Parental care and offspring survival in *Pterostichus anthracinus* (Coleoptera: Carabidae): An experimental study

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Abstract. Parental care in which females attend their offspring is recorded in over 30 species of ground beetles. Despite this, there is no quantitative data on the fate of the offspring when the mother is experimentally removed. This paper investigates parental care in *Pterostichus anthracinus* (Coleoptera: Carabidae). The objectives of the study were to estimate: (1) egg survival when the female is removed; (2) the ability of females to defend their eggs from attack by predators; (3) the ability of females to repair a damaged nest in which egg attendance takes place. In the laboratory, the reproductive activity of *P. anthracinus* lasted four months (from May till August) and peaked in late June. Mean (± SE) number of eggs in each clutch was 25.25 ± 2.19. All of the egg clutches were guarded by a female. Female attendance had no effect on egg mortality due to microbial attack. The duration of embryonic development lasted on average 5.2–5.3 days, and did not differ between the groups with and without maternal care. In *P. anthracinus* maternal care is important in preventing egg mortality due to predators. In the laboratory the percentage mortality of eggs without maternal care due to predators was 100%. In the group in which females attended their eggs, percentage mortality of offspring due to predation was about 51%. Female ability to repair damaged nests is important in preventing dehydration and reducing predation pressure. This laboratory study provides the first quantitative data on the importance of maternal care in ground beetles in determining the survival of their offspring.

INTRODUCTION

Parental care, in a broad sense, can be defined as "any form of parental behavior that appears likely to increase the fitness of a parent offspring" (Clutton-Brock, 1991). In many arthropod groups, parental care is an important life history trait and an effective mechanism for decreasing offspring mortality due to predators and parasitoids (Santos et al., 2016). Among insects, parental care can be divided into pre- and postovipositional. Preovipositional care includes provisioning of nutrients and defensive structures or chemicals to the eggs, selection of oviposition sites, nest building, mass food provisioning, and modification of environmental conditions (Royle et al., 2012). Postovipositional care includes egg and offspring attendance or transport, viviparity, progressive provisioning, and care following nutritional independence (Royle et al., 2012). More than 160 families of insects in 19 orders have evolved some type of postovipositional parental care (reviewed in Machado & Trumbo, 2018) which in turn enabled the origin of subsociality (simple parent-offspring associations) and finally the very complex eusocial societies with overlapping generations, reproductive division of labour and cooperative brood care (Wilson, 1971; Schultner et al., 2017). Subsocial postovipositional care, characterized by prolonged association between adults and young (Eickwort, 1981), is the most primitive level of social interaction involving parents and offspring, and it is of interest because analysis of simple parental responses may elucidate ways toward complex eusocial behaviour (Michener, 1969).

Different schemes are used for classifying parental care in different taxa. A wide variety of subsocial forms are recognized in species of Coleoptera that have no kind of eusocial or "true social" parental behaviour (Brandmayr, 1992). Subsociality in beetles is described for several families including, for instance, Carabidae, Curculionidae, Passalidae, Scarabaeidae, Silphidae and Staphylinidae (Wong et al., 2013). Classification of parental care in ground beetles (Carabidae), in particular, was elaborated by Brandmayr and Zetto-Brandmayr (1979), and it includes two types of parental care.

The first type (Brutfürsorge, egg-watching, egg attendance) implies a strict dependence of the survival of the first stages on the parent. Nevertheless, no direct contact takes place between eggs or larvae and adults, and there is

only passive protection. The second type of parental care (Brutpflege, offspring nursing) consists of a direct care of eggs and larvae, such as cleaning and turning of the eggs, or feeding the larvae with secreted substances. To date there are over 30 species of ground beetles in 10 genera that are characterized by one of these two types of parental care (Table 1). Most species (except *Carterus calydonius*) belong to the tribe Pterostichini, in which the only type of parental care is egg attendance. Offspring nursing is recorded only in *C. calydonius* (Harpalini). In all cases it is only the female that cares for the offspring.

