GENETIC AND ENVIRONMENTAL SOURCES OF VARIATION IN SURVIVAL ON NONNATIVE HOST SPECIES IN THE GENERALIST SEED BEETLE, *STATOR LIMBATUS*

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ABSTRACT—Humans have introduced organisms into many new and often novel environments. These changes in organism distributions provide us an opportunity to directly observe the ecological and evolutionary processes that influence adaptation. Here we examine the suitability of a variety of introduced legume species, plus several non-host native species, for the development of *Stator limbatus* larvae. About half of the species examined were capable of supporting larval development, though larval survival was high on few non-host species. Maternal experiences during egg maturation affected larval survival on seeds of *Parkinsonia aculeata* and *Ebenopsis ebano*, but not on any other non-hosts; females that matured eggs in contact with seeds of *Parkinsonia florida*, a native host of *S. limbatus* in the Sonoran Desert, produced larger eggs and had offspring with higher survival on seeds of these 2 exotic species than if females did not encounter *P. florida* during egg maturation. Using a half-sib breeding design, we detected genetic variation in larval survival on seeds of *P. aculeata*, but found that maternal experience during egg maturation (whether or not females encountered seeds of *P. florida*) was the major factor affecting offspring survival on *P. aculeata*. We conclude that the ability of *S. limbatus* larvae to survive on some non-native plant species depends on the species composition of the local plant community – female experiences with *P. florida* induce changes in egg size and composition that subsequently influence survival of larvae on seeds of the novel host, *P. aculeata*.

RESUMEN—El ser humano ha introducido organismos en una gran variedad de ambientes que con frecuencia son nuevos para las especies introducidas. Estos cambios en las distribuciones de los organismos brindan una oportunidad para observar directamente los procesos ecológicos y evolutivos que influyen la adaptación. En este trabajo se examina la idoneidad de una variedad de especies de legumbres introducidas, más algunas especies autóctonas no-hospederas, para el desarrollo de las larvas de *Stator limbatus*. Aproximadamente la mitad de las especies examinadas fueron capaces de sostener el desarrollo larvario, aunque la supervivencia de las larvas fue alta en pocas especies no-hospederas. Experiencia maternal durante el período de maduración de los huevos afectó la supervivencia de las larvas en las semillas de *Parkinsonia aculeata* y *Ebenopsis ebano*, pero no en ningún otro no-hospedero; las hembras que maduraron huevos en contacto con las semillas de *Parkinsonia florida*, un hospedero nativo de *S. limbatus* en el desierto de Sonora, produjeron huevos más grandes y crías con una mayor tasa de supervivencia en semillas de estas 2 especies exóticas que si las hembras no encontraron a *P. florida* durante la maduración de los huevos. Usando un diseño de cruzar medio-hermanos, se detectó variación genética en la supervivencia en las semillas de *P. aculeata* pero se mostró que la experiencia maternal durante la maduración de los huevos (independientemente de si las hembras estuvieron en contacto con semillas de *P. florida* o no) fue el factor más importante que afectó la supervivencia de las crías en *P. aculeata*. Se concluye que la capacidad de las larvas de *S. limbatus* de sobrevivir en algunas especies de plantas no autóctonas depende de la composición de especies de la comunidad vegetal local – la experiencia de las hembras con *P. florida* induce cambios en el tamaño y composición de los huevos que afecta de manera subsiguiente la supervivencia de las larvas en las semillas de una planta hospedera nueva, *P. aculeata*.

Most parasitic insects are relatively specialized in their diet. Host range seems to be evolution-arily conserved in many species (Mitter et al., 1991), but is evolutionarily dynamic in others

(e.g., nematodes on mycophagous *Drosophila*; Jaenike and Perlman, 2002). The ecological and evolutionary processes that influence host-range evolution, facilitating host shifts in some species but preventing host shifts in others, are poorly understood. However, human activities have caused many species to expand their ranges (Pitelka et al., 1997), and humans have introduced organisms into many new and often novel environments (Vitousek et al., 1996), providing an opportunity to directly observe the ecological and evolutionary processes that influence host range (Thompson, 1998). For example, plants introduced to new localities are often colonized by new herbivores, providing an opportunity to study diet expansion and shifts onto new hosts, processes that could otherwise only be inferred from current patterns (Tabashnik, 1983; Thomas et al., 1987; Bowers et al., 1992; Carroll and Boyd, 1992; Fraser and Lawton, 1994; Leclaire and Brandl, 1994; Fox et al., 1997*a*; Fox and Savalli, 2000).

For herbivorous insects, successful colonization of novel hosts, whether native or introduced, requires that at least some females will oviposit and that their larvae will feed on the potential host (Johnson and Siemens, 1991; Gratton and Welter, 1998). Subsequent adaptation to the new host requires genetic variation in larval growth and survival. An absence of genetic variation in behavior towards, or performance on, the potential host plant will limit the ability of insects to colonize and adapt to the new host. The observations that many taxa of herbivorous insects are highly conserved in their diet, and that most are relative specialists compared to the range of potential hosts available, suggests that genetic variation in the traits required to recognize novel plants as potential hosts, and subsequently adapt to these plants, is lacking (Futuyma et al., 1993, 1994, 1995; but see Blows and Hoffmann, 2005). Recent studies have found mixed results; some have found adequate variation present to allow insects to adapt to potential new hosts (Ueno et al., 1999, 2001, 2003) and even observed rapid adaptation to new host species (van Klinken and Edwards, 2002), whereas genetic variation was lacking in other insects (Schieffer, 1999). This variation in results among studies indicates that the presence of genetic variation in performance on new hosts depends on the specific plant and insect species/population examined (Futuyma et al., 1995;

Nielsen, 1997; Ballabeni and Rahier, 2000; Alleyne and Wiedenmann, 2002).

