

GEOGRAPHIC VARIATION IN CLUTCH SIZE AND A REALIZED BENEFIT OF AGGREGATIVE FEEDING

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Abstract.—We investigated one causal explanation for geographic variation in clutch size and aggregative feeding of the pipevine swallowtail, *Battus philenor*. Populations in California lay larger clutches than those in Texas, and larger feeding aggregations grow at an accelerated rate on the California host plant. Using reciprocal transplant experiments with larvae from California and Texas populations, we found that the benefit of increased growth rate associated with feeding in larger groups occurred only on the California host plant and was observed for larvae from both populations. These results are consistent with the hypothesis that larger clutch size and aggregative feeding are adaptations to characteristics of the California host plant. Future studies on the evolution of clutch size and aggregative feeding of herbivorous insects should consider how these life-history traits affect host plant suitability.

Key words.—Adaptation, clutch size, geographic variation, gregariousness, life-history evolution, plant-insect interactions.

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The adaptive significance of aggregative feeding of Lepidoptera larvae has received a great deal of attention (Sillén-Tullberg 1988; Hunter 2000). Aggregative feeding is of interest to evolutionary biologists because it is usually a consequence of egg clustering by females. Clutch size is an important life-history trait because it represents a proportion of a female's potential fecundity that is invested in a single oviposition event. Numerous hypotheses have been proposed to explain the evolution of large clutch size and subsequent aggregative feeding (Stamp 1980). Nonadaptive explanations posit that large clutch size may be a consequence of female motivational state or egg load when suitable oviposition sites are rare (Courtney 1984; Tatar 1991). Alternatively, many adaptive hypotheses have been proposed for the evolution of aggregative feeding. Aggregative feeding may increase thermoregulatory ability (Bryant et al. 2000), maximize resource use (Le Masurier 1994), circumvent structural plant defenses (Rathcke and Poole 1975), accelerate larval growth due to enhanced plant quality (Fordyce 2003), and function for defense, either through active group defense or enhanced aposematism (Fisher 1930; Stamp 1980).

Two avenues of investigation have commonly been used to infer the adaptive significance and evolutionary history of aggregative feeding. Experimental methods generally consist of manipulating group size of larvae to numbers above or below the naturally occurring densities within a population, and assessing the ecological consequences of group size, such as mortality or growth rate (e.g., Lawrence 1990; Fordyce and Agrawal 2001). Phylogenetic methods of inference compare the degree to which aggregation occurs among lineages as an attempt to reconstruct correlated evolutionary changes in traits presumed to be adaptive (Sillén-Tullberg 1988; Tullberg and Hunter 1996). Using this method, for example, Tullberg and Hunter (1996) concluded that aggregative feeding is more likely to evolve in lineages where larvae are toxic

and warningly colored, thus supporting defense as a factor leading to the evolution of this trait.

Here, we exploited interpopulation variation in clutch size and aggregative feeding of the pipevine swallowtail (*Battus philenor*) to determine if one benefit associated with feeding in larger groups, accelerated growth rate, was only realized in a population characterized by a larger clutch size. This system provided us with a unique opportunity to investigate the adaptive significance of aggregative feeding within a taxon, while permitting us to use populations that naturally feed in small or large groups. Here we present results from a reciprocal transplant experiment of larvae from California and Texas. California females lay significantly larger egg clutches and larvae feed in larger groups compared to populations reported in Texas. We hypothesized that aggregative feeding exhibited by the California population of *B. philenor* represents an adaptation unique to the California host plant whereby groups of larvae effectively manipulate the plant quality so as to enhance its suitability for larval development (Fordyce 2003). Specifically we asked the following questions: Do plant characteristics explain the variation in clutch size observed within the California and Texas populations? Is the increased growth rate associated with larger feeding aggregations observed in California a characteristic unique to California larvae, a response unique to the California host plant, or both?

