

Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate

TOM READER¹ and DIETER F. HOCHULI² ¹Department of Zoology, Trinity College, Dublin, Republic of Ireland and ²School of Biological Sciences, The University of Sydney, New South Wales, Australia.

Abstract. 1. Many moth and butterfly larvae are gregarious early in development, but become solitary in late instars. This ontogenetic variation in behaviour is probably the result of temporal changes in the costs and benefits associated with gregariousness. This study provides observational and experimental evidence that, in one particular moth species, a series of different ecological factors influence larval behaviour at different times during development.

2. Field observations show that young caterpillars of the limocodid *Doratifera casta* form large aggregations while foraging, but that mature larvae are largely solitary.

3. A field experiment revealed that individual first to third instar larvae in larger groups develop more rapidly, but that group size had no detectable influence on survival. The developmental advantage associated with gregariousness is affected by host plant species, but not by predator exclusion, suggesting that group living in these cryptic early instar larvae promotes feeding facilitation, but does not provide individuals with protection from natural enemies.

4. Laboratory experiments revealed that aposematic fourth instar caterpillars in large groups were less likely to be attacked by a generalist insect predator than those in small groups.

5. Field observations provided no evidence that group living affects body temperature, suggesting that microclimatic factors do not favour gregariousness in this species.

6. It is concluded that gregariousness in *D. casta* confers at least two different advantages on larvae at different stages early in development, but that these advantages disappear, or are outweighed by costs associated with intraspecific competition, in final instars.

Key words. Aposematism, caterpillar, development, *Doratifera casta*, facilitation, group living, Limacodidae, ontogeny.

Introduction

Despite the fact that there are costs associated with gregarious behaviour, such as enhanced levels of intraspecific competition (Charnov *et al.*, 1976; Damman, 1991) and disease transmission (Hochberg, 1991; Brown *et al.*, 2001),

many animals choose to live in groups. Assuming that gregariousness is an adaptive trait, individuals living in groups must therefore benefit from the presence of conspecifics. The larvae of many species of Lepidoptera aggregate strongly and there is evidence that individual caterpillars that live in groups may be more likely to survive and produce offspring than they would if they were solitary (Stamp, 1980; Fitzgerald, 1993; Costa & Pierce, 1997; Hunter, 2000). There are a number of reasons why gregariousness may enhance caterpillar survival and fitness. For example, groups of larvae may feed more efficiently than solitary

Correspondence: Tom Reader, Department of Zoology, Trinity College, Dublin 2, Republic of Ireland. E-mail: tom_reader@hotmail.com

larvae as a result of facilitation (Lawrence, 1990; Stamp & Bowers, 1990; Clark & Faeth, 1997; Denno & Benrey, 1997), they may be better defended against predation or parasitism (Lawrence, 1990; Vulinec, 1990; Denno & Benrey, 1997), or they may benefit from a modified local microclimate (Stamp & Bowers, 1990; Klok & Chown, 1999; Bryant *et al.*, 2000). Although there is experimental evidence that each of these factors can individually affect the success of gregarious caterpillars, few studies have simultaneously considered the effects of host plant resistance, predation, and microclimate on a single species. As a consequence, uncertainty still surrounds the relative importance of the different factors that favour group living in the Lepidoptera.

In the larval stage, the univoltine moth *Doratifera casta* Scott (Lepidoptera: Limacodidae) is a generalist herbivore that feeds on the leaves of eucalypts and related tree species of the family Myrtaceae (Common, 1990). Female *D. casta* lay eggs in large clutches on foliage in summer, and first to fifth instar caterpillars forage in groups that consist of up to several hundred individuals (Sutherland, 1997). As *D. casta* caterpillars mature, foraging groups decline in size, and in the final two instars (the sixth and seventh) the large (>4 cm long) larvae are essentially solitary. Such transitions from gregarious to solitary behaviour are common in larval Lepidoptera (Fitzgerald, 1993), and may indicate shifts that occur during development in the balance of the costs and benefits of group living (Stamp, 1981), or the presence of ontogenetic constraints such as those related to body size (Hochuli, 2001).