The evolution of egg attendance in Pterostichini is regarded as an adaptation to the cool climate in the Pliocene-Pleistocene period. During this period, there was an increase in the duration of the embryonic period, which probably led to a greater risk of the eggs being attacked by fungi (Brandmayr & Zetto-Brandmayr, 1979; Brandmayr, 1991). A more detailed study of maternal care in species of Pterostichini leads to the conclusion that egg attendance is usually found in species with: (1) montane distribution, (2) micropterous (short-winged) morphology, (3) two-year life cycle, (4) long embryonic development, and are (5) forest-dwellers and (6) breed during the wet season. However, there are species that do not conform to the above. *Notonomus gravis*, for example, inhabits localities in Australia where temperatures above 40°C are regularly recorded (Horne, 1990). Moreover, some species of the genus *Megadromus* (*M. antarcticus* and *M. guerinii*) for which egg attendance has also been recorded are xerophiles (Larochelle & Larivière, 2001).

Although maternal care in ground beetles is well documented, its benefits in terms of survival of their offspring is unknown. As stated above, egg attendance is hypothesized to protect eggs against fungal attacks, which is the most probable cause of their death (Komarek, 1954; Löser, 1970; Lampe, 1975; Brandmayr, 1977; Horne, 1990). However, the role of females in defending the eggs against predatory arthropods has not been investigated (Löser, 1970). Thus, in spite of the general consensus that parental care increases the probability of offspring survival in ground beetles, there is no quantitative data on the fate of offspring when egg-tending females are experimentally removed.

The objectives of this study were to estimate: (1) egg survival in the absence of females; (2) the ability of egg-tending females to defend their progeny from attack by predators; (3) the ability of egg-tending females to repair a damaged nest. Our study species was *Pterostichus anthracinus* Illiger, which is widespread in Western, Central and Eastern Europe (Turin, 2000). Being a hygrophilous and thermophilous species, *P. anthracinus* is a typical inhabitant of moist parts of flood plains and banks of boggy forest brooks (Lindroth, 1992). In contrast to most of the Pterostichini with maternal care, this species has a relatively short life cycle (28–33 days) and embryonic development (5–12 days) (Brandmayr, 1977; Kolesnikov, 2008). These traits do not conform with the hypothesis of an antifungal role of parental care. Probably, egg attendance in this species is an adaptation to the predation pressure due to ground arthropods.

MATERIAL AND METHODS

Collection and housing

Laboratory and field observations were carried out during the period of activity (from May to October) of adults in 2015–2018. Individuals of *P. anthracinus* used in the laboratory experiments were collected using pitfall traps on the floodplain of the Desna River (53°11′59″N, 34°20′31″E) in southwestern Russia, from 1–5 May. Following transport to the laboratory, beetles were divided into mixed-sex groups of 10 individuals, and each group was housed in $10 \times 7.5 \times 5$ cm plastic ventilated jars filled with fine plant residues. All jars containing beetles were kept outdoors for 24 h in order to induce copulation. Afterwards, copulating pairs were placed in ventilated jars ($10 \times 7.5 \times 5$ cm) made of clear plastic and filled with sand (h = 20–25 mm). The sand was moistened by spraying water daily and replaced once a week. The jars were placed in a room with no special microclimate control and therefore subject to external environmental fluctuations. Adults were fed freshly killed individuals of *Gryllus assimilis* (Orthoptera: Gryllidae).

According to Brandmayr & Zetto Brandmayr (1979), egg attendance in the genus *Pterostichus* is not correlated with the activity of the females. The females are constantly active throughout the favourable season (without an interruption for oviposition), although with oscillations due to meteorological events. In order to test this assumption we recorded the activity of adult *P. anthracinus* using pitfall traps (plastic jars 70 mm in diameter and 85 mm deep filled with 4% formaldehyde as a killing and preserving

Table 1. List of carabid genera in which there are reports of species exhibiting maternal care (the number of cases in each genus is presented in parentheses). There are two main types of parental care in carabids: egg attendance (EA) and offspring nursing (ON).

