*. **B:** *Lecanomerus niger*. **C:** *Selenophorus* sp. from Ecuador. ag, accessory gland; vd, vas deferens; ml, median lobe of aedeagus; ep, epididymis; ed, ejaculatory duct; t, testis.

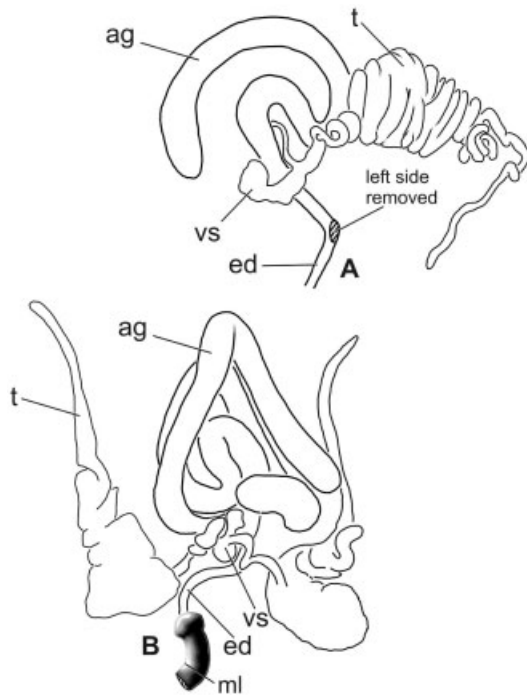


Fig. 4. Male reproductive structures in Scaritini taxa. **A:** *Pasi-machus californicus*, left accessory gland and testis removed. **B:** *Scarites subterraneus*. ag, accessory gland; ml, median lobe of aedeagus; ed, ejaculatory duct; t, testis; vs, vesicula seminalis.

When absent there is no indication of the vas deferens on the accessory gland (Fig. 3A,C). The vasa deferentia are simple tubular ducts and do not have an expanded vesicula seminalis region. Because much of our material was preserved in high concentration EtOH, making the specimens very brittle, it was impossible in some specimens to determine with certainty whether any remnant of the vas deferens was present. The tightly twisted epididymis frequently prevents accurate tracing of the vasa deferentia. Species were confirmed for the absence of the left vas deferens (Table 1) only if fresh material was available. Harpaline accessory glands are of the sinuate form and often occupy a large portion of the abdominal cavity.

The male reproductive tract in all studied species of Abacetini s.str. (i.e., pterostichine-grade taxa sharing eccentrically inserted second antennomeres and markedly short, transverse mentum; Will, 2000; KWW and DRM, unpubl. DNA sequence data) have only one testis (Fig. 1F,G), except for a single specimen of *Abacetus* (*Astigis*) from Kenya, which has a rudimentary left testis and a fully developed right testis. The testis condition of these abetine taxa is either right-present (Indian, Malagasy, and some African *Abacetus* Dejean species) or left-present (Australian, Malaysian, and some African *Abacetus* species). Among the monorchid taxa included in Abacetini s.str., the vesicula seminalis, if present, is only a slight expansion of the vas deferens. The

epididymis is large and tightly compacted between the accessory glands. The accessory glands are large, sinuate-form, and are compressed posteriorly in the abdomen in African and Malagasy *Abacetus* (Fig. 1G). The glands are much looser and positioned farther forward in Australian and Malaysian *Abacetus* (Fig. 1F) and in all other Abacetini s.l., i.e., *Metabacetus*, *Cyrtomoscelis* and *Aristopus*.

The male reproductive tract of taxa within Platynini (Fig. 1C,D), including the subtribe Sphodrina (treated as a distinct tribe by Casale, 1988), may be of either the monorchid or diorchid condition. In all taxa the accessory glands are well developed and of the sinuate form. When two testes are present, they lie laterally in the abdomen with a large, entwined epididymis. Monorchid species have a single testis right of the centerline. This single testis is typically somewhat larger than either of the two testes in diorchid platynines (Fig. 1C,D). An increase of testis size in monorchid taxa could be expected to compensate for reduction in sperm production, as sperm concentration could be a factor limiting reproductive success. On the left side of monorchid males, the vas deferens is present as a short attenuated duct, which is translucent and irregularly margined at its apex.

Examination of reared pupae of the Hawaiian monorchid taxon, *Blackburnia* (*Metromenus*) *erro*, permitted study of the state of the male reproductive system in the pupal instar (Liebherr, 2000). Dissection of the caudal abdominal segments revealed an already formed ejaculatory duct, paired accessory glands, and equally developed vasa deferentia (Fig. 6). No evident testicular mass or sclerotized structures homologous with the median aedeagal lobe or ring sclerite of tergum IX were evident.

DISCUSSION

Phylogenetic Implications in Carabidae

The monorchid condition may be interpreted as a trait shared in those carabids so structured due to common history. However, there is no other character supporting monophyly of a group consisting of only the monorchid carabids (Harpalini, part of

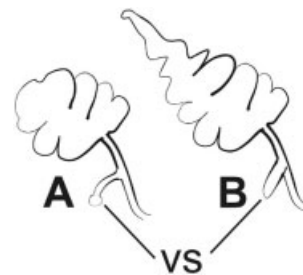


Fig. 5. Carabid testes. **A:** *Omoglymmius* sp. (Rhsodini) and **B:** *Scarites subterraneus* (Scaritini). vs, vesicula seminalis.

TABLE 1. Taxon list for monochid tribes and carabid beetle tribes not previously reported for testis condition