In *D. casta*, two conspicuous developmental changes may be associated with temporal variation in optimal larval group size. Firstly, early instars (first to fourth) skeletonise host plant leaves, while later instars consume the entire leaf structure (they are *leaf-snippers*). It may be that small groups of young, skeletonising larvae are less successful while feeding on physically tough host leaves than large groups, but that the presence of conspecifics has little influence on the foraging success of larger, leaf-snipping larvae. Secondly, young *D. casta* caterpillars are a cryptic brown colour and apparently harmless, but from the third instar onwards individuals are aposematically coloured (with black with yellow markings) and possess stinging spines that produce an irritant chemical upon contact with human skin. Warning coloration and repellent defences are particularly common in gregarious insects, are frequently absent in early instars but present later in development (Booth, 1990), and may be prerequisites for the evolution of gregariousness (Sillén-Tullberg, 1988; Tullberg & Hunter, 1996). If aposematism enhances the benefits of gregariousness, the selective pressure on *D. casta* to live in groups might therefore be expected to be greater from the third instar onwards.

In this paper, the gregarious foraging behaviour of the larvae of *D. casta* is investigated. Firstly, variation during development in larval group size in a wild population of *D. casta* is described. Then, using data collected in the field and the laboratory, the influence of group size on the ability

of individuals of this species to overcome host plant defences, avoid predation, and regulate body temperature is examined. The results of the experiments and observations combine to provide a comprehensive picture of the temporally variable selective environment in which *D. casta* larvae forage.

Methods

Group size observations

Group size and larval instar were recorded in wild populations of *D. casta* in the summers of 2001 and 2002 in woodland at Middle Head in Sydney Harbour National Park, New South Wales (151°15'30"E, 33°49'45"S). Larvae were considered to be members of a group if they were touching, or within one body width of, at least one conspecific. Groups of first to third instar larvae were photographed using a digital camera, and the number of individuals was estimated from the resulting images using previously prepared calibration curves of group surface area versus number of individuals. Groups of older larvae were counted by eye in the field. Since it was not easy to distinguish between sixth and seventh instar larvae, data for these two age groups were combined. Data from the two years were pooled, and mean group size for each instar calculated.

Group size, survival, and development in the field

Interactions between larval gregariousness in *D. casta*, host plant defence, and predation were investigated in a replicated factorial field experiment conducted in January 2002 at the Middle Head field site. Because group size cannot be manipulated in early instars without causing excessive disturbance, larval group size was controlled by manipulating egg clutch size prior to hatching. Eggs laid in large clutches (>200) on the leaves of *Angophora costata* Gaertner and *Eucalyptus botryoides* Smith were collected from the field site and divided using a scalpel into square blocks of standard size (5 mm × 5 mm). Each block contained on average 45.1 intact eggs ($n = 10$, SEM = 1.22). Blocks were assigned randomly to one of three group size treatments: small (one block per group), medium (four blocks = 180.4 eggs), and large (12 blocks = 541.2 eggs). These group sizes reflect clutch size variation in the wild.

Groups of eggs were assigned randomly to branches on one of two host plants (*A. costata* or *E. botryoides*) and to one of four predator treatments. The predator treatments were: *control* (no cage or other restrictions), *tanglefoot* [crawling insect predators excluded from the branch using Tree Tanglefoot Pest Barrier (The Tanglefoot Company, Grand Rapids, MI, U.S.A.)], *vertebrates excluded* [vertebrates excluded from the branch using a coarse (25 mm) cylindrical wire mesh cage (1 m long × 0.4 m diameter)] and *all predators excluded* [all insect and vertebrate preda-

tors excluded using a fine (1 mm) plastic mesh cage (dimensions as above)]. The plastic mesh used for caging was translucent and had no noticeable effects on plant growth during a 3-month period. Each possible combination of group size, host plant, and predator treatment was replicated three times, giving a total of 72 experimental groups of eggs. The blocks of eggs in each group were stapled together on a leaf on one (randomly chosen) of 72 haphazardly selected branches on one of 16 trees of each host species. Some trees were assigned more than one group, but no tree received more than three groups. All branches were inspected prior to the beginning of the experiment, and any predatory insects and non-experimental *D. casta* eggs or larvae were removed. Although the distribution of *A. costata* and *E. botryoides* at the field site overlapped considerably, it was not possible to completely randomise the positions of the trees of the two species with respect to each other. As a consequence, the host plant treatment may not have been completely independent of local spatial variation in soil, water, and light conditions.

Once assigned to a branch, groups of eggs were left to hatch in the field, which occurred one to three days after the beginning of the experiment. After 18 days, cages were removed and the number of larvae on each branch, and the developmental stage that they had reached, was recorded. The effects of group size, host plant species, and predator treatment, and all possible interactions between those factors, on the proportion of the larvae in each group surviving at the time of sampling, and their developmental state (the proportion of larvae that had reached at least the third instar) were analysed using general linear models with appropriate error structures. Model simplification by backward elimination and deletion testing were conducted according to protocols detailed in Crawley (2002).