Genus	Type of parental care	Reference
Carterus (1 sp.)	ON	Brandmayr & Brandmayr Zetto (1974)
Abax (2 spp.)	EA	Löser (1970); Lampe (1975)
Megadromus (8 spp.)	EA	Larochelle & Larivière (2001)
Molops (7 spp.)	EA	Komarek (1954); Leonardi (1969); Brandmayr & Brandmayr Zetto (1974); Brandmayr (1977)
Neoferonia (1 sp.)	EA	Larochelle & Larivière (2001)
Notonomus (4 spp.)	EA	Horne (1990)
Percus (1 sp.)	EA	Kavanaugh (1998)
Plocamostethus (1 sp.)	EA	Larochelle & Larivière (2001)
Pterostichus (6 spp.)	EA	Boldori (1933); Lindroth (1946); Weidemann (1971); Brandmayr & Brandmayr Zetto (1974); Brandmayr (1977); Bousquet (1983); Kolesnikov (2008)
Zeopoecilus (1 sp.)	EA	Larochelle & Larivière (2001)

agent) in 2015. All the adults caught were dissected to determine their reproductive condition and the number of eggs in female ovaries. Four stages were distinguished in the development of the adults: (1) teneral: elytra are soft and pale coloured; (2) immature: ovaries are undeveloped in females and accessory glands of testicles are small and translucent in males; (3) mature: ovaries contain mature oocytes in females and accessory glands of males are swollen and have a nacreous appearance; (4) spent: ovaries have regressed and contain corpora lutea in females and accessory glands of males are greyish and disintegrating.

Observational data

During this laboratory study we observed and examined 132 females of *P. anthracinus*. These females laid a total of 205 egg clutches, of which 155 were used in the experiments. Sixteen clutches were dissected to count the number of eggs in each of them. Owing to the small depth of sand in the jars, females built their nests near the bottom, so that it was possible to check the egg clutches every day by examining them through the clear bottoms of the jars, without disturbing the females. To evaluate the duration of development of eggs we examined clutches daily. The effect of maternal care on egg survival was evaluated using the experiments described below. All experiments were carried out the day after the females laid the egg clutches.

Experiment 1: Survival of offspring in the absence of females

To determine the adaptive value of maternal care in *P. anthracinus* we removed the females. We hypothesized that if offspring survival depends on maternal care, unattended eggs would be less likely to survive than attended eggs. Females were randomly divided into control (n = 19) and experimental (n = 15) groups. Females in the experimental group were removed through a small hole made in the nest. Then the nests were repaired by covering the hole with a piece of plastic and sand. The nests in the control group were disturbed and repaired in the same way, but the females were not removed. The jars with both groups of females were kept outdoors. Percentage mortality of the eggs was recorded daily. During the experiment we recorded the number of egg clutches that had at least one egg attacked by microbes.

Experiment 2: Survival of offspring and predation pressure

This experiment was designed to understand whether egg-tending females are able to defend their eggs from attacks by insect predators. The ground beetle *Platynus assimilis* (Coleoptera: Carabidae), which is the most abundant predatory insect in the area studied, was chosen as the predator for this experiment. It is a Euro-Siberian stenotopic species of deciduous forests on mull-rich soil, occurring in cool and wet, shaded habitats among litter, under bark of tree stumps, often near water. It is a nocturnal, spring breeding species that often co-dominates with *P. anthracinus* in wet habitats (Šustek, 1994).

Two groups of egg clutches were used in this experiment. In the first group, egg clutches (n = 11) in nests were exposed to one individual of *P. assimilis* after the females were removed. In the second group, the females were not removed and each egg clutch (n = 45) was exposed to one individual of *P. assimilis*. Under natural conditions many *P. anthracinus* nests were found under fragments of stones or bark with a hole at the top (see Results). To imitate this situation in both experimental groups the roofs of the nests were opened up before the experiment, thus allowing predators access to the egg clutches. The diameter of the hole in the roof was 5−7 mm. We recorded the number of egg clutches attacked by predators. Percentage mortality of the egg clutches due to predation was recorded daily.

Experiment 3: Ability of nesting females to repair damaged nests

Eggs of soil invertebrates are vulnerable to dehydration (Wilson, 1971; Clutton-Brock, 1991; Klug & Bonsall, 2014). Underground nests protect eggs from desiccating. Moreover, they can serve as an additional line of defence, decreasing the probability of predators finding the eggs. To test the ability of egg-tending females to repair damage to their nests and so decrease the percentage mortality of eggs from dehydration and predation, we observed 18 nesting females. Nests with egg clutches were damaged by making openings (5−6 mm) in their roofs. The jars with nesting females were examined daily to record the number of females that repaired their nests.