MATERIALS AND METHODS

Species Description and Study Populations

The California populations of *B. philenor* are disjunct from the rest of the species range and sometimes are designated as the subspecies *B. p. hirsuta*. Members of the genus *Battus* are restricted to host plants in the genus *Aristolochia* (Aristolochiaceae) and in California only one host plant species is available to *B. philenor*, the endemic *A. californica* (Racheli and Pariset 1992). The average clutch size observed in California populations, including the focal population at Stebbins Cold Canyon, is 13 eggs per cluster and larvae feed in

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dense aggregations following hatching (Fordyce 2003), whereas populations in eastern Texas using other *Aristolochia* species have an average clutch size of 2.5 (Rausher 1995). Geographic patterns of mtDNA variation suggest that *B. philenor* is a recent addition to the California fauna, indicating that the larger clutches observed in California appear to be a recent modification of this life-history trait (Fordyce and Nice 2003). Aggregative feeding in California is beneficial to larvae because individuals in larger groups exhibit an accelerated growth rate in the first instar (Fordyce 2003; Fordyce and Shapiro 2003). Although the mechanism is currently unknown, this accelerated growth rate is a plant-mediated benefit and not the result of a group feeding stimulus, such as tactile cues communicated directly among members of a feeding group (Fordyce 2003). Larger groups of first instar larvae are not better protected against natural enemies, receiving the same percent mortality as smaller group sizes (Fordyce and Agrawal 2001). However, larger groups in California receive an indirect defensive benefit because accelerated growth reduces exposure to natural enemies during the vulnerable early instars (formalized as the slower-growth/higher-mortality hypothesis; Clancy and Price 1987)(Fordyce and Agrawal 2001; Fordyce 2003; Fordyce and Shapiro 2003). No previous work has examined the effect of group size on growth rate in Texas.

Geographic variation in this aggregative-feeding benefit was assessed using two focal populations. The Texas population was studied at Freeman Ranch (Hays Co.), a field station for ecological and agricultural research operated by Texas State University, San Marcos. The host plant at this location is *Aristolochia erecta*, a semi-erect herbaceous perennial. The California population was located at Stebbins Cold Canyon Ecological Reserve (Solano Co.), part of the University of California Natural Reserve System. The host plant in California, *A. californica*, is an endemic climbing liana characteristic of riparian habitats of the Sacramento Valley and surrounding foothills of the Coast Range and Sierra Nevada mountains. All experiments described herein were conducted during the first three weeks of April 2002, during peak flight times of both populations.

Clutch Size and Host Plant Characteristics

We sought to determine if host plant characteristics might explain variation in clutch size observed within the two focal populations. This was motivated, in part, by the fact that Pilson and Rausher (1988) reported that female *B. philenor* in eastern Texas modify their clutch size in response to the amount of foliage available for larval consumption. The number of eggs per clutch was assessed in the field and measurements of the host plants were recorded. In Texas, the number of leaves on the plant, the height of the plant, and the distance to its nearest *A. erecta* neighbor was quantified for all plants where egg clutches were found ($n = 94$). In California, the percent cover of *A. californica* in a circle with a radius of one meter around the plant, the height of the plant, and the distance to the nearest neighboring *A. californica* were determined for plants where egg clutches were found ($n = 198$). Unlike *A. erecta*, which often grow in small patches of individual plants, *A. californica* forms dense patches. Thus,

we decided that percent cover would best describe the amount of potentially available food resources for larvae. Separate multiple regressions were used for each population to determine if any of the plant characteristics measured could predict the number of eggs laid in a clutch by a female. All data were log transformed to meet the assumption of normality, except percent cover of *A. californica* which was arcsin transformed.

Reciprocal Transplant Experiments

We conducted reciprocal transplant experiments to determine if the faster growth associated with larger groups observed in California was an effect unique to the California host plant, larvae of the California population, or both. Eggs were collected from naturally laid clutches and from captive females collected from each of the study locales. Approximately 400 eggs from each of the populations were collected for the experiments at each location. Each experiment consisted of a full factorial design with population (California or Texas) and group size (two or 12 larvae) as factors. Only neonate larvae and plants that had not received previous herbivore damage were used for these experiments. The response variable was the average larval weight (log transformed) of a feeding group after 48 h of feeding in the field. Previous experiments have demonstrated that this time period is sufficient to detect the accelerated growth associated with larger groups in California (Fordyce and Agrawal 2001; Fordyce and Shapiro 2003; Fordyce 2003). Synchronizing the hatch of all eggs used for these experiments was not possible, thus the entire experiment could not be conducted at one time. Rather, full replicates of the factorial design (both populations and group sizes) were performed as egg hatching permitted. To account for variation in growth rate that might be due to different temperatures occurring over the time period in which the experimental replicates were carried out, we used the daily accumulated temperature as a covariate in the ANOVA model. Temperature was recorded at the location of the experiment using HOBO Data Loggers (Onset Computer Corporation, Bourne, MA). Because we were only interested in one benefit of aggregative feeding, accelerated growth rate, predators were excluded by enclosing the experimental plants in spun polyester mesh bags (Kleen Test Products, Brown Deer, WI).

