

Population differences in host use by a seed-beetle: local adaptation, phenotypic plasticity and maternal effects

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Abstract For insects that develop inside discrete hosts, both host size and host quality constrain offspring growth, influencing the evolution of body size and life history traits. Using a two-generation common garden experiment, we quantified the contribution of maternal and rearing hosts to differences in growth and life history traits between populations of the seed-feeding beetle *Stator limbatus* that use a large-seeded host, *Acacia greggii*, and a small-seeded host, *Pseudosamanea guachapele*. Populations differed genetically for all traits when beetles were raised in a common garden. Contrary to expectations from the local adaptation hypothesis, beetles from all populations were larger, developed faster and had higher survivorship when reared on seeds of *A. greggii* (the larger host), irrespective of their native host. We observed two host plant-mediated maternal effects: offspring matured sooner, regardless of their rearing host, when their mothers were reared on *P. guachapele* (this was not caused by an effect of rearing host on egg size), and females laid larger eggs on *P. guachapele*. This is the first study to document plasticity by *S. limbatus* in response to *P. guachapele*, suggesting that plasticity is an ancestral trait in *S. limbatus* that likely plays an important role in diet expansion. Although differences between populations in growth and life history traits

are likely adaptations to their host plants, host-associated maternal effects, partly mediated by maternal egg size plasticity, influence growth and life history traits and likely play an important role in the evolution of the breadth of *S. limbatus*' diet. More generally, phenotypic plasticity mediates the fitness consequences of using novel hosts, likely facilitating colonization of new hosts, but also buffering herbivores from selection post-colonization. Plasticity in response to novel versus normal hosts varied among our study populations such that disentangling the historical role of plasticity in mediating diet evolution requires the consideration of evolutionary history.

Keywords Body size · Egg size · Host size · Insect–plant interactions · *Stator limbatus*

Introduction

Variation among host plants is an important determinant of phenotypic variation in herbivorous insects (Ohsaki and Sato 1994; Mira and Bernays 2002; Singer and Stireman 2003). Species for which host availability varies among populations may become genetically differentiated due to adaptation to their local hosts (Mopper 1996 and references therein). When trade-offs in host use exist, local adaptation can occur at the cost of decreased performance on alternative hosts (Van Zandt and Mopper 1998; Agrawal 2000). Variation in host availability could also result in the evolution of phenotypic plasticity, in which the same genotype expresses different phenotypes on different hosts (Via 1994). Phenotypic plasticity can be an important mechanism of adaptation to variable

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environments (Futuyma 2001), can facilitate colonization and expansion into new environments (Fox and Savalli 2000), and may even influence the evolution of community structure by molding multitrophic interactions (Agrawal 2001). Plastic responses to host plants may be mediated by maternal experiences, a phenomenon called cross-generational (or trans-generational) phenotypic plasticity (Mousseau and Dingle 1991), in which parents modify the phenotype of their offspring in response to environmental conditions (Fox and Mousseau 1998; Lacey 1998; Wade 1998; Mazer and Damuth 2001); e.g., mothers may program developmental changes in their offspring, or change patterns of resource allocation to their offspring, in response to predictive environmental cues (Czesak and Fox 2003 and references therein).

For insects that use discrete resources, such as parasitoids and seed feeders, host size and host quality are major sources of phenotypic variation among host species and may constrain offspring growth, influencing the evolution of body size and life history traits (Hardy et al. 1992; Allen and Hunt 2001; Mackauer and Chau 2001; Tsai et al. 2001). In species with scramble competition, individuals in populations adapted to large hosts are generally larger than those adapted to small hosts, generating genetic variation in body size among populations adapted to different hosts (Toquenaga and Fuji 1990). Also, because resources are more likely to run out in smaller than in larger hosts, insects mature at a smaller size and sooner in small hosts, generating phenotypic variation in body size and development time within populations (Kirk 1991). In our study system, the seed-feeding beetle *Stator limbatus*, beetles in populations adapted to the large-seeded host *Acacia greggii* are about 40% larger than are those adapted to the small-seeded host *Pseudosamanea guachapele*. This difference in body size is likely a consequence of adaptation to large versus small seeds and is associated with differences in a large suite of growth and life history traits.

The objective of this study was to quantify the relative contribution of environmental (host species), genetic (population) and maternal effects to differences in body size and life history traits between populations of *S. limbatus* developing on seeds of the small-seeded *P. guachapele* and the large-seeded *A. greggii*. Specifically, we asked: (1) what is the magnitude of the genetic differences in body size and life history traits between populations that use hosts of different size? (2) What is the influence of rearing host and oviposition host on body size and fitness-related traits? (3) How does maternal rearing host affect the phenotype of their offspring? (4) Do females exhibit adaptive egg size

plasticity in response to the species upon which they were reared or on which they oviposit?

Materials and methods

The beetle

Stator limbatus is a seed-feeding beetle (Coleoptera: Chrysomelidae: Bruchinae) distributed from the north of Argentina to the southwestern United States (Johnson and Kingsolver 1976; Johnson et al. 1989). Populations are found mostly in desert or semiarid environments on ≥ 80 host plant species in at least nine genera throughout its broad distribution. Although *S. limbatus* is considered a generalist because of the large number of hosts it uses, host use varies substantially among localities, and most populations use few hosts and are thus specialists relative to the diversity of plant species available to them (Fox et al. 1995; Morse and Farrell 2005a, 2005b).

Females of *S. limbatus* oviposit directly onto the mature seeds of their hosts. After hatching, the larvae burrow into, and develop completely inside, the seed. Beetles emerge from seeds as adults and start oviposition 12–48 h later. The complete life cycle takes 28–30 days at 28 °C. In another seed beetle, *Callosobruchus maculatus*, pupation represents ~30% of total development, though this is dependent on the host species and the temperature (Chandrankantha and Mathavan 1986).