Tribe	Taxon	L	R	Source
Amarotyptini	<i>Amarotyptus</i> sp	+	+	
Apotomini	<i>Apotomus</i> sp	+	+	
Cymbionotini	<i>Cymbionotum</i> sp	+	+	
Gehringiini	<i>Gehringia olympica</i> Darlington	+	+	
Hiletini	<i>Eucamaragnathus batesi</i> (Chaudoir)	+	+	
Migadopini	<i>Antarctonomus</i> sp	+	+	
Opisthiini	<i>Opisthius richardsoni</i> Kirby	+	+	
Pamborini	<i>Pamborus</i> sp	+	+	
Promecognathini	<i>Promecognathus crassus</i> LeConte	+	+	
Psydrini	<i>Nomius pygmaeus</i> (Dejean)	+	+	
	<i>Psydrus piceus</i> LeConte	+	+	
Siagonini	<i>Siagona</i> sp	+	+	
Zolini	<i>Oopterus</i> sp	+	+	
Subfamily Harpalinae				
Abacetini*	<i>Abacetus (Abacetillus)</i> sp [South Africa]	–	+	
	<i>Abacetus (Astigis) nitidulus</i> Tschitscherine	–	+	
	<i>Abacetus (Astigis)</i> sp [Kenya]	?	+	
	<i>Abacetus (?Caricus)</i> sp [Malaysia]	+	–	
	<i>Abacetus (?Caricus)</i> sp [Australia]	+	–	
	<i>Abacetus (?Caricus)</i> sp [India]	–	+	
	<i>Abacetus (Caricus)</i> nr. <i>obtusus</i> [South Africa]	+	–	
	<i>Abacetus (Caricus) perrieri</i> Tschitscherine	–	+	
	<i>Abacetus (Distrigodes) pygmaeus</i> Boheman	–	+	
	<i>Abacetus (Distrigodes)</i> sp [Kenya]	–	+	
	<i>Aristopus</i> sp	+	+	
	<i>Metabacetus</i> sp [Vietnam]	+	+	
	<i>Cytomoscelis abacetoides</i> (Straneo)	+	+	
Amorphomerini	<i>Amorphomerus raffrayi</i> Chaudoir	+	+	
Anthiini	<i>Anthia</i> sp	+	+	
Caelostomini	<i>Caelostomus</i> 3 spp	+	+	
Catapiesini	<i>Catapiesis</i> sp	+	+	
Cnemalobini	<i>Cnemalobus</i> sp	+	+	
Ctenodactylini	<i>Leptotrachelus dorsalis</i> (Fabricius)	+	+	
Cyclosomini	<i>Tetragonoderus</i> sp	+	+	
Dercylini	<i>Dercylus</i> (s.s.tr.) sp	+	+	
Dryptini	<i>Drypta australis</i> Dejean	+	+	
Galeritini	<i>Galerita forreri</i> Bates	+	+	
Graphipterini	<i>Graphipterus lateralis</i> Boheman	+	+	
Harpalini	<i>Acinopus picipes</i> (Olivier)	–	+	
	<i>Acupalpus</i> sp†	–	+	Ali, 1967
	<i>Allendia chiliensis</i> (Dejean)	–	+	
	<i>Anisodactylus binotatus</i> (Fabricius)	–	+	Bordas, 1900
	<i>Anisodactylus signatus</i> (Panzer)*	–	+	Smrž, 1981
	<i>Anisodactylus</i> sp*	–	+	Ali, 1967
	<i>Axinotoma perrieri</i> (Fairmaire)†	–	+	
	<i>Bradybaenus opulentus</i> Boheman*	–	+	
	<i>Bradycellus ruprestris</i> Say†	–	+	
	<i>Bradycellus</i> sp†	–	+	Ali, 1967
	<i>Carterus</i> sp†	–	+	Smrž, 1981
	<i>Carterus</i> 2 spp	–	+	
	<i>Dichierotrichus</i> sp*	–	+	Ali, 1967
	<i>Dicheirotrichus obsoletus</i> Dejean	–	+	
	<i>Discoderus ?robustus</i> Horn*	–	+	
	<i>Ditomus</i> 3 spp	–	+	
	<i>Euryderus grossus</i> (Say)	–	+	
	<i>Euthenarus promptus</i> Erichson	–	+	
	<i>Harpalus (Pseudoophonus)</i> sp*	–	+	Ali, 1967
	<i>Harpalus</i> (s.str.) 4 spp	–	+	
	<i>Harpalus (Cryptophonus)</i> 2 spp	–	+	
	<i>Harpalus affinus</i> (Schrank)*	–	+	Smrž, 1981
	<i>Harpalus anxius</i> (Duftschmid)	–	+	Bordas, 1900
	<i>Harpalus distinguendus</i> (Duftschmid)	–	+	Bordas, 1900
	<i>Harpalus flavescens</i> (Pitter & Mitterpacher)	–	+	Bordas, 1900
	<i>Harpalus latus</i> (L.)*	–	+	Smrž, 1981
	<i>Harpalus obscurus</i> (Fabricius)	–	+	Bordas, 1900
	<i>Harpalus rubripes</i> (Duftschmid)	–	+	Bordas, 1900
	<i>Harpalus serripes</i> (Quensel)	–	+	Bordas, 1900
	<i>Harpalus smaragdinus</i> (Duftschmid)	–	+	Bordas, 1900
	<i>Harpalus</i> sp*	–	+	Ali, 1967
	<i>Lecanomerus niger</i> Darlington*	–	+	

TABLE 1. (Continued)

Tribe	Taxon	L	R	Source
	<i>Nemoglossa brevis</i> Solier*	-	+	
	<i>Notiobia (Pseudognathaphanus) zabroides</i> (Alluaud)	-	+	
	<i>Notiobia brevicollis</i> (Chaudoir)†	-	+	
	<i>Notiobia germari</i> Castelnau	-	+	
	<i>Notiobia melanaria</i> Dejean	-	+	
	<i>Ophonus azureus</i> (Fabricius)*	-	+	Smrž, 1981
	<i>Ophonus griseus</i> Panzer	-	+	Bordas, 1900
	<i>Ophonus rufipes</i> DeGeer*	-	+	Smrž, 1981
	<i>Ophonus sabulicola</i> (Panzer)*	-	+	Smrž, 1981
	<i>Ophonus</i> 4 spp	-	+	
	<i>Phorticosomus</i> sp	-	+	
	<i>Polpochila capitata</i> (Chaudoir)	-	+	
	<i>Selenophorus</i> sp [Ecuador]†	-	+	
	<i>Siopelus punctatellus</i> (Reiche)†	-	+	
	<i>Stenolophus (Egadroma)</i> sp [Madagascar]†	-	+	
	<i>Stenolophus</i> sp [Madagascar]*	-	+	
	<i>Stenolophus (Stenolophus) proximus</i> Dejean	-	+	
	<i>Stenomorphus convexior</i> Notman*	-	+	
	<i>Scybalicus oblongiusculus</i> Dejean	-	+	
	<i>Trachysarus</i> 2 spp	-	+	
	<i>Trichocellus</i> sp†	-	+	
Helluonini	<i>Helluomorphoides latitarsis</i> (Casey)	+	+	
Licinini	<i>Dicaelus ambiguus</i> Laferté-Sénéctère	+	+	
	<i>Lacordairia</i> sp	+	+	
Loxandriini	<i>Adrimus</i> 2 spp	+	+	
	<i>Loxandrus</i> 11 spp	+	+	
	<i>Homalonesiota straneoi</i> (Darlington)	+	+	
	<i>Stolonis</i> 4 spp	+	+	
	<i>Nebrioferonia strigitarsis</i> Straneo	+	+	
Melanchitonini	<i>Melanchiton rectangulum</i> Chaudoir	+	+	
Metiini	<i>Metius</i> 3 spp	+	+	
Morionini	<i>Morion</i> 2 spp	+	+	
	<i>Moriosomus seticollis</i> Straneo	+	+	
Odacanthini	<i>Pentagonica flavipes</i> (LeConte)	+	+	
Orthogoniini	<i>Orthogonius</i> sp [Malaysia]	+	+	
Peleciini	<i>Pelecium</i> sp	+	+	
Perigonini	<i>Perigona</i> sp	+	+	
Platynini*	<i>Agonidium kenyense</i> (Alluaud)	+	+	
	<i>Agonum</i> 10 spp	+	+	
	<i>Anchomenus</i> 3 spp	+	+	
	" <i>Anchomenus</i> " <i>integratus</i> Broun	-	+	
	<i>Aparupa major</i> Schmidt	-	+	
	<i>Atranus pubescens</i> (Dejean)	+	+	
	<i>Blackburnia (Blackburnia)</i> 2 spp	-	+	
	<i>Blackburnia (Colpocaccus) hawaiiensis</i> (Sharp)	-	+	
	<i>Blackburnia (Metromenus)</i> 2 spp	-	+	
	<i>Calathus</i> 3 spp	+	+	
	<i>Catacolpodes</i> sp	-	+	
	<i>Colpodes helluo</i> Darlington	-	+	
	<i>Colpodes laetus</i> (Erichson)	-	+	
	<i>Ctenognathus bidens</i> (Chaudoir)	-	+	
	<i>Dicranoncus queenslandicus</i> (Sloane)	-	+	
	<i>Euleptus foveolatus</i> Kolbe	+	+	
	<i>Glyptolenus chalybeus</i> (Dejean)	+	+	
	<i>Incagonum</i> 2 spp	+	+	
	<i>Klapperichella comatus</i> (Andrewes)	+	+	
	<i>Laemostenus complanatus</i> (Dejean)	+	+	
	<i>Lepcha pygmaea</i> (Habu)	+	+	
	<i>Liagonum</i> 2 spp	-	+	
	<i>Lorostema</i> 2 spp	+	+	
	<i>Megalonychus madagascariensis</i> Chaudoir	+	+	
	<i>Metacolpodes buchani</i> (Hope)	-	+	
	<i>Metagonum crenatostriatum</i> (Péringuey)	+	+	
	<i>Neomegalonychus</i> sp	-	+	
	<i>Notagonum</i> 6 spp	-	+	
	<i>Notocolpodes euleptus</i> (Alluaud)	-	+	
	<i>Olisthopus parmatius</i> (Say)	+	+	
	<i>Paranchus albipes</i> (F.)	+	+	
	<i>Platyderus</i> sp	+	+	