Statistical analysis

In all experiments Fisher's exact test was used because the expected sample size was less than five in one cell of the 2×2 tables. Mean duration of egg development in the groups with or without females was compared using the Mann-Whitney U-test. A Kruskal-Wallis test (H) was used to test the null hypothesis that the number of eggs in the ovaries of females collected during the whole reproductive period does not differ. We divided the reproductive period when the females contained ripe eggs into four sub periods of ten days.

RESULTS

Observational data

In the laboratory, reproductive activity in *P. anthracinus* lasted nearly four months, from May 10th till August 30th (Fig. 1). During the breeding season females made two types of nests for their clutches of eggs. The first type was built under fragments of stone or bark, and pyramidal in form with a hole at the top, about 20 mm in diameter (Fig. 2A). The second type of nest was located in the ground at a depth of 20–30 mm and did not have a hole at the top. The egg chamber had a diameter of 20 mm. One hundred and thirty two clutches of eggs were laid by the females of *P. anthracinus* during the laboratory study in 2015–2018. Most of egg clutches were laid in June. Each clutch of eggs was about 5–7 mm in diameter (Fig. 2B) and consisted of 17–46 eggs (mean ± SE = 25.25 ± 2.19 eggs per clutch; n = 16). All of these clutches of eggs were guarded by a female, which either sat on the eggs or remained close to the clutch (Fig. 2C, D). About 40% of the females reproduced more than once during the breeding season. Approximately
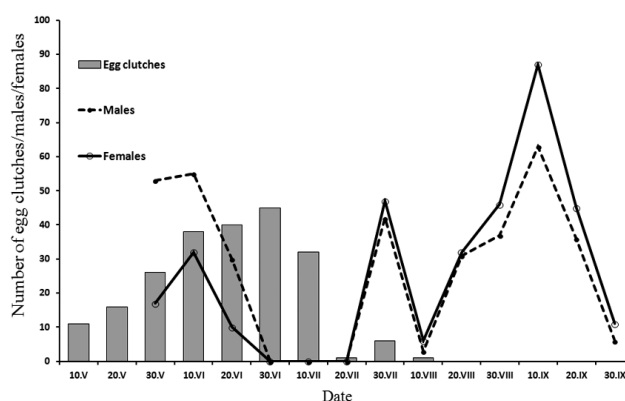

