

Group size in a gregarious tortoise beetle: patterns of oviposition vs. larval behaviour

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Abstract

In laboratory and garden experiments, we tested for the existence of adaptive patterns of oviposition and larval behaviour regarding group size in the gregarious tortoise beetle *Chelymorpha varians* Blanchard (Coleoptera: Chrysomelidae: Cassidinae) on its host plant *Calystegia sepium* L. (Convolvulaceae). Specifically, we addressed the following questions: (i) Which is the more frequent egg cluster size? (ii) Does cluster size fully predict larval group size? (iii) Are newborn larvae attracted or repelled to conspecific groupings? and (iv) Which is the group size associated with enhanced larval development and adult mass? We found that the mean cluster size was 21.4 eggs. Egg hatch time was significantly shorter in larger clusters. A regression analysis of larval group size against cluster size showed non-significant results. Thus, original cluster size did not totally determine the larval group size. The mean larval group size was 17.1. Choice tests in an experimental arena showed that larvae clearly preferred leaves of a host plant rather than moistened papers, and that larvae preferred a small group of conspecifics (four larvae per leaf) over larger groups (12 or 20 larvae). Empty leaves of the host plant showed an intermediate level of preference. Development time and beetle performance (adult mass) were affected by larval group size. Larvae in the smallest group (one per leaf) took four more days to attain adulthood than larvae in the larger groups (12 and 20 larvae). Adult *C. varians* reared in the 12-larvae group were significantly larger than those reared at the other densities. Comparison of patterns across experimental groups, excluding the 12-larvae group, showed a tendency for a greater final mass with slower developmental rate.

Introduction

Gregarious behaviour has been observed in several insect species (Prokopy & Roitberg, 2001). The hypothesized or reported advantages of gregarious behaviour can be grouped in two main types, those related to insect growth and development and those associated with an increased success in defence against natural enemies. There is ample evidence of such benefits of gregariousness (Lawrence, 1990; Clark & Faeth, 1997; Storer et al., 1997; Hunter, 2000; Tullberg et al., 2000; Fordyce, 2003; Reader & Hochuli, 2003; Grear & Schmitz, 2005; Wise et al., 2006). A less-studied aspect of gregarious behaviour in insects is

the relationship between the size of the egg clutch laid by females and the actual group size of developing larvae. Assuming that advantages of gregariousness are density dependent, it is important to elucidate whether there is a fit between clutch size, group size, and the optimum group size. The optimum group size may be defined as the grouping that confers the greatest fitness to individual larvae. If newborn larvae show low mobility, the clutch size produced by the mother would be a good predictor of final group size. Consequently, if there is a fit between actual group size and optimum group size, it could be concluded that there is an adaptive pattern of oviposition. If newborn larvae are rather mobile and able to group themselves, then a fit between actual group size and optimum group size would suggest an adaptive pattern of larval behaviour.

The tortoise beetle *Chelymorpha varians* Blanchard (Coleoptera: Chrysomelidae: Cassidinae) is the sole

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representative of the subfamily Cassidinae in Chile, which has ca. 1600 species in South America (Buzzi, 1988). Larvae and adults feed mostly on morning glory plants (Convolvulaceae) (Artigas, 1973, 1994). Females deposit clusters of eggs on the underside of leaves and emerging larvae show gregarious behaviour (Artigas, 1973, 1994). Larvae of tortoise beetles typically build a shield by storing faeces and exuviae in moveable abdominal spines as a putative defence against natural enemies (Olmstead & Denno, 1993; Vencl et al., 1999; Müller, 2002; Nogueira-de-Sá & Trigo, 2005).

In this study, we tested for the existence of adaptive patterns of oviposition and larval behaviour regarding group size in *C. varians*. When evaluating the fit of observed cluster size or group size to optimum group size, we focused on advantages associated with growth and development. In particular, we addressed the following questions: (1) Which is the more frequent egg cluster size? (2) Does cluster size fully predict larval group size? (3) Are newborn larvae attracted or repelled to conspecific groupings? and (4) Which is the group size associated with enhanced larval development and adult mass?

