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Parental care in a tropical nymphalid butterfly *Hypolimnas anomala*

DONALD M. NAFUS & ILSE H. SCHREINER
Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam, Mangilao, Guam, 96923, U.S.A.

Abstract. A tropical butterfly *Hypolimnas anomala* (Lepidoptera: Nymphalidae) straddles its eggs and guards them. Over 50% of the butterflies remained with their eggs until the eggs hatched. When butterflies were removed from their eggs, there was a significant increase in the number of eggs killed by predators compared to the number of eggs lost when the butterflies were left guarding them. Guarding was effective in reducing egg predation by several species of ants which were too small to remove the eggs whole, but was not effective against the ant *Solenopsis geminata*, which was large enough to carry off whole eggs. The smaller ants were the most common predators at the sites studied. Guarding also did not deter parasitoids from attacking the eggs. Some of the butterflies remained associated with the newly hatched larvae for 1 or more days, until the larvae left the leaf. When the butterfly remained with the larvae, survival of the larvae increased during the 1-day period following hatching. Thus, parental care improved the survival of the eggs and very young larvae from 36% to 61%.

Social behaviour among insects ranges from virtually no contact with conspecifics to fascinating and complex societies as exemplified by the ants and termites. Societies which are truly social occur only in the insect orders of Hymenoptera and Isoptera, but many insects exhibit a degree of parental care. This care can range from passive egg guarding to complex behaviour involving grooming, feeding and protecting young, and constructing nests in the subsocial insects (Tallamy & Wood 1986). Eickwort (1981), following Wilson, defines subsocial behaviour as involving the care of nymphs or larvae by one or both parent insects for some period after hatching. Wilson (1971, page 121) states that subsocial behaviour is often restricted to the first or second instars but involves the carrying or sheltering of the young with the parent's body, or in some cases the parent simply stands nearby. Excluding the truly social species, over 180 insects are known to be subsocial and provide parental care for their eggs and young. These include representatives of at least nine orders (Eickwort 1981; Hinton 1981).

Parental care increases survival of offspring through nutritional improvements (Kidd 1977; Nalepa 1984; Tallamy & Wood 1986), reduced predation or parasitism (Eberhardt 1975; Tallamy & Denno 1981), and improvements in local environmental conditions (Nalepa 1984). The defence of eggs and young can dramatically improve survival (see Eickwort 1981; Tallamy & Wood 1986 for reviews). Here, we document a case of subsocial behaviour in the Lepidoptera and examine some of the benefits for a tropical butterfly, *Hypolimnas anomala* (Wallace) (Nymphalidae).

METHODS

Study sites with host plants, the tree *Pipturus argenteus* (Urticaceae), were located in Barrigada Heights, Guam. The study areas were in successional vegetation near roadways or along powerline right-of-ways, common habitats for this small tree. Nine sites were used. One site was located along a right-of-way cut into the native limestone forest. The rest of the host plants were growing among tall grasses, ferns and other secondary growth vegetation common in successional habitats. Each host tree was monitored daily, and all leaves where butterflies had laid eggs were marked.

To determine how long the butterflies remained associated with clutches or larvae, butterflies that had just laid eggs were selected. Following oviposition, they were checked each day to determine if the butterfly had left and if the eggs had hatched. A total of 108 butterflies were monitored.

To ascertain possible benefits of egg guarding, on each tree some butterflies were removed from their eggs while others were left guarding them. Butterflies that settled close to one another were paired, and one butterfly was selected randomly for removal and the other was left. Butterflies that were not close to another butterfly were selected at random to be left or removed. In each clutch, the eggs were counted at the beginning of the study period. A total of 169 clutches of eggs was studied: 78 were guarded and 91 were unguarded.

The fate of the guarded and unguarded clutches was monitored by checking each clutch every day until the eggs hatched. The species of predators or parasitoids attacking the eggs was noted, and the number of eggs eaten by predators was recorded. Behavioural responses the butterfly made to the attacker were noted. At the end of the brooding period, the number of eggs that hatched, were destroyed by predators, or failed to hatch was tallied. Unhatched eggs were left on the leaf for 3 more days and then removed and incubated in the laboratory to determine how many were parasitized. Eggs were incubated in 30-ml plastic creamer cups at about 28°C until the parasitoids emerged. To analyse the data, we used Wilcoxon's two-sample test (Steel & Torrie 1960).

