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Field Observations Confirming Brood Care in *Percus passerinii* in the Tuscan Apennines, Italy (Coleoptera Carabidae)

Abstract - Field observations made in August, 1996, in the Tuscan Apeninnes, confirm Bargagli's (1874) record of parental care in the Pterostichine Carabid species *Percus passerinii* (Dejean, 1828). A single female was found in an excavated nest with two newly hatched larvae and eight eggs, the latter encased in individual, complete mud capsules. Summer breeding, associated with adult aestivation, low fecundity, and an apparently long embryonic development period are consistent with other Pterostichines exhibiting "brood watching" behavior. Subsequent laboratory observations suggest two distinct egg clutches represented and an unusually long period of embryonic and larval development.

Riassunto - *Osservazioni in natura sulle cure parentali di* Percus passerinii *(Coleoptera, Carabidae) nell'Appennino toscano.*
Le osservazioni compiute in natura, nell'agosto 1996 a Vallombrosa (Firenze), confermano quanto osservato da Bargagli (1874) sulle cure parentali di *Percus passerinii* (Dejean, 1828), una specie di Carabide Pterostichino endemica dell'Appennino centro-settentrionale. Una singola femmina, con due larve schiuse da poco ed otto uova, è stata rinvenuta in un nido scavato nel suolo; ciascun uovo era rivestito da materiale fangoso a formare singole capsule complete. La riproduzione estiva, associata alla estivazione degli adulti, al basso numero di uova, e a uno sviluppo embrionale apparentemente lungo, sembrano congruenti con quanto osservato in altri Pterostichini con comportamento "brood watching". Successive osservazioni condotte in laboratorio suggeriscono la presenza di due distinti eventi di schiusa delle uova e di un periodo di sviluppo embrionale e larvale particolarmente lungo.

Key words: Carabidae, Molopini, *Percus*, parental care, biology, Italy.

SERENDIPITY—NOUN., AN APTITUDE FOR MAKING DESIRABLE DISCOVERIES BY ACCIDENT

It would be difficult, if not impossible, to overstate the role of serendipity in the history of advancement of knowledge in biology. Sometimes, it leads to tremendous leaps forward - as when a lapse in laboratory procedure led to Alexander Fleming's discovery of the pharmacological properties of *Penicillium* mold; or when an unlikely chain of events led to Charles Darwin's participation in the H. M. S. Beagle's voyage of discovery, and, ultimately, to his theory of evolution through natural selection. Usually, it leads to more modest advances, such as that based on the observations reported here.

On August 31, 1996, while attending the XX International Congress of Entomology in Florence, Italy, David Maddison and I took a one-day break from scheduled activities to visit the nearby Tuscan Apennines. On the advice of colleagues Riccardo Sciaky and Paolo Bonavita, we traveled by bus to the town of Vallombrosa, in the Pratomagno range, and visited the beautiful forest of the "Riserva Naturale Biogenetica" di Vallombrosa, where

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we spent the day observing and collecting carabid beetles.

In mid-afternoon, and following a brief but heavy rain and thunderstorm, I discovered a female of *Percus passerinii* (Dejean, 1828) in a large, excavated cell under a stone on the forest floor. My eye had been drawn first, not to the very large (3 cm long) black adult, but to two large (fully half the length of the adult), whitish larvae in the cell with the adult. Finding adult and larval carabids so close together was new to my experience, and it did not occur to me immediately that they might be conspecific. I removed the larvae from the cell and placed them alive in a small container with loose, damp soil. I was about to pick up the adult when I observed a number of large, distinctive, ovoid balls of soil underneath and immediately around her in the cell. One of these, nearest to where the larvae had been positioned, was slightly crushed and cracked open, revealing a large whitish object inside. When I picked up the ball, which I had damaged inadvertently while attending to the larvae, part of its soil coating fell away to reveal a single, huge egg, about 5 mm in length. I carefully removed the adult from the cell and then collected the soil-coated eggs, a total of 8, and placed them in a separate container with loose, damp soil.

By the time Maddison and I had returned to our hotel room in Florence, about four hours later, two additional larvae had hatched in the container of soil, and the two larvae collected at Vallombrosa had already become slightly pigmented. Sometime overnight, two more larvae hatched. The remaining four eggs were watched carefully during the next several weeks, but none of them hatched.