Stator limbatus (Coleoptera: Chrysomelidae: Bruchinae) is a widely distributed seed-feeding beetle distributed from northern South America to the southwestern United States. It is a relative generalist in that it uses seeds of >70 species as hosts, substantially more than most other species in the genus *Stator* and more than most other bruchines (Janzen, 1980; Johnson and Kingsolver, 1976; Johnson et al., 1989; Morse and Farrell, 2005*a*, 2005*b*). However, within any specific location, *S. limbatus* generally uses seeds of only a few species as hosts; it excludes the majority of legumes present from its diet. Because many legumes are popular as ornamental plants, a large variety of non-host species have been introduced into the southwestern United States. Some of these have been incorporated into the diet of *S. limbatus*, whereas others have not. For example, Jerusalem thorn (Mexican paloverde), *Parkinsonia aculeata* (Caesalpinaceae), has been planted as an ornamental in arid regions on multiple continents, but has escaped cultivation and is highly invasive (Paynter et al., 2003). The ancestral distribution of *P. aculeata* in North America is unclear due to its invasiveness; it is native only in parts of the southwestern United States (Isley, 1975) and highly invasive in much of California, Arizona, and Texas (Van Devender et al., 1997), where it is often fairly abundant. Most native species in the genus *Parkinsonia* (and the former genus *Cercidium*) are readily used by *S. limbatus* as hosts (Fox et al., 1996), but *S. limbatus* is rarely found on *P. aculeata* in locations where the plant is native (Johnson and Siemens, 1991). However, in some locations where *P. aculeata* has escaped from cultivation, it has been colonized by *S. limbatus*, though survival of *S. limbatus* larvae on *P. aculeata* seeds is low.

Previous studies have demonstrated that colonization of nonnative hosts by *S. limbatus* is dependent on the host species present in the local community (Fox et al., 1997*a*; Fox and Savalli, 2000; Fox, 2006). This previous work has focused on Texas ebony (*Ebenopsis ebano*; formerly *Chloroleucon ebano* and *Pithecellobium flexicaule*), a mimosoid legume (Mimosaceae). Like *P. aculeata*, *E. ebano* is native throughout much of the range of *S. limbatus* but is only used as a host in areas where the plant is an exotic species. Larval *S. limbatus* survival is low on seeds of *E.*

ebano unless females (mothers) encounter seeds of *Parkinsonia florida* during egg maturation. Females respond to seeds of *P. florida* by changing both the size and composition of their eggs in such a way that larval survival on seeds of *P. florida* is dramatically increased (Fox et al., 1997*b*, 2001). This plasticity in egg size and composition also affects larval survival on seeds of *E. ebano* (Fox and Savalli, 2000; Fox, 2006): females that oviposit on seeds of *E. ebano* without experience with seeds of *P. florida* produce offspring that have low survival on *E. ebano* (<3%) relative to the survival of larvae produced by females that experience seeds of *P. florida* during egg maturation (survival as high as 50% on *E. ebano*). This maternal effect is significant in nature because Texas ebony is widely planted in areas where *P. florida* is abundant (e.g., the Phoenix area of Arizona), and field experiments demonstrate that most females ovipositing on Texas ebony seeds have previously encountered *P. florida* seeds (Fox, 2006).

We have argued that this plasticity in egg size and composition is the likely explanation for why *S. limbatus* is so widespread and such a broad generalist relative to other species in the genus *Stator* (Fox and Savalli, 2000; Fox, 2006). However, it is unknown how widely maternal experience and, thus, the composition of the local community affects the survival of *S. limbatus* larvae on other native and invasive species.

We examined the suitability of a variety of introduced and non-host native legume species for development of *S. limbatus* larvae and tested whether maternal encounters with *P. florida* affect larval survival on these species. We also tested for genetic variation in the ability of larvae to survive on seeds of *P. aculeata* and explored the effect of maternal experience during egg maturation (exposure to *P. florida* seeds) and the resulting plasticity in egg size and composition on the survival of larvae on seeds of *P. aculeata*.

METHODS—*Survival of* S. limbatus *on Non-host Species*— We purchased seeds from 2 suppliers of ornamental plant seeds for the southwestern United States (Sheffield's Seed Company, Locke, New York and S&S Seeds, Carpinteria, California) and 2 suppliers of agricultural and garden seeds (Seedway, Hall, New York and Vermont Bean Company, Randolph, Wisconsin). Collection information on the seeds was not available, but both southwestern seed sources provide desert seeds for gardens and restoration projects in the southwestern United States. All seeds were certified pesticide free. Seeds of Texas ebony, *E. ebano*, were collected in

the field from >50 trees within an approximately 1-ha area in Mont Meta Memorial Park, San Benito, Cameron County, Texas. Seeds were classified as either native non-hosts or exotic (Table 1). Native non-hosts were species that occurred sympatrically with *S. limbatus*, but are not used by *S. limbatus* under natural conditions. Exotic species were those not native to the southwestern United States and not used as hosts by *S. limbatus* in regions where they are native. Naturalized refers to exotics that have escaped cultivation in the southwestern United States. Three of the species we examined are agricultural species commonly grown sympatrically with *S. limbatus* populations: *Vigna angularis*, *V. unguiculata*, and *V. radiata*, each of which is a host species for *Callosobruchus maculatus*, a different seed beetle.