The populations used for this study are from Colombia and Arizona (southwestern US). Each group of populations is in what Morse and Farrell (2005a) show to be different well-supported monophyletic clades: the South American clade and the North American clade of *S. limbatus*. Because the two Arizona and the two Colombia populations are more related to each other than to populations from different clades, we expect genetic differences to be larger between populations of different clades than between populations in the same clade.

The host plants

We compared populations adapted to the large seeds of *A. greggii* (Arizona, US) with populations adapted to the much smaller seeds of *P. guachapele* (Cundinamarca and Tolima, Colombia). *A. greggii* (Fabaceae) is a shrub to small tree distributed throughout much of the southwestern US and northern Mexico (Sargent 1965). It grows in dry areas on gravelly mesas, sides of low canyons and banks of mountain streams. Fruits

contain 1–5 round, laterally compressed, brown seeds with seed mass typically between 60 and 300 mg. Beetles access seeds by entering the pods through holes made by other insects or through cracks in the pods.

Pseudosamanea guachapele (Fabaceae) is a medium to large tree that grows mostly in pastures and dry areas from Guatemala to Ecuador. The dehiscent fruits have 10–25 small, oval, laterally compressed cream-colored seeds that vary in mass from 18 to 46 mg. Because the pods are dehiscent, beetles have direct access to the seeds once the pods mature.

Field collection and establishment of colonies

Beetles were collected on 10–20 August 2002 from *A. greggii* seeds at two localities in Arizona, US: Wenden 33°49'21"N; 113°32'27"W (Yavapai Co., AZ, USA) and Oracle (Pinal Co., AZ, USA) 32°36'39"N; 110°46'13"W, henceforth referred to as the "Arizona" populations. Beetles were collected from *P. guachapele* seeds between 28 December 2002 and 10 January 2003 at two localities in Colombia: Melgar, 4°13'83"N; 74°37'26"W (Tolima) and Anapoima 4°31'13"N; 74°32'22"W (Cundinamarca) in Colombia ("Colombia" populations).

Mature fruits were collected from >20 trees at each locality and brought to the lab. Fruits were opened and seeds bearing eggs were placed individually in petri dishes at 28 °C. Emerging beetles (>200) from each population were used to establish laboratory colonies. To remove any environmental effects (Fox et al. 1995), beetles from all populations were maintained in the laboratory at >100 families per generation at 28 °C, 15:9 light:dark on seeds of *A. greggii* for two generations (nine weeks) prior to beginning this experiment. Survivorship is high on *A. greggii* seeds for all populations studied here (see "Results") such that the rearing of beetles on this host seed imposed at most small amounts of selection on the Colombia populations.

Experimental design

To distinguish between maternal host versus rearing host effects, we used a two-generation rearing design in which half of the beetles from each population were raised on seeds of *A. greggii* and the other half were raised on seeds of *P. guachapele*. The emerging offspring from each host were then split into two groups that were mated and had their offspring raised on *A. greggii* (one group) or *P. guachapele* (the other group; Fig. 1).

The mating procedure for beetles from each population was as follows: 12 h after emergence from

A. greggii, three virgin females and two virgin males, all nonsiblings, were enclosed in a 60 mm Petri dish with ten seeds of a single host (either *A. greggii* or *P. guachapele*); these mating groups formed the *Parental Generation*. Beetles were mated in groups of two males and three females because Colombian females rarely lay eggs when kept in pairs (unpublished data). Mating groups were provided with sugar water. Offspring from each of these groups of five beetles were treated in the analysis as a single data point. The dishes were inspected every day until at least one egg was laid on each seed, for a total of ten eggs per family. Seeds containing eggs were divided into separate 15 mm Petri dishes (one seed/dish) and allowed to develop at a density of one egg per seed (excess eggs were scraped from the seed). Larvae were raised to adult at 28 °C, 15:9 light:dark. These larvae were *Generation 1*.

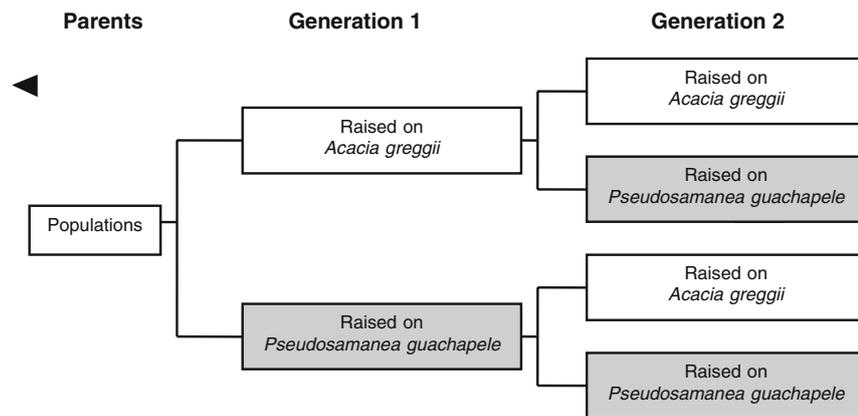
Generation 1 beetles were sexed and weighed within 12 h of emergence from the seed. Half of these beetles had been raised on *A. greggii* and half on *P. guachapele* seeds. For each group, half of the emerging adults were mated and allowed to oviposit on *P. guachapele*; the rest were mated and allowed to oviposit on *A. greggii* seeds. Larvae were again raised to adult at one individual per seed, 28 °C, 15:9 light:dark. These larvae constituted *Generation 2*. Upon emergence, these beetles were weighed and sexed.