To see if the presence of the brooding butterfly made any difference in the survival of first-instar larvae, the percentage of the larvae surviving 24 h after hatching was determined for clutches that were attended or not attended by a butterfly. A total of 44 broods, 22 guarded and 22 unprotected, were followed. Only clutches that had been guarded until the day of hatching were used. The female butterfly was removed on the day the larvae hatched. The number of larvae in each brood was counted after hatching, and then again 24 h later. The broods used in this test were not the same as the ones used for the other experiments.

To see if the presence of the brooding adult altered the chances of the eggs being discovered and exploited by a particular species of predator, we categorized the data into those clutches with at least one egg removed and those with no eggs eaten. Since the species of predator varied by site, the data were grouped according to the species of predator present. We used chi-squared tests to compare the proportion of clutches that were successfully attacked while being guarded with those that were unguarded.

To determine if the butterflies were not simply preventing the discovery of their eggs, but were able to defend their eggs even after predators had found them, we examined the daily survival rate of eggs in clutches known to have been discovered by a predator. Clutches were considered to be discovered if at least one egg was eaten by a predator.

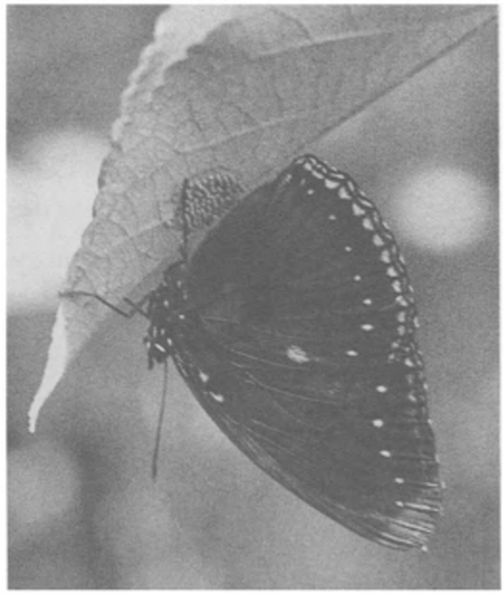

Figure 1. Female butterfly guarding her eggs. Females normally stand centred over the eggs facing the tip of the leaf.

On these clutches, we used linear regression to compare the difference in the rates of removal of eggs that were guarded or unguarded. The independent variable was the number of days from oviposition, and the dependent variable was the difference in the mean percentage of guarded and unguarded eggs surviving each day. Our hypothesis was that if the rate of removal was the same, the regression would have a slope of zero. A total of 77 clutches were followed: 33 guarded and 44 unguarded.

RESULTS

Butterflies typically laid their eggs underneath the leaf near the tip (Fig. 1). Clutches of eggs were generally large, averaging 565 eggs for butterflies that were not removed, but their size varied considerably, ranging from 10 to 1272 eggs. The clutch was irregular in shape. A typical clutch of about 500 eggs was about 2·6 cm in diameter. Individual eggs were between 0·6 and 0·7 mm in diameter. The clutch was small enough that the wing-span (6–7 cm at the leading edge of the forewing) of the butterfly could easily cover it when the wings were spread.

Most of the eggs were laid on the first day, although some butterflies added eggs on the second

Table I. Differences in the number of eggs of *Hypolimnas anomala* that were parasitized or eaten by predators when the clutches were guarded or not guarded by the female butterfly

	Mean ± SD number of eggs per clutch					
				Eaten by		
Status of clutch	Laid	Hatched	Parasitized	All predators	Small ant only	Not hatched
Guarded	565±324	373±371	18±49	136±260	56±146	38±95
Unguarded	492±303	233±310	9±21	223±277	182±261	27±68
Wilcoxon's two-sample *T*-test	7122	7406	6754	5504·5	2694	7049
n1/n2	78/91	78/91	78/91	78/91	57/65	78/91
z	1·550	2·446	0·390	−3·549	−4·164	1·321
P	0·061	0·007	0·35	0·0002	<0·0001	0·0934

day after settling. Consequently, there were fewer eggs where the butterfly was displaced, but the difference was not significant (Table I).