TABLE 1. (Continued)

Tribes	Taxon	L	R	Source
	<i>Platynus (Batenus)</i> 5 spp	+	+	
	<i>Platynus (Dyscolus)</i> 3 spp	-	+	
	<i>Platynus (Glyptolenopsis)</i> 2 spp	-	+	
	<i>Platynus (Platynus) assimilis</i> (Paykull)	+	+	
	<i>Platynus (Platynus) brunneomarginatus</i> (Mannerheim)	-	+	
	<i>Platynus (Platynus) decentis</i> (Say)	+	+	
	<i>Platynus (Platynus) indecentis</i> Liebherr & Will	+	+	
	<i>Platynus (Platynus) opaculus</i> LeConte	-	+	
	<i>Platynus (Platynus) ovipennis</i> (Mannerheim)	-	+	
	<i>Platynus (Platynus) parmarginatus</i> Hamilton	-	+	
	<i>Platynus (Platynus) tenuicollis</i> (LeConte)	-	+	
	<i>Platynus (Platynus) trifoveolatus</i> Beutenmüller	-	+	
	<i>Platynus (Scaphiodactylus) moestus</i> (Dejean)	-	+	
	<i>Platynus (Stenocnemion) acuminatus</i> (Chevrolat)	-	+	
	<i>Pristonychus terricola</i> (Herbst)	+	+	
	<i>Rhadine</i> sp	+	+	
	<i>Sericoda bembidioides</i> Kirby	+	+	
	<i>Synuchus</i> 2 spp	+	+	
	<i>Tanystoma maculicolle</i> (Dejean)	+	+	
	<i>Xestagonum ursulae</i> Schmidt	-	+	
	<i>Violagonum violaceum</i> (Chaudoir)	-	+	
Pseudomorphini	<i>Pseudomorpha</i> sp	+	+	
Zuphiini	<i>Thalpius</i> sp	+	+	

Tribes outside of Harpalinae are listed in alphabetical order, followed by tribes within Harpalinae. Additionally, 84 species of Harpalini listed in the chromosome study of Serrano et al. (1994) are all monorchid (JG unpubl.).

*Both vasa deferentia confirmed present in monorchid taxa; “+”, one vas deferens confirmed absent in monorchid taxa; L, left testis; R, right testis; states are “-” absent; “+” present, “?” reduced but present. Source listed if other than this article.

Platynini, and part of Abacetini). While it is reasonable to presume that large groups of monorchid carabids (e.g., all Harpalini) have this trait due to common ancestry, and thus testis loss would provide evidence for monophyly of each such group independently, it is very unlikely that all monorchid carabids form a clade exclusive of diorchid taxa. In fact, our current knowledge of relationships within Harpalinae, while fragmentary, is resolved enough around the monorchid taxa to indicate separate origins of monorchy within Harpalinae (Fig. 7), as we will now argue.

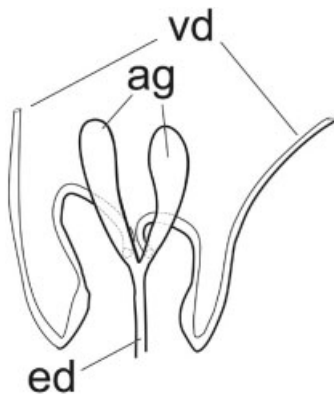


Fig. 6. Male reproductive structures in the pupal instar of *Blackburnia (Metromenus) erro*. ag, accessory gland; ed, ejaculatory duct; vd, vas deferens.

There is evidence that Abacetini s.l. is closely related to Loxandrini (Jeannel, 1948; Deuve, 1990; Arndt, 1993; Bousquet and Laroche, 1993; Will, 2000; KWW, unpubl. data). However, all examined loxandrines and what are likely basal grade abacetine taxa (sensu Deuve, 1990; Will, 2000) have two fully developed testes (e.g., *Loxandrus*, *Metabacetus*, *Aristopus* and *Cyrtomoscelis*). This suggests that monorchy found in Abacetini s.str. has arisen within the Abacetini + Loxandrini clade, independently from other monorchid carabids. It is not clear, however, if monorchy has arisen more than once in abacetines. As noted above, monophyly of Abacetini s.str. is potentially corroborated by testis asymmetry and other data. However, sampled abacetine taxa exhibit more variation in testis configuration than either of the two other monorchid tribes. Within *Abacetus*, a genus of otherwise rather homogeneous-appearing species, all the possible conformations were observed. The tribe as a whole needs taxonomic revision and so a definitive assessment of the evolution of testis form is not possible at this time. Additionally, our sample does not cover the diversity of forms in the tribe, or of potentially closely related but divergent taxa, e.g., *Mireius*, *Chlaeminus*, *Holconotus*, etc. Until a much more comprehensive study of the relationships within abacetine-like taxa is undertaken, it is not possible to determine whether the two monorchid states (right testis absent, left testis absent) in

