The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*

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Summary

1. The California population of the pipevine swallowtail *Battus philenor* is a specialist on the Dutchman's pipe *Aristolochia californica*, an endemic vine that is densely covered with trichomes. Populations of *B. philenor* outside California use other *Aristolochia* species that are largely glabrous. The average clutch size of the pipevine swallowtail is larger in California compared with populations elsewhere and larvae feed gregariously until late in the third instar.

2. In the field, caterpillars consumed more leaf material and showed preference for portions of leaves with trichomes removed. However, large groups of caterpillars were consistently observed feeding on the apical portion of the plant, where trichome density was highest. Smaller groups of caterpillars were observed feeding more often on mature leaves on the lower portions of the plant, where trichome density was lower.

3. Laboratory experiments showed that the walking speed of a commonly observed predator, larvae of the green lacewing *Chrysopa carnea*, was reduced as trichome density increased. Furthermore, lacewing search efficiency and capture rate of a model prey item were compromised by high trichome density.

4. In an additional field experiment, no difference was found in the percentage mortality of groups of four and 12 caterpillars. However, growth rate of the larger group was accelerated by 25% compared with smaller groups. In an experiment using a ladybird beetle larva *Hippodamia convergens* as the predator, no difference was observed in absolute mortality of caterpillars, suggesting that group size does not function directly as a defence against predators.

5. First instar caterpillars are most vulnerable to predators, thus feeding in larger groups may benefit caterpillars by accelerating growth. Feeding in large groups may also be an effective strategy for *B. philenor* to overcome plant trichomes and feed on portions of the plant conducive to faster development. However, feeding on areas with dense trichomes does not appear to provide larvae with a refuge from predators.

Key-words: *Battus philenor* (Papilionidae: Lepidoptera), defence, gregarious behaviour, plant–insect interactions, trichomes.

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Introduction

Herbivorous insects have strong interactions with the plants they eat and the enemies that eat them. Host-plants have developed numerous defences to reduce herbivory or effects of herbivory on plant fitness (Karban & Baldwin 1997; Agrawal 1998, 1999; Strauss & Agrawal 1999). Similarly, predators and parasites have developed strategies to maximize their ability to capture and assimilate their prey (Godfray 1994; Abrahamson & Weis 1997; Olff, Brown & Drent 1999). It is predicted that insect herbivores have evolved morphological, biochemical, behavioural and life-history strategies that will reduce the impact of plant defences and effectiveness of natural enemies.

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Plant defences against insect herbivores may be expressed constitutively or induced following initial attack (Karban & Baldwin 1997; Agrawal, Tuzun & Bent 1999). Plants employ chemical defences including phenolics and alkaloids (Duffey & Stout 1996), mechanical defences such as latex and lignin (Dussourd & Eisner 1987) and structural defences such as trichomes and thorns (Dimock & Tingey 1988; Ågren & Schemske 1993) to deter herbivores. Insect herbivores have developed a number of strategies to overcome these defences. For example, defences can be avoided spatially or temporally through modifications in feeding behaviour (Dussourd & Eisner 1987; Stamp & Casey 1993). Some insects have also developed enzymatic pathways to detoxify or overcome plant defensive compounds (Lindroth 1991; Broadway 1995), while others sequester these compounds and use them for their own defence against predators and parasites (Duffey 1980; Malcolm 1992). Structural defences, such as trichomes, can be overcome and removed by some herbivores prior to feeding (Rathcke & Poole 1975; Young & Moffett 1979).

Although predators and parasites can have strong negative impacts on herbivores, aspects of plant defences may interact with natural enemies of herbivores (Price *et al*. 1980; Hare 1992; Thaler 1999). Some of these interactions present the plant with challenges because structural plant defences against herbivores may act antagonistically with predation, or conversely plant defences may only be effective in the presence of predators. For example, plant structural defences can act to deter herbivores in isolation, but in some instances these same structures can offer herbivores refuge from predation (Gross & Price 1988). Plant structures can impede the searching ability of predators and at times be lethal to predators and other insects that are beneficial to the plant (Obrycki 1986; van Lenteren *et al*. 1995). For example, plant trichomes can offer protection to herbivores by reducing the threat of parasitoid attack (Kauffman & Kennedy 1989).