In 2 sequential experiments, we screened the suitability of seeds of these species for development of larval *S. limbatus*. In the first experiment, each replicate ($n = 5$ per plant species) represented a mated pair confined with 15 to 20 seeds of a single plant species in a 25-mm Petri dish. The female was allowed to lay eggs until death. We scored whether females laid any eggs and then raised larvae to adults at one larva per seed (extra eggs were scraped from the seed prior to hatching) at 30°C and 15:9 light:dark. This experiment included all species listed in Table 1.

In the second experiment, we again screened the suitability of seeds for larval development, but we modified our design to consider the influence of maternal egg size plasticity on the ability of larvae to survive on seeds of the novel hosts. Each replicate ($n = 10$ per plant species) represented one mated pair that was first confined on 8 seeds of either *A. greggii* or *P. florida* until the female laid at least one egg. During this period, females responded to the seeds with which they were confined and started laying larger eggs if in contact with *P. florida* and smaller eggs if in contact with *A. greggii* (Fox et al., 1997*b*). This design was intended to mimic the situation in which females emerge from a native host species (either *A. greggii* or *P. florida*), or at least experience the normal host during the early part of the life of the adult female, and then colonize the nonnative species or the native non-host. Females were then transferred to a dish containing 15 to 20 seeds of their test host (species listed in Table 2) and allowed to lay eggs for 24 h. Eggs laid within this 24-h period were reared to adult at 30°C and 15:9 light:dark.

This second experiment was replicated with 2 populations of beetles, one collected from seeds of *P. florida* along Highway 17 at the north end of Phoenix, Maricopa County, Arizona, and the other collected from seeds of *A. greggii* near Clear Creek-Cape Verde in the Verde Valley, Yavapai County, Arizona. Only Phoenix beetles were used in the first experiment.

Genetic Variation in Survival on P. aculeata—Beetles for this experiment were collected from *A. greggii* at 2 locations, the first along American Avenue in Oracle, Pinal County, Arizona (Oracle population), and the second near Clear Creek-Camp Verde, Yavapai County, Arizona (Verde Valley population). *Parkinsonia aculeata* seeds were obtained from 2 seed suppliers (Sheffield's Seed Company and S&S Seeds). Though collection information is unavailable for these seeds, both companies are regular suppliers of seeds for ornamen-

TABLE 1—Preliminary screening of non-host seed suitability for *Stator limbatus* larval growth. *n* is the number of females that laid eggs on the host/number of pairs tested. Mean survivorship is calculated by average across the families.

Plant species	Native or exotic	*n*	Larval survivorship (mean ± *SE*)
Caesalpiniaceae			
Bauhinia purpurea	Exotic	4/5	0
B. variegata	Exotic	3/5	0
Caesalpinia pulcherrima	Exotic	5/5	0.323 ± 0.162
Cercis canadensis	Exotic[2]	3/5	0
C. occidentalis	Native, non-host	5/5	0
Parkinsonia aculeata[3]	[5]	3/5	0
P. aculeata [3]	[5]	2/5	0.161 ± 0.089
Mimosaceae			
Acacia baileyana[3]	Exotic, naturalized, colonized	4/5	0.428 ± 0.077
A. baileyana[3]	Exotic, naturalized, colonized	3/5	0.611 ± 0.223
A. longifolia	Exotic, naturalized	0/5	—[4]
A. mearnsii	Exotic	4/5	0.408 ± 0.069
Albizia julibrissin	Exotic, naturalized, colonized	5/5	0.579 ± 0.173
Delonix regia	Exotic	5/5	0
Leucaena leucocephala	Native, non-host	5/5	0
Prosopis glandulosa	Native, non-host	0/5	—[4]
P. juliflora[1]	Native, non-host	4/5	0.583 ± 0.150
Papilionaceae			
Amorpha fruticosa	Native, non-host	0/10	—[4]
Cytisus scoparius	Exotic, naturalized	1/5	0
Olneya tesota	Native, non-host	2/5	0
Robinia neomexicana	Native, non-host	5/5	0

[1] Unknown whether this was *P. juliflora* var. *velutina* or *P. juliflora* var. *torreyana*, which are often considered separate species.

[2] Native to eastern USA, but not in the range of *S. limbatus*.

[3] Seeds from different suppliers: Sheffield's Seed Co. and S & S Seeds, respectively.

[4] No females laid eggs on these seeds and, thus, larval survival could not be scored.

[5] Native non-host in part of the range of *S. limbatus*; exotic, naturalized in other parts.

tal and restoration projects in the southwestern United States. All seeds were certified pesticide-free.

We used a standard half-sib design (Roff, 1997) to test for genetic variation in the ability of larvae to survive on *P. aculeata* seeds. A half-sib design is one in which each male (sire) is mated to multiple females (in our case, 4 females) to create 4 full-sib families per sire. These families thus share the same father (and are, thus, half-sibs to each other) but have different mothers, such that we have 4 independent groups of offspring per male. Variation among full-sib families in the survivorship of offspring might be due to variation in the genotypes of the mothers (dams) or due to variation in egg size or composition (i.e., a maternal effect). However, because males contribute primarily genes to their offspring, variation among sires in the survival of their offspring is due to additive genetic variation among those sires. We can, thus, test for the presence of genetic variation in survival on *P. aculeata* by testing for a significant sire effect in an analysis of variance (Roff, 1997).