As soon as I had realized that there were intact eggs in the excavated cell with the larvae and the adult female, it had become clear to me that I was observing some sort of parental care by at least one female of *P. passerinii*. Figure 1 is a copy of the quick sketch I made in my field notebook at the time to record my impressions of the cell and relationships of the adult, larvae, and eggs within it. I did not know if this kind of behavior had been described previously for this or any related species of *Percus*, nor was I aware of the known distribution of such behavior among carabid beetles in general. So I wrote to Pietro Brandmayr and Tullia Zetto Brandmayr (at the University of Calabria), who have investigated such behavior extensively in carabids and other beetles, and briefly described my observations to them. In an enthusiastic response, they provided me with pertinent references, many of them their own publications, and urged me to publish a detailed account of my observations. They noted that, as early as 1874, Bargagli (1874) had reported almost identical observations of brood care by *P. passerinii* adults, but also that these findings had not been confirmed subsequently (Thiele, 1977). It is in response to the encouragement from the Brandmayrs that I provide the following more detailed account.

FIELD AND SUBSEQUENT LABORATORY OBSERVATIONS AND MEASUREMENTS

FIELD OBSERVATIONS OF *PERCUS PASSERINII* ADULTS. Field obervations reported here were made in the lush forest of the Riserva Naturale Biogenetic di Vallombrosa, at an elevation of about 970 meters, and within 50 meters of a small stream, the Fosso dei Bruciati. Forest structure featured a nearly closed canopy with sparse understory development (fig. 2). Tree species included deciduous forms (e.g *Tilia*, *Acer*, and *Fagus* spp.), peripherally in old canopy gaps, and interior stands of conifers, mainly of *Abies alba* Mill., with smaller plan-

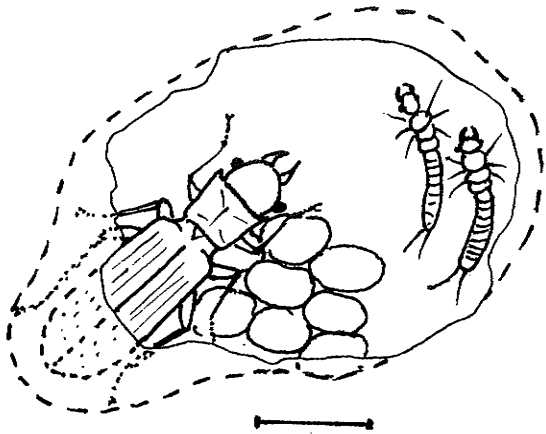

Fig. 1. Sketch of excavated nest, with covering stone removed, showing location and relative sizes of eggs, larvae, and adult female *Percus passerinii* (Dejean); redrawn from field notebook.

ted clumps of *Picea sitchensis* (Bong.) Carrière, and *Chaemaecyparis lawsoniana* (A. Murray) Parl. *Percus passerinii* were found only in areas where deciduous trees predominated. Other carabid species found in the same area, all in greater numbers than *P. passerinii*, included *Nebria tibialis* (Bonelli, 1809), *Abax ater curtulus* Fairmaire, 1856, and *Pterostichus micans* Heer, 1841.

A total of seven adult specimens of *P. passerinii* were found on August 31, 1996, all of them female, and all but one of them in cells excavated beneath stones on the forest floor. Only one of these, the fourth specimen collected, was found in association with larvae and/or eggs. It is certainly possible that there were soil-coated eggs, which I overlooked, in the cell(s) of one or more of the first three females collected, but I think that this is unlikely. Careful search of the cells of the last three females produced neither eggs nor the remains of same.

Most of the stones under which these adult females were found were large, between 30 and 50 cm in diameter, with flattened undersides, and resting on loosely compacted soil. In contrast, but perhaps only by chance, the cell of the female with larvae and eggs was under a smaller, flatter stone, about 15 cm in diameter and 6 cm thick, positioned in the center of a small trail through the forest, on more compacted soil.