Fig. 1. Seasonal activity of adults of *P. anthracinus* (data for 2015) and reproductive activity of the females (data for 2015–2018).
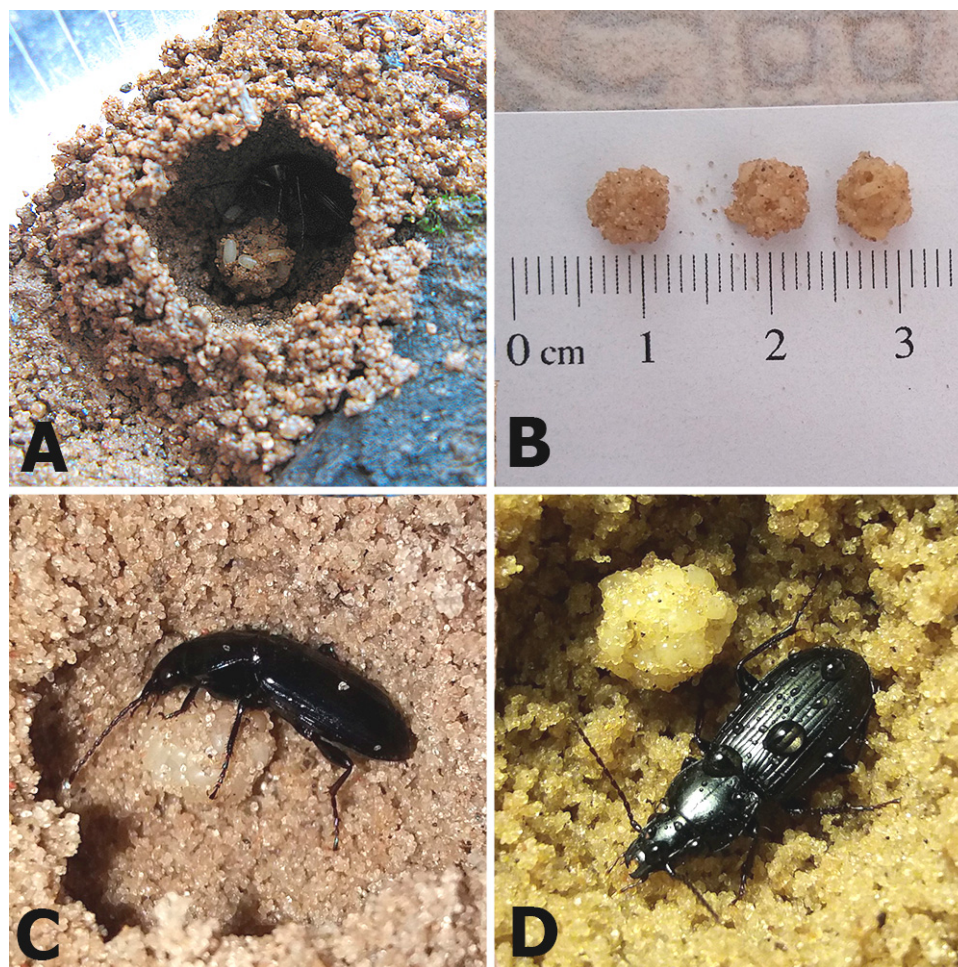
Fig. 2. Nest, nesting female and egg clutches of *P. anthracinus*: A – nest with a clutch of eggs; B – clutches of eggs; C, D – females with their clutches of eggs.

23% of the females laid 2 clutches, 9.2% laid 3 clutches and 2.3% 4 or 5 clutches. Embryonic development of eggs lasted 4–8 days. The interval between clutches in the females that laid more than one clutch of eggs varied from 5 to 32 days (mean ± SD = 14.53 ± 6.17, n = 83). Newly hatched larvae initially remained in the nest, were inactive and non-aggressive towards one another.

Based on pitfall trapping and manual collections, *P. anthracinus* adults were active from the first week of May till October, with two peaks in activity, one at the beginning of June and the other at the beginning of September (Fig. 1). The first peak was mainly associated with the activity of mature adults and the second peak with the activity of juvenile and immature adults. During the first peak, activity of males was more than double that of females. During the second peak females were more active than males. Females with mature eggs were recorded up to the first week of August. Mean number of mature eggs in the ovaries changed during the reproductive period from 9.55 to 17.31 eggs per female, however this difference was not significant (Kruskal-Wallis test (H) = 5.36; df = 3, n = 51, p = 0.148).

The various parameters of fecundity and embryonic development in *P. anthracinus* are summarized in Table 2.

Experiment 1: Offspring survival in the absence of females

The results of the first experiment revealed that the presence of females did not reduce the incidence of the eggs being attacked by microbes (Fisher's exact test: df = 1, p = 0.681). In the experimental (n = 15) and control (n = 19) groups, 86.7% and 78.9% of clutches of eggs, respectively, survived to complete their embryonic development. Mean duration of egg development in the control and experimental groups was 5.27 ± 0.14 (mean ± SE; n = 15) and 5.33 ± 0.23 days (mean ± SE; n = 13), respectively (Mann-Whitney U-test, U = 108, p = 0.596).

Experiment 2: Offspring survival and predation

In the group in which females were removed from the nests, 100% of the clutches of eggs (n = 11) were quickly

Table 2. Summary of the reproductive parameters that determine the fecundity of *P. anthracinus*.

