Colonization of a new host by a seed-feeding beetle: Genetic variation, maternal experience, and the effect of an alternate host

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Human introductions of non-native plants, and subsequent colonization of these plants by herbivores, can be used to study the ecological processes influencing the evolution of insect diet. The seed beetle *Stator limbatus* has colonized non-native Texas ebony (*Ebenopsis ebano*) that is grown as an ornamental in Arizona (USA). Texas ebony is not used as a host by *S. limbatus* where the beetle and plant are naturally sympatric but is used by the sister species to *S. limbatus* (*S. beali*). Lines created in an artificial selection experiment were used to test for genetic variation in the ability of *S. limbatus* larvae to survive on Texas ebony and to examine the role that a native host (blue paloverde, *P. florida*) plays in facilitating the colonization of Texas ebony. Genetic variation in survival on Texas ebony was present in this *S. limbatus* population, but the major factor affecting survival was maternal experience with the alternate host — females that encountered paloverde during egg maturation produced offspring that survived > 10-times better on Texas ebony as compared with offspring of females that did not encounter paloverde. This difference was because paloverde stimulated a plastic change in egg composition. Absence of paloverde in the community will reduce larval survival and limit the ability of *S. limbatus* populations to incorporate Texas ebony into their range of suitable hosts. Blue paloverde provides a stepping stone for colonization of Texas ebony; exposure of mothers to *P. florida* seeds during egg maturation can increase offspring survival enough that populations do not go extinct before evolving increased survival on the new host, Texas ebony.

Introduction

Human activities have introduced organisms into many new and often novel environments (Vitousek *et al.* 1996) and caused many other species to expand their ranges (Pitelka *et al.* 1997). These changes in distribution create natu-ral experiments through which we can study ecological and evolutionary processes that otherwise could only be inferred (Carroll & Boyd 1992). For example, the evolution of insect diet can be studied using recent changes in plant distributions and the expansion of herbivores onto these plants, or the introduction of herbivores

into areas containing only novel hosts (Tabash-nik 1983, Bowers *et al*. 1992, Carroll & Boyd 1992, Fraser & Lawton 1994, Leclaire & Brandl 1994, Singer & Thomas 1996, Fox *et al*. 1997a, Gratton & Welter 1998, Fox & Savalli 2000).

Colonization of novel hosts, and subsequent adaptation to those hosts, can be influenced by species composition of the local community (Fox & Savalli 2000), composition of the community from which the colonist migrated (Secord & Kareiva 1996), and the metapopulation structure of the colonizing species (Hanski & Singer 2001). For example, the relationships between an insect and plant in their natural range are not always the same as the relationships that will be formed following plant or insect colonization of a new environment (Secord & Kareiva 1996). This is partially because the plants/insects have not co-evolved with species in the new locality but also because interactions between a predator, parasitoid, or herbivore and its hosts are influenced by other species in the community. These can be direct interactions with natural enemies, competitors, alternate host species (Strong *et al*. 1984, Strand & Obrycki 1996), and abiotic conditions (McEnvoy 1996), but also indirect interactions with other organisms in the community (i.e., organisms that interact with predators, parasitoids or competitors but not directly with the focal species; Secord & Kareiva 1996). Thus, the responses of insects to novel hosts, and the subsequent evolution of insect diet, are affected by complex interactions between the insect, its hosts, and the diversity of other species present in the community (Gould 1979, Secord & Kareiva 1996).

Following colonization, subsequent adaptation to a new host requires genetic variation in survival and growth. The observations that many taxa of herbivorous insects are highly conserved in their diet (Mitter *et al*. 1991), 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 often lacking (Futuyma *et al*. 1993, 1994, 1995). Some recent studies have indeed found genetic variation lacking (Schieffer 1999) whereas others have found adequate variation present to allow insects to adapt to potential

new hosts (Ueno *et al*. 1999, 2001, 2003). This discrepancy is consistent with the expectation that the presence of genetic variation is dependent on the specific plant species (Futuyma *et al*. 1995, Ballabeni & Rahier 2000, Alleyne & Wiedenmann 2002) or the insect population examined (Nielsen 1997).

In this paper I test for the presence of genetic variation in the ability of a seed beetle, *Stator limbatus*, to survive on seeds of a new host species (Texas ebony, *Ebenopsis ebano*) that was recently introduced into southwestern United States and colonized by this beetle. I then examine the mechanism generating a previously observed result that female encounters with a native host species (blue paloverde, *Parkinsonia florida*) improve offspring survival on Texas ebony.