In this study, the role that plant trichomes play in the feeding behaviour, growth and vulnerability to predators of the gregariously feeding larvae of California populations of the pipevine swallowtail butterfly *Battus philenor* (L.) (Papilionidae) was examined. An emphasis was placed on gregarious feeding as a strategy of herbivores to exploit the plant and defend themselves. Gregarious feeding in other Lepidoptera has been suggested to play a role in thermoregulation, group-feeding stimulus and group defence (Stamp 1980). Specifically, the following questions were asked. (i) How does the presence of plant trichomes influence caterpillar feeding? (ii) How do natural variation and manipulated densities of plant trichomes influence predator walking speed, searching efficiency and capture rate? (iii) How does caterpillar group size influence feeding behaviour and growth rate? (iv) How does caterpillar group size influence predation by crawling and flying predators?

Materials and methods

STUDY SYSTEM AND NATURAL HISTORY

Northern California populations of pipevine swallowtails [currently recognized as a taxonomic subspecies, *B. philenor hirsuta* (Skinner)] emerge in April and May from diapaused pupae (Shapiro 1984). Upon mating, females lay clusters of eggs (mean ± SE, $13·1 ± 0·3$, $n = 621$) on the Dutchman's pipe *Aristolochia californica* (Torrey) (Aristolochiaceae), a perennial endemic riparian vine that reproduces largely by vegetative growth. The plant contains aristolochic acids, bitter toxic alkaloids, and is densely covered in non-glandular trichomes (Fordyce 2000). Eggs are typically laid on the apical portion of the stem and larvae feed on young apical leaves together in a large group. Eggs and larvae can be extremely abundant, with up to 300 individuals m^{-2} in some patches (J.A. Fordyce, personal observations). On average, first instar larvae feed in groups of 11 (mean ± SE, $11·1 ± 0·7$, $n = 20$) in California populations. Other populations of pipevine swallowtails in Texas and Mexico lay two to six eggs per clutch (Pilson & Rausher 1988; Spade, Tyler & Brown 1988; Rausher 1995), and these larvae feed on largely glabrous host-plants (Pfeifer 1966, 1970; Spade *et al*. 1988). Thus, laying eggs in large clutches and subsequently feeding in large groups may be unique to the California population of *B. philenor*. Larvae of *B. philenor* are aposematically coloured and in California each clutch feeds gregariously in large groups until late in the third instar. In these early instars, a silk mat is laid down by large groups of larvae, which may help them anchor down and feed on foliage; smaller groups are unable to lay down a conspicuous silk mat. By the fourth and fifth instar, larvae can defoliate entire vines, they are highly mobile and rarely seen feeding as groups.

Feeding larvae sequester aristolochic acids from the plant (Brower 1958; Rothschild *et al*. 1970) and are presumably defended against predators. In addition, all of the instars have reversible defensive glands (osmeteria) that can protrude from the prothorax and deliver toxic or repellent compounds (Stamp 1986). However, first instar larvae are vulnerable to attack by crawling predators (authors' personal observations). Lacewing larvae (*Chrysopa* spp.) and ladybird beetle larvae (Coccinelidae) have been observed feeding on first instar *B. philenor* larvae in the field. Although each lacewing larva can consume several first instar caterpillars, they refuse to feed when offered second or later instars. Newly emerged second instars are slightly smaller than late first instars, except for the head capsule and the region of the thorax directly adjacent to the head capsule, and thus size does not seem to be a determinant of the lacewings' selectivity. Thus, it appears that the first instar is the most vulnerable to attack by these crawling predators. Given that second instar larvae are found in smaller groups (mean ± SE, $6·2 ± 0·7$, $n = 46$), predation on first instars is implicated as a source of mortality.