Sample sizes for each generation were as follows: The Parental Generation consisted of 268 groups (families), giving rise to 1,543 adult offspring in Generation 1. From these Generation 1 beetles, we created 211 groups (families) that produced 1,388 offspring in Generation 2.

Data collection

We collected both reproductive data and survival/growth data. Reproductive data were collected for Generation 1 beetles. These beetles differed in their rearing host (*A. greggii* versus *P. guachapele*) and in the host upon which they oviposited. We scored adult body mass, age at first reproduction, egg size, and the number of eggs laid during the first 24 h of oviposition (the 24 h after the female's first egg was laid). Survival and growth data were collected on Generation 2 beetles. These beetles differed in both the host upon which they were raised and the host upon which their mother was raised. We recorded egg hatch, survivorship at different developmental stages (embryo, inside of seeds and total egg-to-adult), egg-to-adult development time (time between when the egg was laid and when the adult beetle emerged from the seed), and adult body mass.

Fig. 1 Three-generation rearing design used to evaluate maternal, oviposition and rearing host effects in four populations of the seed-feeding beetle *Stator limbatus*. Beetles were reared on either *Acacia greggii*, a large seeded host, or *Pseudosamanea guachapele*, a small-seeded host



All beetles were weighed on an electronic balance (Mettler Toledo AT261 Delta range; Columbus, OH, USA) to 0.01 mg. We also measured the lengths of two eggs for each dish using an ocular micrometer; egg length was the average of these two eggs (i.e., one mean egg size per group).

Analysis

For Generation 1 we used ANOVA (type III, sums of squares) to examine clade (country), population (nested within clade), sex, rearing host and oviposition host effects on age at first reproduction, egg size, and the number of eggs laid in the first 24 h of oviposition. We used group means as our lowest level of independence. Analyses in which interactions between variables were nonsignificant were repeated without the interactions. When the ANOVA yielded significant results, we performed specific post hoc comparisons between pairs of populations. For Generation 2 we used ANOVA to examine clade, population (nested within clade), sex, rearing host and maternal host effects on body mass and egg-to-adult development time. Survivorship was analyzed using logistic regression.

We used analysis of covariance to determine whether differences in egg size among treatments remained significant after controlling for the body size of the females laying those eggs. All statistical tests were done using SAS (SAS Institute 1985).

Results

Population effects (genetic effects)

Generation 1

There were significant differences between the two clades (Colombia versus Arizona) for age at first

reproduction and the number of eggs laid in the first 24 h of oviposition. Females from Arizona started to lay eggs sooner after emerging from their host seed than did females from Colombia, regardless of oviposition host (Fig. 2; Colombia $\bar{X}=3.2\pm 0.2$ days; Arizona $\bar{X}=1.6\pm 0.1$ days; $F_{(1,193)}=41.1$, $P<0.0001$). Egg size did not differ between clades (Fig. 3; $F_{(1,191)}=0.15$, $P=0.69$) but did differ between populations within clades; Oracle females laid the largest eggs and Wenden females laid the smallest eggs in all treatments. Arizona females also laid more eggs (twice as many) during the first 24 h of oviposition than did Colombia females (Fig. 4; Colombia $\bar{X}=5.9\pm 0.3$ eggs; Arizona $\bar{X}=12.9\pm 1.1$; $F_{(1,186)}=43.8$, $P<0.0001$).

Generation 2

Egg-to-adult development time and body mass varied among populations. Arizona beetles took longer to

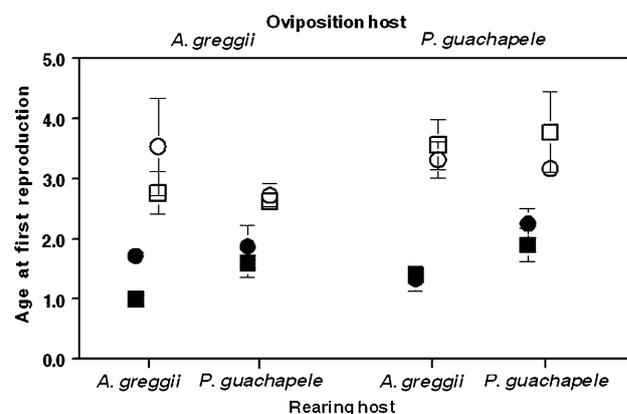


Fig. 2 Effect of oviposition host and rearing host on age at first reproduction of females from four populations of *Stator limbatus*. Solid symbols indicate populations from Arizona, USA [Oracle (filled squares), Wenden (filled circles)]. Open symbols represent populations from Colombia [Anapoima (open squares), Melgar (open circles)]. Standard error bars for some points are smaller than the symbols. The means presented are averages of group means for each treatment–population combination

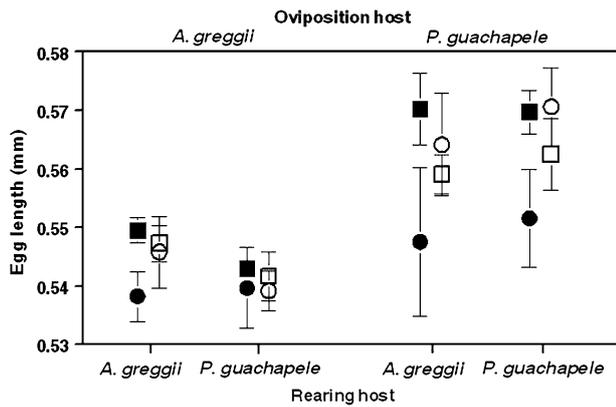


Fig. 3 Effect of oviposition host and rearing host on the size of eggs laid by females of four populations of *Stator limbatus*. Solid symbols indicate populations from Arizona, USA [Oracle (filled squares), Wenden (filled circles)]. Open symbols represent populations from Colombia [Anapoima (open squares), Melgar (open circles)]. Standard error bars for some points are smaller than the symbols