Group size and thermal ecology

In order to assess the effects of group size and position within a group on body temperature in *D. casta*, individuals in 30 groups of second instar larvae and 18 groups of third instar larvae were examined. Group size was estimated from digital photographs (as above). For each group of larvae visited, two sets of temperature readings were taken in immediate succession, and the averages of each pair of readings were used in analyses. Larval surface temperatures for three categories of caterpillar in each group sampled were measured; larvae on the edge of the group (T_{ext}), larvae in the centre of the group (T_{int}), and the nearest solitary larvae not associated with the group (T_{sol}). The surface temperature of the leaf upon which larvae were foraging (T_{leaf}) was also recorded.

Leaf and larval surface temperatures were measured using a Type T surface probe (ECEFast Pty. Ltd, Sydney, Australia) attached to a hand held digital thermometer (COMARK Evolution N9001, Hertfordshire, U.K.). The flat, 3 × 3 mm tip of the probe was gently pressed onto the back of the larva, or the surface of the leaf. Temperature

readings were made between 10.00 and 13.00 hours on fine days during the summer of 2000 at the Middle Head field site. Ambient air temperatures, which were recorded throughout sampling, ranged from 23 °C to 36 °C and were not significantly different from T_{leaf} (results not shown).

The temperature data were analysed in three ways. Firstly, any gross effects of *D. casta* physiology or behaviour on body temperature were sought by comparing temperature measurements for the three categories of larva with each other and with T_{leaf} . Secondly, regressions of body temperature on T_{leaf} for each category of larva were examined. If *D. casta* is a thermal conformer, the intercept and slope of such regressions should not be significantly different from 0 and 1 respectively (see Bryant *et al.*, 1997 and references therein). Finally, the significance of the relationship between group size and the difference between body temperature of larvae within groups and T_{leaf} was assessed.

Group size and predator choice

Since warning coloration and repellent defences are most evident in late instar *D. casta* larvae, it was suspected that the effects of group size on predation rates would be strongest in older larvae; however, field experiments like that described above were unfeasible with older larvae because, from the fourth instar onwards, they become very mobile, consume prodigious amounts of leaf material and are thus difficult to maintain in field cages. As an alternative to field experiments, laboratory choice tests were conducted to establish whether the behaviour of a typical generalist insect predator, *Pristhesancus plagipennis* Stål (Hemiptera: Reduviidae), was affected by larval gregariousness. Although little is known about the predators of *D. casta* in the wild, *P. plagipennis* is a native of eastern Australia that is a voracious generalist predator of lepidopteran larvae. Indeed, it has been used to control pest populations of caterpillars on crops (Grundy & Maelzer, 2000). Furthermore, this species happily consumes *D. casta* in the laboratory: captive *P. plagipennis* nymphs introduced to *D. casta* larvae locate their prey rapidly, apparently by sight, before impaling them with the rostrum, sucking out the body contents, and discarding the empty exoskeleton.

Two experiments were conducted with *P. plagipennis* and *D. casta*. *Pristhesancus plagipennis* nymphs were supplied by Pisces Enterprises, Brisbane, Australia. Firstly, *P. plagipennis* was offered a choice of *D. casta* larvae in different sized groups. Three fourth instar *P. plagipennis* nymphs were placed in each of 11 cylindrical, clear plastic arenas (40 cm high × 20 cm diameter) that contained one group of fourth instar *D. casta* larvae from each of three size categories. Small (five larvae), medium (10 larvae), and large (25 larvae) groups of *D. casta* larvae were created by removing individuals from randomly selected large groups (>30 larvae) on leaves of *A. costata* collected from the wild. Each arena contained a cutting of *A. costata* to which leaves carrying groups of larvae were stapled. *Pristhesancus plagipennis* was

allowed to attack and consume *D. casta* larvae for 36 h, at which point the number of larvae surviving in each group was assessed. Groups of three predators were used in each arena in order to increase the number of prey consumed during the experiment, the duration (36 h) of which was constrained by the time for which plant material could be kept fresh in laboratory conditions.

In a second experiment, *P. plagipennis* nymphs were given no choice as to the prey group sizes upon which they could feed. Three fourth instar *P. plagipennis* nymphs were placed in each of 21 arenas (as above), each of which contained 30 *D. casta* larvae. In seven of these arenas (selected at random), the larvae were introduced as a single group. Of the 14 remaining arenas, half contained five groups of six larvae and half contained 10 groups of two larvae. Groups were created as above. After 36 h, surviving larvae were again counted.

