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Developmental Biology of the Aquatic Beetle *Berosus alternans* Brullé (Coleoptera: Hydrophilidae) in the Laboratory

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Abstract

A stage-specific vertical life table of the aquatic beetle *Berosus alternans* Brullé was constructed based on a laboratory study. Embryonic development time was estimated as 3.5 days. Eggs hatched between the 4th and 14th days after being laid. Females constructed, on average, 2.46 egg cases per day during 84 consecutive days. The first oviposition occurred on the 6th day after mating, extending until the 84th day. The mean durations of instars and stages were: egg, 6.12; L1, 4.93; L2, 5.13; L3, 11.17; pre-pupa, 109.12 and pupa, 7.31 days. Preadult development time was completed in 127.6 days for males, and in 154.4 days for females. When we tested mean duration by developmental stages, pre-pupa was significantly longer for females than for males ($P < 0.01$). Relative mortality was highest in the third instar, followed by the pupal stage.

The aquatic-beetles of the genus *Berosus* Leach have a worldwide distribution with 113 species in the Neotropical region (Hansen 1999). Ecological observations indicate that the species of *Berosus* are inhabitants of lentic and lotic habitats, and they are swimmers, divers and climbers. The trophic status of adults are: piercers-herbivores, collectors-gatherers and shredders, while the larvae are predators (White and Brigham 1996). However, the most conspicuous ecological characteristic is the benthic habit of their larval instars, which possess tracheal gills that permit them to obtain oxygen from water (Bøving and Henriksen 1938). Pupation occurs in the soil near the edge of pools, as in the other genera of the Berosini (Wilson 1923).

The most extensive research about the biology of *Berosus* spp. was conducted by van Tassell (1966), who studied the egg case construction, biology of all stages of development, genetics, cytology, behavior and sound production of Nearctic species. With respect to Neotropical species, few details are known. Archangelsky (1999) included biological notes referring to habitats, development time of the immature stages, and oviposition sites in a study describing the larvae of *B. aulus* Orchymont and *B. auripes* Boheman.

Berosus alternans is endemic to Argentina (provinces of Tucumán, San Luis, Buenos Aires, Neuquén and Río Negro) (Oliva 1989; Fernández and Bachmann 1998). After our recent description of the immature stages of *Berosus alternans* Brullé (Fernández and Campos 2002), we initiated a study to elucidate its life history in the laboratory. In the present paper, we show the results of the life table and reproductive parameters of the oviposition and egg-hatching behavior of *B. alternans*.

Materials and Methods

Berosus alternans adults were collected in an ephemeral pool located in Florencio Varela (34°46′S; 58°16′W) (Buenos Aires, Argentina) and reared in the laboratory. Specimens were conditioned in an aquarium of 10 × 10 × 10 cm and fed with algae and decaying plant matter collected in the same pool. Those females, which had mated in the field, laid eggs on aquatic plants provided for this purpose. Egg cases were removed from the aquarium every day, and larvae allowed to hatch in individual 30 ml containers with tap water. The progeny was reared individually to prevent cannibalism, and fed *ad libitum* with *Aedes aegypti* L. larvae (Diptera: Culicidae) of equivalent size of *B. alternans* instars larvae. After *B. alternans* third instar larvae stopped feeding, they were placed for pupation in a container as was described in our previous paper (Fernández and Campos 2002).

The embryonic development time was estimated from dissection of five groups of egg cases during five consecutive days after eggs were laid. An embryo was considered fully developed when the pigmented stemmata were visible through the transparent shell of the egg, as this is a conspicuous feature of the embryo in the later stages, before the pharated first larval instar is attained (van Tassell 1966).

Four individual females mated in nature were reared in the laboratory to study oviposition patterns. Every day the number of egg cases constructed was recorded as well as the number of eggs per case; time of hatching, and the delay between first and second eclosion of the pair of eggs in each case.

Horizontal survivorship was estimated in laboratory from egg to adult from 46 oothecae laid by four *B. alternans* females. Following Service (1973), S_i was defined as the number of individuals entering instar *i*; D_i as deaths in the instar *i*; P_i as the relative death proportion in instar *i*, and calculated by D_i/S_i; and Pd_i as the daily death proportion in instar *i*. The relative mortality in different stages was quantified by key-factor analysis and expressed as killing power (*k*), obtained by the subtraction of successive values of log S_i (Varley and Gradwell 1960). The immature developmental rates by sex were estimated from the number of individuals surviving to adult.