RESULTS AND DISCUSSION

The average clutch size of the Texas population was 5.0 ± 0.3 (mean \pm SE; $n = 95$ clutches) with a range of 1–17. None of the plant characteristics we measured explained variation in clutch size ($F_{3,90} = 0.57$; $P = 0.63$). These findings are contrary to those reported by Pilson and Rausher (1988) who found that *B. philenor* populations in eastern Texas lay more eggs on larger plants that could support more larvae. However, their study populations use other species of *Aristolochia*. Perhaps more importantly, our observations of the Texas population began shortly after the plants had flushed. Large female egg load early in the season may have influenced the motivation of females whereby they were less discriminatory among host plants (Courtney 1984). Increased female motivation may also explain why we observed an

TABLE 1. Analysis of variance of larval weights after 48 h of feeding. None of the interactions with temperature approached significance in either experiment and subsequently were dropped from the model. (A) Experiment in California on *Aristolochia californica*. (B) Experiment in Texas on *A. erecta*.

Factor	df	MS	F	P
(A) California (<i>A. californica</i>)				
Population (P)	1	1.471	69.333	<0.0001
Group size (G)	1	0.214	10.109	0.0029
P × G	1	0.035	1.658	0.2055
Temperature	1	0.008	0.385	0.5385
Error	39	0.021		
(B) Texas (<i>A. erecta</i>)				
Population (P)	1	0.282	16.352	0.0003
Group size (G)	1	0.005	0.282	0.5992
P × G	1	0.001	0.060	0.8079
Temperature	1	0.053	3.089	0.0889
Error	33	0.017		

average clutch size that was larger than those previously described for other Texas populations (Rausher 1995). The average clutch size in California observed over the course of this study was 12.7 ± 0.5 eggs (mean \pm SE; $n = 198$ clutches) with a range of 2–38. This is consistent with the clutch size observed at various locations throughout the range of *A. californica*-feeding *B. philenor* (Tatar 1989, 1991; Fordyce and Agrawal 2001; Fordyce 2003).

We did find that some of the clutch size variation in California could be explained by the host plant characteristics ($F_{3,194} = 7.73$, $P < 0.0001$). Specifically, clutch size increased with the height of the plant (forward stepwise regression; $F_{1,196} = 22.0$, $P < 0.0001$). However, plant height explained only a small portion of the variation ($R^2 = 0.09$). The average plant height sampled in Texas was 16 cm and in California was 18.5 cm. In California, even plants less than 5 cm high had 10.2 ± 1.02 (mean \pm SE) eggs per clutch, notably larger than the average clutch size observed in Texas. Thus, it seems unlikely that the relationship between clutch size and stem height sufficiently explains the comparatively larger clutches in California compared to Texas.

The benefit of accelerated growth associated with larger feeding aggregations was detected in California, where females lay larger clutches, and was not detected in Texas, where females lay small clutches. Larger feeding aggregations grew faster on *A. californica* regardless of the population origin of the larvae (Table 1a; Figure 1a). In contrast, we found no effect of group size on growth of *B. philenor* larvae feeding on *A. erecta* in Texas (Table 1b; Figure 1b). That the observed geographic variation in clutch size and aggregative feeding is congruent with the observed variation in this benefit of aggregative feeding is consistent with the hypothesis that the larger clutches observed in California are an adaptation to *A. californica*. Previous studies have determined that the faster growth observed for larger feeding aggregations in California is a consequence of a group-size-dependent induced response in the plant that enhances the quality of *A. californica* for larval development (Fordyce 2003). Larger groups of larvae grew faster, regardless of their population origin. Faster growth reduces the amount of time larvae remain in vulnerable early stages (Fordyce and Agraw-