TRICHOMES: HERBIVORE PREFERENCE TEST

An experiment was conducted to assess whether early instar larvae show a preference for pubescent leaves. On each of 18 intact stems, two newly emerged larvae were placed on a single leaf of *A. californica*, with trichomes removed from one half of the leaf (i.e. on one side of the mid-vein) with a shaving razor. Trichomes were removed on both the top and bottom sides of the leaves and it was decided randomly from which half of the leaf to remove trichomes. This shaving did not visibly damage the leaf surface, and has been used successfully by other workers (Schmitz 1994). One larva was placed on the trichome-removed half of the leaf and the other on the half with trichomes intact. Both larvae were permitted to move freely on the leaf between the trichome-removed and trichome-intact side so that preference regarding trichome presence could be assessed. A paired design was employed so that any systemic changes in the leaf associated with shaving the trichomes would be present in both treatments. Larvae were confined to this leaf using Tanglefoot Pest Barrier (The Tanglefoot Company, Grand Rapids, MI) around the petiole. After 24 h it was recorded which half of the leaf (with or without trichomes) sustained more herbivory, as an indicator of larval preference with regards to the presence of trichomes. These data were analysed using a sign-test (Zar 1999). All larvae used in this and subsequent experiments were newly emerged from eggs collected on wild plants in Mix Canyon, Solano County, California, USA. This experiment was conducted in Stebbins Cold Canyon, University of California Ecological Reserve, Solano County, California, USA, within the natural range of *A. californica* and *B. philenor*.

TRICHOMES: HERBIVORE NO-CHOICE TEST

To examine the effects of trichomes on larval feeding rate, a no-choice test was conducted at Stebbins Cold Canyon on 40 intact stems. Trichomes were removed from a randomly chosen half of a leaf on each stem and a newly emerged larva was placed on each half. Again trichomes were removed from both the top and bottom of one side of the leaf. Larvae were constrained to either half of the leaf using a barrier of Tanglefoot that coated the midrib of the leaf and encircled the petiole where it attached to the blade. Leaves were collected after 24 h and digitized using a scanner, and leaf area removed was measured on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). A paired *t*-test was used to determine differences in leaf area consumed for replicates where both larvae survived ($n = 24$ pairs). Where one or both of the paired larvae died (usually found in the Tanglefoot),

McNemer's test (Zar 1999) was used to determine if larval death was more likely on the half of the leaf with or without trichomes.

TRICHOMES: PREDATOR WALKING SPEED, SEARCHING EFFICIENCY AND PREDATION

Here the goal was to use natural variation in trichomes and also to manipulate them to demonstrate their role in affecting predators. Third instar larvae of the green lacewing [*Chrysopa carnea* (Stephens)] (obtained from A-1 Unique Insect Control, Citrus Heights, CA) were used. Fifteen pipevine stems were selected from a population in Davis, California, USA, and measured for mean trichome length and density using an ocular micrometer. Single lacewing larvae were allowed to acclimate to the stem for 2 min and then walking speed over 3 cm was measured. Linear regression was used to examine the relationship between length and density of trichomes and lacewing walking speed. The trichomes on those same 15 stems were then removed by gently rubbing them with sticky tape. Walking speed was again assessed on the stems and regressed against the trichome measurements obtained before removing the trichomes.

In the next experiment 26 arenas, in which the predators had access to prey on 5 cm of pipevine stem bounded by water on the bottom, were constructed. Half of the stems were assigned randomly to have their trichomes removed with the sticky tape. Herbivorous mites [*Tetranychus urticae* (Koch)] were used as a model prey organism as there was an explicit interest in determining the effects of trichomes on predator foraging ability and *B. philenor* were no longer available in the field. Ten spider mites were introduced from a laboratory colony as prey and a single lacewing was introduced to each pipevine stem. Lacewing larvae naturally consume both first instar *B. philenor* and mites. The time to first attack was recorded as a measure of searching efficiency, and the number of prey consumed in 60 min was recorded as a measure of predation rate. These data were natural log-transformed to meet the assumption of normality of residuals. The influence of trichomes on the predator-searching parameters was analysed using *t*-tests.