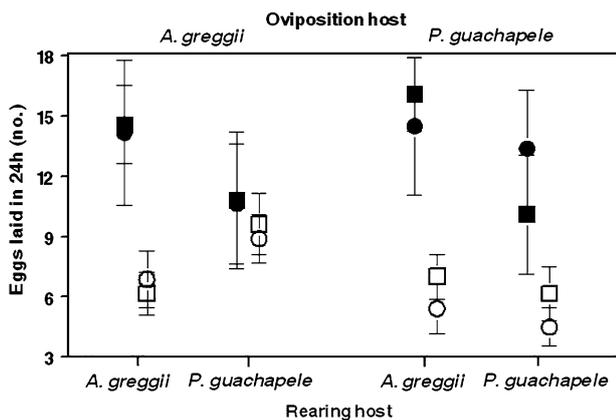


Fig. 4 Effect of oviposition host and rearing host on the number of eggs laid during the first 24 h of oviposition for females from four populations of *Stator limbatus*. [Oracle (filled square), Wenden (filled circle)]. Open symbols represent populations from Colombia [Anapoima (open square), Melgar (open circle)]. Standard error bars for some points are in some cases smaller than the symbols

develop to adult than did beetles from Colombia (Fig. 5; Colombia $\bar{X}=23.2\pm 0.1$ days; Arizona $\bar{X}=23.9\pm 0.2$ days; $F_{(1,369)}=4.29$, $P=0.039$). Also, regardless of treatment, beetles from Arizona were substantially larger than beetles from Colombia (Fig. 6; least squares means after removing treatment effects: Colombia $\bar{X}=1.53\pm 0.01$ mg; Arizona $\bar{X}=2.27\pm 0.04$ mg; $F_{(3,364)}=387.0$, $P<0.0001$). Males were larger than females in all populations (sex effect $F_{(1,364)}=49.2$, $P<0.0001$), as has been shown in other studies with this species. However, the degree of dimorphism differed between clades—Colombian

beetles were more sexually dimorphic than Arizona beetles (clade by sex interaction $F_{(1,368)}=8.23$, $P=0.0044$). The mean body size difference between sexes in Arizona beetles was 2.4%, but was 10% for Colombian beetles.

Rearing host and oviposition host effects

Generation 1

There was no significant effect of rearing host on the age at first reproduction ($F_{(1,193)}=0.02$, $P=0.9$), but females started laying eggs sooner when ovipositing on *A. greggii* (Fig. 2; laying on *A. greggii* $\bar{X}=2.6\pm 0.2$ days; laying on *P. guachapele* $\bar{X}=2.9\pm 0.2$ days; $F_{(1,193)}=4.7$, $P=0.032$). There was no significant effect of either rearing or oviposition host on the number of eggs laid in the first 24 h of oviposition (rearing host effect: $F_{(1,186)}=2.33$, $P=0.13$; oviposition host effect $F_{(1,186)}=0.24$, $P=0.62$).

Females exhibited egg size plasticity in response to their oviposition host. Irrespective of the population of origin, females laid larger eggs on seeds of *P. guachapele* than on *A. greggii* (Fig. 3; average size of eggs laid on *A. greggii*= 0.54 ± 0.004 mm; average size of eggs laid on *P. guachapele*= 0.57 ± 0.007 mm; $F_{(1,185)}=37.41$, $P<0.0001$). This difference was still highly statistically significant after controlling for female body size (i.e., including female body mass as a covariate; host effect on egg size, $F_{(1,188)}=42.56$, $P<0.0001$).

Generation 2

In general, seeds of *A. greggii* were a much better substrate for larval development than were seeds of *P. guachapele*; beetles from all populations experienced higher survivorship and matured sooner and larger when raised on seeds of *A. greggii*.

When performing the logistic regression containing all terms, survivorship at all stages of development was significantly higher when eggs were laid on *A. greggii* (Fig. 7; survivorship of embryo $X^2_1=9.3$, $P<0.002$; egg hatch $X^2_1=21.2$, $P<0.0001$; survivorship of larvae and pupae inside the seed $X^2_1=21.1$, $P<0.0001$; survivorship from egg to adult; $X^2_1=8.5$, $P<0.004$). However, survivorship was fairly high at all stages of development and thus effect sizes were small (Fig. 7). Also, the effect of rearing host differed between maternal host treatments (see ‘‘Maternal rearing host effect’’ section below).

Egg-to-adult development time was longer inside of *P. guachapele* seeds than inside *A. greggii* seeds (Fig. 5; 2.1 days longer in males and 2.2 days longer in females; $F_{(1,369)}=103.6$, $P<0.0001$). This pattern was still signifi-