To create half-sib families, males were mated sequentially to 4 virgin one-day-old females (seeds were isolated to ensure that emerging females were virgin). Prior to mating, the females were exposed to seeds of either *P. florida* (to condition them to lay large eggs; *n* = 59 sires for the Oracle population, *n* = 51 sires for the Verde Valley population) or *A. greggii* (to condition females to lay small eggs; *n* = 60 sires for the Oracle population, *n* = 58 sires for the Verde Valley population) for 24 h prior to the addition of a male. As in the previous experiment, this design was intended to mimic the situation in which females emerge from a native host species (either *A. greggii* or *P. florida*), or at least experience the normal host during the early part of the adult females life (e.g., mating on these hosts), and then colonizes *P. aculeata*. *Parkinsonia florida* and *A. greggii* are 2 of the 3 most common hosts for *S. limbatus* in Arizona; the third common host is small-leaf paloverde (*Parkinsonia microphylla*), on which females lay small eggs; i.e., the size of eggs laid on *P. microphylla* is not different than the size on *A. greggii* (Fox et al., 2001).

After a male was added to the dish with the female, the pair was confined with the conditioning host until the female laid at least one egg (dishes were checked

TABLE 2—The effect of maternal (*Stator limbatus*) experience during egg maturation on the survival of her larvae on a range of host and non-host species. *n* is the number of females that laid eggs on each host/number of pairs set up on each host. *Parkinsonia aculeata* seeds were from Sheffield's Seed Co.

| | *Acacia greggii*-conditioned | | | | *Parkinsonia florida*-conditioned | | | |
| | Phoenix | | Verde Valley | | Phoenix | | Verde Valley | |
Plant species	Larval survivorship (mean ± SE)	*n*	Larval survivorship (mean ± SE)	*n*	Larval survivorship (mean ± SE)	*n*	Larval survivorship (mean ± SE)	*n*
Caesalpiniaceae								
Bauhinia purpurea	0	10/10	0	7/10	0	6/10	0.05 ± 0.05	3/10
B. variegata	0	5/10	0	4/10	0	8/10	0	1/10
Caesalpinia pulcherrima	0.25 ± 0.13	8/12	0.21 ± 0.14	4/10	0.33 ± 0.17	6/7	0.04 ± 0.04	6/10
Cercis canadensis	0	7/10	0	4/10	0	5/10	0	7/10
C. occidentalis	0	8/10	0	7/10	0	7/10	0	8/10
Parkinsonia aculeata	0	10/10	0.01 ± 0.01	9/10	0.13 ± 0.05	7/10	0.10 ± 0.04	7/9
P. florida	–	–	0.05 ± 0.03	8/10	–	–	0.39 ± 0.14	9/9
Mimosaceae								
Acacia gregii	–	–	0.79 ± 0.10	10/10	–	–	0.85 ± 0.04	10/10
Albizia julibrissin	0.92 ± 0.05	10/10	0.92 ± 0.02	9/9	0.73 ± 0.08	10/10	0.90 ± 0.03	10/10
Ebenopsis ebano	0.03 ± 0.03	10/10	0	10	0.14 ± 0.06	10/10	0.29 ± 0.07	10/10
Delonix regia	0	9/10	0	7/10	0	9/10	0	9/10
Olneya tesota	0	5/10	0	5/10	0	9/10	0	4/10
Prosopis glandulosa	–[1]	0/10	–	–	–[1]	0/10	–	–
P. juliflora	0.42 ± 0.19	6/10	0.59 ± 0.16	5/10	0.83 ± 0.10	5/10	0.53 ± 0.19	4/10
Papilionaceae								
Vigna angularis var. *angularis*	–	–	0	3/10	–	–	0	3/10
V. unguiculata[2]	–	–	–[1]	0/10	–	–	–[1]	0/10
V. radiata var. *radiata*	–	–	0	5/10	–	–	0	6/10

[1] No females laid eggs on these seeds and, thus, larval survival could not be scored.

[2] California blackeye.

twice per day), after which the female was transferred to a dish containing 20 *P. aculeata* seeds and the male was transferred to a new female. Half of the females were transferred to dishes containing Sheffield-variety *P. aculeata* seeds and the other half to dishes containing S&S-variety *P. aculeata* seeds. Some females did not mate or failed to lay eggs. We discarded families if the male failed to fertilize at least 2 females.

Females were allowed to lay eggs for only 24 h because the effect of conditioning host declines rapidly after 24 h (Fox et al., 1997b; Savalli and Fox, 2002). Larvae were raised to adult at one egg per seed (extra eggs were scraped from the seeds) at 30°C and 15:9 light:dark. We also measured the size (length) of 3 eggs per female to test for egg-size effects on larval survival. Egg size is a major determinant of larval survival on a variety of hosts (Fox and Mousseau, 1996; Fox et al., 2001). We used analysis of variance to test for variation in mean survivorship among sire families (a random effect), with the mean survivorship of each full-sib family treated as a single data point (survivorship means were arcsine-square root transformed for the analysis). We used analysis of covariance to test for

effects of egg size on larval survival, and we used logistic regression for all other analyses.