Pre-pupal "stage" was considered as the "larva which pupated" of van Tassell (1966). Pre-pupa differs from the active third instar larva because it accumulates fat and turns opaque, obscuring the outlines of the gut and the main tracheal trunks. Their reactions are slower and their locomotion movements are sluggish. They stopped feeding, and explored for burrow sites in moist soil.

Statistical comparisons were made by *t*-test, and when assumptions of parametric test were violated, Mann-Whitney *U*-test was used.

Results

Egg-cases of *B. alternans* contained two eggs, as described in our previous paper (Fernández and Campos 2002). The cases were built separately or in groups on stems and leaves of aquatic plants, below the water surface, as well as on the side of decaying tree leaves. No egg case was attached to the container wall when any natural substrate was provided. Embryonic development time was estimated to be 3.5 days ($n_{egg} = 56$, embryonated egg: 51.8% and 48.2% on the 3rd and 4th day respectively), fully non-embryonated eggs were observed before the third day. During dissections made on oothecae, a micro-bubble inside the case was observed around both eggs, even when the first instar larvae had emerged.

From 342 egg-cases observed, 38 (11%) contained only one egg. Eggs hatched between the 4th and 14th day after deposition, occasionally until the 17th day (mean = 6.12, SD = 1.18, n = 500). Pairs of eggs from the same case hatched synchronously, or the second egg hatched no more than two days later. First instar larvae may

Table 1. Estimation of duration of *Berosus alternans* immatures by sex, reared in laboratory.

Life stage	Stage duration in days (Mean ± SE) ♂ n = 20	Stage duration in days (Mean ± SE) ♀ n = 16
L1	5.00 ± 0.29	4.81 ± 0.23
L2	4.85 ± 0.23	5.50 ± 0.22
L3	12.35 ± 1.30	13.63 ± 1.52
Pre-pupae	100.1 ± 3.96*	125.06 ± 8.71*
Pupae	5.95 ± 0.22	5.37 ± 0.26
Time to adulthood	128.25 ± 3.97*	154.37 ± 8.00*

* $P < 0.01$. Comparison by *t*-test.

opportunistically remain inside the egg case until the day after eclosion. Emergence occurs by cutting the silk strands around the cover on the base of the egg case.

Females mated in nature and reared in the laboratory constructed 1 to 10 egg cases per day (mean = 2.46, SD = 1.90, n = 339) during 86 consecutive days. We observed the same females constructing more than one egg case daily for several consecutive days, or at intervals of 1 to 9 days. At the end of the oviposition period, females either laid eggs (occasionally one) covered by scarce silk, or constructed incomplete cases composed of the basal plate only, but containing no eggs. Although this interval of ovipositions did not represent the complete life history of each female, the frequency distribution of laying was near a normal distribution (Kolmogorov-Smirnov normality test: $P < 0.01$); the maximum of eggs was laid between the 30^{th} and 54^{th} day extending until the 84^{th}day. On the other hand, observations from nulliparous females bred in laboratory from eggs, showed that the first oviposition occurs 6 days after mating.

Records of the ratio of sexes in each pair of eggs (n = 12) confirmed that each case could contain both male (n = 3), both female (n = 3), or one male and one female (n = 6).

A life table was constructed from a laboratory study of 92 eggs (46 egg cases) of *B. alternans*, of which 84 hatched and the remaining died in the egg stage. Laboratory observations showed that the mean durations of instars and stages were: egg: 6.12, L1: 4.93, L2: 5.13, L3: 11.17, pre-pupa: 109.12 and pupa: 7.31 days. Preadult development by sex ranged from 105 to 173 days (males), and 106 to 239 days (females). On average males emerged sooner than females (Table 1), and the effects of sex on developmental time was significantly different ($t_s = -3.11$, df = 34, $P < 0.01$). When we tested mean duration by developmental stages, pre-pupa was significantly higher for females than males ($t_s = -2.79$, df = 34, $P < 0.01$). However, no significant differences were detected for the remaining stages (Mann-Whitney test: L1: U = 283.5, $P = 0.70$; L2: U = 348, $P = 0.10$; L3: U = 329, $P = 0.30$; Pupa: U = 247, $P = 0.12$; $n_{♀} = 16$, $n_{♂} = 20$) (Table 1).

The survivorship of *B. alternans* from egg to adulthood, was estimated to be 39%. The relative death proportion (P_i) was highest in the third instar, followed by the pupal stage. Considering the rate of daily mortality (Pd_i), third instar and pupa were consistent with P_i. However, the daily mortality in the pre-pupal stage was lower than the relative death proportion (Table 2). The *k* values ("killing power" of Valey and Gradwell 1960) were consistent with P_i.