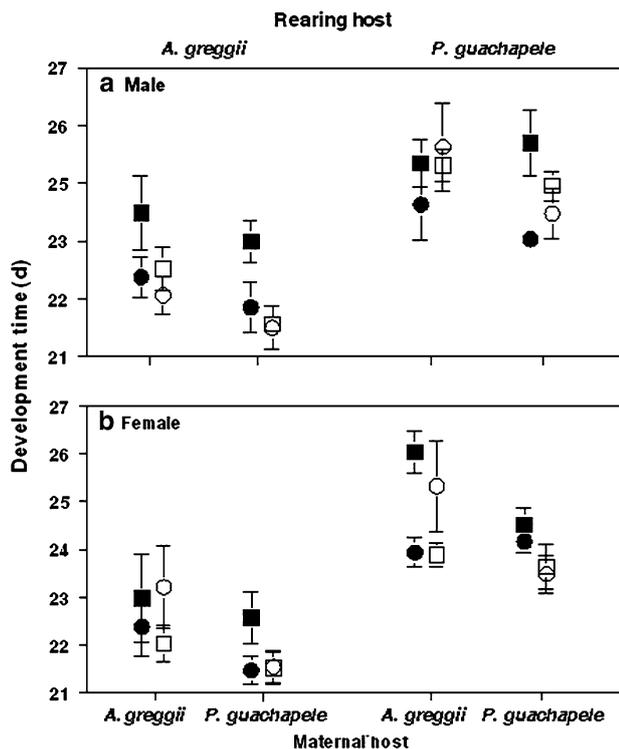


Fig. 5a–b Effect of maternal host and rearing host on egg-to-adult development time of (a) male and (b) female beetles from four populations of *Stator limbautus*. Solid symbols indicate populations from Arizona, USA [Oracle (filled squares), Wenden (filled circles)]. Open symbols represent populations from Colombia [Anapoima (open squares), Melgar (open circles)]. Standard error bars for some points are smaller than the symbols

cant after controlling for maternal egg size (host effect after controlling for maternal egg size, $F_{(1,375)}=103.15$, $P<0.0001$) and offspring body mass (host effect after controlling for body mass, $F_{(1,378)}=38.56$, $P<0.0001$), though beetles that matured larger also matured sooner (slope= -0.17 day/mg; $P=0.0006$).

Despite taking longer to reach maturity, beetles raised on *P. guachapele* were smaller than beetles raised on *A. greggii*, regardless of their native or maternal host (Fig. 6; average size of beetles emerging from *A. greggii*= 1.94 ± 0.37 mg; average size of beetles emerging from *P. guachapele*= 1.61 ± 0.02 mg; $F_{(1,364)}=367.8$, $P<0.0001$). Although beetles from all populations were larger when raised on *A. greggii*, the effect of rearing host differed between beetles from the two clades and differed between the sexes (clade by rearing host by sex interaction $F_{(1,368)}=8.23$, $P=0.0044$). Beetles from Arizona, which are much larger than beetles from Colombia, were more negatively impacted by rearing on *P. guachapele* seeds than were the smaller-bodied Colombian beetles; Arizona beetles were >20% smaller when raised on *P. guachapele* (relative to being reared on *A. greggii*; females were

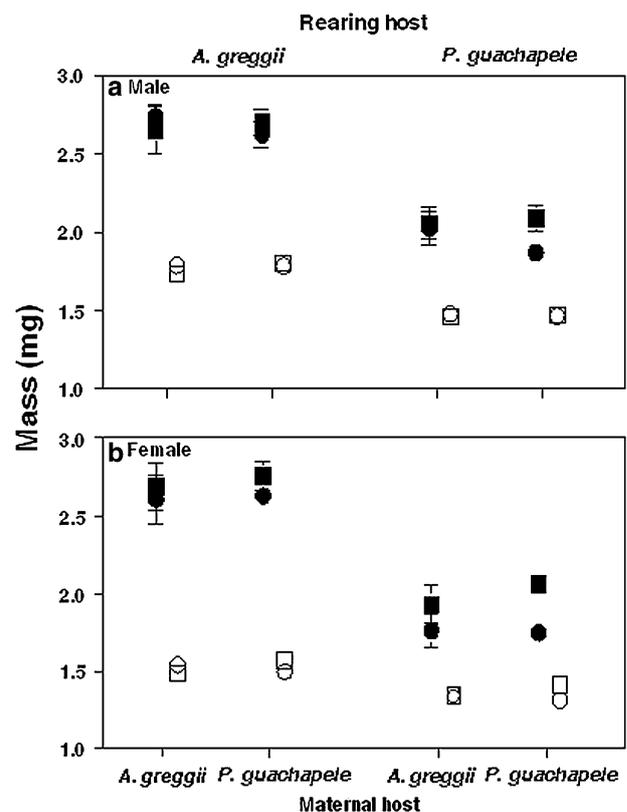


Fig. 6a–b Effect of maternal host and rearing host on body mass of beetles from four populations of *Stator limbautus*. a Males; b females. Solid symbols indicate populations from Arizona, USA [Oracle (filled square), Wenden (open circle)]. Open symbols represent populations from Colombia [Anapoima (open squares), Melgar (open circles)]. Standard error bars for some points are in some cases smaller than the symbols

29.5% smaller and males were 24.5% smaller), whereas Colombian beetles were only 11.0% (females) and 16.5% (males) smaller when raised on *P. guachapele*.

Maternal rearing host effect

A significant maternal host \times clade interaction was found for egg hatch ($X^2_1=4.72$, $P=0.02$), survivorship inside of the seed ($X^2_1=8.12$, $P=0.004$) and egg-to-adult survivorship ($X^2_1=8.44$, $P=0.004$). However, though statistically significant, the patterns are unclear (Fig. 7).