RESULTS—*Survival of* S. limbatus *on Non-host Species*—Females laid eggs on seeds of most of the exotic and non-host native legume species (Tables 1 and 2). Notable exceptions were *Prosopis glandulosa*, *Amorpha fructicosa*, *Acacia longifolia*, and *Vigna unguiculata*, upon which females did not lay eggs. Despite laying eggs on many non-host species, most species of seeds were either unsuitable or only marginally suitable for larval development (Tables 1 and 2). However, larvae had moderately high survivorship on a few species, including *Albizia julibrissin*, *Prosopis juliflora*, and all of the *Acacia* species upon which females oviposited. Exposure of females to *P. florida* seeds during egg maturation improved survival of their offspring on only 3
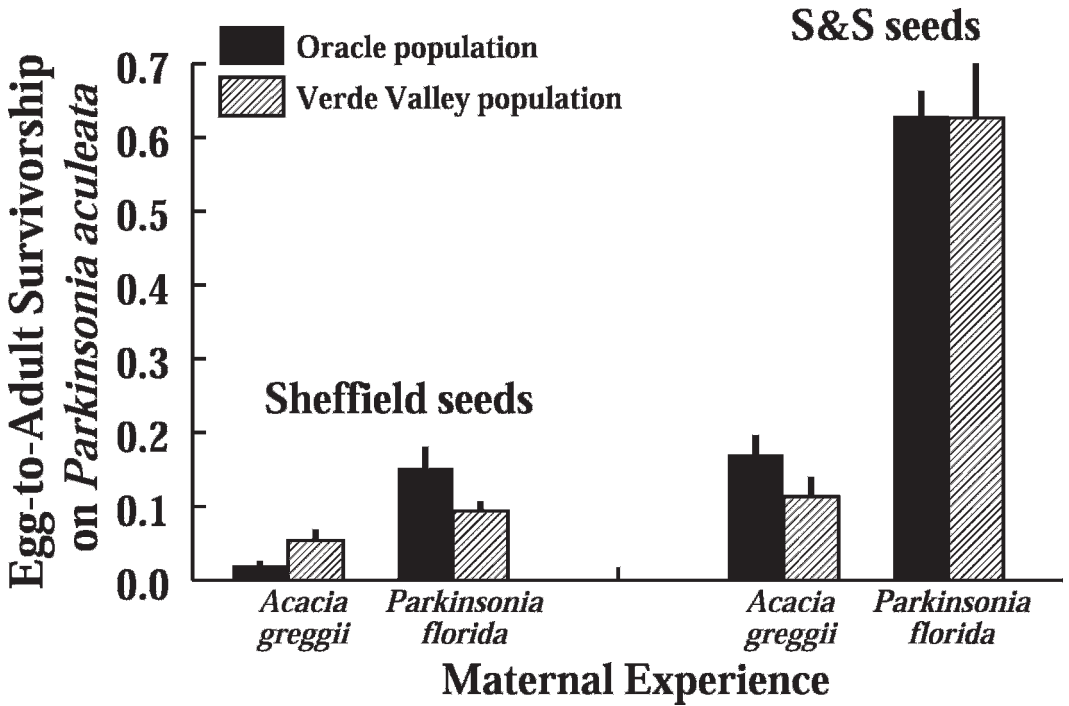
FIG. 1—Egg-to-adult survivorship ($\pm$ SE) of beetles reared on seeds of *Parkinsonia aculeata*. Sheffield and S&S seeds refer to sources of *P. aculeata* seeds; Oracle and Verde Valley are 2 populations of beetles reared on these seeds. Females were confined with seeds of either *Acacia greggii* or *Parkinsonia florida* during egg maturation, which influences both the size and composition of their eggs (see Fig. 2). All larvae were then reared on seeds of *P. aculeata*.

host species: *P. florida* (a native host), *E. ebano*, and *P. aculeata* (Table 2; $P < 0.05$ for each).

Genetic Variation in Survival on P. aculeata—Survivorship on seeds of *P. aculeata* was primarily influenced by the seed to which females were exposed during egg maturation (Fig. 1); females exposed to *P. florida* seeds laid significantly larger eggs (analysis of variance; $F_{1,499} = 100.2$, $P < 0.001$) and larvae hatching from these eggs had much higher egg-to-adult survivorship than larvae hatching from eggs laid by females exposed to *A. greggii* (Wald $\chi^2_1 = 150$, $P < 0.001$). The source of *P. aculeata* seeds (S&S versus Sheffield) had a significant effect on the survivorship of larvae; larvae had higher survivorship on S&S seeds than Sheffield seeds (Wald $\chi^2_1 = 157$, $P < 0.001$; Fig 2). There was a significant seed × treatment interaction (Wald $\chi^2_1 = 28.9$, $P < 0.001$), indicating that the relative advantage to larvae of maternal exposure to *P. florida* differed between the 2 sources of *P. aculeata*; specifically, the effect of maternal exposure to *P. florida* was much larger on S&S seeds (Figs. 1, 2).

Overall, Verde Valley females laid slightly larger eggs than did Oracle females ($F_{1,499} = 15.8$, $P < 0.001$), but there was no significant difference between beetle populations in their mean egg-to-adult survivorship on *P. aculeata* seeds (Wald $\chi^2_1 = 0.78$, $P = 0.38$).