Results

Group size observations

Early instar *D. casta* caterpillars in the populations surveyed typically formed large groups of more than 100 individuals (Fig. 1). Group size declined dramatically as caterpillars aged, and after the fifth instar, the majority (54.1%) were found foraging as solitary individuals.

Group size, survival, and development in the field

Larvae of *D. casta* suffered high levels of mortality (52.3% overall) during the 18 days for which the field experiment ran. The results show that levels of survival were affected strongly by host plant species (GLM with quasi-binomial errors: $F_{1,67} = 18.236$, $P < 0.001$), with larvae being much more successful feeding on *E. botryoides* (62% survival) than they were on *A. costata* (37% survival).

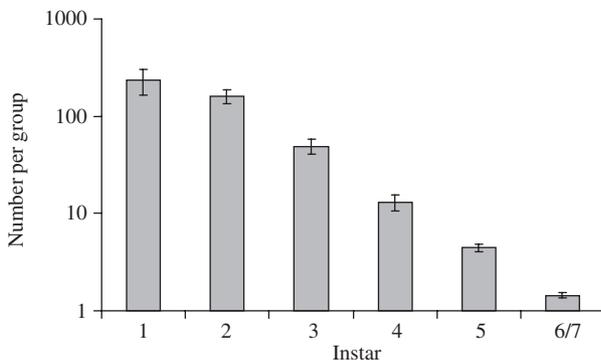


Fig. 1. Results of a field survey recording average larval group size in *Doratifera casta* at first (5), second (38), third (32), fourth (36), fifth (179), and sixth/seventh (218) instars (number of groups sampled in each instar is given in parentheses).

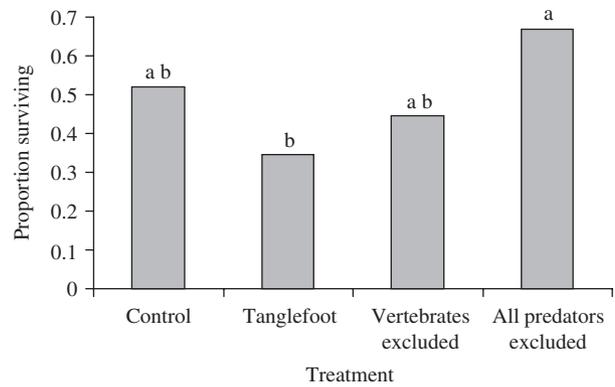


Fig. 2. The effect of predator exclusion treatments on the survival of early instar *Doratifera casta* larvae. Lower case letters indicate results of *post hoc* Tukey comparisons between group means. Columns that do not share a letter are significantly different ($P < 0.05$).

The predator treatment also significantly affected survival (GLM with quasi-binomial errors: $F_{3,67} = 5.337$, $P = 0.002$; Fig. 2). Overall, larval survival was greatest in the cages that excluded all vertebrate and invertebrate predators, but only the difference between this treatment and the tanglefoot treatment was statistically significant.

Initial group size had no effect on the survival of larvae ($F_{2,65} = 0.501$, $P = 0.608$), and there were no significant interactions between group size and the other experimental factors. This could be a consequence of the fact that it was difficult to maintain group size manipulations over time. During the course of the experiment, initial groups of hatchling larvae gradually fragmented. By the time sampling took place, although the total number of larvae on an experimental branch was strongly dependent on initial group size (ANOVA: $F_{2,60} = 27.97$, $P < 0.001$), the average size of the group fragments remaining was not significantly different between initial group size treatments (ANOVA: $F_{2,254} = 0.465$, $P = 0.628$).

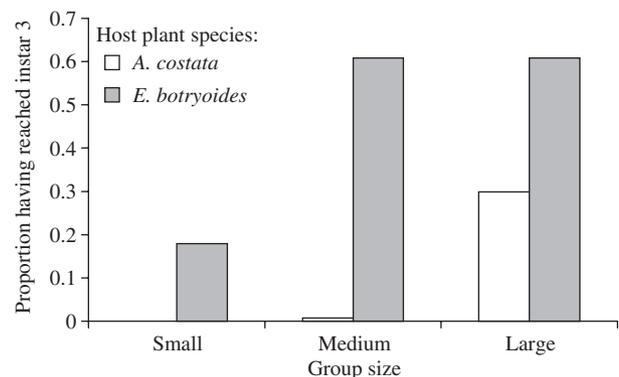


Fig. 3. The effect of host plant species and group size on the development rates of early instar *Doratifera casta* larvae.

