

SHORT COMMUNICATION

Host plant trichomes and the advantage of being big: progeny size variation of the pipevine swallowtail

JAMES A. FORDYCE,¹ ROMINA D. DIMARCO,¹ BONNY A. BLANKENSHIP¹ and CHRIS C. NICE² ¹Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, Tennessee, U.S.A. and ²Department of Biology, Population and Conservation Biology Program, Texas State University, San Marcos, Texas, U.S.A.

Abstract. 1. Plants possess numerous traits that confer resistance against insect herbivores, and herbivores, in turn, can evolve traits to ameliorate the effectiveness of these traits. The pipevine swallowtail, *Battus philenor*, is an extreme specialist on plants in the genus *Aristolochia*. The only host plant available to the California population of *B. philenor* is *A. californica*. *Aristolochia californica* is distinct from most other *B. philenor* host plants in that it is pubescent.

2. The progeny of *B. philenor* are larger in California compared with populations examined in Texas. Size differences persist throughout larval development.

3. Regardless of maternal host plant, population differences in progeny size persist, and crosses between California (large progeny) and Texas (small progeny) *B. philenor* populations resulted in offspring producing intermediate sized progeny, indicating a heritable component to progeny size variation.

4. California neonate caterpillars more easily overcame the trichomes of *A. californica* compared with Texas neonates. When trichomes were removed from *A. californica*, time to feeding establishment was reduced for caterpillars from both populations. Texas caterpillars established feeding sites on *A. californica* with trichomes removed, in the same time required to establish feeding on their non-pubescent host plant, *A. erecta*.

5. This study shows that plant trichomes might impose selection pressure on progeny size.

Key words. Adaptation, *Battus philenor*, caterpillar, egg size, trichomes, variation.

Introduction

Plant traits that confer resistance against herbivores can serve as strong selective agents on herbivore physiology, life history, behaviour, and morphology. Variation in plant resistance traits can lead to locally adapted herbivore phenotypes. A majority of the work on local adaptation of insect herbivores has emphasised the physiological mechanisms by which insects cope with plant chemical defences (e.g. Broadway, 1995; Berenbaum *et al.*, 1996; Feyerisen, 1999; Glendinning, 2002). However, plant structural defences, such as trichomes or leaf toughness, can also affect the evolution of insect traits

(Levin, 1973; Bernays, 1991; Hanley *et al.*, 2007;). One trait associated with leaf toughness is egg size and, in particular, the resulting size of neonates. For example, geographical variation in egg size of the pine processionary moth, *Thaumetopoea pityocampa* Denis & Schiffermür, is positively correlated with the toughness of its host plant's needles (Zovi *et al.*, 2008), and the skipper *Parnara guttata* Bremer & Grey manipulates egg size in response to host plant leaf toughness (Mizumoto & Nakasuji, 2007). Although larval size has been identified as an important trait associated with leaf toughness, less attention has been paid to the role that plant trichomes might play in the evolution of egg size.

The pipevine swallowtail, *Battus philenor* L., is an extreme specialist on plants in the genus *Aristolochia* L. (Aristolochiaceae). There exists striking geographical variation in egg size and hatching weight of caterpillars, with California populations

Correspondence: James A. Fordyce, Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, U.S.A. E-mail: jfordyce@utk.edu

being nearly twice as large as other populations (Fordyce & Nice, 2004). In California, only one naturally occurring host plant is available, the endemic *A. californica* Torr. *Aristolochia californica* differs from most other *Aristolochia* species used by *B. philenor* outside of California, in that it is densely covered in trichomes (Fordyce & Agrawal, 2001). The present study was undertaken to illustrate a possible link between progeny size variation and host plant pubescence by (1) examining the relationship between caterpillar feeding performance, caterpillar size, and host plant pubescence, and (2) assessing whether progeny size variation was heritable.