The study system

Stator limbatus (Coleoptera: Chrysomelidae: Bruchinae) is a seed parasite that uses seeds of some mimosoid and caesalpinioid legumes as hosts (Johnson & Kingsolver 1976, Janzen 1980, Johnson *et al*. 1989, Morse & Farrell 2005a, 2005b). In the southwestern United States, *S. limbatus* primarily uses seeds of *Acacia greggii*, *Parkinsonia florida* (previously *Cercidium floridum*) and *P. microphylla* (*C. microphyllum*). However, a large variety of non-host legumes have been introduced into the range of *S. limbatus*, some of which have been incorporated into the beetle's diet. Texas ebony, *Ebenopsis ebano* (previously *Chloroleucon ebano* and the junior synonym *Pithecellobium flexicaule*) is a common ornamental in central Arizona and has been colonized in most locations where it has been introduced (Fox *et al*. 1997a). Arizona females readily accept seeds of Texas ebony for oviposition, and larval densities are very high on Texas ebony seeds in Arizona, though larval survival is very low on these seeds. The colonization of Texas ebony is especially interesting because this species is not used as a host by *S. limbatus* in locations where the beetle and plant are naturally sympatric (southern Texas and the gulf coast of Mexico). However, Texas ebony is used as a host by the sister species to *S. limbatus* (*S. beali*) that is a specialist on Texas ebony.

Diet evolution and adaptation to novel hosts is more complicated in *S. limbatus* than in many herbivores because females exhibit plasticity in egg size that influences larval survival on seeds of a variety of plant species. Females that encounter seeds of the natural host blue paloverde (*Parkinsonia florida*) during egg maturation increase the size of their eggs relative to the size of eggs laid by females that mature eggs in contact with any of the other native southwestern hosts (Fox *et al*. 1997b, Savalli & Fox 2002). This plasticity has presumably evolved because larvae hatching from large eggs have a substantial survival advantage over larvae hatching from small eggs when developing on seeds of *P. florida*, but this survival advantage is small or non-existent on other natural hosts (e.g., Fox & Mousseau 1996, Fox *et al*. 2001). In many locations *P. florida* is the primary host of *S. limbatus* and is likely the source of females that colonize non-native plants, including Texas ebony (Fox *et al*. 1997a). Survival of larvae on seeds of Texas ebony is greatly enhanced when females are exposed to seeds of *P. florida* during egg maturation (Fox & Savalli 2000), though females do not recognize Texas ebony as a host upon which they should lay large eggs to improve survival of their larvae (the traits on seeds that stimulate egg size plasticity are not the same traits that cause larval mortality; Fox *et al*. 1997c). Egg size appears to influence the survival of larvae of Texas ebony, but it is unclear that the observed maternal host effect is due to plasticity in egg size rather than some other as yet unidentified mechanism.

Here I test four hypotheses: (1) there is genetic variation within *S. limbatus* populations in their ability to survive on the novel host, Texas ebony (*Ebenopsis ebano*), (2) evolutionary changes in egg size affect survival of larvae on seeds of Texas ebony, (3) changes in egg composition in response to maternal experience during egg maturation affect the survival of larvae on seeds of Texas ebony, and (4) females laying eggs on Texas ebony in the field are encountering seeds of *P. florida* prior to colonizing Texas ebony. To test the first three hypotheses I take advantage of *S. limbatus* lines created in a recent laboratory selection experiment in which we (Czesak & Fox 2003) imposed selection on egg size. This experiment produced lines of beetles

that lay large eggs (UP lines) or small eggs (DOWN lines) as compared with an unselected CONTROL line (details in Czesak & Fox 2003). Because Fox and Savalli (2000) found that egg size affects larval survival on *E. ebano*, we expect the UP lines to evolve increased survival on seeds of Texas ebony and the DOWN lines to evolve reduced survival on seeds of Texas ebony. Because all selected lines were initiated with a single population of *S. limbatus*, differences among selected lines in larval survivorship on Texas ebony necessarily indicate two things: that egg size, or a trait genetically correlated with egg size, affects larval survivorship on seeds of Texas ebony, and that genetic variation in survivorship on Texas ebony is present in the populations from which the selection lines were created (otherwise the lines could not have evolved differences in survivorship in response to selection). I then manipulate maternal host exposure during egg maturation of females in the selected lines to disentangle the effect of egg size and egg composition on larval survival.