Butterflies assumed a position centred over the eggs facing downwards toward the tip of the leaf. The wings were held in the standard resting position, closed over the back of the insect. Only female butterflies were observed on the eggs. Although a few females left the eggs soon after laying them, most remained on the clutches for several days (Fig. 2). Over 50% of the butterflies stayed on the eggs until they had hatched and half of these remained on the leaf while the larvae were on it. During their early instars the larvae aggregated in a closely packed cluster while feeding, resting and moulting. Feeding began at the hatching site underneath the adult. The brood of larvae left the leaf 1–3 days after hatching, depending on how long it took them to consume the leaf. Butterflies were never observed to follow the larvae to a new leaf. The position of the butterfly on the leaf varied during the period when the larvae were present. Sometimes the female was over the caterpillars, while at other times she was near the leading edge of the feeding larvae. Many females tended to remain near the original oviposition site. Butterflies that left the leaf at any time during the brooding period did not return.

Those butterflies that stood over their eggs did not leave them in response to disturbance. The butterflies could be pushed with a finger or otherwise provoked, but they remained over the eggs. The 'staying' behaviour was sufficiently strong that the leaves with the butterflies and eggs could be picked and carried several kilometres back to the laboratory without causing the butterfly to leave. Normal escape reactions, such as quickly flying away in response to disturbance, were absent during this brooding phase.

In response to disturbance, two types of behaviour were observed: wing-beating and wing-spreading. The wing-beating behaviour consisted of the butterfly vigorously flapping her wings against the leaf while standing over the eggs. Minor disturbances, such as gentle brushing against the wings or antennae, evoked this behaviour. This was normally the response to disturbance. In the wing-spreading behaviour, the butterfly flattened her wings against the leaf surface and held them there. The wings covered the eggs and formed a barrier

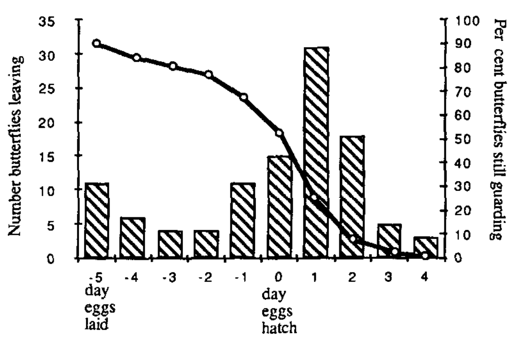

Figure 2. The day on which butterflies left the leaf on which they laid eggs. Because the incubation time varies, hatching is used as day 0. Data from days −5 and −6 are combined. Bars represent the number of butterflies leaving by the end of the day out of a total of 108 butterflies. The line is the percentage of butterflies remaining at the end of the day.

between them and the base of the leaf. Although this behaviour occasionally took place at an initial disturbance, it occurred more commonly after persistent disturbances. The behaviour could last for only a few seconds or for periods as long as 30 min or more. In certain exceptional cases, the butterflies may have held their wings spread for several days, as they were never observed to have them closed during visits to the tree.

The eggs were attacked by four parasitoids, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), *Trichogrammatoyia tortricis* Girault (Hymenoptera: Trichogrammatidae), *Ooencyrtus* sp. (Hymenoptera: Ooencyrtidae) and *Telenomus* sp. (Hymenoptera: Scelionidae), and by three species of ants (Hymenoptera: Formicidae), *Solenopsis geminata* (F.), *Monomorium floricola* (Jerdon) and *Tapinoma minutum* Mayr. When the butterfly was left on the eggs, significantly more eggs survived to hatch (66%) than when the butterfly was removed (47%; Wilcoxon's two-sample test: $P=0.007$; Table I).

The primary source of egg mortality was predation by ants, principally *S. geminata* and *M. floricola*. Other predators were rare. Nearly one-fourth of the guarded eggs were removed by ants. In the unguarded eggs, the proportion removed increased to 45%. Ants evoked both wing-beating and wing-spreading defensive behaviours. Response to the ants was not always immediate.

The effectiveness of the defensive behaviour varied with the species of ant attacking the clutch. Against *S. geminata*, a fire ant, the defence was ineffective. The fire ants were not easily deterred by the wing-beats and showed little response to them. They also avoided the spread wings by coming around the bottom edge of the leaf or skirting around the edge of the wing. The butterflies were not able to prevent the fire ants from discovering and removing their eggs. *Solenopsis geminata* was the primary predator on one tree, but was not present on the other trees. The ants found and removed all the eggs from 30 of the 35 unguarded clutches (86%) laid on that tree. Guarded eggs suffered the same fate, as 19 of 22 (86%) clutches were discovered and removed. The difference was not significant ($\chi^2=0.005$, $P>0.90$). These ants (length 3·2–4·5 mm, head width 0·6–1·0 mm) were larger than the other species attacking *H. anomala* eggs. Their heads were as large as, or larger than, the diameter of the eggs and they were able to pry