Table 1. The results of paired *t*-tests comparing measurements of surface body temperature from *Doratifera casta* larvae on the interior (T_{int}) and exterior (T_{ext}) of groups and solitary larvae (T_{sol}).

Instar	Statistic	T_{int} vs T_{leaf}	T_{ext} vs T_{leaf}	T_{sol} vs T_{leaf}	T_{int} vs T_{sol}	T_{ext} vs T_{sol}	T_{int} vs T_{ext}
Second ($n = 30$)	<i>t</i>	0.693	-0.482	-1.328	1.497	0.902	1.281
	<i>P</i>	0.494	0.634	0.195	0.145	0.375	0.201
Third ($n = 18$)	<i>t</i>	-0.621	-0.727	-0.659	0.387	0.222	0.338
	<i>P</i>	0.542	0.477	0.519	0.704	0.827	0.740

When experimental branches were examined, most larvae surviving (93.2%) had reached either the second or third instar. Rates of development were significantly affected by host plant and group size treatments (Fig. 3). Larvae feeding on *E. botryoides* were much more likely to have reached at least the third instar than those feeding on *A. costata* (GLM with quasi-binomial errors: $F_{1,59} = 23.971$, $P < 0.001$), and larvae in large groups developed more rapidly than those in small groups (GLM with quasi-binomial errors: $F_{2,59} = 4.741$, $P = 0.012$). There was a significant interaction between host plant species and group size (GLM with quasi-binomial errors: $F_{2,57} = 6.136$, $P = 0.004$), with the host plant effect being most noticeable in medium-sized groups of larvae. There was no significant effect of predator treatment on development (GLM with quasi-binomial errors: $F_{3,54} = 1.420$, $P = 0.247$).

Group size and thermal ecology

There were no significant differences between the surface body temperatures of solitary and gregarious *D. casta* larvae and ambient temperatures in either the second or third instar, and the position of larvae within groups had no significant effect on body temperature (Table 1). The intercepts of the regressions of larval body temperature on ambient temperature were not significantly different from 0 in gregarious or solitary second or third instar larvae, and slopes were not significantly different from 1 (Table 2 and Fig. 4). Group size did not have a significant effect on the difference between larval body temperature (T_{int}) and ambient temperature (T_{leaf}) in either the second (linear regression: $n = 22$, $r^2 = 0.15$, $P = 0.111$) or third instar (linear regression: $n = 18$, $r^2 = 0.05$, $P = 0.366$).

Table 2. The results of regressions of the surface body temperature of *Doratifera casta* larvae on the interior (T_{int}) and exterior (T_{ext}) of groups, and solitary larvae (T_{sol}), on the ambient temperature (T_{leaf}). Figures in parentheses indicate standard error. In all cases, $r^2 > 87\%$ and $P < 0.001$.

Instar	T_{int} vs T_{leaf}		T_{ext} vs T_{leaf}		T_{sol} vs T_{leaf}	
	Slope	Intercept	Slope	Intercept	Slope	Intercept
Second ($n = 30$)	1.01 (0.04)	-0.53 (1.07)	1.07 (0.05)	-2.10 (1.35)	1.09 (0.10)	-2.85 (3.21)
Third ($n = 18$)	0.97 (0.06)	0.91 (1.88)	0.93 (0.07)	1.93 (2.06)	0.88 (0.15)	3.27 (4.83)

Group size and predator choice

Pristhesancus plagipennis readily attacked and consumed *D. casta* during choice and no-choice experiments. When given a choice, *P. plagipennis* ate significantly more *D. casta* individuals from small groups than from large groups (GLM with quasi-binomial errors: $F_{2,30} = 4.509$, $P = 0.019$; Fig. 5). When no choice was available, the pattern was the same. Individuals in smaller groups of prey were more likely to be attacked and consumed by *P. plagipennis* than those in larger groups (ANOVA: $F_{2,18} = 7.614$, $P = 0.004$; Fig. 6).

Discussion

First to fifth instar *D. casta* larvae are conspicuously gregarious. The aggregation of newly hatched larvae could simply be a consequence of the fact that eggs are laid in large batches, with apparently gregarious behaviour being a by-product of the selective pressures acting on the clutch-laying behaviour of adult females (see Stamp, 1980; Courtney, 1984); however, *D. casta* larvae forage in groups throughout the early stages of development. During this time, since larvae regularly move from leaf to leaf in search of new food, there are ample opportunities for individuals to leave groups, or at least not to re-form them. Furthermore, early instar individuals separated artificially from groups are known to search actively for and join foraging conspecifics (Sutherland, 1997). Gregarious behaviour in early instar *D. casta* larvae is thus very likely to have adaptive significance.