More evident is the result that beetles whose mothers were reared on *P. guachapele* emerged about one day sooner than beetles whose maternal host was *A. greggii*, regardless of the host on which the progeny were reared (Fig. 5; maternal host *P. guachapele*, $\bar{X}=23.0\pm 0.1$ days, maternal host *A. greggii*, $\bar{X}=23.8\pm 0.2$ days; $F_{(1,369)}=12.6$, $P=0.0004$). This effect of maternal host was still highly significant after controlling for egg size ($F_{(1,375)}=15.4$, $P=0.0001$) and for

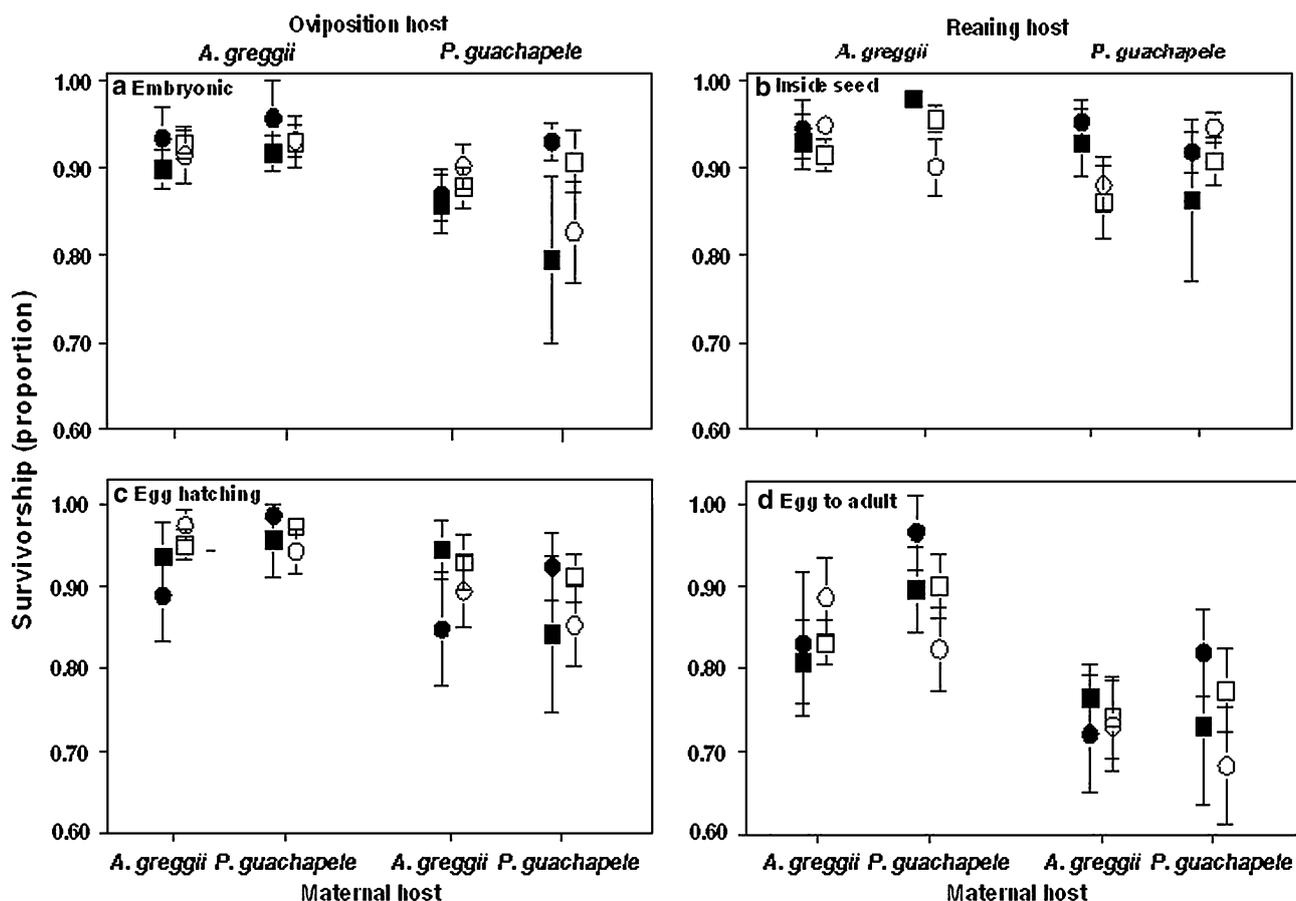


Fig. 7a–d Effect of maternal host and oviposition-rearing host on survivorship at different developmental stages (a–c) and total egg-to adult survivorship (d) for four populations of *Stator limbatus*. Solid symbols indicate populations from Arizona, USA

[Oracle (filled squares), Wenden (filled circles)]. Open symbols represent populations from Colombia [Anapoima (open squares), Melgar (open circles)]. Standard error bars for some points are smaller than the symbols

the mass of offspring ($F_{(1,378)}=18.7, P<0.0001$). Despite maturing sooner, offspring from mothers reared on *P. guachapele* were not smaller ($F_{(1,368)}=0.49, P=0.48$), indicating that maternal host affected development rate and not just development time. This result is contrary to the effect of rearing host on development time; beetles from all populations matured sooner (Fig. 5) and at much larger body size (Fig. 6) when raised on *A. greggii*.

Discussion

Population differences and plastic responses to host species

Even though populations from the two clades (Colombia and Arizona) exhibited significant genetically based differences in body size and life history traits, all populations of *S. limbatus* were phenotypically plastic in response to host species; they

developed faster and matured at a larger size inside *A. greggii* seeds than inside *P. guachapele* seeds. This plasticity may be in response to seed size or seed quality—*A. greggii* are substantially larger seeds, but may also be a better nutritional source. In agreement with the usual expectations for scramble-competing species (Hardy et al. 1992; Tsai et al. 2001) beetles matured at larger size when developing on large seeds. However, contrary to the typical host size effects, beetles also matured sooner on the large-seeded species (and thus had a higher growth rate). This is consistent with results from studies showing that development time decreases and adult mass increases when insects develop on high-quality hosts (Lindroth et al. 1991; Stockhoff 1993). We thus believe that many of the host effects observed here are due to nutritional differences between the species rather than just seed size effects. Further experiments are in progress to distinguish the relative effects of seed size from seed quality on responses to host species.

