Flexible oviposition behavior in the golden egg bug (*Phyllomorpha laciniata*) and its implications for offspring survival

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Female golden egg bugs show unique flexibility in their oviposition behavior. Females can lay eggs on plants, where they are left unattended, or on the back of conspecifics, where they remain until hatching. In this article we show that eggs have greater survival rates when carried by an adult than when laid on plants. The main causes of egg mortality are predators and a parasitoid wasp. Our results suggest that, although predation pressure is similar, fewer eggs are attacked by parasitoid wasps when carried by an adult than when laid on plants. In addition, we show that, when given a choice, females prefer to lay eggs on the backs of conspecifics than on plants. Thus, female oviposition choice is adaptive and minimizes individual offspring mortality. The factors that may maintain such behavioral variation in current populations are discussed. Key words: golden egg bugs, oviposition behavior, parasitoids, *Phyllomorpha laciniata*, predation risk. [Behav Ecol 13:70–74 (2002)]
icance of egg carrying in *Phyllomorpha laciniata* by answering the following questions: To what extent does this behavior affect offspring survivorship? And, if so, which are the factors responsible for the increase in egg survival when carried? To answer these questions we used an experimental approach both in the laboratory and in the field. First, to find out which proportion of eggs are laid by females on plants versus on conspecifics, we confined males and females to plastic boxes and analyzed their oviposition behavior. Second, in the laboratory we tested the effect of different temperatures on egg survival. And finally, we used an experimental approach in the field to test whether the survival rate of eggs is the same when left unattended on plants as when carried by adult bugs.

MATERIALS AND METHODS

Experimental designs in the laboratory

Selection of oviposition sites by females

To determine the proportion of eggs laid by females on plants versus conspecifics, we collected individuals from a field population in Boca de los Frailes (Almeria, Spain), during April 1998. We kept in each of 10 plastic boxes (17 × 17 × 17 cm) 2 males and 2 females during 5 days. Adults were provided with fresh *P. argentea* flowers as a food resource and oviposition surface every day. The boxes were kept in the laboratory at 25°C and a photoperiod of 16:8 h light:dark. After 5 days the number of eggs on plants and on adult bugs were counted.

Effect of temperature on egg hatching rate

*Phyllomorpha laciniata* females were collected from the field in El Espinar (Segovia, Spain) during May and April 1997. Females were kept isolated in plastic boxes (17 × 17 × 17 cm) in the laboratory on a 16:8 h light:dark photoperiod. Each box contained potting soil with live *Paranychia argentea* as a food resource and oviposition surface.

At the field site a thermometer placed on the soil in the shadow can reach 36.6°C. To study the effect of temperature on the survival of developing eggs, we checked the boxes every day to look for the eggs that females had laid on *P. argentea*. Eggs were taken out from each terraria every day and incubated on plastic petri dishes without lids at three temperature treatments: 25 ± 5, 35 ± 5, and 41 ± 5°C. The sources of heat were three lamps of 45, 60, and 100 W, respectively. We drew squares on each petri dish, and we only set one petri dish at each temperature treatment. Every egg in each treatment was randomly assigned to one square. The experiment was designed so that eggs from the same female were incubated at the three temperature treatments. The experiment lasted 18 days, and the petri dishes were checked every day to determine egg hatching. We removed nymphs from each petri dish daily.

Experimental design in the field

We conducted a field experiment investigating the impact of environmental conditions, predators, and parasitoids on egg mortality rates when laid on plant versus on adults. The experiment was performed at the park El Soto in Villaviciosa de Odon (Madrid, Spain) during June and July 1997.

Eggs of *P. laciniata* are difficult to find on plants because they are very small and cryptic, so we collected females of *P. laciniata* in the park and we enclosed each of them within a green net over a *P. argentea* plant. This prevented females from escaping and forced them to lay eggs on the host plant. The females, each in individual nets, were placed 2 m apart in four parallel transects. Each transect was 2 m apart from each other. We enclosed 40 females under individual nets. Females were kept inside the nets during 2 days and were then released only if they had laid eggs on the plant. Otherwise, females were kept in the nets for another day. Some females laid eggs on the net instead of on the plant. We followed only the fate of the eggs laid on plants.