Materials and methods

Butterfly size variation

Two focal populations, one in California and one in Texas, were used to compare egg, larval, pupal, and adult size variation. To examine the role host plant species might play in size variation, *B. philenor* caterpillars were reared on three host plant species, *A. californica* (California host plant), *A. erecta* L. (Texas host plant), and *A. macrophylla* Lam. (an abundant host plant in the eastern part of the butterfly's range that served as a novel host plant for both focal populations). Eggs were obtained from wild caught females collected in Solano County, California and Hays County, Texas. In total, more than 1000 eggs were obtained from more than 40 females from each population. A sample of neonate caterpillars was weighed to assess hatching weight differences between the populations. Neonate caterpillars from both populations were evenly distributed across the three host plant species, and size variation throughout development was quantified by measuring the width, at its widest point, of caterpillar head capsules using digital images and the software package ImageJ (v 1.42; U.S. National Institutes of Health, Bethesda, Massachusetts). Caterpillars were reared in groups without regard to family identity, and the number of caterpillars in a group was reduced as caterpillars developed ($n \approx 20$ per container for first instars, $n \approx 2$ per container for sixth instars). Measurements on head capsules for each stage were made on a random subset collected from cages after each molt. Differences in head capsule size between populations were examined using *t*-tests. Pupal weight and adult dry weight were obtained. Adult head width at its widest point, and forewing length from the thorax to the apical edge of the M_1 vein were measured using digital calipers. Comparisons of adult size between populations were performed using ANOVA and included sex as a factor.

Matings within and between California and Texas populations were accomplished via hand-pairing of lab-reared individuals. First instar head capsule size was measured across three captive generations to assess the genetic component of between-population progeny size variation. Mean head capsule size for the progeny of each female was used as a response variable. Initial matings were as follows: California \times California ($n = 11$), Texas \times Texas ($n = 13$), California female \times Texas male ($n = 3$), and Texas female \times California male ($n = 6$).

Table 1. Morphological measurements of *Battus philenor* from California and Texas populations.

	California		Texas	
	n	Mean (SE)	n	Mean (SE)
Egg size (mm)	50	1.318 (0.008)	50	1.121 (0.009)
Larvae				
Neonate weight (g)	50	935 (10)	25	535 (14)
Head capsule width (mm)				
First instar	25	0.834 (0.006)	25	0.687 (0.006)
Second instar	25	1.212 (0.019)	25	1.060 (0.011)
Third instar	25	1.762 (0.025)	25	1.616 (0.021)
Fourth instar	25	2.800 (0.060)	25	2.570 (0.062)
Fifth instar	25	3.910 (0.082)	25	3.547 (0.060)
Sixth instar	10	5.031 (0.079)	2	4.81 (0.320)
Pupal weight (g)	61	1.238 (0.029)	51	1.167 (0.032)
Adults				
Male head width (mm)	17	4.356 (0.047)	31	4.547 (0.051)
Female head width (mm)	27	4.324 (0.039)	45	4.367 (0.004)
Male forewing length (mm)	17	39.54 (0.597)	31	42.206 (0.763)
Female forewing length (mm)	27	42.00 (0.335)	45	44.148 (0.519)
Male dry weight (g)	17	0.118 (0.007)	31	0.115 (0.006)
Female dry weight (g)	27	0.149 (0.006)	45	0.130 (0.004)

Host plant variation

The host plant used by the focal Texas population in Hays County is *A. erecta*, which is glabrous, although other species of *Aristolochia* are used by *B. philenor* in the region (Rausher, 1995). The California host plant, *A. californica*, is highly pubescent. Average leaf toughness for the two plant species was accessed using a force gauge penetrometer (Type 516; Chatillon, Largo, Florida). The toughness of the five most apical leaves of individual plants was averaged, providing an average leaf toughness for *A. californica* ($n = 16$) and *A. erecta* ($n = 20$).

Trichomes, head size, and feeding establishment

To assess the effect of trichomes on the feeding performance of neonate larvae from each population, neonate larvae were challenged with *A. erecta*, *A. californica*, and *A. californica* with trichomes removed. Trichomes were removed from *A. californica* using a shaving razor (cf. Fordyce & Agrawal, 2001). Time from the initiation of the experiment to the time when caterpillars began feeding on the leaf was recorded.

Results and discussion

The progeny of California females were larger than those from Texas (Table 1) based on egg diameter ($t_{73} = 22.95$, $P < 0.01$), neonate weight ($t_{98} = 15.91$, $P < 0.01$), and first instar head capsule width ($t_{94} = 11.63$, d.f. = 94, $P < 0.01$).