There was a significant effect of egg size on larval survivorship (analysis of covariance with egg length as the covariate and population, seed type, and maternal treatment as fixed effects; $F_{1,499} = 77.1$, $P < 0.001$; Fig. 2). The effect of egg size on larval survival differed between seed sources (Fig. 2; $F_{1,499} = 33.6$, $P < 0.001$; significantly higher slopes on S&S seeds), but did not differ between beetle populations (Fig. 2; $F_{1,499} = 2.74$, $P = 0.10$).

In an overall mixed-model analysis of variance, including all terms except egg size, we saw no evidence for genetic variation in larval survivorship (sire nested within population × seed source; $F_{210,293} = 0.95$, $P = 0.67$). However, this analysis had low power due to the large effects of conditioning host (*A. greggii* vs. *P. florida*). Thus,
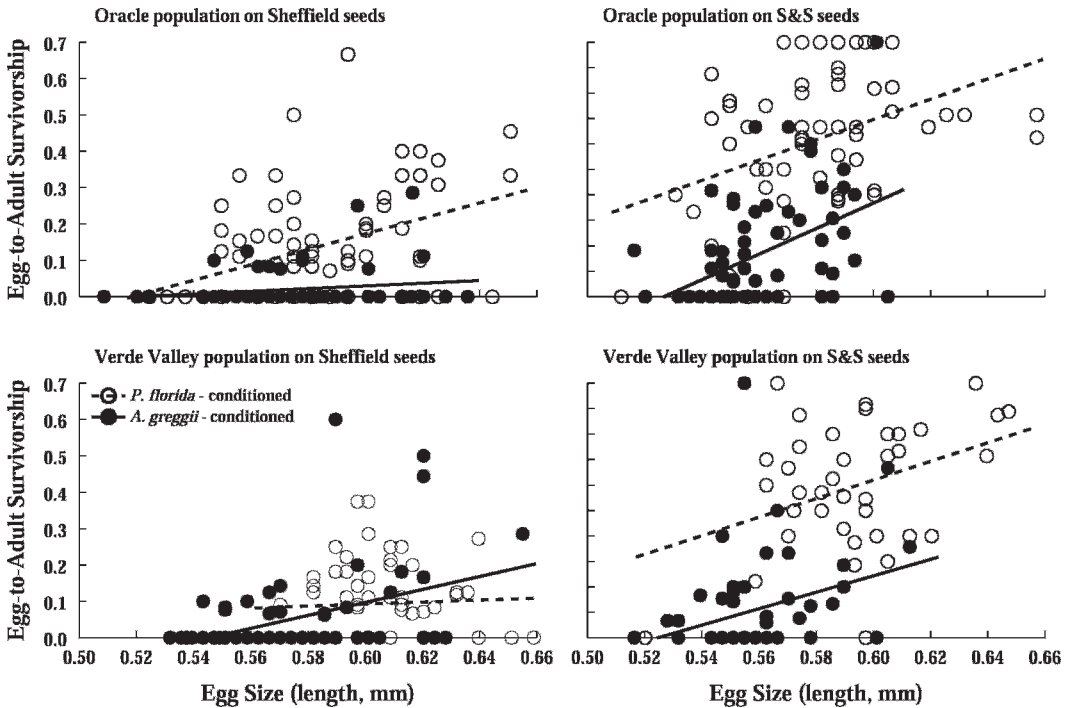
F ig. 2—The relationship between the size of eggs laid by female *Stator limbatus* and the survival of their larvae on seeds of *Parkinsonia aculeata*. Sheffield and S&S seeds refer to sources of *P. aculeata* seeds; Oracle and Verde Valley are 2 populations of beetles reared on these seeds. Note that females laid larger eggs when they were exposed to *Parkinsonia florida* during egg maturation and that offspring from these eggs had higher egg-to-adult survival.

we performed 2 other types of analyses. First, we split our full mixed-model analysis of variance into 2 separate models, one for each conditioning host. In this case, the effect of sire (nested within population × seed source) was significant when females were exposed to *A. greggii* during egg maturation ($F_{107,147}$ = 1.50, P = 0.01), but not when females were exposed to *P. florida* during egg maturation ($F_{103,146}$ = 0.85, P = 0.81). Second, we removed the effect of egg size from our full model by including egg length as a covariate. This model should remove the effects of variation in egg length among females (within sires) and the causal effect of the shift in egg length due to conditioning host (*A. greggii* vs. *P. florida*) on larval survivorship. In this full model, the sire effect (nested within population × treatment × seed source) was significant ($F_{162,119}$ = 1.82, P < 0.001), indicating the presence of genetic variation in survival that was otherwise obscured by phenotypic variation in egg size. Interestingly, this analysis also detected a significant egg length × sire in-

teraction ($F_{162,119}$ = 1.77, P < 0.001), suggesting that the effect of egg size on larval survivorship depended on the genotype of the offspring.

D iscussion—The results of this study suggest that 1) *S. limbatus* larvae are capable of developing from egg-to-adult on seeds of a few of the non-host species examined; 2) genetic variation present within populations affects the ability of larvae to survive on seeds of at least one of these hosts, *P. aculeata*; and 3) maternal plasticity in egg size or composition (in response to seeds of *P. florida*) has a large effect on the ability of larvae to survive on seeds of *P. aculeata* but not on most of the other non-host species.