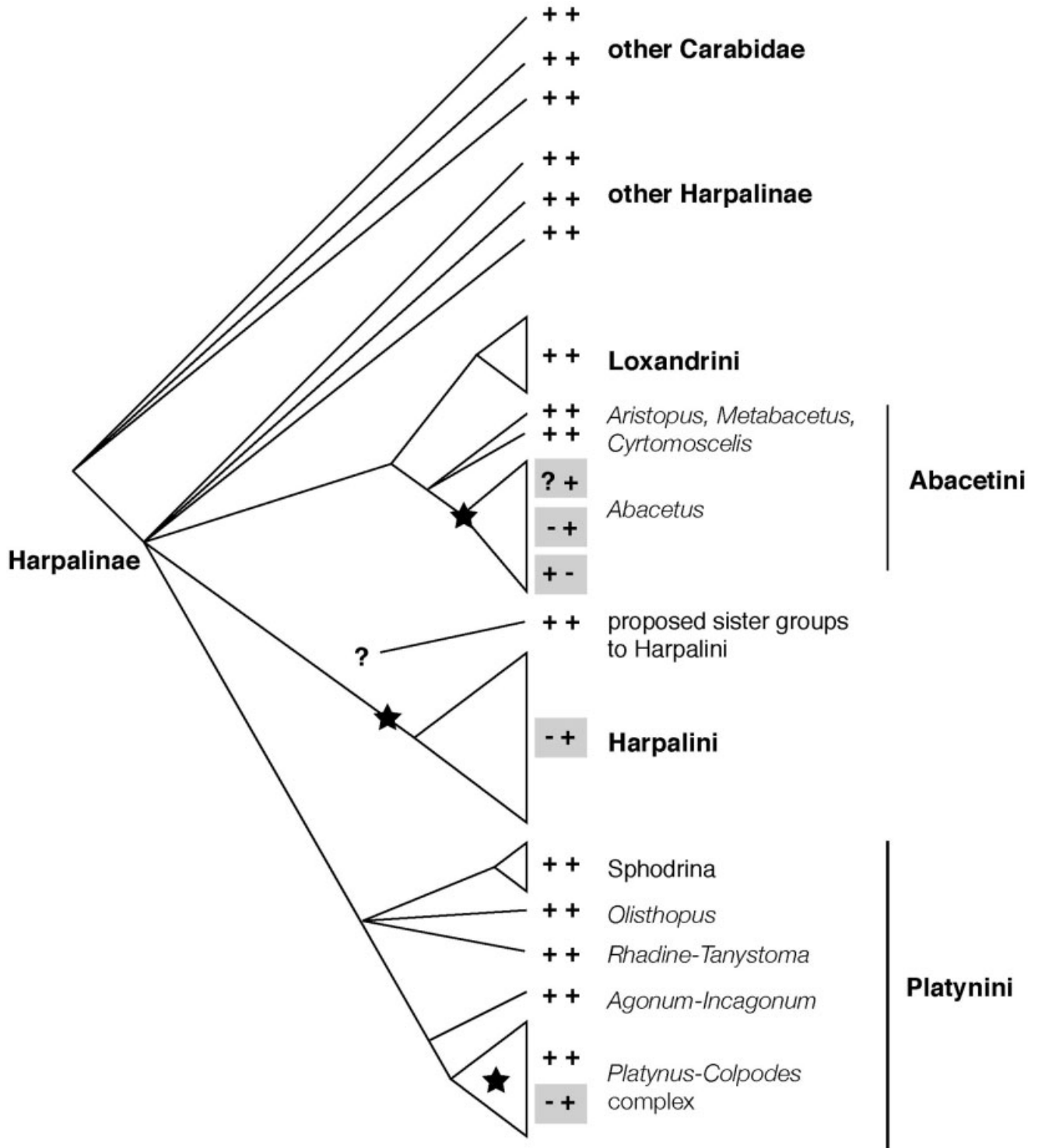


Fig. 7. Phylogeny of carabids relevant to the evolution of monorchy; see text for references supporting the phylogenetic structure. ++: males possess two testes; -+: males possess only a right testis; + -: males possess only a left testis; ?+: males possess a larger right and smaller left testis. Stars indicate proposed locations of origin of monorchy.

this group are the result of two independent losses or lateral inversion in a unified monorchid abacetine lineage.

Although no decisive character evidence has been presented to specify the adelphotaxon to Harpalini, a number of possible hypotheses of affinity have

been implied in published classifications. Kryzhanovskiy (1976) placed Harpalini in the supertribe Harpalitae along with Cnemalobini, Amorphomerini, Agonicini (the latter now included in Peleciini) and Amblystomini (presently placed within the subtribe Harpalina). *Sugimotoa parallela*, an isolated taxon known only from a small number of islands in the Austral and Oriental regions, was originally described as a harpaline taxon, suggesting that this species may have affinities to harpalines. Testis state could not be checked for *Sugimotoa*, as only dried specimens not suitable for testis examination were available. However, Ball et al. (1995) showed clearly that other character evidence places *Sugimotoa* in Lebiini. We examined specimens of an *Amblystomus* species from Australia and found it to be typical for harpalines, i.e., possessing only one testis (Table 1). No properly preserved specimens of the Australian agonicine genera *Agonica*, or *Pseudagonica* were available for study. Given the current placement of agonicines in the tribe Peleciini it is unlikely that they have a close relationship to harpalines. However, we cannot discount the possibility, as we examined only a single *Pelecium* species, which was found to have well-developed, paired testes.

The single species attributed to *Amorphomerus* may be sister to or a member of Harpalini. Jeannel (1948) considered it to be closer to harpalines than to any other member of derived carabids he called "conchifera." *Amorphomerus* is similar in general form and shares many characters with Harpalini, but also has many unique apomorphies of mouthparts and the terminal abdominal structures used to deliver pygidial gland defensive chemicals (Deuve, 1993). The latter structures formed the basis for Erwin's (1991) placement of Amorphomerini in the supertribe Orthogonitae. These structures, although similar in function, are formed differently in each of the tribes included in Erwin's Orthogonitae and so they are not likely synapomorphic for the group (Will et al., 2000). In the pinned specimens of *Amorphomerus*, a pair of lateral structures corresponding in position and structure to testes was clearly present. As all suggested adelphotaxa to Harpalini that have been examined possess two testes, it is likely that the monorchid condition of Harpalini is autapomorphic for the tribe. Harpalini is apparently an isolated taxon that primitively evolved the monorchid condition and has subsequently diversified. The tribe's species level diversity and breadth of distribution surpasses the two other monorchid groups, which unlike harpalines have well-supported sister-group relationships with diorchid taxa.