Our data also suggest that large-bodied beetles (e.g., from Arizona) suffer greater fitness costs than do small beetles when raised on small seeds—although beetles from all populations matured smaller when raised on the small seeds of *P. guachapele*, beetles from Arizona (which are larger) were affected most by host species. This result does not directly demonstrate selection on body size, but is suggestive. The seed-beetle for which the effects of host size on body size are best studied is *C. maculatus*, in which intense larval competition inside small seeds drives the evolution of contest competition favoring large larvae and leading to the evolution of large body size (Messina 1991a, 1991b, 2004; Toquenaga 1993). In contrast, populations adapted to larger-seeded hosts evolve scramble competition with larvae feeding at the periphery of the seeds, where the probability of encountering other larvae decreases. The absence of contest competition allows the evolution of small adults (Credland et al. 1986), possibly because maturing sooner (and thus smaller) reduces the probability of encountering potential competitors and reduces generation time.

We have no evidence that contest competition evolves in *S. limbatus*. Larval survival is high, even at high larval density on small seeds (A. Amarillo and Fox, unpublished data for both Arizona and Colombian populations). Also, beetles matured both sooner and at a smaller size when reared at high density, a response typical of species that exhibit scramble competition (Ode et al. 1996).

Maternal host effects

Maternal effects are widespread among all types of organisms (Gil et al. 1999; McIntyre and Gooding 2000a; Agrawal 2002; Reinhold 2002). In insects, they influence a large number of traits including larval survival, development time, wing morph, and sex ratio. Maternal effects also provide a mechanism by which organisms can deal with variable environments (Fox and Mousseau 1998). In generalist herbivorous insects, different host plants represent different sets of chemical and physical conditions with which offspring must cope. Female rearing environments, and their oviposition experiences, provide females with information on the hosts that their offspring will encounter. Females thus have the opportunity to modify traits such as egg size and composition (e.g., maternally derived proteins and mRNAs) in order to prepare offspring for the expected host species. Although many studies have now shown effects of maternal diet on offspring growth and development (reviews in Fox et al. 1995; Spitzer 2004) few have demonstrated that maternal effects

based on resource use are adaptive (Spitzer 2004). Those examples of adaptive resource-based maternal effects are largely cases in which females respond to host species or host quality to regulate offspring flight morphs (review in Fox and Mousseau 1998) or for which females manipulate egg size in response to oviposition substrate (see “Egg size plasticity” section, below) or in response to food stress (e.g., many cladocerans; discussed in Fox and Czesak 2000).

In this experiment *S. limbatus* offspring matured sooner (shorter egg-to-adult development time), regardless of rearing host, when the maternal rearing host was *P. guachapele*. This result is contrary to the effect of rearing host on development time; beetles from all populations matured sooner when raised on *A. greggii*. However, this result is similar to a maternal effect found for *S. limbatus* by Fox et al. (1995), in which offspring matured sooner when mothers had been reared on *Parkinsonia florida*, rather than *A. greggii*, regardless of offspring rearing host. That maternal effect was also contrary to the direct effect of rearing host on offspring—offspring reared on *P. florida* matured later than offspring reared on *A. greggii*. Fox et al. (1995) also found that maternal rearing host affected offspring body size (offspring were larger when their mothers were raised on *P. florida*), but no such effect was found in this current study. Neither Fox et al. (1995) nor ourselves in this current study found any evidence that offspring have higher fitness (higher survivorship, reduced development time or larger body size) when raised on the same host as their mother (i.e., no significant maternal host \times offspring host interactions). Our data thus indicate that maternal rearing host affects offspring through some as-yet unclear mechanism, but we have no evidence that *S. limbatus* mothers prepared their offspring for the specific host that the mothers had encountered (no evidence of adaptive “conditioning” or “acclimatization,” following the terminology of Via 1991 and Spitzer 2004, respectively). However, the observed maternal effect may be adaptive—though females do not prepare their offspring for a specific host, they may respond to the poor quality of their rearing substrate by changing their allocation to eggs, so that their offspring are better prepared to tolerate food stress or a lower quality host. This type of maternal effect has been observed in many cladocerans (e.g., food-stressed females lay larger and more energy-rich eggs; references in Glazier 1992). However, the adaptive significance, if any, of the maternal host effect observed in *S. limbatus* needs to be examined further.

The mechanism for the maternal rearing host effect in *S. limbatus* is not known. Previous studies have

shown that maternal effects on offspring development time are often due, at least in part, to effects on egg size (Fox 1997a, 1997b; Fox et al. 1999). Despite the regular result that variation in egg size mediates variation in development time, the maternal rearing host effect on development time observed in the current study is apparently not due to changes in egg size; egg size was not affected by maternal rearing host and the maternal host effect on development time was still statistically highly significant after including egg size as a covariate in the statistical model. The observed maternal rearing host effect is thus more likely due to changes in egg composition, such as egg energy reserves, maternally produced proteins (such as regulatory proteins or enzymes), or maternal mRNAs. Unfortunately, how maternal effects influence the composition of eggs is an area that is poorly studied in arthropods other than *Drosophila* (Rushlow et al. 1987; Girton and Jeon 1994). For herbivores, we know that egg energy reserves change with maternal age (McIntyre and Gooding 2000a) and female nutritional status (Murphy et al. 1983; Wallin et al. 1992; Fox and Dingle 1994), and that compounds sequestered by parents during development can be passed to offspring (Sime et al. 2000; Hartmann et al. 2004), but we know little else.