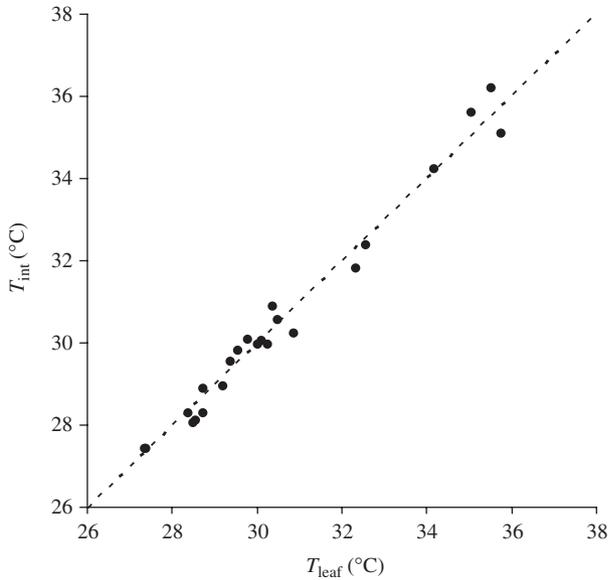


Fig. 4. A typical example of the relationship between ambient temperature (T_{amb}) and the surface body temperature of *Doratifer a casta* larvae. In this case, data shown are for second instar larvae from the interior of groups (T_{int}). The dotted line is the expected relationship for thermal conformers ($y = x$).

Gregariousness and development

The results of this study show that there are at least two ways in which individual *D. casta* larvae benefit from foraging gregariously. Firstly, young larvae in large groups develop more rapidly. Group size is likely to affect a number of biotic and abiotic factors that will in turn influence caterpillar development. Several studies in which it has been shown that individuals in larger groups grow more rapidly have concluded that this is probably because facilitation by

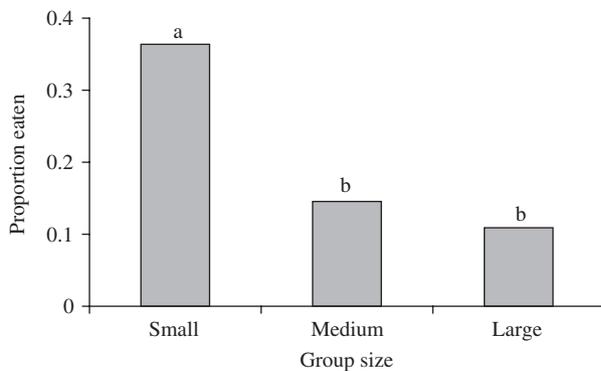


Fig. 5. The effect of prey group size on levels of predation by *Pristhesancus plagipennis* on fourth instar *Doratifer a casta* larvae in a choice experiment. Lower case letters indicate results of *post hoc* Tukey comparisons between group means. Columns that do not share a letter are significantly different ($P < 0.05$).

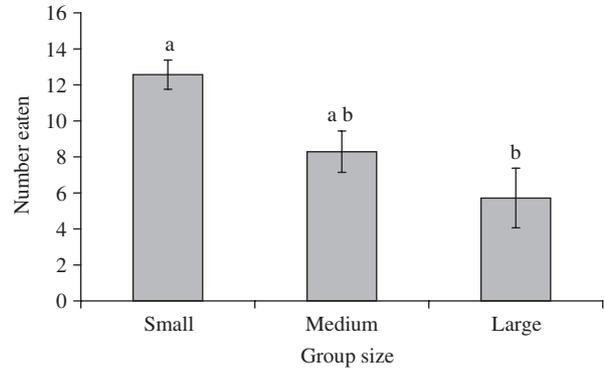


Fig. 6. The effect of prey group size on levels of predation by *P. plagipennis* on fourth instar *Doratifer a casta* larvae in a no-choice experiment. Error bars represent ± 1 SEM. Lower case letters indicate results of *post hoc* Tukey comparisons between group means. Columns that do not share a letter are significantly different ($P < 0.05$).

conspecifics raises food consumption rates (Lawrence, 1990; Stamp & Bowers, 1990; Denno & Benrey, 1997). In some cases, such a conclusion has been justified by data that show how the benefits of gregariousness are reduced when larvae feed on host plant tissues that are of higher quality or easier to consume (Tsubaki, 1981; Lawrence, 1990). The results presented here appear to provide further support for the facilitation hypothesis, since there was a significant interaction between host plant species and group size in the field experiment (Fig. 3). The benefits of gregariousness were most marked at low group sizes when *D. casta* larvae were feeding on a high quality host (*E. botryooides*), but were only detected at high group sizes on a lower quality host (*A. costata*).