Methods

Selection lines

Details of the experiment creating the selected lines are presented in Czesak and Fox (2003; *Acacia* lines). In brief, we imposed artificial selection on egg size for nine generations in two replicate groups of lines, each with an UP, DOWN and CONTROL line. Both replicates were initiated from a single population of beetles collected in central Arizona, in an area close to colonized trees of Texas ebony (Apache Junction population). Each replicate was created from a single sample of 200 females at the beginning of the selection experiment. We selected one egg from each of these 200 females to create the CONTROL lines, then selected 10 eggs from each of the 40 females that laid the largest eggs (UP lines) or the smallest eggs (DOWN lines) on seeds of catclaw acacia, *Acacia greggii* (a common native host in Arizona). Subsequently, we maintained CONTROL colonies at about 100 families per generation. In the selection lines we raised ~200 females each generation, always

raising 10 eggs from each of the top 40 females (UP lines) or bottom 40 females (DOWN lines).

Six generations after we completed selection on egg size I measured the survivorship of larvae from the selected and control lines on seeds of Texas ebony. I used a full-sib design because I am testing for variation among selected lines and between treatments, and am not testing for genetic variation within selected lines. Each virgin female was paired with a single virgin male from the same selected line, creating an average of 54 families per line (total = 647 families). Half of the pairs were confined on seeds of Texas ebony (in a 35 mm Petri dish) as they mated and matured eggs to simulate direct colonization of this new host by virgin females. The other half of the pairs were confined on seeds of *P. florida* seeds as they mated and started maturing eggs to simulate females that encounter this native host before migrating to the new host. Females were checked once per day until they had laid at least one egg, after which they were transferred to a 60 mm Petri dish containing ≈ 20 seeds of *E. ebano*. Females were allowed to lay eggs in this 2nd dish for 24 h. These eggs were allowed to hatch and were raised to adult at 30 °C, photoperiod 15:9 light:dark. I measured egg length of 2–3 eggs per dish, and scored egg-to-adult survivorship of all developed eggs.

The Texas ebony seeds used in this experiment were collected in the field from > 50 trees within an ≈ 1 hectare^2 area in Mont Meta Memorial Park, San Benito, Cameron County, TX. Seeds were frozen (to kill parasites). Only seeds undamaged by other organisms were used for this experiment.

Egg size in nature

I have argued previously, and do so here, that patterns of survivorship of larvae on Texas ebony in the field indicate that this host is a sink for beetles; females are colonizing Texas ebony from other hosts, rather than populations cycling through multiple generations on Texas ebony. However, I have never directly tested this hypothesis. In the areas around Phoenix, AZ, where Texas ebony is widely planted as an ornamental and attacked heavily by *S. limbatus*,

the most likely source host from which adult females migrate to Texas ebony is *P. florida*. To test whether *P. florida* was indeed the source of females that laid eggs on Texas ebony in Papago Park we compared the size of eggs laid on Texas ebony seeds in Papago Park with the size of eggs laid on both *A. greggii* and *P. florida* in a variety of locations in Arizona. Specifically, in 2004 we collected seeds bearing *S. limbatus* eggs from five populations of *P. florida* and two populations of *A. greggii*, and compared these eggs with those laid on *C. ebano* in Papago Park, Phoenix, AZ. Eggs that had been laid in the field were measured in the lab using an ocular micrometer (as above). We know from laboratory experiments that females lay small eggs on *A. greggii*, relative to the size of eggs laid on *P. florida*, and that females encountering only Texas ebony during egg maturation lay *Acacia greggii*-sized eggs (Fox & Savalli 2000; *see* also Results, below). For females to lay large eggs on Texas ebony they must encounter *P. florida* during egg maturation.

Results

For both replicates, females from the UP lines laid larger eggs on Texas ebony seeds than did females from the DOWN lines (Fig. 1A and B; there was significant heterogeneity in egg size among lines, $F_{2,635} = 922$, $P < 0.001$). As previously observed, females that matured their eggs while in contact with *P. florida* seeds (open circles in Fig. 1) laid much larger eggs than did females that matured their eggs without contact with *P. florida* seeds (closed circles in Fig. 1A and B; $F_{1,635} = 1467$, $P < 0.001$). There was no detectable interaction between selection treatment and the host experienced during egg maturation on the size of eggs laid by females ($P = 0.35$).