GROUP SIZE: EFFECT ON CATERPILLAR FEEDING BEHAVIOUR

To determine how group size affects feeding behaviour, groups of 1, 2, 4, 6, 8, 10, 12, 14 and 16 neonate larvae were placed on separate stems (10 replicates of each group size = 730 larvae distributed over 90 stems) in Stebbins Cold Canyon. Newly emerged larvae were placed on the leaf directly below the growing tip, where newly hatched larvae typically feed. These stems (*c.* eight leaves) were enclosed in Fibe-Air spun polyester mesh bags (Kleen Test Products, Brown Deer, WI) to exclude the potential effects of predators. Four days

after the larvae were placed on the plants, their position on the plant was recorded, with '0' recorded as larvae on the growing tip, '1' as larvae on the first leaf, '2' as larvae on the second leaf, and so on. Where larvae from a single replicate were feeding on more than one leaf, an average of the number at each leaf position was calculated. Linear regression was used to determine the effect of larval group size on larvae position on the stem. In addition, the length and density of trichomes on the top surface of the youngest leaf and three leaves lower (covering the range where most of the larvae were found) were measured using an ocular micrometer. Paired *t*-tests were used to compare the length and density of trichomes on high vs. lower leaves.

GROUP SIZE: EFFECTS ON PREDATORS AND CATERPILLAR GROWTH

To examine the effects of caterpillar group size on predation, a $2 \times 2 \times 2$-factorial field experiment was conducted where three factors were manipulated: group size (four or 12 larvae), access to crawling predators (mesh bags open or closed on the bottom) and access to flying or jumping predators (mesh bags open or closed on top). There were six replicates of each treatment for a total of 48 experimental stems. This experiment was conducted in a patch of dense pipevine in Davis, California, USA. Treatments were assigned haphazardly and all replicates received a spun polyester mesh bag. Freshly hatched first instar larvae were placed on the tips of the growing stem, where eggs are laid and first instar larvae typically feed. After 4 days (the typical period of growth from first to second instar) we counted the surviving larvae and weighed them to the nearest microgram (averaging weights where more than one larva survived). Percentage survival data were arcsine square-root transformed and analysed with a fully factorial three-way ANOVA, with group size, access to crawling predators and access to flying (or jumping) predators as the main fixed effects. Percentage survival

was used as the response variable because one motivation of this experiment was to examine the effect of predators from the perspective of *B. philenor* fitness. If laying large clutches confers higher fitness, it is predicted that large groups will have a higher percentage survival than smaller groups. Larval weights between the two group sizes were analysed using a model identical to that used above. Because newly hatched larvae were put on the plant, it was assumed that initial differences in larval weight were negligible.

A second experiment was conducted to test for the effects of caterpillar group size on defence against predators. Here, the number of larvae consumed was the response variable of interest because the goal was to examine the effect of caterpillar group size from the perspective of the predator. In this experiment, newly emerged larvae were placed onto growing stems in groups of four or 12, with 12 replicates of each treatment. All replicates were enclosed in a mesh bag and closed at the top and the bottom, with each bag receiving a single ultimate instar ladybird beetle larva collected from the same field site. At this site ladybird beetle larvae were commonly seen preying on first instar *B. philenor* larvae. After 24 h the number of surviving larvae was counted. The number of larvae consumed was compared between the treatments using a *t*-test.