Based on the distribution of testis conditions across the tribe Platynini, it is clear that the diorchid condition is part of the tribal groundplan, i.e., present in the common ancestor of all extant species. Genera classified as more basally divergent in a tribal cladistic

analysis (Liebherr, 1986)—*Olisthopus*, the subtribe Sphodrina, and the *Anchomenus* and *Rhadine-Tanystoma* clades (Table 1) are all characterized by males with paired testes. The genus *Euleptus*—a diorchid taxon—shares the derived absence of a median mentum tooth with *Olisthopus*, though the relative proximity of phylogenetic relationship for these taxa has not been established. The fourth clade recognized by Liebherr (1986) includes the preponderance of species- and genus-level taxa in Platynini. In this group, species of the genera *Agonum* and *Incagonum* are more generalized, as they are characterized by tarsi suitable for terrestrial locomotion, and their bodies are not ornamented with cuticular appurtenances. In contrast, the very diverse *Platynus-Colpodes* complex (Liebherr, 1998) comprises numerous taxa with extreme cuticular modifications, including expanded tarsal pads (putatively adaptive for life in tropical and semitropical montane, arboreal microhabitats; Liebherr and Zimmerman, 1998), elongate mandibles and antennae, apical and/or subapical elytral spines, and distinctly metallic, brightly colored dorsal body surfaces.

Evolutionary transformation from the plesiomorphic diorchid to the derived monorchid platynine condition occurred within this *Platynus-Colpodes* complex (Fig. 7), and more specifically among taxa now placed in the genus *Platynus* (Bousquet and Laroche, 1993; Bousquet, 2003). A group of three northern hemisphere species considered plesiomorphic among this complex (Moret, 1989), including the type species of the genus name, *Platynus assimilis*, are characterized by diorchid males. A second group of diorchid taxa have been classified as *Platynus* subgenus *Batenus* (Liebherr, 1989) (Table 1). However, numerous other species currently treated as members of the subgenus *Platynus*—*P. brunneo-marginatus* to *P. trifoveolatus* (Table 1)—plus numerous other subgeneric- and generic-level taxa in the complex have monorchid males. The monorchid platynines geographically span North and South America, Africa, Madagascar, Asia, Australia, New Zealand, and the various Pacific Island groups. This very broad distribution points to a relatively ancient origin of the monorchid condition if it characterizes a monophyletic group; ampho-Atlantic relationships would suggest a Cretaceous origin (Hallam, 1994). At present, there is little reason to reject such a claim; however, a comprehensive cladistic analysis of worldwide platynine generic-level taxa must be conducted to settle the question. At the least, phylogenetic reclassification of *Platynus* is warranted, taking the new information on testis condition into account.

The presence of equally developed, although small, vasa deferentia in the pupa of the monorchid platynine taxon *Blackburnia (Metromenus) erro* (Table 1) establishes that testis and the associated vas deferens asymmetry progressively develops during the pupal stage. The rudimentary left vas deferens

of adult monorchid platynines suggests that development on this side aborts or reverts early in the pupal stage, with any substantial development of the epididymis and testis restricted to the right side. The accessory gland configuration of the early *Blackburnia* pupa (Fig. 6) is of the simple type (Figs. 2C, 6), even though adults possess the sinuate type (Fig. 2B). As the simple type is found in both highly derived taxa like Lebiomorphs and various basal grade taxa, this ontogenetic transformation may imply two different evolutionary scenarios for accessory glands in the Lebiomorph lineage. If Lebiomorphs were derived from an ancestor that possessed adult sinuate type glands (e.g., Pterostichini or Platynini), then the adult Lebiomorph gland configuration may be interpreted as a neoteneous condition, i.e., a reversion to the more generalized form occurring earlier in ontogeny of the common ancestor. Conversely, if Lebiomorph lineages fall outside a clade defined by the derived sinuate gland form, then the Lebiomorph gland configuration may represent the primitive state shared with Patrobini and basal taxa of the subfamily Harpalinae (Smrž, 1981). Again, cladistic analysis will inform the developmental interpretation for evolution of this structure.

Within-species variation of bilateral structures has been demonstrated experimentally to be influenced by the presence or absence of imaginal discs in nymphalid butterflies (Klingenberg and Nijhout, 1998). Failure of germ cells to migrate or develop in the embryo could cause similar alterations in internal organ symmetry, e.g., presence or absence of vas deferens, or loss of the testis in monorchid beetles could be caused by such a mechanism. However, the point in development at which testis formation fails is presently unknown.

Asymmetry

The basic bilateral symmetry of all Bilateria was apparently established very early in the evolution of the metazoan clade (Martindale et al., 2002). Major breakdown of left–right symmetry during development is considered very difficult, i.e., bilateral symmetry is strongly conserved (Coyne, 1987; Tuinstra et al., 1990), even though there are multiple independent phylogenetic origins of symmetry loss (Palmer, 1996). Two general types of asymmetry are recognized: directional asymmetry and fluctuating asymmetry (Sumner and Huestis, 1921; Van Valen, 1962). Fluctuating asymmetry, which is found in all organisms, is a nondirectional, statistically recognizable departure from perfect symmetry. Directional asymmetry occurs when one side consistently differs from the other. Antisymmetry is a special case of directional symmetry (Van Valen, 1962), whereby there is a significant difference between sides, but the state a side has, e.g., the larger side, varies randomly. We use the term *absence asymme-*

try here to designate cases in which the difference is presence–absence of primitively bilateral organs, rather than a statistically significant difference based on a distribution of measures between mirrored or paired structures.

Symmetrical Monorchy

Within the many invertebrate groups that exhibit symmetrical presence of a single testis—Entoprocta, Gnathostomulida, Nematoda, Rotifera, Platyhelminthes, Gastrostichta, Pterobranchia, Tardigrada, and Crustacea—there are some to many hermaphroditic species (Brusca and Brusca, 2003). In the case of hermaphrodites, optimal resource partitioning (Angeloni et al., 2002) between different sex gonads could be the selective pressure that drives testes to a unitary structure. Very few hexapods are structural hermaphrodites, but the condition is known in Plecoptera, Isoptera, Blattodea, and Hemiptera (Rościszewska and Soldán, 1999; Normark, 2003). However, the presence of viable gametes of both sexes in a single individual is only reported from a few species of Hemiptera (Hughes-Schrader, 1963; Hughes-Schrader and Monohan, 1966). None of these hermaphroditic hexapods are known to be monorchid.

Fusion of the testes, either at their apex or into a unitary structure, is reported from several hexapodan clades; e.g., many Lepidoptera (Howe, 1975); Coleoptera: Elateridae (Vats and Vasu, 1993); Coccinellidae (Maffei et al., 2001); Chrysomelidae (Suzuki, 1988; Devasahayam et al., 1998). In all of these hexapods there is little question that they represent derived fusion of paired testes, whereas other structures such as accessory glands remain symmetrical.

Transformation to Monorchy

Within ptiliid beetles, the single, medial testicular mass of *Nossidium pilosellum* appears to be an intermediate condition between diorchy and possession of a single lateral testis. Based on this condition plus a generalized sperm structure, de Marzo (1992) suggested that *N. pilosellum* represents the ancestral condition for testis configuration for the family. This claim is supported by the apparent sister-group relationship between *Nossidium* and the remaining sampled ptiliids as suggested by exoskeletal characteristics (Dybas, 1976; W.E. Hall, pers. commun.). If a single, medial testicular mass is ancestral within ptiliids, it is possible that the origin of monorchy in ptiliids came about by fusion of the testes rather than a loss of one of the contralateral pair.