Because egg size was previously observed to be correlated with offspring survival on seeds of Texas ebony (Fox & Savalli 2000) larval survival should be highest for UP line offspring raised on seeds of Texas ebony and lowest for DOWN line offspring. Indeed, offspring survival varied among lines ($F_{2,635} = 26.9$, $P < 0.001$); survival on Texas ebony was higher for larvae hatching
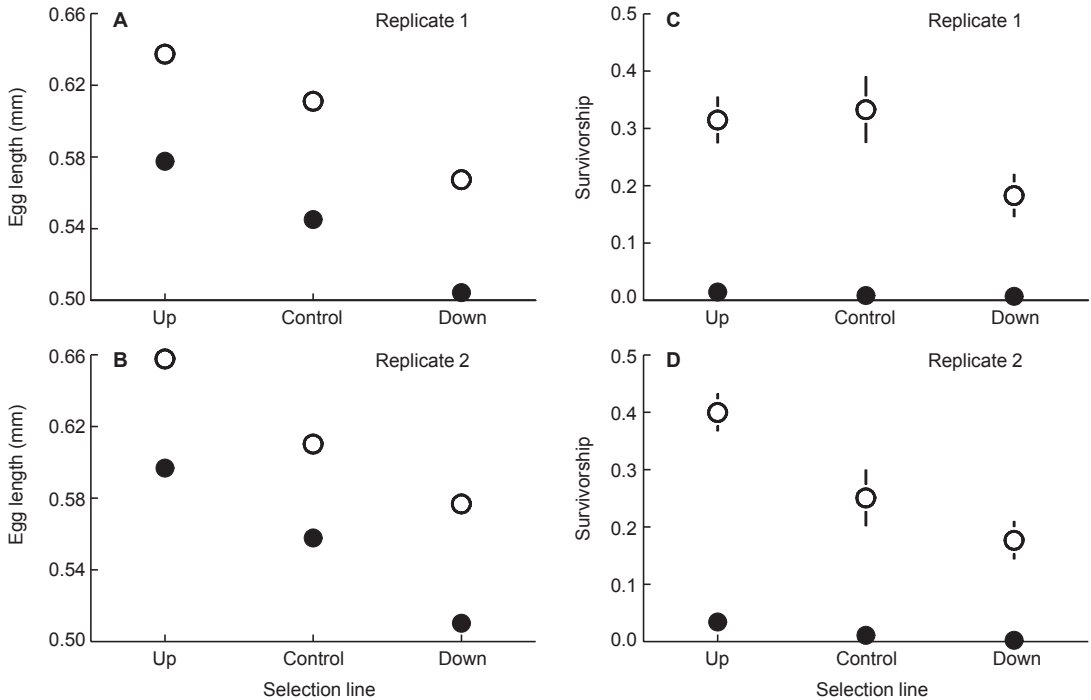
Fig. 1. Length of eggs laid by females on seeds of Texas ebony (**A**, **B**) and egg-to-adult larval survivorship on seeds of Texas ebony (**C**, **D**) in lines selected for large eggs (UP) or small eggs (DOWN) and unselected controls. Standard errors are present but are smaller than some points and thus not visible. Females were confined on seeds of either *Acacia greggii* (closed circles) or *Parkinsonia florida* (open circles) during egg maturation.

from eggs laid by females of the large egg (UP) lines than for offspring hatching from eggs laid by females in the small egg (DOWN) lines (Fig. 1C and D). Also, in an analysis of covariance including all terms, the effect of egg length on survival of offspring was significant ($F_{1,637}$ = 5.44, $P = 0.020$), suggesting a small causal relationship between egg length and larval survival. When including egg size as a covariate, the variance among lines became non-significant ($F_{2,637}$ = 1.18, $P = 0.31$) indicating that the variation in larval survival *among* the selected lines is due largely or entirely to variation in egg size among the lines.

Although the selected lines differed in larval survival on seeds of Texas ebony, by far the largest effect on offspring survival was the host upon which females matured their eggs; when females matured their eggs in contact with seeds of *P. florida* their offspring had up to 16 times higher survivorship on seeds of Texas ebony than did offspring of females that matured their eggs without access to *P. florida* (conditioning host

effect, $F_{1,635}$ = 383, $P < 0.001$). This effect was partly due to phenotypic plasticity in egg size (females lay much larger eggs when maturing their eggs on *P. florida*). However, after removing the effect of egg size on larval survival via analysis of covariance the effect of conditioning host was still highly significant ($F_{1,637}$ = 107, $P < 0.001$). This is evident when comparing Figs. 1A and B with 1C and D; note the large effect of selection line on egg size (steep left-to-right slope of points in Fig. 1A and B) but relatively small effect of selection line on larval survivorship (weak left-to-right slope of points in Fig. 1C and D). This indicates that very little of the variation in larval survival can be explained by variation in egg size among females.