Egg size and egg size plasticity

Despite their much smaller body size, females from Colombia laid eggs similar in size to those laid by the much larger bodied Arizona beetles. Arizona *S. limbatus* are largely capital breeders—they use primarily larval-acquired resources for producing eggs, such that producing large eggs comes at a substantial fecundity cost to females (adult females will feed, and feeding does prolong their life, but it has very little effect on total fecundity). Though we did not quantify lifetime fecundity in this study, our data do show that fecundity in the first 24 h of oviposition is much lower in Colombian beetles than in Arizona beetles, as expected from their large egg size relative to their body size. Also, unpublished data (A. Amarillo) indicate that lifetime fecundity in the lab is very low for Colombian beetles and females do not lay eggs unless food is provided. Because selection for high fecundity is strong, the selection for high fecundity may be balanced by very strong selection favoring large eggs in Colombia beetles and, unlike Arizona beetles, Colombian beetles may use (and even require) adult food sources to produce eggs (i.e., they are income breeders). Such variation in allocation strategies (capital versus income breeding) within a species provides

an exciting opportunity to study the evolution of allocation strategies.

Within the Coleoptera and Lepidoptera there are a number of species that exhibit egg size plasticity in response to host species and/or quality (Leather and Burnand 1987; Nylin and Gotthard 1998; Awmack and Leather 2002; Ekbom and Popov 2004; Takakura 2004). Plastic responses to host quality, like the responses we observed in *S. limbatus*, are a strategy that allows organisms to cope with variation among hosts. Previous studies with *S. limbatus* have shown that females adjust the size of eggs they lay in response to the oviposition host species, but not in response to variation in the size of seeds within species (Fox et al. 1997; Savalli and Fox 2002). Specifically, populations of *S. limbatus* from Arizona and Texas (USA) lay larger eggs on seeds of *P. florida* (which produces seeds that are very resistant to larval penetration) than on seeds of either *A. greggii* or *P. microphylla* (which produce nonresistant seeds). This plasticity appears to be adaptive. Offspring from larger eggs have much higher survival during penetration of *P. florida* seed coats (thus selection favors large eggs on this host), but females laying larger eggs have substantially reduced fecundity relative to females laying smaller eggs (thus, selection favors small eggs on *A. greggii* and *P. microphyllum*, on which larval survival is high for small eggs).

In this current study we found that females from all populations laid larger eggs when ovipositing on *P. guachapele* than when ovipositing on *A. greggii* (note that we found no effect of maternal rearing host on egg size, but did find a large effect of maternal oviposition host on egg size). This is the first time plasticity in *S. limbatus* has been demonstrated to increase egg size in response to a host species other than *P. florida*. In contrast to the egg size plasticity exhibited by Arizona beetles in response to *P. florida*, the host effect on egg size observed here does not appear to be due to selection in order to overcome seed coat defenses. Larval mortality on *P. guachapele* was not affected by egg size. The larger eggs laid on seeds of *P. guachapele* may be an adaptive strategy that helps larvae compensate for the low quality and/or size of *P. guachapele* seeds; females may lay larger eggs either (a) as a mechanism to promote development on a poor quality nutritional source or (b) to prepare larvae for the small size of their host seed and the larval competition they are likely to experience. These hypotheses have yet to be tested.

Stator is a genus of beetles that mainly use seeds of legumes. Most species are specialists in that they use just a couple of species as hosts. In contrast, *S. limbatus*

has colonized ~80 legume species across all three legume families. They thus must cope with wide variation in seed quality, chemistry and size. Specialization on *Acacia* appears to be the ancestral trait in the genus *Stator*, and the generalist diet of *S. limbatus* appears to be derived from *Acacia*-specialized ancestors (Morse and Farrel 2005a). It is likely that the phylogenetic constraint on diet evolution (feeding on *Acacia*) was overcome in *S. limbatus* by the evolution of egg size plasticity, allowing the species to colonize a wide diversity of host species and become a relative generalist. The Colombian and Arizona populations both responded to *P. guachapele* by increasing egg size (relative to the size of eggs laid on *A. greggii*). Both populations also respond to *P. florida* by laying large eggs (unpublished data). That Colombian and Arizona populations are located on very divergent clades (Morse and Farrell 2005a) supports the hypothesis that egg size plasticity is ancestral within *S. limbatus*; that egg size plasticity evolved before the divergence between clades is more parsimonious than the alternative hypothesis that egg size plasticity evolved separately in each clade. Recent studies of *S. limbatus* colonization of non-native (ornamental or invasive plants) species in the southwestern US support this hypothesis—the survival of offspring on novel hosts following colonization is influenced by female experiences pre-colonization and the effects of these experiences on the size and composition of eggs laid by females (Fox 2006; Fox et al. 2006). We propose that egg size plasticity is an adaptive trait that has played an important role in diet expansion and diversification in *S. limbatus* and may be the feature of this beetle's life history that allowed it to evolve a generalist lifestyle.

In conclusion, we have demonstrated that populations of *S. limbatus* that use different hosts have diverged in body size and life history traits. However, all *S. limbatus* populations exhibited substantial host-associated phenotypic plasticity. This plasticity, both by offspring (e.g., development time and body size) and their mothers (egg size plasticity, which affects offspring as a maternal effect) likely buffers these beetles from high mortality or low fitness that they would otherwise experience when encountering novel hosts, and thus likely facilitates colonization of novel hosts. However, phenotypic plasticity also buffers organisms from selection post-colonization, reducing the rate at which populations adapt to novel hosts (Strauss et al. 2006). In addition, plasticity in responses to novel environments (e.g., host species) may be asymmetrical, with some populations (e.g., large-bodied Arizona populations of *S. limbatus*) experiencing greater fitness costs than others (e.g., small-bodied

Colombia populations) when exposed to lower quality (e.g., smaller-seeded) hosts. Disentangling the historical role of plasticity in mediating the colonization of new environments, and subsequent adaptation to those environments, requires consideration of the phylogenetic history of the species and populations being studied.

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