Facilitation is not the only possible explanation for the significant interaction between group size and host plant species in the field experiment. Larval development rates may have been affected by properties of the host plants other than leaf toughness or palatability. For example, perhaps larvae in small groups on the poorer host plant species were more *restless* and spent more time searching for conspecifics to feed alongside. Alternatively, the result could be connected with the fact that mortality was higher on *A. costata*, and that group size would therefore have been reduced on this host during the course of the experiment. Finally, the interaction may have a non-biological explanation. The data collected on larval development were categorical (larvae either had or had not reached the third instar), but maturation is in reality a continuous process. A linear relationship between a continuous measure of maturity and group size, with a similar slope but different intercepts for larvae feeding on each of the two host plants, could still lead to a significant interaction in analysis of variance if data were categorised. Ultimately, experimental manipulation of host plant properties would be required in order to clarify the role of facilitation in this study system.

The expectation is, however, that gregariousness does enhance the feeding efficiency of *D. casta* in the field, since laboratory experiments have shown that solitary larvae find it very difficult to initiate a feeding site in comparison with individuals in groups (Sutherland, 1997).

It has been suggested that caterpillars in large groups grow faster because of the effects that the presence of conspecifics has on local microclimate. Larvae in dense aggregations have been shown to benefit from increased temperatures and humidity (Joos *et al.*, 1988; Klok & Chown, 1999), and in some lepidopteran species microclimatic factors are thought to have been important in driving the evolution of complex forms of social behaviour such as tent building (Knapp & Casey, 1986; Joos *et al.*, 1988); however, the results of this study show that *D. casta* larvae do not experience a thermal advantage when foraging in groups in the wild. Indeed, since body temperatures closely track ambient temperatures, it appears that *D. casta* larvae are strict thermal conformers. In addition, *D. casta* does not roll leaves or build tents, and does not appear to perform any obvious form of basking behaviour. Being active in the warm and humid conditions of Sydney in summer, there is perhaps little incentive to *D. casta* to indulge in the kinds of social behaviour that produce modifications of local microclimate in many temperate-zone species of Lepidoptera. While temperature regulation may have played an important role in the evolution and maintenance of gregariousness in regions of the world where ambient temperatures are typically well below the optimum for caterpillar growth and development, microclimatic factors are unlikely to provide a strong selective advantage to group living in species found nearer the equator (Bryant *et al.*, 1997).

It is conceivable that gregarious behaviour enhances rates of development in caterpillars because it increases the amount of time available for foraging and reduces the amount of energy spent performing predator avoidance behaviour. At any one time, some individuals might engage the enemy with head shaking, chemical excretion etc., leaving others free to feed. If *D. casta* benefits from such an effect, gregariousness should only enhance development in the presence of natural enemies. The results of the field experiment show that this is not the case, since there was no significant interaction between the group size effect on development rates and the predator exclusion treatment.

Gregariousness and survival

Various attempts have been made to understand how gregariousness in larval Lepidoptera may affect levels of predation and parasitism in the field. While some studies have found that individuals in groups are less susceptible to attacks by natural enemies (Vulinec, 1990), others, like this one, have found no pattern (Le Masurier, 1994; Denno & Benrey, 1997; Fordyce & Agrawal, 2001), and some have found that gregariousness actually enhances

rates of attack (Stamp, 1981; Cappuccino *et al.*, 1995). The inconclusive nature of these results is probably a consequence of intrinsic differences between species, as well as differences between studies in experimental design. Caterpillars of species like *D. casta* are mobile animals, with groups continually moving, coalescing, and fragmenting, especially in the later stages of development, and as a result experimental field studies in which group size is manipulated will always be limited in their ability to address the question of the effect of gregariousness on predation and parasitism.