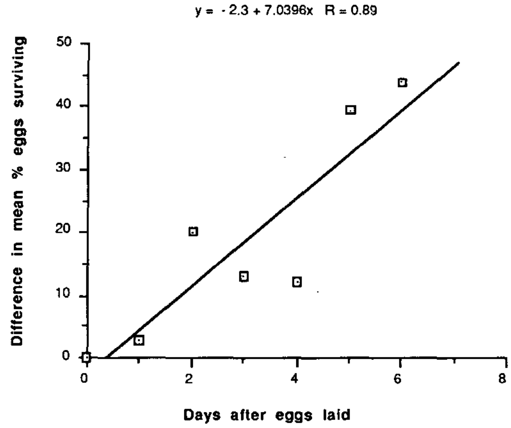

Figure 3. The difference in the percentage of eggs surviving each day after oviposition between guarded clutches and unguarded clutches. Only clutches that had at least one egg successfully removed by an ant are included. Points were computed by subtracting the mean survival of the unguarded eggs from the mean survival of the guarded eggs for each day ($\text{Mean}_{\text{guarded}}-\text{Mean}_{\text{unguarded}}$). The slope is positive indicating significantly more eggs were removed by ants when the eggs were unguarded ($t=4.435$, $df=5$, $P<0.01$).

the eggs off the leaf and carry them away. Recruitment of worker ants and removal of the eggs was rapid once the clutch was discovered. Over 90% of the clutches were entirely removed within 24 h of discovery, and, in the remaining cases, the eggs were gone within 48 h.

In contrast, the other species of ants were tiny (length 1·8 mm, head width 0·35 mm for the largest workers) and could not pry the eggs off the leaf. Instead they chewed through the chorion and removed the contents of the egg, leaving the bottom part of the shell attached to the leaf. The process of attacking an egg took several minutes from initiation to completion. Defence against these ants was more successful. Wing-beats disrupted the attack on an egg and caused the ant to become agitated. When the butterfly was removed from her eggs on trees where the tiny ants were the primary predators, 46 out of 65 clutches (71%) had at least one egg successfully attacked. When the butterfly was left on her clutch, only 19 of 57 (33%) lost one or more eggs. This difference is significant ($\chi^2=17.098$, $P<0.001$).

There are at least two ways egg guarding can prevent exploitation of the eggs: the guarding adult can prevent discovery of the eggs (keep the ant from finding the eggs and communicating the discovery to other workers) or the adult can successfully defend eggs that have been discovered and attacked. To see if the butterflies could actually defend eggs against the tiny ants, we examined the rate of removal of eggs only from clutches known to have been discovered and attacked. Eggs were removed at a slower rate from clutches that were guarded than from the unguarded ones (Fig. 3). The difference in the rate of removal was significant as shown by the positive slope, indicating that the defensive behaviour (wing-beating) was successful in driving some of the tiny ants off the clutches.

The tiny ants were the only predators or the dominant predators at seven of the nine sites and were present at eight of the sites. If the seven sites where these tiny ants dominated are examined separately, the impact of these ants can be seen more clearly. Nearly three times as many eggs were preyed upon in the untended clutches (Table I) and 53% more eggs hatched in the guarded clutches than in the unguarded ones.

None of the egg parasitoids was particularly abundant, and there was no significant difference in the parasitization rate between clutches that were guarded or unguarded. Unguarded clutches tended to have slightly lower rates of parasitization, although this was an artefact due to removal of parasitized eggs by ants. Overall, rates of parasitization were typically less than 5%, but occasionally, individual clutches were parasitized entirely. Parasitoids were frequently seen working on the eggs and crawling around the leaf under the butterflies, but we never saw the butterflies respond to them.

The presence of the tending female also improved the survival of first-instar larvae. The female often remained on the eggs for at least 1 day after the majority of the eggs hatched (Fig. 2). An average of 93% of the larvae were alive 1 day after hatching if the butterfly remained on the brood during that period compared to 76% survival when the butterfly was not present. The difference was significant ($S_{\text{guarded}} \pm \text{SD} = 1{\cdot}35 \pm 0{\cdot}15$, $S_{\text{unguarded}} \pm \text{SD} = 1{\cdot}11 \pm 0{\cdot}43$ where $S = \text{arcsine} \sqrt{(\text{Proportion surviving})}$; unpaired $t = 2{\cdot}525$, $df = 42$, $P = 0{\cdot}01$). Overall, if the female butterfly guarded her eggs from oviposition through the first 24 h after hatching, the percentage of her offspring surviving this period increased from 36% to 61%.