In contrast, the only carabid that shows what might be interpreted as an intermediate form is a single specimen of a species of *Abacetus* (*Astigis*), which has two testes present but one significantly smaller. The scarcity of intermediates makes it difficult to determine the transformational pathway

that led from diorchy to asymmetrical presence of a single testis within carabids. However, the fact that monorchid carabids have only a single testicular follicle, rather than the two follicles one would expect (at least temporarily) if there were a fusion of two testes, is consistent with a simple loss of one testis.

Optimal Visceral Packing

All organ systems located in the abdominal cavity must “compete” for space, for an increase in one system can only be accommodated by stretching intersclerotic membranous cuticle, or shrinking another system. There is no indication that the stiff cuticular exoskeleton in insects is altered in form to contain specific organs systems. The opposite would seem to be the case: viscerae are expanded or contracted according to the form of the abdomen. One thus might expect that in the competition for space, some of these organs or structures might be subject to a loss on one side, i.e., absence asymmetry.

Smrž (1981: 469) suggested that the “exceeding development” of the gut—an adaptation to herbivory—as the selective pressure that led to degeneration of the left testis in Harpalini. However, we see no evidence of a relationship between gut size or form and reduction in the male reproductive structures. The alimentary canal in harpaline taxa is similar to that in predacious groups (Yahiro, 1990, 1998). Additionally, the crop is the most expanded portion of the gut, and it lies well forward of the accessory glands and testis, not near the space that would be filled with a left testis (Fig. 1B).

Most members of Harpalini are thought to be at least facultative, and in some cases for at least a portion of their diet, obligatory herbivores (Larochelle, 1990; Bracht-Jorgensen and Toft, 1997). This feeding habit does not have an obvious connection to testis loss, as other carabids that are well known as frequent seed and pollen feeders, e.g., *Amara* (Thiele, 1977: 120) (Fig. 1E) and *Calathus*, retain paired testes. The two other lineages exhibiting a single testis, abacetines and platynines, are generally thought to be primarily or exclusively predatory. Species of both groups have been successfully reared for two generations on *Tenebrio* larvae (Tenebrionidae) without supplemental feeding of vegetable material (Liebherr, 1983). Finally, there is no obvious reason that males should require additional space to accommodate increased gut capacity, whereas females undergo no such adaptive response. There is no corresponding asymmetry in female reproductive systems in monorchid taxa (Liebherr and Will, 2000; KWW, unpubl. data) and no reports that feeding preferences differ between the sexes.

While Smrž’s hypothesis of herbivory with associated crowding by an expanded gut is not the explanation for loss of a testis in monorchid carabids,

competition for limited space in the abdominal cavity might still be the cause of absence asymmetry.

Carabid beetles typically have heavily sclerotized sterna; therefore, the volume of the carabid beetle abdominal cavity is essentially fixed in adults. These beetles have only a modest capability for expansion by stretching the relatively flexible intersegmental and tergal cuticle. Given this constraint, the maximum development of the internal organs must be balanced over the life of the individual, with each system giving way as the season dictates. We have noted an interaction of this type in an ongoing study of pygidial gland chemical products where gravid females are much more likely to have nearly empty pygidial gland reservoirs than conspecifics that are not egg laden (KWW, unpubl. data). It is reasonable to assume that external and common internal selective pressures (e.g., predation and physiological requirements, respectively) would be sufficient to keep most organ systems somewhere near optimal size, e.g., defensive glands and gut. However, reproductive structures in males could be subject to additional pressures such as sperm competition and female-choice (Eberhard, 1985, 1996). The quantity of accessory gland fluid produced might be a significant factor for reproductive success in these beetles, as has been shown for bruchid seed beetles (Savalli et al., 2000). If so, during the carabid beetle breeding season testes and accessory glands would need to simultaneously optimize space use in the abdomen.

The accessory glands in male carabid beetles, particularly in monorchid taxa, appear to occupy a large portion of the abdominal volume. However, whether monorchid taxa consistently possess relatively larger accessory glands was not measured in our study. Our comparative sample shows that testes are much more variable in size, shape, and position in carabids with sinuate accessory glands than in carabids with simple or mirror-recurved glands, suggesting that these structures are directly impacting testis development. Unfortunately, we have little information on the reproductive biology in carabids and no comparative sample of relative organ sizes over the course of the adult instar. Such studies could shed light on any compensatory relationships between the various internal organ systems. It is possible that there is the inverse causal correlation between accessory glands and loss of testes: i.e., reduced testicular output is compensated for by increased size and output of the accessory glands. A robust phylogenetic hypothesis for Harpalinae taxa would elucidate these alternate historical associations of testis loss and gland size increase.

Why Testes Are Lost

If limited space for the viscera is causing absence asymmetry, an explanation is still needed for why a testis would be lost rather than some of the other structures in the abdomen.

There are nine organs or structures within the carabid beetle abdominal cavity: fat body, tracheal system, muscular system, nervous system, dorsal vessel, digestive system, pygidial defensive structures, and two developmentally separate components of the reproductive system, genitalia plus accessory glands and gonads. Each has different functions and forms that are subject to different adaptive forces and various evolutionary constraints. In examining each of these systems in turn, it is evident that some cannot or are unlikely to be subject to absence asymmetry for various reasons: they are unitary and so do not consist of paired structures, each of the bilateral pair functions unilaterally, or as they are developmentally constrained.

Given its form, the fat body cannot by definition be subject to absence asymmetry. This organ is a distributed mesh of adipocytes filling spaces between organs. Expansion of the fat body within the abdomen is accomplished through stretching membranous abdominal cuticle, with well-fed adults in breeding condition exhibiting abdomens expanded dorsolaterally so that the subelytral space is largely taken up by the abdomen.

The tracheal system consists of spiracles and main tracheal branches that are typically symmetrical. The tracheal system is formed as invaginations of the external cuticle and so is developmentally tied to the external body form. It would be possible to have significant asymmetry in this system if there was a dramatic change in the external body form. Internally, the main branches ramify into smaller tracheae and further into terminal tracheoles that are distributed to provide gas exchange for all tissues, particularly tissues with high metabolic output (Chapman, 1982). As such, much of the system is anastomosing, in some cases forming a unitary medial structures and/or nonsymmetrically distributed clusters of tracheae (Snodgrass, 1993).

Musculature in the abdomen is attached to cuticular structures to protract, contract, rotate, etc., the tergum, sternum, postabdomen, and genitalia (Bils, 1976). Muscle symmetry matches the underlying structure, but typically must provide antagonistic and symmetrical movement. Most muscles are mirrored on either side of the true centerline, but may be unequally developed, as in the case of those that serve the rotated male genitalia.