Females of *S. limbatus* readily oviposited on seeds of most non-host legumes, regardless of their suitability. Thus, female behavior toward seeds is not likely a major factor limiting diet expansion in this beetle. A few of the species tested were acceptable to only some females, but only seeds of golden wattle (*A. longifolia*), indigo-bush (*Amorpha fruticosa*), honey mesquite (*P.*

glandulosa), and cowpea (*V. unguiculata*) were unacceptable to all females. Cowpea seeds have a rough seed coat relative to *S. limbatus* native hosts. Previous experience (C. Fox, pers. observ.) indicated that *S. limbatus* females avoided seeds with rough seed coats but laid eggs on many objects that were generally round and smooth (including non-seeds). This likely explains why many females accepted seeds of mung (*V. radiata*) and azuki (*V. angularis*), but not seeds of the congener cowpea. Although females refused to lay eggs on honey mesquite (*P. glandulosa*), seeds of velvet mesquite (*P. juliflora*) were acceptable to females (and suitable for larval development). Both of these species are native in the natural range of *S. limbatus*, but are unsuitable as hosts because they have indehiscent fruits and, thus, seeds are not exposed to egg-laying females. Indigo-bush (*A. fruticosa*) is also native in the southwestern USA, but not used in nature. Thus, it is not surprising that seeds were unacceptable to females in the lab, but nonetheless curious why other native non-hosts were acceptable. We suspect that use of hosts is the result of multiple sequential behavioral decisions by females, only the last of which involves acceptance of seeds. For example, we know little about how females recognize hosts from a distance, though host finding might explain why some native and apparently suitable hosts are not used in nature. Females of *Stator vachelliae* have been observed ovipositing on seeds of *P. aculeata*, but only in areas where seeds of *P. aculeata* are commingled with seeds of the normal host of *S. vachelliae* (Johnson and Siemens, 1991), suggesting that females distinguish hosts before encountering seeds, after which they might be less selective.

Of the seeds that females would accept, about half were at least marginally suitable for larval survival, and a few species were good hosts for larvae. However, the only native non-host that was suitable was velvet mesquite (*P. juliflora*), which produces fruits that make seeds inaccessible to beetles. Larval survival was high on seeds of mimosa (silktree; *A. julibrissin*), an invasive species established throughout much of the United States. This plant has been colonized by *S. limbatus* in regions where the 2 species now overlap in distribution (pers. observ.). Although *A. julibrissin* is native to China, other species in the genus and closely related genera (e.g., *Pseudosamanea*) are natural hosts for *S. limbatus*

in the New World, so it is not surprising that it is both a good host and has been colonized. Texas ebony (*E. ebenopsis*) is native in part of the range of *S. limbatus*, but is not used as a host anywhere it naturally co-occurs with *S. limbatus*. Interestingly, it is used as a host by *S. beali*, a closely related species of *Stator* that evolved from *S. limbatus* about 1.2 to 1.6 million years ago (Morse and Farrell, 2005*b*). Although *E. ebano* is not used by *S. limbatus* in its natural range, it has been colonized by *S. limbatus* in areas where the plant is grown as an ornamental (e.g., in central Arizona, outside its natural range and outside the range of *S. beali*). It is likely that *E. ebano* is not used as a host in regions where this plant is native either because of larval competition between *S. limbatus* and *S. beali* or because the ecological conditions were unsuitable for *S. limbatus* to shift to this host in Texas and Mexico.

Despite being considered a generalist insect because it attacks about 70 host species, most of the host species fall into only a few, distinct clades. For example, *S. limbatus* uses species in the genera *Harvardia*, *Lysiloma*, *Ebenopsis*, and *Albizia*, all of which are phylogenetically recently diverged (Miller and Bayer, 2001). *Stator limbatus* has been collected from at least 18 species of *Acacia* native in its range, all in the same subgenus (*Aculeiferum*). However, it has also colonized at least 8 species of Australian *Acacia* that have been introduced as ornamental plants in the New World (Morse, 2003), despite the distant relationship between the Australian *Acacia* and New World *Acacia* (subgenus *Phyllodineae*) (Miller and Bayer, 2001). *Acacia baileyana* is a common, large, street acacia (e.g., grown along highways) and *A. mearnsii* is an invasive shrub, both introduced from Australia. However, despite being phylogenetically quite distant from the host species in the New World, both are suitable as hosts, and *S. limbatus* has been collected from *A. baileyana* in the field. It is likely that *A. mearnsii* also will be colonized by *S. limbatus*.

Females of *S. limbatus* readily oviposited on seeds of many of the exotic species and even many of the native non-hosts. These oviposition ''mistakes'' provide the opportunity for selection on the new host and might provide the opportunity for host range expansions (Courtney and Kibota, 1990). Despite often poor survival on the mistakenly chosen host, larvae on novel hosts often experience much lower

rates of attack by natural enemies (Jeffries and Lawton, 1984), potentially counterbalancing the poor survival caused by poor host suitability. As stated earlier, *P. aculeata* is occasionally used by *S. vachelliae*, but almost exclusively in a narrow zone where seeds of *P. aculeata* and seeds of the normal host, *Acacia flexuosa*, are mixed on the ground beneath overlapping canopies (Johnson and Siemens, 1991). Survival of *S. vachelliae* is low on *P. aculeata*, but larvae are largely free of parasitism. Over multiple generations of selection, larval survival on novel hosts could increase enough so that the reduction in parasitism actually favors offspring laid on this new host rather than the normal host, thus selecting for changes in female oviposition preference and increased use of the new host in nature.