The cells in which the beetles were found were clearly excavations, not simply fissures in the soil. All of them were broadly open when their overlaying stones was removed, indicating that soil had been removed right up to the underside of the rock over much, if not most, of the cell. Cell diameters ranged from about 30 to 60 mm, with all but one cell smaller than 50 mm. The one noticeably larger cell was that housing the brooding female (fig. 1).

None of the adults uncovered were quick to move when exposed to light, and most of them remained motionless until collected. This slow reaction to discovery, location in

excavated cells rather than simply in crevices adequate for hiding during daylight hours, and seasonal timing (*i.e.*, late summer) all suggest that these beetles were aestivating when discovered. Brandmayr & Zetto Brandmayr (1979) discussed an evolutionary correlation between brood care and adult aestivation in *Molops* spp., a genus closely related to *Percus*. Observations reported here support this hypothesis. That only one of eight females found aestivating was also brooding eggs suggests that not all, and perhaps only a small percentage, of the females in this local population were reproducing in 1996. Perhaps most of the females found were reproductive immatures; and this species may have, in effect, a two-year life cycle, as is indicated for at least some *Molops* spp. (Brandmayr & Zetto Brandmayr, 1979).

FIELD AND LABORATORY OBSERVATIONS OF EGGS. As noted above, the eggs of *P. passerinii* collected at Vallombrosa were very large and somewhat oval in shape (fig. 3A). Measurements of the eggs were made in the laboratory, so no measurements were obtained for the eggs that had hatched already. One of the four intact eggs was destroyed by fungus shortly after collection, but the three remaining eggs measured 4.8, 4.9, and 5.1 mm long and 3.6, 3.7, and 3.7 mm wide, respectively, which corresponds reasonably well with dimensions reported by Bargagli (1874).

When I discovered them, the eight eggs in the cell were coated completely with a thick layer of sculpted, compacted soil. Close examination of the coatings in the laboratory revealed them to be slightly varied in thickness, from 0.7 to 1.2 mm, over the entire egg surface. With a coating of such thickness, the dimensions of the capsule for each egg were about 7 mm by 5.5 mm. The coatings were composed of a mixture of clay and other fine soil particles, including small white granules, presumably of quartz. The capsules did not appear to be attached to the substrate in any way but, rather, were resting freely on the soil surface in the cell. The coatings disintegrated to some degree in transit from the field, before I had an opportunity to examine their intact structure under a microscope (fig. 3A). However, in transferring the balls from the cell to the storage container, I did not detect any obvious seams or divisions into a base and lid, as have been described for the capsules around eggs of *Abax* species (Brandmayr, 1977).

Counting the two unpigmented larvae that were found in the cell, probably having hatched minutes, or at most within a few hours, before their discovery, a total of six eggs hatched within a 24-hour period; and all of these new larvae were fully pigmented within 30 hours of the initial discovery. In contrast, the eggs that did not hatch during that first 24-hour period were kept in the laboratory (at room temperature ranging from 18 to 22°C) for slightly more than an additional four weeks without hatching. Because no sign of development within the eggs was evident externally after that extended period, I concluded that they had not been fertilized. Consequently, I preserved the eggs (by dropping them in just-boiled water, leaving them in the water until it cooled to room temperature, and transferring them to 75% ethanol for storage). On subsequent microscope examination, however, I found that two of the three preserved eggs had ruptured slightly during processing, and that differentiated, but totally unpigmented, structures were visible internally through the split chorion. It is now clear that the eggs were still viable and probably continuing to develop at the time they were preserved.

Fig. 2. Photograph of habitat for *Percus passerinii* (Dejean) in the "Riserva Naturale Biogenetica di Vallombrosa", Pratomagno, Tuscan Apeninnes, Italy; arrow denotes location of stone under which a female was found with eggs and larvae.

These findings suggest that the brood present in the single cell may have represented two different, temporally distinct, clutches of eggs - the first including six eggs, all of which hatched on August 31 or early on September 1, and a second including four eggs, none of which had hatched after more than 28 additional days. It is also possible that the second clutch of eggs may have been incomplete, if the female had been interrupted in the middle of production of that clutch on August 31. Embryonic development in at least some *Molops* spp. requires 30 days or more (Brandmayr, 1977, 1992); and it would appear that a similar period may be required for such development in *P. passerinii* as well.