Materials and methods

Adults and larvae of *C. varians* were collected from plants of *Convolvulus arvensis* L. (Convolvulaceae) at the edge of roads in central Chile and they were reared under room conditions. Adult beetles from this collection were used to yield egg clusters and experimental larvae. Entire shoots of *Calystegia sepium* L. (Convolvulaceae), which is another common host plant, were collected on the Universidad de Concepción campus and placed in plastic 2-l containers to mimic the dense plant cover where beetles are usually found. Shoots were replaced every second day.

To determine clutch size frequency (Question 1), egg clusters were recovered from the rearing containers and the number of eggs in each cluster was counted. We also recorded the hatching time of eggs from each cluster and compared mean hatching time of classes of cluster size using analysis of variance (ANOVA). To examine whether clutch size predicts larval group size under natural conditions (Question 2), 20 egg clusters were chosen at random (from a total of 724 clusters obtained from the rearing containers) and were placed individually on detached *C. sepium* leaves in a garden on the Universidad de Concepción campus. The initial number of eggs and the final number of larvae in each group were recorded 24 h after hatching. We evaluated whether the relationship between larval group size and cluster size was significant using a regression analysis. There were no events of larval predation or egg parasitism during this outdoors assay.

To determine whether *C. varians* larvae are able to orient themselves to their feeding sites and to explore their behaviour in response to conspecific groupings (Question 3), two different preference tests were performed in a simple arena (a rectangular plastic pot, 20 × 20 × 7 cm). In the first bioassay, leaves of *C. sepium* were placed in opposite corners and pieces of moistened paper were used as controls in the other two corners. Thirty larvae were placed in the centre of the arena using a fine camel-hair brush. The location of the larvae was recorded 2 h later. We performed 20 replicates of this test and used 2-day-old larvae. Larvae that did not choose a substrate or remained in the centre of the arena (average 12%) were not considered in the analysis. A Wilcoxon matched pairs test was used to compare larval choice. In the second bioassay, leaves of *C. sepium* were placed in the corners with four, 12, and 20 larvae each, and a leaf without larvae was used as control. A single larva was placed in the centre of the arena and its location was recorded 1 h later. The test was replicated 25 times and 2-day-old larvae were used. Larvae were manipulated using a fine camel-hair brush. Paired χ^2 tests were used to compare the frequency of choice of the different treatments.

Finally, we evaluated the relationship between group size and *C. varians* performance (Question 4). Larvae were confined in Petri dishes (10 cm in diameter) and were fed with single fresh leaves of *C. sepium* in four group sizes: (i) one larva, (ii) four larvae, (iii) 12 larvae, and (iv) 20 larvae ($n = 25$ replicates per treatment). Leaves were of the same size range for all groups (~30–40 cm²) and were changed daily. Beetle feeding was not limited by food supply because no group consumed a significant proportion of the leaf within a day. Development time was recorded during the five larval instars and the pupal stage (includes prepupa and pupa), and total development time from hatching to adult emergence was also determined. Larval instars and pupal stage were identified following a morphological description (Artigas, 1973). Adult beetle weight was determined immediately after emergence. Development and performance in the different treatments of group size were compared using a one-way ANOVA and Tukey's honest significant difference (HSD) test as post hoc analysis. Variables were log transformed [$\log(x + 1)$] to meet ANOVA assumptions. All tests were performed with Statistica 6.0[®] (StatSoft Inc., Tulsa, OK, USA).

Results

Clusters obtained from rearing containers had 7–48 eggs ($n = 724$ clutches), with a mean value of 21.4 eggs per cluster and ca. 77% of the clusters were in the 11–30 eggs range (Table 1). Egg hatch time varied with clutch size

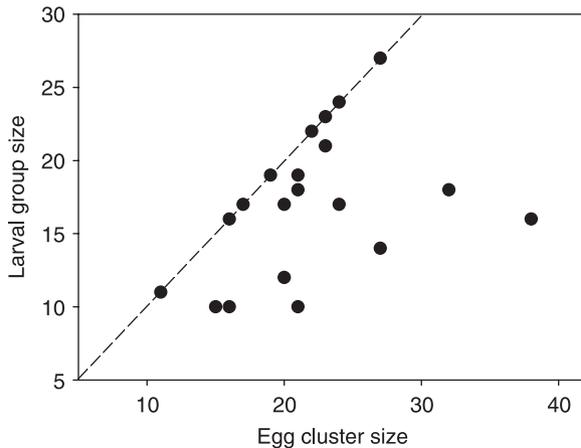


Figure 1 Plot of larval group size against original egg cluster size in *Chelymormpha varians* ($n = 20$). The dotted line is the identity function $y = x$. Points should lay close to this line if group size is largely determined by the cluster size. Larval group size refers to the main group formed 24 h after egg hatch.