Laboratory experiments perhaps offer a better opportunity to investigate the ways in which natural enemies influence foraging behaviour in the Lepidoptera. The experiments with *P. plagipennis* show that this predator has a marked tendency to avoid *D. casta* caterpillars in large groups. Although *P. plagipennis* is a representative generalist predator whose impact on wild prey populations remains unknown, the results clearly demonstrate the potential for group living to benefit *D. casta* caterpillars by reducing mortality. Together with reports documenting the response of generalist vespids (Stamp & Bowers, 1988) and vertebrate predators (Sillén-Tullberg, 1990; Hatle & Salazar, 2001) to aggregations of insect prey, these data suggest a general trend: gregariousness is advantageous to aposematic insects because it reduces rates of attack by a variety of natural enemies. There is experimental evidence that groups of insects with warning colours more effectively convey an aposematic signal to potential predators than solitary individuals (Gamberale & Tullberg, 1998; Hatle & Salazar, 2001), and it has been postulated that gregariousness can only evolve in the presence of the selective advantages that are associated with aposematism (Sillén-Tullberg, 1988; Tullberg & Hunter, 1996). The fourth instar *D. casta* larvae that were fed to *P. plagipennis* were brightly coloured and armed with an irritant sting, and it is quite possible that the generalist insect predator avoided large groups of prey because of a strong aposematic signal. Although little is known about the ability of insect predators to respond to warning coloration in other invertebrates, some insects are certainly capable of learning to avoid noxious prey on the basis of prior experience (e.g. Traugott & Stamp, 1996). In the context of this study, it would be interesting to examine the behaviour of *P. plagipennis* nymphs towards smaller, cryptic *D. casta* larvae. If choice experiments showed that *P. plagipennis* only responds to gregariousness in the presence of aposematic coloration, this might help to provide an explanation for the failure here to find an interaction between group size and survival in the field experiment.

There are ways in which group size in *D. casta* may influence predator behaviour that do not involve aposematic coloration. When disturbed, groups of *D. casta* larvae sometimes rear up and move their heads from side to side in unison in a manner that is presumably off-putting to predators or parasitoids. Such head-shaking behaviour may be more effective at deterring the attacks of natural enemies in larger groups of larvae (Cornell *et al.*, 1987). Alternatively,

perhaps predators such as *P. plagipennis* mistakenly perceive a tightly packed group of *D. casta* larvae as a single prey individual. In such a case, larger groups of prey would be more likely to escape predation on the grounds that they were too large to attack.

Solitary behaviour in late instar caterpillars

Since the progress of specific groups of larvae over time was not followed here, it was not possible to assess to what extent the developmental decline in group size that was observed (Fig. 1) was a product of the fragmentation of groups, and to what extent it was simply a consequence of mortality during development. However, anecdotal evidence that fragmentation does indeed happen in the fifth to seventh instars is strong. In early summer, a small number of leaves on affected host plants hold tightly packed groups of sessile first to third instar larvae, but, by the end of the season, the leaves of the same infested trees are covered with active fifth to seventh instar larvae, either in small diffuse groups or foraging on their own. The transition to the solitary way of life is likely to be a consequence of a rapid increase in the severity of intraspecific competition for food as the caterpillars approach maturity. It was found that late instar larvae kept in the laboratory consumed prodigious amounts of food (a single larva typically consuming many leaves per day), and towards the end of the season affected host plants at the field site were suddenly and almost completely defoliated. In these circumstances, any advantages associated with gregariousness will be outweighed by the need to search for unexploited patches of food.

Conclusions

It is becoming increasingly apparent that no single factor is alone responsible for the evolution and maintenance of gregarious foraging behaviour in the Lepidoptera (Clark & Faeth, 1997). This study, and other similar ones in which the benefits of group living have been assessed experimentally in the field, have shown that early instar caterpillars in large groups do not gain significantly from the presence of conspecifics in terms of survival (Denno & Benrey, 1997; Fordyce & Agrawal, 2001). Instead, it seems that gregariousness is advantageous because individuals in groups are able to develop more rapidly, probably as a consequence of the effects of facilitation. This study has also shown that later in development, gregariousness is a strategy that can help protect aposematic caterpillars from predation, indicating that the selective pressures acting on older larvae are probably different from those acting on cryptic early instars. In the latest developmental stages of the species that was studied here, caterpillars become solitary, and this behavioural switch almost certainly indicates a further change in the balance of the costs and benefits associated with gregariousness. On the basis of the findings

of this study, and evidence presented elsewhere, it is concluded that it is impossible to understand the reasons why group living is such a successful strategy in insects without taking account of ontogenetic variation in the selective environment with which individuals are presented. A suite of different factors, some more important at each developmental stage, some more trivial, make group living in young and older caterpillars, and clutch laying by adults, advantageous.

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