We marked the plant where females had laid eggs by staking a piece of wood in the soil next to the plant; flowers carrying eggs were also marked by a small spot of green typist’s correction fluid (Tipp-ex brand) placed underneath the flower. We checked egg survivorship on plants daily during 18 days, allowing enough time for the eggs to complete their development (Reguera and Gomendio, unpublished data).

We marked with typist’s colored correction fluid 42 adults carrying eggs (18 females and 24 males) in the same area where females had been enclosed within the net. We recorded the sex of the bug, the number of eggs they carried, the stage of development of the eggs, and the position of the eggs on the body of the bug. Three distinct stages of egg development could be distinguished according to their color pattern: the eggs are white for some days, then turn to yellow with red spots, and finally become golden yellow (see Kaitala, 1996). Only recently laid eggs (the white ones) were considered for the analyses because more developed eggs could no longer be susceptible to further parasitism due to the presence of a hard cuticle (Baker and Pigott, 1993; Gauld and Bolton, 1988; Gross, 1993) or because the contents of the eggs are no longer fluid enough to support the development of the parasite (Gross, 1993; Lewis and Redlinger, 1969; Salt, 1998). We made censuses every day during 18 days in the study area trying to find as many marked adults as possible. Every time we found a marked adult we recorded whether there was any change in the number or in the stage of development of the eggs they carried. The development of the eggs on plants and adults were checked during the same days in June and July to assure that the number of predators, parasitoids, and the environmental conditions were identical for both groups of eggs.

Eggs that complete their development hatch before 18 days, and the shell remains glued to the adult or the plant for a long time. Eggs that did not hatch after 18 days were considered to have failed in their development. Eggs that disappeared during the study period were considered to have been preyed upon by other insects. It is highly improbable that the eggs laid by females on plants and bugs had fallen down because they glue firmly to the surface where the females lay them. In the same way, egg removal by the adult bugs is highly unlikely to cause disappearances because it causes severe damage to the wings. Finally, parasitized eggs are easy to recognize because the eggs turn black as the parasitoid develops inside the eggs.

RESULTS

Experimental designs in the laboratory

Selection of oviposition place by females

The females that were kept with males in plastic boxes laid a total of 29 eggs after 5 days. From these 29 eggs, 20 (69%) were laid on an adult bug, and only 9 (31%) eggs were laid on plants. The proportion of eggs laid on plants was significantly lower than the proportion of eggs laid on other adult bugs (n = 10, df = 1, χ² = 4.172, p < .04), despite the fact that the surface covered by the plant was always larger than the surface represented by backs of conspecifics. A larger proportion of eggs were laid on males (80%) than on females (20%; n = 10, df = 1, χ² = 7.20, p < .007).
Twenty-one out of 40 females placed within the net laid eggs. The experimental design in the field was set up to observe the effect of temperature on egg hatching. The figure 1 shows the mean percentage of hatched eggs incubated at 25 ± 5°C, 33 ± 5°C, and 41 ± 5°C.

Effect of temperature on egg hatching rate
Eggs of 15 females were used in the three temperature treatments. We found a significant relationship between the temperature and the hatching success of the eggs. We used a logistic regression where the hatching success of the eggs was the dependent variable with two possible values (one egg could hatch or not), and the independent variables were the females and the temperature. The whole-model test showed a significant relationship (df = 16, χ² = 83.36, p < .0001), with temperature being the significant effect (df = 2, Wald χ² = 3.173, p < .0001), whereas the females had no effect (df = 14, Wald χ² = 6.48, p < .05). Figure 1 shows that when the temperature reaches 41°C, only a small proportion of eggs hatch.