Egg size in nature

The size of eggs laid on seeds of Texas ebony in Papago Park was significantly greater than the size of eggs laid by females on *A. greggii*, but
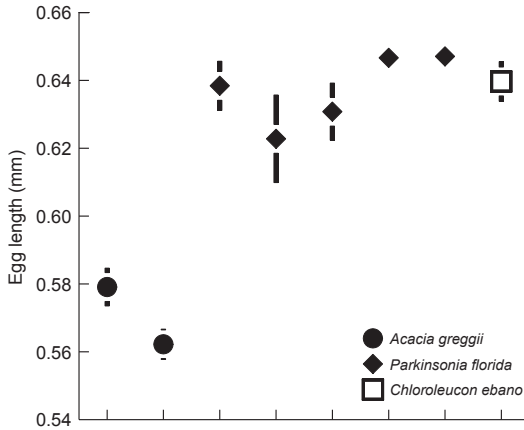
Fig. 2. The size of eggs laid by female *S. limbatus* in the field on seeds of *A. greggii*, *P. florida*, and *E. ebano*.

not significantly different from the size of eggs laid on seeds of *P. florida* (Fig. 2). Laboratory experiments have demonstrated that Papago Park females lay *Acacia greggii*-sized eggs on Texas ebony unless they encounter *P. florida* during egg maturation (Fox *et al.* 1997a); females do not recognize Texas ebony as a species upon which they should lay large eggs. I thus interpret these field patterns as evidence that females ovipositing on Texas ebony in Papago Park are experiencing seeds of *P. florida* at some point during egg maturation. At a minimum, females are dispersing from Texas ebony and encountering seeds of *P. florida* during egg maturation before returning to Texas ebony. More likely, females emerging from Texas ebony seeds at this site disperse and new females, reared from *P. florida*, migrate to the Texas ebony patch.

Discussion

Previous experiments have shown that females of *Stator limbatus* respond to the presence of *Parkinsonia florida* seeds by increasing the size of their eggs and that this egg size plasticity increases the survival of *S. limbatus* larvae on seeds of Texas ebony (Fox *et al.* 1997a, Fox & Savalli 2000). This current study extends our previous results in two ways. First, it clearly demonstrates that genetic variation is present within populations of *S. limbatus* for their ability to survive on seeds of Texas ebony. If genetic

variation was absent, the selected lines could not have evolved to differ in larval survival on this novel host. The presence of genetic variation indicates that *S. limbatus* populations using Texas ebony in nature can adapt to this host. In fact, larval mortality is very high, imposing substantial selection on traits that improve larval survival on this host. Alternatively, because mortality is very high selection should favor females that avoid laying eggs on Texas ebony when alternate hosts are available. I have not tested for genetic variation in oviposition behavior, but it likely exists since genetic variation in oviposition behavior has been detected in most studies of other insects and in numerous studies with seed beetles (e.g., Fox 1993, Tucić *et al.* 1999). However, Texas ebony seeds are readily accepted by central Arizona *S. limbatus* and even preferred by many females in choice tests with seeds of native hosts (C. Fox unpubl. data; likely due to the large size of Texas ebony seeds). It will thus require substantial evolutionary change for *S. limbatus* to lose its acceptance of Texas ebony seeds.

Second, this study indicates that, although egg size does indeed influence the ability of larvae to survive on the exotic host, most of the effect of maternal experience on offspring survival is due to changes in egg composition — maternal experience with seeds of *P. florida* had a large effect on offspring survival on Texas ebony that could not be explained by variation within or among lines in egg size. That females modify egg composition in response to contact with *P. florida* seeds was originally suggested by the results of Fox and Savalli (2000) but not confirmed until this current study. This plasticity affects survival of larvae on seeds of three plant species, the native *P. florida*, the non-native (but naturalized) *P. aculeata* and non-native Texas ebony, but not on a variety of other species (C. Fox unpubl. data). Unfortunately, the specific cause of poor larval survival on these seeds is unknown. It is possible that seeds of these three species contain similar secondary compounds that need to be detoxified by larvae and that females are packaging into their eggs specific proteins, or mRNAs used to produce proteins, that help detoxify defensive compounds in the seeds. Both *P. aculeata* and *P. florida* are con-