Parameters	n	Range	Mean	SD
Number of clutches per female	132	1–5	1.64	0.97
Number of eggs per clutch	16	17–46	25.25	8.79
Number of eggs in ovaries	51	1–36	14.53	9.67
Embryonic development (days)	15	4–8	5.27	0.54
Interval between egg clutches (days)	83	5–32	14.53	6.17

Fig. 3. Roofs of nests of *P. anthracinus*: A – damaged roof; B – roof that has been repaired by a female.

found and eaten by adult *P. assimilis*. In the group in which females were not removed the percentage mortality of their clutches of eggs (n = 45) due to predation was about 51%. It was observed that egg-tending females were very sensitive to their nests being invaded by predators. Egg-tending females usually kept their fore legs on their clutch of eggs and turned around and tried to protect them with their body. Then the females attacked the predator and drove it away from the nest, after which they sealed the hole in their nest using sand (Fig. 3). Based on the experimental data, it may be concluded that maternal care results in a reduction in attacks by predators, which has a positive effect on the survival of the eggs (Fisher's exact test: df = 1, p = 0.004).

Experiment 3: Ability of nesting female to repair damaged nest

There was no difference in egg survival recorded in the group in which nesting females were in damaged nests (n = 18) and in the control group (n = 19) (Fisher's exact test: df = 1, p = 0.354). Within a few hours of a nest being disturbed, the tending females repaired the damaged roof with sand particles (Fig. 3). Then the loss of clutches of eggs was due to cannibalism by females, not dehydration.

DISCUSSION

According to Brandmayr and Zetto-Brandmayr (1979), the seasonal activity of adult *P. anthracinus* is not influenced by parental behaviour. The adults are continuously active throughout the favourable season, with small fluctuations due to meteorological conditions (Weidemann, 1971; Lampe, 1975). The sharp decrease in adult activity recorded in late June (Fig. 1) could be due to the flash floods that occurred at that time in 2015. The low activity of females compared to that of males during May–June

may be related to the females attending their eggs. However, the differences in activity between males and females may also be related to annual rhythms in the development of each sex and their regulation by external factors (Thiele, 1977).

Based on laboratory observations, the number of eggs in clutches laid by a female range from 17 to 46. This is similar to that recorded for other carabid species of the tribe Pterostichini (Boldori, 1933; Komarek, 1954; Leonardi, 1969; Löser, 1970; Weidemann, 1971; Lampe, 1975; Bousquet, 1983). However, we recorded females that lay 2–5 clutches in the course of a breeding season. Based on a mean number of eggs per clutch of 25, the overall number per season may equal 50–100 eggs. Estimates of the fecundity of *P. anthracinus* based on using a special equation is 65–95 eggs per female (Grüm, 1984). This number of eggs is extraordinarily high for carabids with parental care, as the better offspring survival is thought to balance a reduced number of clutches (Thiele, 1977; Brandmayr & Zetto-Brandmayr, 1979).

The first experiment in which the female was removed revealed that egg mortality due to microbial attack does not depend on the presence of a female. Also there is no difference in duration of the development of eggs in clutches unattended by females and that of eggs in the control group. Insects that rear their progeny in subterranean nests are frequently exposed to infection by microbes or deprivation of oxygen (Tallamy, 1984). Microbial infection of eggs is an important factor leading to the evolution of parental care in arthropods. In subsocial species that nest in the soil, parental behaviour often functions as a countermeasure against micro-organisms (Wong et al., 2013; Klug & Bonsall, 2014). However, quantitative data on egg mortal-

ity due to microbial infection are rare. Female attendance in different species is not equally important for protecting eggs from microbial infection. Studies on dung beetles reveal that brood balls that are not cared for by adults are attacked by fungi, which negatively affects the survival of their offspring (Favila, 1993; Halffter et al., 1996). Field and experimental studies on earwigs reveal that exposure to fungi may significantly decrease the hatching success of unattended clutches but has no significant effect on the hatching success of attended clutches (Klostermeyer, 1942; Lamb, 1976; Boos et al., 2014). On the other hand, field experiments on *Acutisoma longipes* (Opiliones) reveal that females are unable to protect their eggs against attack by fungi (Machado & Oliveira, 1998).