Even if females accept new species as potential hosts and larvae have a marginal ability to survive on those plants, populations can only adapt to these new hosts if sufficient genetic variation is present for the evolution of increased survival. Lack of genetic variation might be a major constraint in the adaptation of insects to new hosts, and thus, a constraint in the evolution of insect diets (e.g., Futuyma et al., 1995; but see Blows and Hoffmann, 2005). We tested for genetic variation in survival on seeds of the marginally suitable nonnative host, *P. aculeata*, and detected genetic variation in larval survival. In a previous study of colonization and adaptation to a different nonnative, Texas ebony (*E. ebano*), on which larval survival is poor, we also detected genetic variation in survival of larvae on seeds (Fox, 2006). Though we have only tested for genetic variation in adaptation to 2 novel hosts so far, we suspect such genetic variation is present for larval survival on all of the species that are at least marginally suitable for *S. limbatus* larvae.

One constraint on the interpretation of our results is that we only tested for genetic variation in the ability of larvae to survive on *P. aculeata*; we did not test for genetic variation in behavior towards this or other potential hosts. However, most females were willing to oviposit on *P. aculeata* in our study, and beetles have been collected from *P. aculeata* in the field (Morse and Farrell, 2005b). Because our focus here was on the suitability of seeds for larval development rather than oviposition behavior, we did not record how long females delayed before oviposition, nor whether females would have readily migrated from dishes containing non-host seeds had they not been confined. However, it was clear that females generally delayed oviposition and showed reduced fecundity on *P. aculeata*, indicating that it is not a preferred host. We suspect that *P. aculeata* would be a low-ranked species in paired preference tests. However, recent studies on other insects have demonstrated rapid evolution of host preference and specificity (reviewed in van Klinken and Edwards, 2002; Messina, 2004). If *P. aculeata* continues to increase in abundance in the southwestern United States, it is likely that female encounters with this host will become frequent enough to influence the evolution of oviposition preference.

In our study, we did not test whether larval performance on native host plants was genetically correlated with performance on non-host plants. If genetically based trade-offs exist between performance on host and non-host plants, then adaptation to a newly colonized host will be counteracted by selection on the ancestral host (Ueno et al., 1999, 2003). However, there is little evidence that genetic correlations between performance on pairs of species are negative, suggesting that the ability of insects to exploit novel hosts is rarely constrained by genetic trade-offs (Ueno et al., 1999, 2003).

Females of *S. limbatus* respond to the presence of *P. florida* seeds by increasing the size of their eggs (Fox et al., 1997b). In a previous study (Fox and Savalli, 2000), we demonstrated that this egg size plasticity facilitates the expansion of beetles onto ornamental Texas ebony (*E. ebano*) in central Arizona. When females encounter *P. florida* during egg maturation, they lay larger eggs and their offspring have improved survival on seeds of *E. ebano* relative to the survival of offspring from parents never encountering *P. florida*. Ornamental *E. ebano* in central Arizona are surrounded by native *P. florida* (Mitchell, 1977). Survivorship of field-collected larvae and the size of eggs laid by females on seeds of *E. ebano* in the field both indicate that females are encountering this nearby *P. florida* prior to laying eggs on *E. ebano* (Fox et al., 1997a; Fox, 2006).

As with ornamental *E. ebano*, many invasive populations of *P. aculeata* are in areas where *P. florida* is abundant. Here we demonstrate that egg size plasticity also has large effects on the survival of offspring reared on seeds of *P. aculeata*, just as for *E. ebano*, but that maternal

encounters with *P. florida* did not affect larval survival on seeds of a variety of other plants (Table 2). Maternal experience with seeds of *P. florida* affected larval survival on only a few non-host species, but on both *P. aculeata* and *E. ebano* the effect was large. It is likely that populations of *S. limbatus* cannot successfully colonize these species except in areas where *P. florida* is a common host and can provide immigrants; larval mortality would be too high and populations on the new hosts would quickly go extinct. However, exposure to *P. florida* during egg maturation could increase offspring survival enough that populations do not rapidly go extinct and have an opportunity to adapt to the new host via the evolution of egg size, egg composition, or other traits that influence larval survival. In areas where *P. aculeata* and *E. ebano* are native, high mortality generates substantial selection for females to avoid seeds of these species. This likely explains why *S. limbatus* populations in these areas do not use these species – they have evolved host avoidance. However, in areas where females have not evolved in sympatry with *P. florida* or *E. ebano*, they have not been selected to avoid these species. We suggest that because migrants to these nonnative species come from *P. florida*, mortality is reduced and, thus, selection for behavioral avoidance is relaxed, such that increased survival might evolve on these hosts before females evolve host avoidance.

More generally, our results indicated that the ability of insects to incorporate nonnative plants into their diet depended on the species composition of the local plant community. The ability of insects to colonize empty patches depends on the experiences of migrants, which in turn depend on the species composition of the local or surrounding patches. Similar effects of local species composition on colonization dynamics have been observed in metapopulation studies (Singer and Thomas, 1996; Hanski and Singer, 2001). The substantial changes in plant and animal distributions occurring in modern times provide an opportunity to study these ecological processes that influence insect colonization of new plant species and subsequent adaptation by herbivores to those species.

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