Results

TRICHOMES: HERBIVORE PREFERENCE TEST AND HERBIVORE NO-CHOICE TEST

When given access to trichome-intact and trichome-removed portions of a leaf, larvae fed almost exclusively on the half of the leaf where trichomes were removed in 16 out of 18 cases (Fig. 1; $P < 0.025$, sign test). After 24 h of feeding, larvae consumed 70% more leaf tissue when they were confined (i.e. no choice) to the portion of the leaf where trichomes were removed compared with where trichomes were present (Fig. 1;
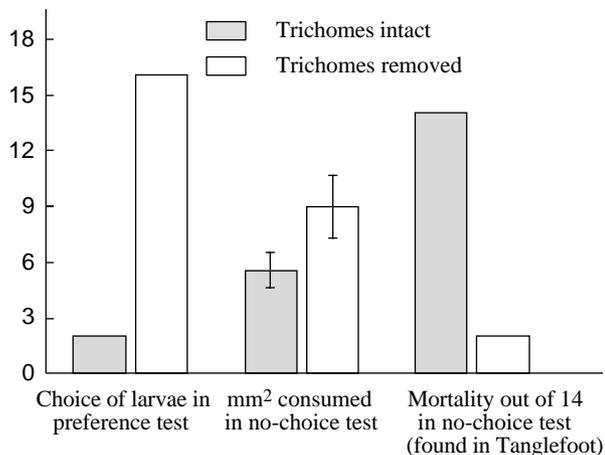
Fig. 1. The effect of removing trichomes on pipevine leaves in the larval preference test, leaf area consumed in the no-choice test, and mortality in the no-choice test. Leaf area consumed is only reported for replicates where both larvae survived. Bars are means ± SE. Note that units for the *y*-axis are given below each pair of bars.
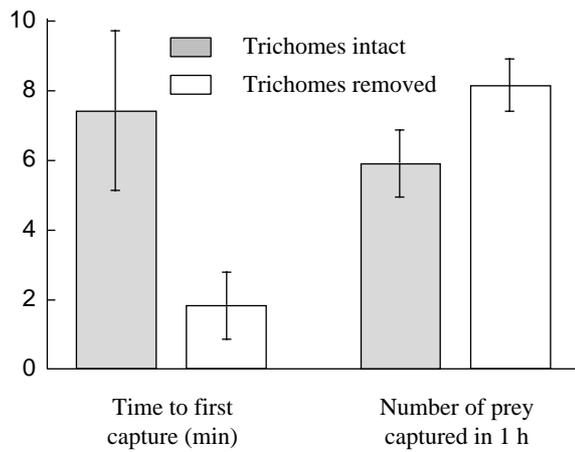
Fig. 2. The effect of trichomes on time to first prey capture and capture efficiency (total number of prey captured in 1 h) for lacewing larvae. Untransformed data are presented (natural log-transformed data used for analysis). Bars are means ± SE. Note that units for the *y*-axis are given below each pair of bars.
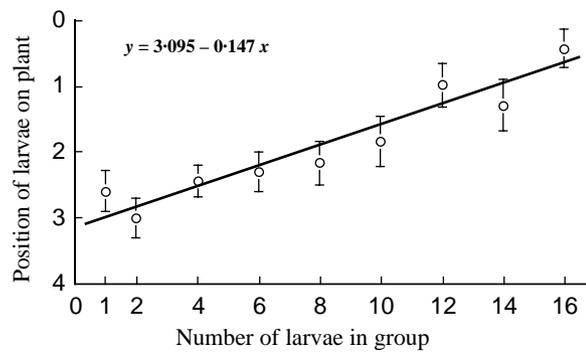
Fig. 3. Relationship between *Battus philenor* larval group size and larval feeding behaviour (feeding position on plant). Each group size was replicated 10 times on separate stems; bars are means ± SE. Note that the *y*-axis is labelled from high values to low values. Zero indicates the apical meristem and subsequent numbers indicate which leaf below the meristem. We chose this scheme to help visualize that, as larval group size increases, larvae feed on leaves closer to the apical portion of the plant stem.

$t = 2.119$, d.f. = 23, $P = 0.045$, paired *t*-test). Sixteen of the 40 original replicates had at least one dead larva found in the Tanglefoot. Significantly more of these deaths (14 out of 16) occurred where larvae were placed on the half of the leaf with trichomes intact (Fig. 1c; $\chi^2 = 12.07$, $P < 0.001$). Although these deaths were not a direct result of the trichomes, they further support the results of the preference test because larvae found in Tanglefoot were presumably trying to leave areas with high trichome density.

TRICHOMES: PREDATOR WALKING SPEED, SEARCHING EFFICIENCY AND PREDATION

Increased trichome density was negatively associated with predator walking speed ($R^2 = 0.343$, $F_{2,12} = 5.194$, $P = 0.024$). Length of trichomes was not associated with predator walking speed ($R^2 = 0.001$, $F_{1,13} = 2.712$, $P = 0.126$). When trichomes were removed from the same stems, predator walking speed was nearly four times faster ($t = -4.688$, d.f. = 14, $P < 0.001$, paired *t*-test) and the negative slope disappeared when we regressed former trichome density with walking speed ($F_{1,13} =$

0.015, $P = 0.905$), suggesting that trichome density was the factor that affected predator walking speed.

Searching efficiency of lacewing larvae was compromised and total number of prey captured was lower on stems with trichomes intact compared with treatments with trichomes removed. Time to first capture was 75% faster in treatments where trichomes were removed from the stem (Fig. 2; $t = 2.928$, d.f. = 13, $P = 0.012$). The total number of prey consumed in one hour was 22% higher on stems with trichomes removed (Fig. 2; $t = 3.000$, d.f. = 24, $P = 0.006$).