On average, California adults had a higher dry weight ($F_{1,97} = 4.63$, $P = 0.03$) and females from both populations weighed more than males ($F_{1,97} = 10.90$, $P = 0.01$). Texas adults had a greater forewing length ($F_{1,97} = 16.11$, $P < 0.01$) and a marginally greater head width ($F_{1,97} = 3.35$, $P = 0.07$). Forewing length and head width were greater for females than males (forewing length: $F_{1,97} = 10.95$, $P < 0.01$; head width: $F_{1,97} = 4.84$, $P = 0.03$). Host plant species consumed during the larval stage had no effect on any of the adult size measurements, and none of the interaction terms in the ANOVA were significant ($P > 0.1$). The lack of a host plant effect indicates that the size differences that exist between these two populations are not a plastic response to differences in host plant quality. The larger size of California larvae persisted through development ($F_{1,240} = 55.98$, $P < 0.01$). The interaction between larval instar and population was marginally significant ($F_{4,240} = 2.23$, $P = 0.07$), owing to the fact that differences between the two populations decreases in later instars (Table 1). The sixth instar was not included in this analysis, because the head capsule fractures prior to pupation, making it difficult to accurately measure its width. The weights of pupae, however, were not different between the two populations ($t_{110} = 1.62$, $P = 0.11$). Overall, these results indicate that California caterpillars are larger than those from Texas.

The lab crosses between California and Texas *B. philenor* show a genetic component to progeny size variation, with the parentage of the female explaining 32% of first instar head capsule width ($F_{3,28} = 4.10$, $P = 0.02$). Progeny size was apparently an extension of the female phenotype. That is, California females crossed with Texas males had the expected California progeny size [mean head width of population crosses (\pm SEM) = 0.897 (0.047)], and Texas females crossed with California males had the expected Texas progeny size [mean head width of population crosses (\pm SEM) = 0.714 (0.025)] (Table 1). However, the progeny of females produced from crosses between populations (i.e. the offspring of F1s) were of intermediate size compared with offspring from matings between individuals from the same source population. The progeny of F2 females were similarly of intermediate size, suggesting that progeny size is not a simple, single locus Mendelian trait.

Comparison of leaf toughness between *A. californica* (the California host plant) and *A. erecta* (the Texas host plant) showed no difference ($t_{34} = 0.62$, $P = 0.54$). However, qualitatively the plants differ in that *A. californica* is highly pubescent and *A. erecta* is glabrous. The trichomes of *A. californica* impede the foraging of arthropods (Fordyce & Agrawal, 2001), and this extends to *B. philenor*. The large neonates from California consistently established feeding sites in a shorter time period compared with Texas neonates ($F_{1,59} = 10.94$, $P < 0.01$; Fig. 1), although larvae from both populations took longer to establish feeding on *A. californica* ($F_{2,59} = 10.23$, $P < 0.01$). The interaction between caterpillar population and plant was not significant ($F_{2,59} = 0.02$, $P = 0.98$; Fig. 1). The experimental removal of trichomes from *A. californica* resulted in feeding establishment times

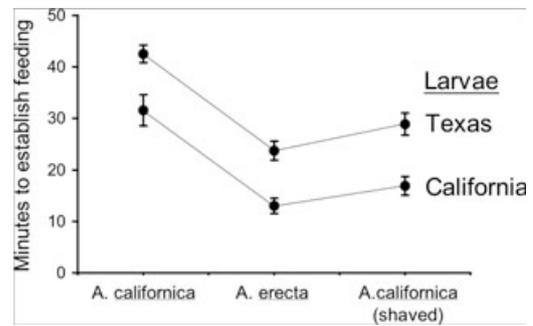


Fig. 1. Time to feeding establishment for California and Texas *Battus philenor* neonate larvae.

similar to that on *A. erecta* ($F_{1,59} = 1.32$, $P = 0.25$). Interestingly, the time to feeding establishment for each population on its normal host plant was not different ($F_{1,59} = 1.78$, $P = 0.19$), indicating they both perform similarly on their respective host plant species.

The adaptive significance of progeny size variation as it relates to host plant characteristics has largely focused on leaf toughness as the agent of selection favouring larger progeny (Bernays & Janzen, 1988; Fox & Czesak, 2000; Zovi *et al.*, 2008), although other factors, such as climate, can favour larger progeny (Ayres & Scriber, 1994; Fischer *et al.*, 2003). It is unknown if a tradeoff exists between fecundity and progeny-size for California *B. philenor*, as has been observed for other insects (Fischer & Fiedler, 2001; Hassall *et al.*, 2006; also see Bauerfeind & Fischer, 2007; Karl *et al.*, 2007). Time to feeding establishment, and the ability to forage, has a direct effect on larval developmental rate. This is particularly important for *B. philenor*, because the first instar is the developmental stage that suffers the highest mortality (Fordyce & Agrawal, 2001; Fordyce & Shapiro, 2003; Fordyce & Nice, 2008). This study demonstrates that plant trichomes might, similar to leaf toughness, impose selection pressure for increased progeny size.