DISCUSSION

The parental care we found in *H. anomala* is subsocial as defined by Eickwort (1981), and it shows the pattern of behaviour expected for brooding insects. While on the clutch, the normal escape behaviour of flying away was suppressed and, instead, the butterfly remained on and defended the eggs despite disturbances or potential threats to the life of the butterfly. Moreover, some individuals of *H. anomala* continued the guarding behaviour beyond the egg-incubation stage by standing over the first-instar larvae, a behaviour characteristic of subsocial insects. This improved the survival of these larvae, at least during the first day after hatching, although not as much as for some other subsocial insects. Maternal defence by the lacebug, *Gargaphia solani*, can reduce nymphal losses from predation sevenfold (Tallamy & Denno 1981). In *Antiteuchus tripterus*, a phytophagous pentatomid bug, nymphal survival increases about 30% when the female guards the nymphs (Eberhardt 1975).

Unlike many of the other subsocial insects, the parental care exhibited by *H. anomala* is not absolute, and some individuals readily abandon their clutches in response to disturbance. Conflicting selection pressures may be present. The effectiveness of the defence varied depending on the species of predator attacking the eggs. The guarding behaviour provided no protection against egg parasitoids or fire ants, but it was fairly effective against two tiny ant species which were the most common predators of eggs and young larvae in the study area. When attacked by fire ants, butterflies that leave immediately and lay any remaining eggs elsewhere would have a better chance of having at least some surviving offspring. This is particularly true since fire ants are not present at most sites. On the other hand, if the butterflies are attacked by the tiny ants, females that remain on the eggs and defend them will increase the number of eggs surviving attack. In this case the benefits of moving to another tree would not be as certain since there are tiny ants on most trees. In many other species of subsocial insects the defence is far more effective and is universal among the adults. When *A. tripterus* was removed from 48 egg clusters, no eggs survived to hatch (Eberhardt 1975). *Elasmucha grisea* and *E. fieberi*, acanthosomid bugs, also provide essentially 100% protection for the eggs (Melber & Schmidt 1975a, b; Maschwitz & Gutmann 1979), but the reduviid bug, *Zelus* sp., is less effective. When guarded, 21% of the eggs were parasitized, compared to 55% parasitized when the guarding adult was removed (Ralston 1977).

In the animal kingdom, the degree of investment in parental care is related to a balance between the costs of providing care and the benefits derived from increased fitness (Wilson 1975). We have shown some of the benefits to the butterfly, but we have not evaluated what the costs might be. At least one of the potential costs, predation on adults, does not seem to be important on Guam. We rarely found any evidence of predation on the adults, such as unconsumed wings, and observed only one instance of predation on a butterfly guarding her eggs. That predator was a species of preying mantid. None of the ants attacking the eggs was observed to attack the adult butterflies. All the potential bird predators on Guam have become rare or extinct (Savidge 1987), so we could not determine whether bird predation might be a problem for a conspicuous, sitting butterfly.

Larval aggregations or clustering of eggs is not uncommon in the Lepidoptera (Stamp 1980; Eickwort 1981), but tending of the eggs is extremely rare. In addition to the example described here, it also occurs in the closely related *Hypolimnas antilope* (Rothschild 1979) but is otherwise not known among the Lepidoptera. Why parental care evolved in this species of butterfly, and not among other Lepidoptera, is not known at this time. Wilson (1975) suggests there are four prime movers which can lead to parental care. These are: stable, structured environments in which species have low fecundity (K-selected species); physically extreme environments which are difficult to invade; use of certain types of food resources which are scarce or difficult to exploit; and predation pressure. Predation pressure is probably the primary driving factor in this instance, but other factors are also involved. One of these factors may be the size of the host plant, which is a tree capable of supporting large clutches of larvae. This allows the egg clusters of *H. anomala* to be large and to represent a significant proportion, if not all, of a female's reproductive capacity. The female has little to lose and may gain substantially by remaining and guarding her eggs from predators. Although other species of butterflies lay their eggs in clusters, often these clusters contain fewer eggs than those of *H. anomala* (Stamp 1980). In these species the female presumably benefits more by dispersing small clutches than she does by guarding the eggs she has already laid. For these species, potential predators may not be as universally present as are ants on the host of *H. anomala*, and thus the probability of an individual cluster surviving would be higher.

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