Table 1 Size, frequency, and hatching time (days, mean \pm SE) of *Chelymormpha varians* egg clusters

Class	Frequency	Percentage	Hatching time
1–10 eggs	53	7.31	11.36 \pm 0.12a
11–20 eggs	333	45.98	10.37 \pm 0.04b
21–30 eggs	224	30.94	9.48 \pm 0.04c
31–40 eggs	88	12.15	9.18 \pm 0.04d
41–50 eggs	26	3.62	9.08 \pm 0.05d

Means followed by different letters are significantly different [$P < 0.05$, Tukey's honest significant difference (HSD) test after a one-way analysis of variance (ANOVA)].

(one-way ANOVA: $F_{3,96} = 8.25$, $P < 0.001$), and it was significantly shorter in larger clusters (Table 1). The regression analysis of larval group size against cluster size showed non-significant results ($y = 0.319x + 10.08$; $P = 0.084$; $n = 20$) (Figure 1). Thus, original cluster size

did not precisely determine the larval group size. Some minor groups of larvae were formed separately from the main group. The average cluster size in this experiment was 19.5, which is very close to the overall mean cluster size, and the mean larval group size was 17.1. In 40% of cases, the final larval group was of the same size as the original cluster size. Larvae were rather mobile. First instars were able to move within a shoot and third instars could move between individual plants.

In the choice experiments, larvae clearly preferred leaves of *C. sepium* (79.6%) to moistened papers (9.2%) (Wilcoxon matched pairs test: $Z = 3.92$, $P < 0.001$). Larvae showed a slight preference for the smallest group (four larvae per leaf, 44% of cases) over larger groups (12 or 20 larvae per leaf, 16% of cases each one) ($\chi^2 = 4.67$, $P = 0.031$). The control treatment (empty leaves) showed an intermediate level of preference (24% of cases), which was not significantly different from either the smallest group ($\chi^2 = 2.23$, $P = 0.136$) or the larger groups ($\chi^2 = 0.50$, $P = 0.479$).

Development time was affected by larval group size (Table 2). Larvae in the smallest group (one per leaf) took four more days to attain adulthood than larvae in the larger groups (12 and 20 per leaf). Our estimate of beetle performance (adult mass) was also affected by larval group size (Table 2). Adult *C. varians* reared in the 12 per leaf group were significantly larger than those reared at the other densities. Comparison of patterns across experimental groups, excluding the 12-larvae group, showed a tendency for a greater final mass with slower developmental rate (Table 2).

Discussion

We found ample variation in egg cluster size in *C. varians*, with a seven-fold increase from the smallest to the largest clutch recorded, but with a clear modal range of 11–30 eggs. Egg-hatching time was consistently shorter in larger clusters. This suggests a disadvantage of smaller clutches with regard to temporal escape from egg parasitism by

	Size of group			
	1 larva	4 larvae	12 larvae	20 larvae
Larval stages	20.2 \pm 1.07a	18.0 \pm 0.45ab	17.2 \pm 0.24b	17.0 \pm 0.41b
Pupal stage	7.9 \pm 0.21a	7.7 \pm 0.26a	6.8 \pm 0.26b	7.1 \pm 0.20ab
Total time	28.1 \pm 1.11a	25.6 \pm 0.54ab	24.1 \pm 0.22b	24.0 \pm 0.44b
Adult weight	29.6 \pm 1.81a	29.4 \pm 1.61a	35.2 \pm 1.52b	26.2 \pm 1.16a

Means followed by the same letter within a row are not significantly different [$P > 0.05$, Tukey's honest significant difference (HSD) test after a one-way analysis of variance (ANOVA)].