Experimental design in the field
Twenty-one out of 40 females placed within the net laid eggs on P. argentea within 3 days. Out of 33 eggs laid by females on flowers, only 1 survived until hatching, 10 of them were predated, and 22 were parasitized. Out of the 42 adults marked, we could follow the fate of 36 eggs carried by 18 adults: 9 eggs survived until hatching, 13 eggs were predated, 13 eggs were parasitized, and 1 of the eggs failed to hatch. The mean number of eggs that each female laid on plants (1.57 ± 0.2 SE) did not differ significantly from the mean number of eggs carried by adult bugs (2.00 ± 0.3; n = 39, Mann-Whitney U, Z = −1.43, p = .15). The proportion of hatched eggs was lower in broods on plants than in broods carried by individuals (n = 39, Mann-Whitney U, Z = −2.359, p = .0183).

The number of broods in which at least one egg was predated did not differ between those on plants and those on adult bugs (n = 39, df = 1, χ² = 0.0001, p > .9). The mean proportion of predated eggs was the same for eggs on plants and eggs on adults (n = 39, Mann-Whitney U, Z = −0.68, p = .5). There was no correlation between the number of eggs in each brood and the number of eggs predated, regardless of whether they were on plants or on adult bugs (n = 39, on plants r = −.18, p = .43; on adults r = .40, p = .10).

There were no significant differences between the number of broods on plants and on adults with at least one egg parasitized (n = 39, df = 1, χ² = 0.007, p = .9). Although eggs on plants tended to show higher parasitism rates than on individuals, the difference did not reach statistical significance. However, although the number of eggs parasitized increased with the size of broods placed on plants, no relationship was found when broods where carried by adult bugs (n = 39, on plant r = .66, p = .0034; on adults r = .993, p = .70).

DISCUSSION
This study shows that egg hatching success in P. laciniata is higher when eggs are carried by adults than when they are left unattended on plants. In addition, our results show that, when given a choice, females prefer to lay eggs on conspecifics rather than on plants and thus improve egg survival. Females improve their reproductive success by laying eggs on the backs of conspecifics.

Phyllomorpha laciniata females can lay eggs on males or on other females. It is clear that females never carry their own offspring, raising the question of whether egg carrying is this species is a form of parental care or a result of intraspecific parasitism. The available evidence supports the idea that males are likely to carry some of their offspring because all males in natural populations end up carrying eggs, and carrying males accept more eggs that carrying females (Gomendio and Reguera, 2001; Reguera and Gomendio, 1999). In addition, the proportion of males carrying eggs varies with current levels of parasitoid pressure, whereas the proportion of females carrying eggs does not (Reguera and Gomendio, unpublished data). Given that egg carrying is costly for adults because it increases predation risk (Reguera and Gomendio, 1999), it seems likely that egg acceptance is so widespread among males because of the chances that they will have fathered at least some of them, although it will be in the females’ interest to lay eggs on conspecifics regardless of paternity. Thus, some degree of intraspecific parasitism is also expected (for a detailed discussion, see Gomendio and Reguera, 2001).

Egg carrying has a different impact on the three main factors that influence egg mortality rates in P. laciniata. The first factor is temperature. When eggs are left unattended, egg mortality is temperature dependent, but we still do not have data indicating that parents try to optimize the developmental temperature of the eggs. The second factor is predators. Predation on eggs was high both on plants and on adult bugs, but no differences were found between the two groups. A final factor is parasitoids. Eggs on plants tend to suffer higher parasitism rates than on adults, but the difference did not reach statistical significance due to the small sample size. Moreover, a higher number of eggs are parasitized on plants as the number of eggs per brood increases, but this is not true for eggs on adults. Thus, although adult bugs do not seem to be able to avoid being detected by parasitoids, they do seem to prevent parasitoids from attacking as many eggs from the same brood as they do on plants.