The dorsal vessel, ventral nerve cord, and abdominal ganglia are unitary structures and cannot lose a symmetrical half without loss of function. Similarly, the gut, including Malpighian tubules, is based on and functions as a bilaterally symmetrical structure. However, as in vertebrates, the gut is enlarged, lengthened, and asymmetrically positioned in the body to provide storage space and a greater surface area for nutrient absorption. In carabids the crop is frequently enlarged, sometimes asymmetrically, and lies far anterad the reproductive structures. The

mid- and hindguts meander and often loop (Yahiro, 1990, 1996). The coils of the gut usually lie along the central axis.

The pygidial or defensive glands are paired, symmetrical structures that occupy a significant portion of the abdomen (Fig. 1A,B) (Forsyth, 1972; Will et al., 2000). The relative amount of the abdomen that the pygidial gland reservoirs occupy is correlated more with defensive chemical compound than with size of the beetle (Forsyth, 1972; KWW, unpubl. data). All three groups of monorchid carabids are known to use formic acid as their primary defensive irritant (Dazzini Valcurone and Vita Finzi, 1974; Dazzini Valcurone and Pavan, 1980; KWW, unpubl. data). When turgid, the reservoirs in these taxa can occupy nearly one-third of the volume of the abdomen (Fig. 1C). Pygidial glands are homologous and probably synapomorphic for all Adephaga. Their evolution and maintenance is tightly tied to predation avoidance, and this defense is probably one of the major contributing factors to the success of Carabidae (Erwin, 1985; Will et al., 2000). The spray emitted by a beetle in response to predation is directional and unilateral (Rossini et al., 1997). Therefore, the glands are nonredundant: each works to protect its respective side. This excludes the possibility of absence asymmetry evolving for these structures.

The reproductive tract, genitalia, and other terminal- or postabdominal sclerites are ectodermally derived structures that are often highly modified and frequently asymmetrically shaped. The abdominal segments that telescope into the abdomen (Deuve, 1993) and external genitalia of female carabid beetles are typically symmetrical. However, the female internal tract is rarely symmetrical, with the bursa dramatically asymmetrical and the accessory gland and spermatheca directed to the left or right (Liebherr and Will, 1998; Will, 2002). Male genitalia, and to a lesser degree the last ventrite, are always asymmetrically formed. In all carabid males the penis (median lobe of the aedeagus) is rotated 90° when in repose. Typically, the rotation is clockwise; however, a rotation 90° counterclockwise is found in some or all species of several groups (tribes Scaritini and Caelostomini, and subtribe Sphodrina of Platynini). The parameres of the median lobe are typically unequally developed, with the upper (eusinistral) larger than the lower (eudextral). In species with inverted genitalia this is reversed, and the development of all associated muscles is also inverted. Despite this dramatic change, the reproductive structures anterad the male genitalia and other internal organs do not noticeably differ between left-side-up and right-side-up taxa. As the terminalia in carabid beetles are directly involved in copulation, they are presumed to be under strong selective pressure, e.g., mate selection or species isolation mechanisms (Eberhard, 1985, 1996). There is a large amount of variation and frequently direc-

tional asymmetry in the male genitalia; however, no true absence asymmetry has been found in these well-sclerotized cuticular structures. The extremely small vestigial right paramere in some Zuphiini (Baehr, 1998) approaches this condition. The endophallus, which is everted to interact with the female bursa copulatrix, like the bursa, has no fixed symmetry. Typically, the elaborate patterns of spines and plates found on this structure vary at the species level and coincide to a degree with aspects of the bursa copulatrix of the conspecific female.

The anterior portion of the cuticular structures of the male reproductive system consists of paired accessory glands. Unfortunately, there is no information on the contents or the precise function of the accessory glands in carabid beetles. It is assumed that the male deposits this substance together with sperm in the female's bursa, perhaps to form a spermatophore (Takami, 2002). As a paired structure it may initially seem more likely that one side of the accessory glands would be lost or reduced rather than a testis. However, loss of symmetry in this structure could be constrained by two factors. First, the accessory glands may develop from the same imaginal disc as the external genitalia, seminal vesicles, and a portion of the vasa deferentia, as thought to be the case in some Coleoptera and Lepidoptera (Matsuda, 1976; Kaulenas, 1992). Actual developmental origins are known for *Drosophila*, where there is a single genital imaginal disc from which the anal plates + hindgut, external genitalia, vasa deferentia, seminal vesicles, anterior ejaculatory duct, ejaculatory bulb, and accessory glands originate (Brody, 1996). Most of these structures could not exist or function in the absence of a symmetrical half, and the whole unit must be symmetrically paired across the midline. Although one side may differ in size and shape due to fluctuating asymmetry, directional or absence asymmetry is not known in carabid beetle accessory glands. Second, it is likely that production and certainly storage of the accessory gland fluid is related to the area and volume of the glands. Like the gut, greater internal surface is achieved by the highly convoluted and asymmetrically positioned accessory glands in many species (Fig. 1).

In all three monorchid groups it is usual to find dense populations consisting of large numbers of individuals, suggesting that females would have easy access to males and may benefit from multiple matings (Parker, 1984). In such a case sperm may be in direct competition, predicting that sperm number, testes size, and/or ejaculate quantity would increase to enhance the chance of fertilization. The monorchid condition might initially seem to suggest that sperm competition does not occur in these beetles. However, if mechanical displacement, rather than direct competition, of rival sperm proceeds through flushing by accessory gland fluids, then absolute sperm number becomes unimportant and in-

creasing the volume of nonsperm ejaculate becomes increasingly significant (Parker, 1984). Under this scenario the importance of producing more accessory gland fluid could balance high sperm production. It is also possible that females mate only once and the male ejaculate chemically induces a refractory period or acts as a mating plug to prevent further copulation (Simmons and Siva-Jothy, 1998). In all of these possible mating systems an increase in accessory gland ejaculate could lead to avoidance of direct sperm competition, reduce the need for maximizing sperm production, and so eliminate the pressure to maintain a redundant testis. As long as a sufficient number of sperm are produced, these could also be mechanisms for the increasing size and/or importance of the accessory glands. Alternatively, the accessory gland could be acting to counterbalance the effect of having reduced testicular output and the testis loss would have been due to some other force.

In many monorchid taxa, e.g., *Selenophorus* (Fig. 1A,B), the accessory glands are extremely large and fill the region of the abdomen normally occupied by the left testis. However, it is impossible to establish this as a cause or result of the testicular degeneration since accessory gland size varies greatly between species, among individuals, and even over the course of the season (Smrž, 1981: 468; and observations made for present study).

The testes are clearly functionally redundant and, after migration of the gonad primordium, developmentally independent. Given the assumption that a male produces more sperm than needed to successfully inseminate any encountered female (Cohen, 1973; Parker, 1984), a single testis is likely sufficient for reproduction even in two-testis species. If true, it is surprising that a testis has not been lost in more taxa. The analogous occurrence of monorchy in ptiliids is probably also best explained in terms of optimal visceral packing, but in this instance it may be due to the beetles' extremely small size and peculiarly large sperm (Dybas and Dybas, 1987). We expect that a broader survey of other very small insects will result in a significant increase in the number of monorchid cases consistent with visceral packing constraints within the confines of the cuticular insect abdomen. The set of organs that interact may differ in each lineage, but if testes are involved, they are likely to lose one of the bilateral pair.