GROUP SIZE: EFFECT ON CATERPILLAR FEEDING BEHAVIOUR

Initial caterpillar group size linearly influenced where the larvae were observed feeding on the plant. Larvae that were part of a larger group were more likely to be observed feeding on the apical portion of the stem, either on the apical bud or on the apical leaf. Feeding was more concentrated on leaves distal from the apical bud as initial group size was decreased (Fig. 3; $R^2 = 0.356$, $F_{1,84} = 46.448$, $P < 0.001$). The ultimate

Table 1. Factorial ANOVA for the effects of crawling and flying predators on *Battus philenor* survival in larval group sizes of four and 12

Variable	d.f.	Mean square	F	P
Group size	1	140·227	0·184	0·670
Crawling	1	8120·399	10·682	0·002
Flying	1	5441·233	7·158	0·011
Group size × crawling	1	327·478	0·431	0·151
Group size × flying	1	293·484	0·386	0·538
Crawling × flying	1	116·007	0·153	0·698
Crawling × flying × group size	1	4·904	0·006	0·936
Error	40	760·201		
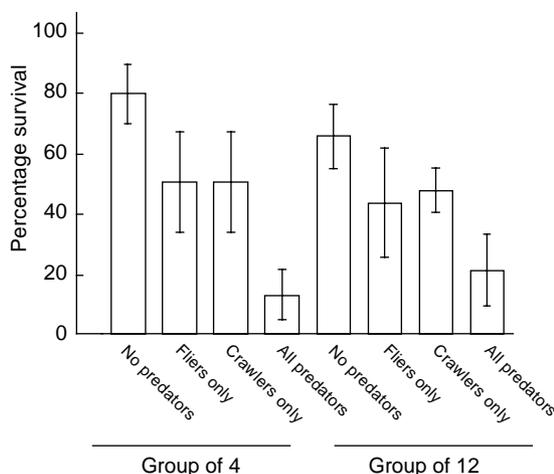
Fig. 4. The effect of flying and crawling predators on percentage survival of *Battus philenor* larvae in groups of four and 12. Bars are means ± SE.

(upper) leaves closest to the growing tip were found to have more than twice the density of trichomes compared with the third leaf (trichomes per mm^2 ± SE: top leaf 34 ± 7, third leaf 15 ± 2; paired $t = -7.422$, d.f. = 14, $P < 0.001$), as would be expected because the leaf blade grows and increases in surface area, while total trichome number does not increase with leaf expansion. Upper and lower leaves did not differ in the lengths of the trichomes (mean length mm ± SE, top leaf 0·52 ± 0·04, third leaf 0·51 ± 0·03; paired $t = 1.175$, d.f. = 14, $P = 0.259$).

GROUP SIZE: EFFECTS ON PREDATORS AND CATERPILLAR GROWTH

Caterpillar group size did not influence the percentage survival in the field experiment (Table 1 and Fig. 4). Access to larvae by crawling predators or flying predators each reduced the percentage survival of both group sizes by 30% (Table 1 and Fig. 4). The effects of crawling and flying predators were equal and additive (i.e. a slightly greater than 60% reduction in survival where both flying and crawling predators had access to larvae). Sih, Englund & Wooster (1998) suggested log-transforming percentage survival data for examining the impact of multiple predators on prey, to utilize the

Table 2. Factorial ANOVA for the effects of crawling and flying predators on larval weight of *Battus philenor* in group sizes of four and 12. Sample size is smaller than analysis in Table 1 because of larval deaths

Variable	d.f.	MS	F	P
Group size	1	6·473	5·946	0·021
Crawling	1	1·550	1·424	0·243
Flying	1	0·051	0·047	0·830
Group size × crawling	1	1·635	1·502	0·231
Group size × flying	1	0·818	0·752	0·393
Crawling × flying	1	0·002	0·002	0·964
Crawling × flying × group size	1	0·069	0·064	0·802
Error	28	1·089		
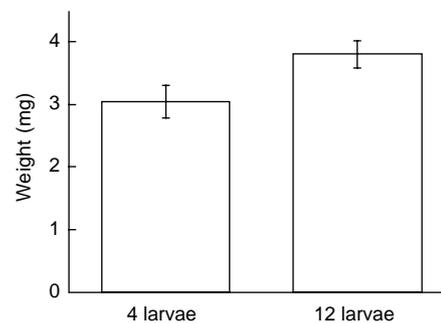
Fig. 5. The effect of *Battus philenor* larvae group size on larval weight after 4 days of feeding in the field. Bars are means ± SE.