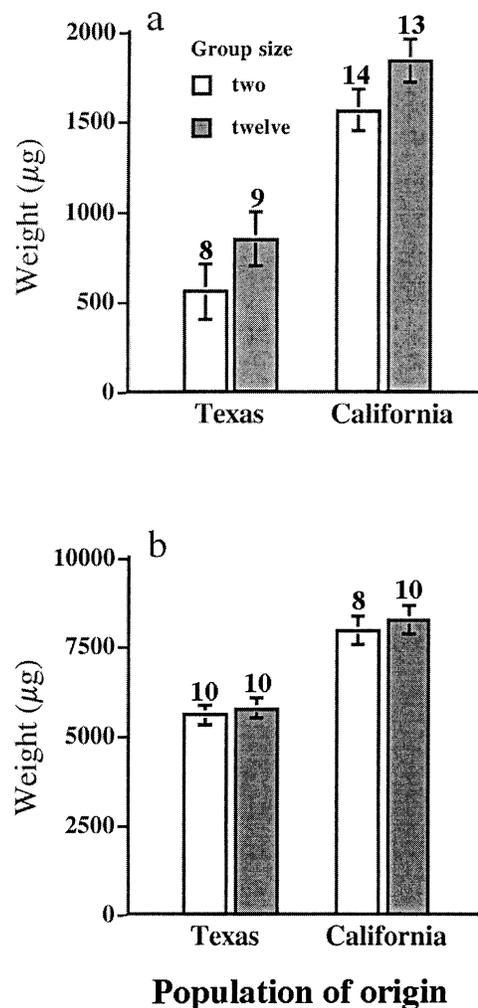


FIG. 1. Mean weight of larvae from California and Texas after feeding for 48 hours in groups of two or twelve individuals. (a) Experiment in California on *Aristolochia californica*. (b) Experiment in Texas on *A. erecta*. Numbers above bars indicate number of replicates for each treatment. Error bars are \pm one standard error.

wal 2001; Fordyce and Shapiro 2003). Geographic variation in clutch size may represent a form of phenotype matching (sensu Berenbaum and Zangerl 1998), where clutch size has been modified to exploit variation in this consequence of aggregative feeding.

The significant population effect for both experiments was due to a significant size difference of eggs and larvae of both populations. Larvae from the California population were larger than the Texas population (unpaired t -test; $t = 22.58$, $df = 72$, $P < 0.0001$). Hatching weight of California larvae was 934.9 ± 10.0 µg (mean \pm SE; $n = 50$) and of Texas larvae was 539.5 ± 14.4 µg (mean \pm SE; $n = 24$). Subsequent captive rearing experiments of both populations have demonstrated that this observed size difference is apparently genetically determined, as eggs and first instar larvae reared from laboratory cultures maintain their size differences and eggs and larvae of F_1 crosses are intermediate in size (J. A. Fordyce, unpubl. data).

Overall, the *A. erecta* from Texas may be a superior food

for developing larvae compared to *A. californica*. Larvae from both populations gained appreciably more weight on *A. erecta* compared to *A. californica* over the 48-hour period of each experiment. Although ambient temperature can have a significant impact on larval developmental rates, this is an unlikely explanation as the experiments in Texas and California were carried out under similar temperature conditions. Furthermore, the accelerated growth rate on *A. erecta* compared to *A. californica* was also observed under controlled lab rearing conditions, with individuals reared on *A. erecta* molting into the second instar three to four days earlier compared to larvae reared on *A. californica* (J. A. Fordyce, pers. obs.). It is unknown if this difference is a consequence of nutritional differences between the two plant species, leaf trichomes (which are present on *A. californica* but not on *A. erecta*), or some other unknown differences in resistance to herbivory. It is possible that accelerated growth associated with increased group size might be present on both plants, however the overall rapid growth on *A. erecta* might obscure its detection and negate any realized ecological benefit of accelerated growth.

Conclusions

This system provided us with the opportunity to examine variation in a benefit associated with a life-history character, specifically clutch size and aggregative feeding, within a taxon between populations employing decidedly different strategies. The population in California lays larger clutches, and on the California host plant accelerated growth is a consequence of larger feeding aggregations. This is consistent with the hypothesis that interpopulation variation in clutch size is an adaptation to local host plant characteristics, specifically the induced responses of the plant to various degrees of herbivore damage (Fordyce 2003). Ultimately, it demonstrates that the life histories of insect herbivores may be modified in ways that effectively manipulate or circumvent herbivore induced responses in plants.

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