Table 2 Development time (days, mean \pm SE) from egg hatching to adulthood and final adult weight (mg) in *Chelymormpha varians* reared in larval groups of different size

microwasps, which may be considerable for *C. varians* in nature (Olivares-Donoso et al., 2000; JF Costa, pers. obs.). Large clusters are further thought to protect eggs from desiccation (Clark & Faeth, 1998). Therefore, small egg clusters should be counterselected in this tortoise beetle, at least during the egg stage. Given that larvae were rather mobile and were able to group themselves (see below), the egg cluster size was not a very good predictor of larval group size. Consequently, considering the posthatching stages, egg cluster size should not be the target of strong selection.

Larvae of *C. varians* exhibited gregarious behaviour. Newborn larvae tended to aggregate even before starting to feed (JF Costa, pers. obs.). Interestingly, the size of the original cluster did not wholly determine larval group size. Minor, satellite groups were formed and remained separate throughout the larval stages (JF Costa, pers. obs.). This suggests that the optimal larval group size is smaller than the modal egg cluster size (~20). Larvae were able to orient themselves to their feeding site. Furthermore, when larvae were offered different substrates, they preferred the smallest group (four larvae per leaf) over the larger groups (12 and 20 larvae per leaf). These patterns suggest that an aggregation stimulus was produced in the smallest group. There is evidence in chrysomelid beetles that aggregation is enhanced by chemical cues associated with feeding by conspecifics (Peacock et al., 2001; Tansey et al., 2005; Kendrick & Raffa, 2006). If larvae in the smallest group are actually attracting their conspecifics, and this does not occur in the 12 per leaf group, then it could be interpreted as evidence that the optimal larval group size is greater than four per leaf. In summary, results of these assays suggest that the optimal larval group size is >4 and <20 .

Larval rearing density affected both larval and pupal developmental rate and final adult mass. Rapid larval development is thought to minimize the exposure time of in particular the younger, more vulnerable instars to either natural enemies or abiotic stress (Clancy & Price, 1987; Fordyce & Shapiro, 2003; Medina et al., 2005). Adult size is highly correlated with fitness in insects (Honěk, 1993; Nylin & Gotthard, 1998). Overall, the worst performance was observed in the one larva per leaf group, which showed slower development and smaller adults. The best performance was found in the 12 larvae per leaf group. This group showed the largest adult size without the penalty in developmental rate that is often found in life-history studies (Roff, 1992). Earlier work on Lepidoptera species that are gregarious as larvae has shown faster larval development associated with larger group sizes (Clark & Faeth, 1997; Fordyce, 2003; Reader & Hochuli, 2003), particularly during the first instars because feeding by older, larger larvae may decrease resource availability and

hence cause sibling competition (Clark & Faeth, 1997; Gibbs et al., 2004). Some of these studies suggested that aggregative feeding enhanced host-plant suitability. Although the present study lacks a mechanistic approach, we might infer that a similar phenomenon of enhanced suitability could be occurring with *C. varians* larvae feeding on *C. sepium*. Possible underlying mechanisms include the overcoming of either physical defences (e.g., Clark & Faeth, 1997) or induced responses of plants (e.g., Fordyce, 2003). It is important to note that the experimental conditions left out the possible benefits related to protection against natural enemies or abiotic stress, which cannot be excluded in a natural context.

From the data on cluster size and the assay on larval group formation in the field, it can be extrapolated that the most frequent larval group size in the field should be ~17. As discussed above, the 12-larvae group rendered better performance than the 20-larvae group, which is closer to the average group size in the field. It can, therefore, be asked whether larvae of *C. varians* are forming optimal groups. Taken together, our results would suggest that the modal larval group is suboptimal. However, no definite conclusion on the optimality of larval group size in *C. varians* can be drawn without taking into account the selective pressures of predation and parasitism or environmental stress in the field. There are reports of both a good fit (Crowe, 1995) and no clear correspondence (Nahrung et al., 2001) between the average group size in the field and larval survival in gregarious chrysomelid beetles. The present study allows a finer approach to those group size benefits related to host-plant suitability interaction in future research, and has contributed to determine the relative importance of oviposition and behaviour on gregariousness in this tortoise beetle.

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