There are at least two ways in which egg carrying can prevent exploitation of the eggs by parasitoids. The guarding adult can prevent the discovery of the eggs, or the adult can successfully defend the eggs that have been discovered (Gross, 1993; Salt, 1938). In the golden egg bug, the number of broods with at least one egg parasitized was not significantly different between carrying adults and plants. It seems, therefore, that adults carrying eggs cannot avoid being discovered by the parasitoid. However, we have found that the number of eggs parasitized increases with the number of eggs per brood on plants but not on adults. We still do not know why the parasitoid wasp is less successful when eggs are carried by an adult than when they are laid on plants, but it is probably related to the fact that the parasitoid needs some time to drill the eggs, making it possible for the adult to get rid of the parasitoid, or to escape, before the parasitoid completes the process again (Godfray, 1994; Gross, 1993; Salt, 1938; Tobias, 1965). Alternatively, the mobility of adults could make han-
calculated time (the interval between first contact with the host and completion of oviposition) too long to be beneficial for the parasitoid (Gross, 1993; Takasu and Hirose, 1991). Whatever the causal factors may be, it is clear that laying eggs on adults has an adaptive value.

In *Phyllomorpha lacinia* an egg not carried by an adult bug has a low chance of survival. If offspring survival among different oviposition sites varies, natural selection should favor females who choose those sites in which performance of the progeny will be highest. It is rather surprising, therefore, that females sometimes opt for no parental care even when the survival of the eggs on plants is very low. There could be two reasons for this behavior: (1) if predation and parasitism rates vary throughout the reproductive season, females could maximize reproductive success with a flexible pattern of oviposition behavior. (2) There may be a trade-off between the time and energy invested in looking for a receptive bug to lay the eggs on and the increase in fecundity achieved by laying more eggs even if not in the best possible sites. In fish, it has been suggested that the costs of looking for the optimal sites may result in a loss of fecundity (see Warner et al., 1995). Given that *Phyllomorpha lacinia* females lay one or two eggs every 2–3 days throughout the whole season, the choice of location for each egg is not as crucial as it may be for species that lay large broods. In other words, the golden egg bug follows a reproductive strategy in which a few eggs are laid at short intervals of time. Thus, every time a female oviposits, only a small proportion of all the eggs is laid, and the decision about where to lay eggs must be made many times. In this context, it may pay to lay more eggs than to look for the best oviposition site for each of them.

Obviously, it would only pay females to lay some eggs on plants if they have some chances of surviving, as our results suggest. In a study on another population of *Phyllomorpha lacinia*, Kaitala (1996) argued that no eggs survived when laid on plants. In Kaitala’s study, eggs were removed from adult bugs and then placed on branches with inflorescences which had also been removed from the original plant. Not surprisingly, all the eggs had disappeared after 1 week. It was assumed that all disappearances were the result of egg predation. However, this result is likely to be the consequence of an incorrect experimental manipulation. When females lay eggs they are covered by a sticky substance that glues them firmly to the surface on which they are laid (plant or conspecific). The eggs become attached to the extent that experimental removal of the eggs is difficult and, in the case of eggs laid on conspecifics, egg removal may result in damage to the wings or other body structures. By the time an egg is removed, it has lost its original stickiness. Thus, in Kaitala’s study eggs which were placed on flowers after removing them from the backs of conspecifics were unlikely to remain attached to the plant and may have fallen to the ground or may have been blown away by the wind. The results of our study show that egg survival on plants is low, but not zero. This explains why females do oviposit on plants, despite being a risky alternative.

In conclusion, our results suggest that females do increase their reproductive success by laying eggs on conspecifics, where hatching rates are much higher than on plants. However, because females lay eggs continuously throughout the active season, the costs of looking for a receptive adult for each egg have to be balanced against losses in fecundity, and these opposing forces result in females following a flexible strategy in which some eggs are laid on plants and some on conspecifics.

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**REFERENCES**