Discussion

The biology of *B. alternans*, in comparison to other *Berosus* species, shows some differences, such as the number of eggs per ootheca, as discussed in our previous paper

Table 2. Estimates of horizontal life stage survivorship for *Berosus alternans* immatures reared in the laboratory.

Stage (i)	Age in days at beginning of stage (t_i)*	No. entering stage (S_i)	Deaths in stage (D_i)	Relative proportion dying (P_i)	Proportion dying daily (Pd_i)	k
Eggs	0	92	8	0.087	0.015	0.040
L1	6.12	84	1	0.012	0.002	0.005
L2	11.05	83	1	0.012	0.002	0.005
L3	16.18	82	31	0.378	0.042	0.206
Pre-pupae	27.35	51	3	0.059	0.001	0.027
Pupae	136.47	48	12	0.250	0.039	0.125
Adults	143.78	36				
		S 0.39				K 0.408

L1 to L3 = larval instars; S = eggs to adulthood survivorship (adult/egg); K = sum of "killing power" (k).
* Values estimated from males and females together.

(Fernández and Campos 2002). The presence of single eggs per ootheca, and the lax and loose weaves of the cases coincided with the end of the oviposition period. This anomaly presumably occurs as a consequence of physiological fatigue of the females.

The egg case of *Berosus* is made into a micro-bubble provided from the ventral air bubble of the female. Its microfilm of air temporarily covers the fresh case including the mast (van Tassell 1966). Our results from study of *B. alternans* agree with these observations. Moreover, the dissections from old cases showed that the micro-bubble remains inside the case covering both eggs until the first instar larvae abandon the case. This micro-atmosphere may allow the pharated first instar larvae to survive at least for a short period in a humid environment if for any reason the ootheca stays exposed out of the water. This may be facilitated by the compact weave of the case (preventing rapid leakage). On the other hand, the female's behavior of ovipositing in the underside of submerged leaves of the aquatic plants also facilitates egg survival.

In a previous study, we observed an ample variation in the length of the egg case's masts (Fernández and Campos 2002), for which a function is unknown. In the present study, we observed eggs surviving from oothecae with different mast lengths. This suggests that this structure might not have a specific function. Presumably, the variation in the size of the mast may be associated with the capacity of the female to produce silk.

Egg case construction of *Berosus* spp. begins at approximately 24 hours after copulation. Alternation between egg laying and copulation occurs in at least some species (van Tassell 1966). By contrast, nulliparous *B. alternans* females laid 6 days after mating, and females collected in the field that were reared in laboratory in absence of males constructed egg-cases and laid during 84 days with intervals of no more than 9 days.

In a laboratory study, developmental time of *Berosus pugnax* LeConte was estimated to be approximately two months, with an extended third instar larval period (25–30 days), and short pre-pupal (6–8 d) and pupal stages (4–5 d) (Archangelsky 1994). On the other hand, van Tassell (1966), studying other unidentified *Berosus* species, observed that a single larva required 74 days from hatching to adult emergence, with a long period as a third instar larva and 8 days as a pupa. Our results show that complete development of *B. alternans* immatures occurs in four months for males and five months for females. As compared with *B. pugnax*, *B. alternans* remained in the pre-pupal stage longer (±3 months) than the third instar larva (±11 days).

Aouad (1988) reported that the length of time in the pupal stage of *Berosus affinis* (Brullé) in temporary habitats is variable and regulated by the drought period of the habitat. By contrast, in permanent habitats pupation occurs within a month. We know that *B. alternans* occurs in temporary pools (observations of L. Fernández), but its biological cycle in nature is unknown. Although our results were obtained from laboratory records, they show ample capacity for variation in the length of the pre-pupal period as compared to pupal stage (See SE in Table 1). We think that this conspicuous characteristic, plus the burying behavior of the pre-pupal stage, could be adaptations to survive in ephemeral pools when the drought occurs sooner than normal. Van Tassell (1966), who studied other species of *Berosus*, observed a similar behavior and verified in the laboratory that mature third instar larva placed on moist sand burrowed themselves immediately.

In this paper we show the proportion of individuals dying by stages. Daily death and "*k*" factor are considerably higher in the third instar larva than in the other stages, however, causes of mortality in the laboratory are unknown and not attributable to competence or enemies because each individual was reared separately. Intrinsic causes could provoke the observed deaths.

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