Female ovaries show no asymmetry in carabid beetles; however, it is expected that the two systems evolved independently, as the male and female gonad primordium probably develop from different parts of the imaginal disc as in *Drosophila* (Chen and Barker, 1997). Additionally, although the ovaries of the female, like testes, are independent and redundant, total egg output can be considered approximately equal to reproductive success. In carabids this contrasts strongly with the males' presumed overabundance of sperm. Assuming that adequate resources are available to the female, more

eggs would typically be better and two ovaries would increase the rate of egg production over the adult beetle's relatively short life.

Other Cases of Organ Absence or Extreme Asymmetry

For an organ system to completely lack one of a contralateral pair is extremely rare; however, there are a number of notable cases of vestigial and non-functional organs, with some groups having an apparently derived loss of one organ. These are analogous to the case in carabid beetles and serve as additional tests of the idea of optimal visceral packing.

In nearly all birds the left ovary is reduced and nonfunctional, and it has been proposed that ovary reduction in birds could: 1) be a weight-saving adaptation; 2) prevent damaging interaction between adjacent eggs in the oviducts during movement; or 3) be the result of pressures exerted by other internal organs (Kinsky, 1971). A well-developed pair of ovaries and oviducts that are sometimes fully functional occurs in a significant number of birds, particularly in Falconiformes species. Consistent with the influence of organ packing, the right lobe of the liver is not relatively larger in Falconiformes as it is in other birds. Flight may or may not have a significant influence on organ loss, given that in the other flight-capable vertebrate clade, Chiroptera (bats), members have two functional ovaries (Volleth and Tidemann, 1993). However, implantation in bats occurs only in the right uterine horn. Improved flight is not an issue in *Ornithorhynchus* (platypus), which have two ovaries, but the right one is vestigial and nonfunctional (Grant, 1989).

Female scarab beetles in the subfamily Scarabaeinae are known to have only a single ovary, and in the most extreme cases a single ovariole (Halffter and Mathews, 1966; Halffter and Edmonds, 1982). This is thought to be a synapomorphy for the subfamily and found in all member taxa (C. Scholtz in litt.). This syndrome is, according to Halffter (1977) and Halffter and Edmonds (1982), related to the evolution of nidification, k-strategy life history, and markedly low fecundity in dung beetles. A trend toward reduction of ovariole number in symmetrically paired ovaries and unilateral egg production in non-Scarabaeinae coprophagic beetles is cited as evidence for the repeated occurrence of this phenomenon.

Several other insect groups include species that have alternating or unilateral egg production and asymmetrically developed ovaries, e.g., Chrysomelidae (Coleoptera) (Suzuki, 1974), Chloropidae (Diptera) (Meier et al., 1999), and Aphididae (Hemiptera) (Halffter and Mathews, 1966; Halffter and Edmonds, 1982). Most of these, like the scarabaeine beetles, probably have a significant maternal investment per offspring. In the case of some aphids a single female has only one ovariole and produces only one egg (Woodward et al.,

1970). This suggests that ovarian asymmetries may develop under a number of relatively extreme reproductive strategies not likely linked to space constraints imposed on internal organs. However, as far as is known, testes are paired and symmetrical in all of these taxa.

In the female insect, the tradeoff between flight musculature and gonad development is a recurring phenomenon, so much so that it is called the oogenesis-flight syndrome (Johnson, 1969), and a similar explanation for monorchy in carabids could be entertained. However, none of the known instances of this type of tradeoff have led to asymmetrical ovary development. Moreover, monorchid carabid beetles exhibit a variety of metathoracic flight wing configurations, from fully macropterous to vestigially winged, suggesting that testis asymmetry is based on some other evolutionary force. The notion that absence asymmetry in internal organs is an adaptation to flight in any flight-capable clade seems only weakly supported.

The dramatic asymmetry found in the cephalocordate *Asymmetron* includes the presence of a single lateral gonad (Beklemishev, 1969), but this animal's structure is due to a generally asymmetrical developmental program involving more than just its internal organs. Visceral packing may be invoked to explain the peculiar arrangement of organs in these animals, but it would be difficult to separate this from their overall departure from symmetry.

An extreme shift in body-form can dramatically change the space available to internal organs and create a situation likely to result in absence asymmetry. The tubular body-form in Serpentes (snakes), amphisbaenians, and caecilians shows similar internal organ adaptations. In snakes the left lung is reduced but functional in plesiomorphic groups like boids, and vestigial and nonfunctional or absent in more derived groups (Bellairs, 1970; Greene, 1997). Similarly, amphisbaenians have the right lung reduced (Gans, 1992) and caecilians have the left lung reduced (Junqueira et al., 1999). Lung reduction is also known in some burrowing skinks (Bellairs, 1970). These groups do not, however, show absence asymmetry in reproductive organs.

The beetles in Ptiliidae are analogous to snakes and other serpentiform animals in having a dramatic shift in body-form. For the ptiliid beetles it is a move to miniaturization that is likely to have driven the loss of one testis. Size reduction creates a situation where organs must compete, especially within the confines of the cuticular exoskeleton of insects.

The context within which monorchy evolved repeatedly within Carabidae is not like that in snakes or ptiliid beetles. Body forms vary across monorchid carabid taxa but are never extreme, and the beetles are of similar body size to diorchid relatives. In fact, these groups have been considered to typify the average-looking, generalized member of the family.

Clearly, abdominal configuration and the interaction of the internal organs due to the external form of the abdomen in monorchid carabid beetles are not substantially different than in diorchid taxa. Making room for expansive accessory glands is perhaps the most significant element associated with testis loss in carabid beetles.

Whether larger accessory glands in monorchid taxa are the cause or result of testes loss in a space-limited abdomen still requires explanation as to why this absence asymmetry, so rare in animals, has happened at least three times within the carabid subfamily Harpalinae. Related systems in the abdomen and behaviors involved in the reproductive biology of carabid beetles need to be more intensively studied and coupled with a phylogenetic hypothesis for Harpalinae to address the question of what elements in these taxa predispose them to testis loss.

CONCLUSIONS

In summary, given the assumption that the internal viscera are subject to adaptive pressure for optimal packing, a testis, redundant and independently developed, is the most likely element to be lost. Other internal organs are not symmetrically paired (fat body), are unitary and/or functionally constrained (tracheae, nerve cord, dorsal vessel, gut, genitalia, muscles, pygidial glands), or probably developmentally and functionally constrained (tracheae, accessory glands). Observations of gross morphology of the male reproductive system and distribution of the monorchid condition allow us to propose this scenario and pressures that could lead to testis loss, but suggest no obvious functional explanation for the reduction of a testis.

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