LABORATORY OBSERVATIONS OF LARVAE. The two larvae taken from the field, as well as the four that hatched subsequently from the eggs collected, fit the brief description and illustrations of Bargagli (1874) (fig. 3B). Mean body length (not including the urogomphi) of these six first instar larvae is about 17 mm. Full pigmentation, to a light brown over the head capsule and all body sclerites, was achieved within 36 hours of hatching.

Over a four-week period following their hatching, the six larvae were kept alive in the laboratory in two groups of three larvae each. Each group was housed in a small plastic petri dish (50 mm diameter by 10 mm deep) with slightly damp peat moss. Although the larvae moved about their container occasionally, they remained unusually limited in

their movements, and no cannibalism or other aggressive behavior was observed among them during this entire period. On several occasions throughout this period, I attempted to feed the larvae small bits of dissected mealworms (larvae of the tenebrionid species, *Tenebrio molitor* Linnaeus), a food source that I have used successfully in rearing thousands of carabid larvae over the past 25 years. The larvae of *P. passerinii* showed no attack response to this fresh food when it was presented and absolutely no interest in feeding, even four weeks after hatching. Perhaps, like the larvae of at least some *Abax* spp., which apparently feed exclusively on earthworms (Lampe, 1975; Löser, 1970, 1972), *P. passerinii* larvae may require some specific prey.

DISCUSSION

Melber & Schmidt (1977) proposed a classification of levels of parental care below the true social ["eusocial" (Wilson, 1971)] level and used it for comparing different forms of such behavior in Heteroptera. Brandmayr & Zetto Brandmayr (1979) and Brandmayr (1992) applied this classification scheme to the different forms of parental care found in carabid beetles. Among carabids, the kind of parental care reported here for *P. passerinii* is most similar to (but not identical with) that known for several *Molops* spp., called "brood watching [type IIb]" (Brandmayr & Zetto Brandmayr, 1979; Brandmayr, 1992) or *Brutfürsorge* (Melber & Schmidt, 1977).

Brood watching in *Molops* is characterized by the female (1) excavating a subterranean nest deep beneath a stone during the summer, this coupled with an adult aestivation period; (2) producing a small number (5-12) of eggs that will require a month or more for embryonic development; and (3) guarding over the eggs and newly-hatched larvae until the latter leave the nest two or three days after hatching. Encasement of eggs in mud capsules is unknown among *Molops* spp., but it has been recorded for many *Abax* spp. and for *Percus navaricus* Dejean (Lumaret, 1971), as well as for a few other Pterostichines and some non-Pterostichine Carabids. There is no evidence that *Molops* adults provide any nourishment to or direct care of the larvae once they have hatched. Nonetheless, the parental investment in the production and care of such a few eggs represents the most markedly K-selected reproductive strategy known among Carabid beetles (Brandmayr & Brandmayr, 1983), except perhaps for *P. passerinii* females, which both encase their eggs in mud and watch over them.

Interestingly, Lumaret (1971) observed no brood watching behavior in *P. navaricus* females, which also differ from *P. passerinii* females in producing smaller eggs, and more of them, over a seemingly much longer egg-laying period. Under laboratory conditions, he recorded variation in the incubation period for eggs of *P. navaricus*, ranging from 9 to 28 days, depending on soil humidity (Lumaret, 1971). This too would seem to differ with the even longer incubation period suggested by the observations reported here for *P. passerinii* eggs.

An obvious difference in brood care between *Percus passerinii* females and those of *Molops* spp. is that the former encase their eggs in mud capsules while the latter do not. But there may also be other differences of a more profound nature. Brandmayr & Zetto Brandmayr (1979) note that suppression of predatory behavior (*i.e.*, cannibalism) among