Two strategies are potentially used by egg-tending adults to protect their offspring against infection by microbes: mechanical removal of spores and an antimicrobial secretions (Costa, 2006). Caussanel (1970) reports that females of the earwig *Labidura riparia* brush their eggs with their front tarsi. Females of other species of earwigs manipulate eggs with their mouthparts, and this "licking" may be interpreted as cleaning behaviour (Lamb, 1976). In fact, Shepard et al. (1973) record that radioactively labelled material is transferred from *L. riparia* female to their eggs, which indicates that tending females apply secretions to their eggs. In the harvestman *Quindina albomarginis,* egg-tending males remove mould by eating it and by doing so prevent the massive spread of mycelia (Mora, 1990). Females of the European beewolf (*Philanthus triangulum*) provide antifungal protection by transferring an inoculum of bacteria (*Streptomyces philantii*) from glands located on their antennae to the brood nest in which they lay their eggs. These bacteria produce antibiotics that protect the larvae against fungal attack (Kaltenpoth et al., 2005). For burying beetles, Rozen et al. (2008) show that *Nicrophorus vespilloides* applies substances (e.g. lysozyme) that inhibit microbial growth and thus protect their offspring by limiting the development of microbes. According to Brandmayr & Zetto-Brandmayr (1979) and Komarek (1954), adults of species of the Pterostichini (except *Molops piceus*) neither clean the eggs with their mouth parts nor manage them in any other way. This may explain why egg-tending females of *P. anthracinus* are ineffective in preventing their egg clutches being attacked by microbes.

Relatively short embryonic development in *P. anthracinus* (6–7 days) compared with other species of Pterostichini, in which egg development lasts more than three weeks, is an important factor in decreasing egg mortality due to microbial attack (Ferron, 1978). The choice of an appropriate oviposition site is also crucial for offspring survival (Clutton-Brock, 1991). For instance, field studies on the egg-attending harvestman *A. longipes* show that the females are unable to protect their eggs against fungal attack, but the choice of suitable oviposition sites by the females can reduce the attack within the cave habitat of this species (Machado & Oliveira, 1998). *P. anthracinus* was very abundant at the sparsely vegetated river bank studied, and the individuals preferred light, sandy soils. According

to Andersen & Skorping (1991), ground beetles living in open microhabitats are almost completely free of parasites (such as fungi and nematodes), whereas species in densely vegetated microhabitats are commonly infected by parasites. Differences in the microclimate are unlikely to account for this difference, since temperature, substratum moisture, pH-value and light intensity may be very similar in open and protected microhabitats. The most likely cause of the low infection rate of species at open sites is that flooding regularly washes away the infective stages of parasites (Andersen & Skorping, 1991). There is only limited information in the literature on the susceptibility of ground beetles to microbes (fungi) in field and laboratory experiments (Kabacik-Wasylik & Kmitowa, 1973; Steenberg et al., 1995; Traugott et al., 2005). These studies indicate that percentage mortality in carabids due to fungi is low, but there is no quantitative data on the effect of fungi or other microbes on the survival of the eggs of ground beetles.

The experiment on the effect of predation on offspring survival showed that parental care is of paramount importance in *P. anthracinus*. Percentage mortality of unattended eggs was 100% whereas that of attended eggs was 50%. Similar results are recorded for many other species of arthropods with parental care: shield bugs (Melber & Schmidt, 1975; Tallamy & Denno, 1981; Mappes & Kaitala, 1994; Filippi-Tsukamoto, 1995; Mappes et al., 1997), harvestmen (García-Hernández & Machado, 2017), staphylinid beetles (Wyatt & Foster, 1989) and burying beetles (Scott, 1990). For carabid beetles, however, there is little information on the effect of predators on egg survival. In *Pterostichus oblongopunctatus*, a species with no parental care, percentage mortality of eggs in undisturbed soil is 70%, but when predators are removed it decreases to 7% (Heessen, 1981). In fact, predation is often cited as a significant ecological factor in the evolution of parental care (Tallamy & Wood, 1986; Royle et al., 2012).