Acknowledgements

We thank the University of California Natural Reserve System for permitting access to Stebbins Cold Canyon, Texas State University for access to Freeman Ranch, and the helpful comments provided by two anonymous reviewers. This work was supported by the U.S. National Science Foundation (DEB-0614223) and by a Texas State University Research Enhancement Grant.

References

- Ayres, M.P. & Scriber, J.M. (1994) Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera, Papilionidae). *Ecological Monographs*, **64**, 465–482.
- Bauerfeind, S.S. & Fischer, K. (2007) Maternal body size as an evolutionary constraint on egg size in a butterfly. *Evolution*, **61**, 2374–2385.
- Berenbaum, M.R., Favret, C. & Schuler, M.A. (1996) On defining “key innovations” in an adaptive radiation: cytochrome

- P450s and Papilionidae. *The American Naturalist*, **148**, S139–S155.
- Bernays, E.A. (1991) Evolution of insect morphology in relation to plants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **333**, 257–264.
- Bernays, E.A. & Janzen, D.H. (1988) Saturniid and sphingid caterpillars—2 ways to eat leaves. *Ecology*, **69**, 1153–1160.
- Broadway, R.M. (1995) Are insects resistant to plant proteinase inhibitors? *Journal of Insect Physiology*, **41**, 107–116.
- Feyereisen, R. (1999) Insect P450 enzymes. *Annual Review of Entomology*, **44**, 507–533.
- Fischer, K. & Fiedler, K. (2001) Egg weight variation in the butterfly *Lycaena hippothoe*: more small or fewer large eggs? *Population Ecology*, **43**, 105–109.
- Fischer, K., Brakefield, P.M. & Zwaan, B.J. (2003) Plasticity in butterfly egg size: why larger offspring at lower temperatures? *Ecology*, **84**, 3138–3147.
- Fordyce, J.A. & Agrawal, A.A. (2001) The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. *Journal of Animal Ecology*, **70**, 997–1005.
- Fordyce, J.A. & Nice, C.C. (2004) Geographic variation in clutch size and a realized benefit of aggregative feeding. *Evolution*, **58**, 447–450.
- Fordyce, J.A. & Nice, C.C. (2008) Antagonistic, stage-specific selection on defensive chemical sequestration in a toxic butterfly. *Evolution*, **62**, 1610–1617.
- Fordyce, J.A. & Shapiro, A.M. (2003) Another perspective on the slow-growth/high-mortality hypothesis: chilling effects on swallowtail larvae. *Ecology*, **84**, 263–268.
- Fox, C.W. & Czesak, M.E. (2000) Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, **45**, 341–369.
- Glendinning, J.I. (2002) How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata*, **104**, 15–25.
- Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology Evolution and Systematics*, **8**, 157–178.
- Hassall, M., Walters, R.J., Telfer, M. & Hassall, M.R.J. (2006) Why does a grasshopper have fewer, larger offspring at its range limits? *Journal of Evolutionary Biology*, **19**, 267–276.
- Karl, I., Lorenz, M.W. & Fischer, K. (2007) Energetics of reproduction: consequences of divergent selection on egg size, food limitation, and female age for egg composition and reproductive effort in a butterfly. *Biological Journal of the Linnean Society*, **91**, 403–418.
- Levin, D.A. (1973) The role of trichomes in plant defense. *The Quarterly Review of Biology*, **48**, 3–15.
- Mizumoto, M. & Nakasuji, F. (2007) Egg size manipulation in the migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperidae), in response to different host plants. *Population Ecology*, **49**, 135–140.
- Rausher, M.D. (1995) Behavioral ecology of oviposition in the pipevine swallowtail, *Battus philenor*. *Swallowtail Butterflies: Their Ecology and Evolutionary Biology* (ed. by J.M. Scriber, Y. Tsubaki and R.C. Lederhouse), pp. 53–62. Scientific Publishers, Gainesville, Florida.
- Zovi, D., Stastny, M., Battisti, A. & Larsson, S. (2008) Ecological costs on local adaptation of an insect herbivore imposed by host plants and enemies. *Ecology*, **89**, 1388–1398.

Accepted 17 September 2009

First published online 10 November 2009