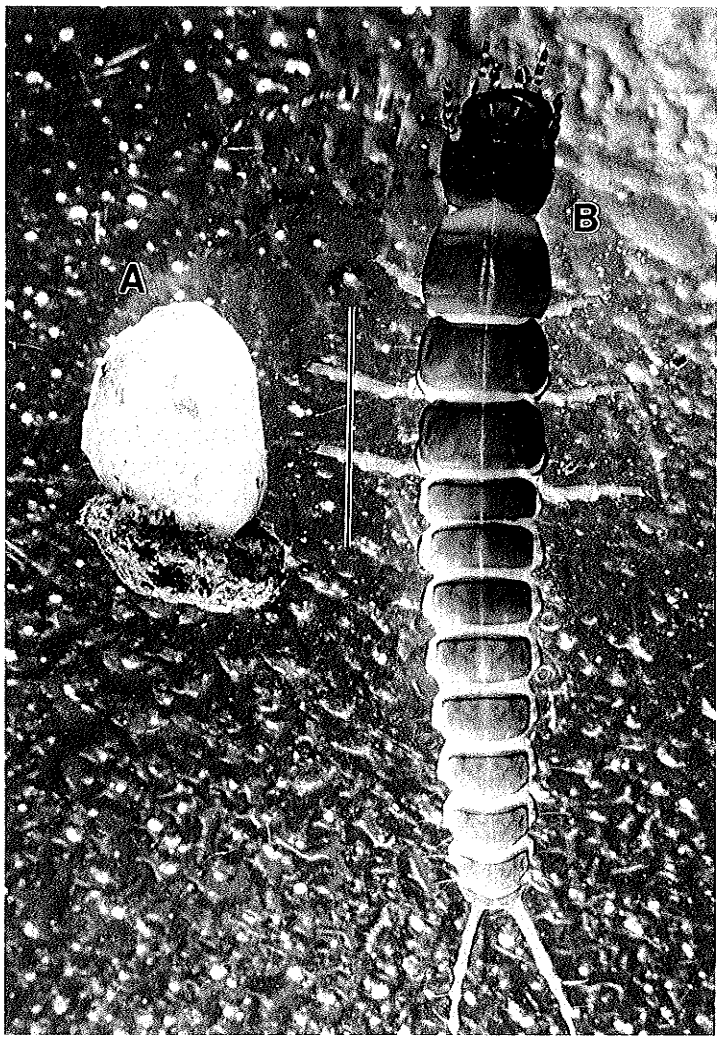

Fig. 3. Photograph of egg and first instar larva of *Percus passerinii* (Dejean); scale line = 5.0 mm. A. Egg (slightly ruptured, with contents protruded on left), with a small portion of its mud capsule still attached. B. First instar larva, dorsal aspect.

siblings is probably due to the presence of the last residual yolk supplies in the gut of the larva. Larvae of *Abax* and *Molops* spp. are thought to disperse from the brood chamber two or three days after hatching (Brandmayr & Zetto Brandmayr, 1979), so this suppression of aggressive or other predatory behavior in the larvae would need to last only a few days. One would assume that, after leaving the nest, the larvae would soon begin to forage and feed aggressively for themselves. However, at least under laboratory conditions with lar-

vae prevented from dispersing from one another, this does not appear to happen with *P. passerinii* larvae. How long could their yolk reserves be expected to last? Is there some other food source and/or feeding stimulus within the nest that they require? Or is it possible that the parent provides some nourishment or other stimulus to the larvae that they require before leaving the nest to feed on their own? Perhaps prolonged suppression of predatory behavior facilitates co-existence in the nest with a second clutch of eggs, which the larvae from the first clutch might otherwise attack and eat.

Of greater interest, perhaps, is the apparent production of two clutches of eggs. Brandmayr and Zetto Brandmayr (1979) reported that, in all instances known, brood watching (and aestivating) females do not feed, after laying eggs, until after the larvae have hatched and left the nest. If egg production by the female *P. passerinii* reported here included two distinct clutches, separated, as it seems, by more than 28 days, how were the resources required for egg production apportioned among these clutches? Did the female begin aestivation with all the resources required for both clutches already stored in her fat-body, or did she obtain the resources for the second clutch after producing the first clutch? If the former, then the amount of resource material stored in the fat body must be impressive indeed. Just such an abundance has been confirmed through hundreds of dissections of *Molops* and *Percus* females during summer months by Brandmayr & Brandmayr [personal communication]. The female would also require the ability to partition resources between the first clutch and second clutches (or perhaps, alternatively, to reserve some for her own internal needs). There is no evidence at present to support a break in aestivation for the female to feed between production of the first and second clutches. In either case, it is clear that the observations reported here raise at least as many new questions as they answer about parental care in *P. passerinii*.

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