geric and thus may share similar seed chemistries (though it is known that *P. microphylla* does not share the same defensive chemistry as *P. florida*; Siemens *et al.* 1992, 1994). However, *E. ebano* is in a different family of legumes (Mimosaceae). Also, larval mortality on *P. florida* is largely due to the death of first instar larvae while trying to penetrate the seed coat, whereas larval death on both *P. aculeata* and *E. ebano* seeds occurred primarily inside the seed indicating that any common defensive chemistries among species are sequestered in different locations in the seed (but *see* Johnson & Siemens (1991) who show that the majority of *S. vachelliae* death on *P. aculeata* occurs at the seed coat). Alternatively, females could be increasing the nutritional content of eggs (e.g., increasing lipid or protein content), which could explain both the egg size (more material in the eggs) and composition effect (McIntyre & Gooding 2000). However, changes in egg nutritional content in response to host species should have more general positive effects across a larger range of plant species. The chemistry of seed resistance to *S. limbatus* in these three species, and the mechanism by which maternal experience prepares larvae to survive on these seeds, is under current investigation.

Field estimates of egg-to-adult survival on Texas ebony range between about 10% and 15% (Fox *et al.* 1997a). In contrast, egg-to-adult survival tends to be very high in the laboratory on non-resistant hosts such as *Acacia greggii* (> 90%; Fox *et al.* 1997b) or *Parkinsonia microphylla* (> 75%; Fox *et al.* 2001). However, the rate of egg parasitism is fairly high on native hosts (e.g., ~40% on *P. florida* in southern California; Mitchell 1977) such that switching to a novel host may provide an escape from natural enemies (Jeffries & Lawton 1984) potentially counterbalancing the poor survival on Texas ebony. Interestingly, another species of *Stator*, *S. vachelliae*, attacks seeds of the non-host Mexican paloverde, *P. aculeata*, and larvae developing on the exotic are largely free of parasitism (Johnson & Siemens 1991). Possibly escape from parasitism following *S. limbatus* colonization of Texas ebony along the gulf coast of Texas and Mexico provided the selective benefit that allowed *S. limbatus* to locally adapt to this host, leading to

speciation and the origin of *S. beali* (the sister species of *S. limbatus*).

The effect of maternal exposure to seeds of *P. florida* on the survival of larvae on seeds of Texas ebony was very large. Thus, even in the absence of competition with *S. beali* it should be difficult for populations of *S. limbatus* to successfully colonize Texas ebony except in areas where *P. florida* is the source of immigrants. Larval mortality is so high that 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 instead have an opportunity to adapt to the new host via the evolution of egg size, egg composition, or other traits that influence larval survival. My results thus demonstrate that colonization of novel hosts, and subsequent adaptation to those hosts, can be influenced by the species composition of the local community and/or the composition of the community from which the colonist migrated. In this case, a third species in the interaction (*P. florida*) provides a stepping stone for colonization of Texas ebony. Absence or low densities of *P. florida* in the community will dramatically reduce larval survival on the new host and should limit the ability of populations to incorporate Texas ebony into the repertoire of suitable host plants. Interestingly, *S. limbatus* has also colonized the invasive *P. aculeata* throughout the northern parts of its range (Morse 2003). Laboratory studies (C. Fox unpubl. data) demonstrate that exposure of females during *P. florida* during egg maturation influences larval survival on *P. aculeata*, on which survival of larvae is also very low when mothers do not encounter *P. florida* seeds (just as observed for Texas ebony). I thus believe that egg size plasticity is a major component of diet expansion in *S. limbatus* and likely is the explanation for why this species is a relative generalist (using > 70 species as host) in a genus of dietary specialists (Morse & Farrell 2005a).

Evolution of the diet of *Stator limbatus* is also limited by the presence of other seed/fruit predators. Female *S. limbatus* lay eggs only on seeds, not on fruits, and thus require direct access to the seeds of their host (Mitchell 1977). They are thus restricted to hosts that produce dehis-

cent pods (such as *Parkinsonia microphylla*) or to hosts that suffer substantial fruit damage by other insects or rodents that provide access to the seeds (e.g., *P. florida*). Texas ebony has dehiscent fruits; its seeds are thus available to *S. limbatus*. However, a variety of other legume species have been introduced into the southwestern United States whose seeds are more suitable for *S. limbatus* development (compared with those of Texas ebony), but they are not colonized likely because their fruits are indehiscent and other seed/fruit feeders are not present to create access to seeds for *S. limbatus*. The ecology and evolution of the current diet expansion onto Texas ebony, and the ecology and evolutionary processes that effect *S. limbatus* use of other non-native hosts throughout its distribution are under on-going study.

Acknowledgements

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