'multiplicative risk' model (i.e. not allowing individual prey to be killed twice). Log-transformation of our data did not affect the results. In particular, the interaction term (between access to flying and crawling predators) did not approach significance in any of the analyses.

Larvae in groups of 12 were 25% larger than those in groups of four at the end of the experiment (Table 2 and Fig. 5), indicating positive density-dependent growth. Exposure to crawling or flying predators had no effect on larval weight of the survivors (Table 2). When a ladybird beetle larva was enclosed with caterpillars in groups of four or 12, group size did not affect larval defence, as an equal absolute number of larvae was consumed in both treatments (mean ± SE, group of four 1·5 ± 0·5, group of 12 1·6 ± 0·5; d.f. = 22, separate variance $t = -0.093$, $P = 0.927$).

Discussion

Trichomes on the leaves and stem of *A. californica* may provide a line of defence against herbivory by its specialist herbivore, *B. philenor*. Although aristolochic acids (bitter alkaloids) are probably effective at deterring generalist herbivores (Park *et al.* 1997), it is likely that this investment confers increased fitness on *B. philenor* larvae and adults through sequestration of these compounds for use in anti-predator defence (Brower 1958; Rothschild *et al.* 1970). The presence of trichomes, however, is effective at reducing the rate of herbivory, as demonstrated in our trichome-removal experiment. Similarly, Ågren & Schemske (1993) showed that *Brassica rapa* plants selected for low trichome density experienced more damage from *Pieris rapae* larvae compared with plants selected for higher densities of trichomes. Subsequently, the density of trichomes throughout the plant may influence where on the plant mobile herbivores are likely to feed.

The youngest leaves, presumably the most nutritious for developing caterpillars, have the highest density of trichomes. Although this may simply be a result of incomplete leaf expansion, the net effect is that these young tissues are the best defended by trichomes. Mortality in the no-choice trichome-removal experiment was due to larvae trying to leave the side of the leaf with trichomes intact and subsequently ending up in the Tanglefoot barrier. This may be because the experimental leaves were young and trichomes provided an adequate barrier to efficient larval feeding. In the preference test, which was also carried out on young leaves, larvae did not try to leave the leaf, rather they fed on the portion of the leaf with trichomes removed. Similarly, Yencho & Tingey (1994) found that the Colorado potato beetle preferentially feeds on leaves with trichomes removed. Thus, trichomes acting as structural defences may have the capacity to influence greatly where *B. philenor* feeds on the plant.

The experiment on the effect of group size on feeding behaviour suggests that feeding in large groups by the California populations of *B. philenor* may be an adaptation to overcome the effectiveness of trichomes. Other populations (i.e. those in Texas and Mexico) that feed in small groups primarily feed on glabrous species of pipevines. In California, large groups of larvae feed on leaves at the tips of stems where trichome density is highest, and smaller groups of larvae feed lower on the plant, where trichome density is proportionally lower. A silk mat created by larger groups of larvae may help them feed on foliage with high trichome density. This thin veil of silk may provide the larvae with an adequate anchor to feed from while they consume the leaf from the edge inwards, thus effectively circumventing the leaf trichomes by attacking the leaf from the side (below the bases of the trichomes). Similarly, Rathcke & Poole (1975) described the gregarious feeding behaviour of *Mechanitis isthmia* (Bates) (Ithmiidae) caterpillars that effectively overcome trichome defences

of their host-plant by constructing silk scaffolding. Larvae of *B. philenor* in smaller groups are unable to lay down significant mats of silk (authors' personal observations). Although Young & Moffett (1979) found that gregarious feeding of *M. isthmia* also increased the efficacy of trichome removal, there was no evidence that *B. philenor* larvae remove trichomes prior to feeding on the leaf.