Insects protect their offspring from predators in several different ways. True bugs (Hemiptera) straddle their eggs to shield them from predators and parasites, and by spreading their wings increase their size and then aggressively attack predators and evict them from the area (Tallamy & Denno, 1981; Kudo et al., 1989). The same behaviour is reported in earwigs, burying beetles and dung beetles that invariably attack intruders and eject them from their nests (Caussanel, 1970; Lamb, 1976; Klemperer, 1982; Scott, 1990). A tropical butterfly *Hypolimnas anomala* responds to disturbance in two ways: wing-beating and wing-spreading (Nafus & Schreinder, 1988). In harvestmen, males or females depending on the species guard their eggs by sitting on or remaining next to them. They frequently attack an aggressor with their pedipalps and try to bite it with their chelicerae (Machado & Oliveira, 1998; Machado, 2002). Egg-tending females of *P. anthracinus* are highly aggressive and use their well-developed mandibles to drive potential egg predators from their nests. This is all the more remarkable given that the females of other species of Pterostichini do not exhibit any escape or defence reactions (Brandmayr & Zetto-Brandmayr, 1979).

Another significant feature of females of *P. anthracinus* is their ability to repair damaged nests. Nest repair is well known in some arthropods with subsocial parental care, such as passalid beetles, dung beetles and some arachnids. Males of the harvestman *Q. albomarginis* build cup-like mud nests and repair the walls of nests destroyed by the action of rain, wind or larger animals (Rodrigues & Guerrero, 1976; Mora, 1990). In passalid beetles, young adults cooperate with their parents in repairing sibling pupal cases, which are important in protecting offspring from predation or parasitism (Schuster & Schuster, 1985). Adults of dung beetles also repair damage to brood balls or nests (Klemperer, 1982). Experiments with burying beetles reveal that damage of the animal carcass in the form of holes accelerate the decline in the quality of this resource for the brood of the beetle as they make it more accessible to fly larvae. This is minimized by the parents repairing the holes (Trumbo, 2017). Males of the desert tenebrionid beetle *Parastizopus armaticeps* dig and extend breeding burrows in order to maintain a suitable moisture level for the development of their offspring (Rasa, 1998). Females of the subsocial intertidal rove beetle *Bledius spectabilis* remain with their eggs and prevent them from being inundated by blocking the entrances to their burrows when the tide comes in (Wyatt, 1986). These examples show that for many arthropods the preservation of nest integrity is very important. In *P. anthracinus,* preservation of underground nest integrity probably helps not only prevent dehydration of eggs, but it is also important in decreasing the chance of potential predators gaining access as they are often very abundant in coastal and riverside habitats (Manderbach & Reich, 1995; Hering & Plachter, 1997; Paetzold et al., 2005).

Active parental protection in subsocial arthropods is not sufficient to deter all of the natural enemies that may attack their offspring. In many cases some individuals readily abandon their clutches when disturbed or even eat their eggs (Giles, 1953; Bharadwaj, 1966; Caussanel, 1970; Knabke & Grigarick, 1971; Lamb, 1976; Filippi-Tsukamoto et al., 1995). The effectiveness of the defence varies depending on the species of predator attacking the eggs (Nafus & Schreiner, 1988). In some arthropods additional lines of defence may protect the offspring by either reducing the likelihood of a predator detecting their brood or by decreasing the predator's consumption rate during periods when the parents are temporarily absent. Examples of such additional defences include: nest building, coating eggs with either mucus, offensive chemicals or debris and silk (Santos et al., 2016). According to an analysis of parental care in arthropods, additional defences play a crucial role in reducing the level of mortality due to natural enemies (Santos et al., 2016). Offspring mortality is consistently lower in species exhibiting additional defences. Based on our observations, there are two lines of offspring defence in *P. anthracinus*: the nest per se and the protection provided by the female. For a better understanding of the evolution of maternal care, however, future studies need to concentrate on the relative importance of these two lines of offspring defence.

CONCLUSIONS

1. It was found that female attendance in *P. anthracinus* did not affect the mortality of eggs due to microbial attack. The presence of female also did not affect the embryonic development of the eggs.

2. For the first time this study revealed that egg attendance in *P. anthracinus* significantly decreases offspring mortality due to arthropod predation. This is because egg-tending females act aggressively if a potential predator enters their nest.

3. Their underground nests are an additional line of defence in that they reduce the risk of the eggs desiccating and being eaten by predators, and females of *P. anthracinus* are capable of repairing their nests.

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