Several other studies have investigated the role that plant structural defences may play in the evolution of life-history traits of herbivores. For example, Gross & Price (1988) suggested that the stellate trichomes of horsenettle *Solanum carolinense* (L.) are probably responsible for the obligate endophytism of *Tildenia inconspicuella* (Murtfeldt) (Lepidoptera: Gelechiidae), a phenomenon rarely observed for this group. Larvae were unable to penetrate leaves with firm stellate trichomes, whereas they could easily penetrate and feed on leaves where trichomes were thin and flexible. Others have investigated and discussed how plant architecture may directly or indirectly affect herbivore vulnerability to predators and parasites (Obrycki 1986; Agrawal & Karban 1997). Specialist insects on plants with trichomes have evolved morphological or behavioural characteristics to overcome these defences (Rathcke & Poole 1975; Young & Moffett 1979). The small cost associated with trichome production (Ågren & Schemske 1993) makes it unlikely that they will be selected against, even if their effectiveness is lessened by herbivore adaptations. If, however, high trichome densities offer herbivores that are adapted to these defences 'enemy-free space', we would assume that the cost of herbivory would favour lower trichome densities. The aristolochic acids of *A. californica* are effective defences against most other herbivores and thus trichomes may be one of the primary defences against the specialist on this plant. At this stage it is unclear if the negative effect of trichomes on predators is more important than the negative effect on *B. philenor*.

The effect of trichomes on predator and caterpillar feeding behaviour led to the prediction that larvae in smaller groups should be more vulnerable to predation because they feed in areas that are more accessible to walking predators (i.e. leaves with low trichome density). In addition, smaller groups may also be less defended against predation simply because of the lack of an enhanced aposematic display (Stamp 1980). The defensive osmeteria of *B. philenor* may also be more effective in large groups. Contrary to these predictions, when groups of four or 12 larvae were enclosed with a single predator, equal numbers of larvae were consumed in groups of both sizes. Thus, caterpillar defence, from the perspective of the predator, does not appear to be dependent on group size. This is in contrast with one predicted function of gregarious feeding for toxic caterpillars (Stamp 1980; Sillen-Tullberg 1988). However, first instar larvae exhibit less of the aposematic coloration characteristic of later instars, lacking the stark contrast between orange spines and black background colour, thus group feeding may

provide an effective defence through aposematic display in later instars.

In the open bag experiment, percentage mortality of larvae was also independent of group size. It is possible that groups of 12 were more apparent or attractive to predators than groups of four, because a greater absolute number of larvae was consumed in the larger group (but an equal percentage). Two non-exclusive hypotheses, both involving the recruitment of predators, could explain this pattern. First, large groups of larvae on the tips of plants may simply be more conspicuous to flying predators than smaller groups that feed lower on the plant. This could lead to an equal percentage mortality higher and lower on the plant, even though a greater absolute number of larvae is preyed upon on the tips. Secondly, larger groups may feed in denser aggregations than smaller groups, leading to an increase in the absolute number of larvae consumed once a feeding group is discovered by a predator. In other systems, larger, more conspicuous, groups feeding on plant tips had not only higher absolute mortality, but also higher percentages of mortality, than less conspicuous groups (Damman 1987). From the perspective of butterfly fitness, the overall percentage mortality of larvae is the key unit of interest, not the absolute number of larvae killed in a particular clutch. Thus, for the pipevine – *B. philenor* system, group size and the related location of feeding does not seem to affect predation rates directly.

We observed that larger groups gained more weight over the course of our 4-day field experiment, which encompassed a large part of the typical period of first instar growth. Faster growth may, in turn, result in less overall mortality for larvae feeding in larger groups (the slow-growth-high-mortality hypothesis; Clancy & Price 1987). Working with *Pieris rapae* (L.) larvae, Benrey & Denno (1997) demonstrated that slower growth rate extended the 'window of vulnerability' to a wasp parasitoid, and thus increased parasitism. Although larger groups do not have a better direct defence against predators and suffer the same percentage mortality as smaller groups over the same period of time, larvae in larger groups grow faster. Fast growth may be an important indirect benefit of living in large groups, as the first instar larvae of *B. philenor* appear to be the most susceptible to predation. The large clutch size characteristic of the California population of *B. philenor* may be an adaptation to overcome the trichome defences of *A. californica*, facilitating increased larval growth rate.

Acknowledgements

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