

Variation in inbreeding depression among populations of the seed beetle, *Stator limbatus*

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Abstract

Inbreeding depression has been documented in many insect species, but the degree to which it varies among traits within populations and among populations within species is poorly understood. We used a single-generation factorial breeding design to examine variation in inbreeding depression among three populations of the seed-feeding beetle, *Stator limbatus* Horn (Coleoptera: Chrysomelidae: Bruchinae), from the southwestern USA. Eggs from sib matings were less likely to develop and hatch, and larval hatch-to-adult mortality was higher for offspring of sib matings. Overall, inbreeding resulted in a reduction in the proportion of eggs that produced an adult from >80% for outbred matings in all three populations to an average of only 54% for inbred matings. Of those larvae that survived to adult, inbred beetles took ~1.5 days (>5%) longer to reach adult. The only measured trait not affected by inbreeding was adult body mass. The degree to which inbreeding increased mortality varied among the populations – inbreeding depression was lowest in the population that is most isolated. Although populations of *S. limbatus* are generally large in nature our results suggest that increased inbreeding associated with population fragmentation can have substantial effects on fitness of *S. limbatus*.

Introduction

Many insect species are experiencing substantial population declines and/or fragmentation due to habitat destruction, pollution, and climatic changes (van Nouhuys, 2005). Although the major consequences of population fragmentation and reductions in population size are ecological, inbreeding depression associated with small and fragmented populations influences the extinction rate of populations both in the laboratory (Bijlsma et al., 2000) and in nature (Saccheri et al., 1998; Nieminen et al., 2001). Inbreeding depression is a decline in the mean value of traits, generally fitness-related traits, with inbreeding (Charlesworth & Charlesworth, 1987; DeRose & Roff, 1999). It is generally due to increased homozygosity, which can affect offspring fitness both by reducing the beneficial effects of being heterozygous (due to overdominance) and by increasing the frequency of expression of recessive deleterious alleles (Carr & Dudash, 2003). In insects, inbreeding has been demonstrated to negatively affect egg hatch (Higashiura et al., 1999; Morjan et al., 1999;

Armbruster et al., 2000; van Oosterhout et al., 2000; Nieminen et al., 2001; Haikola, 2003), juvenile survival (Armbruster et al., 2000; van Oosterhout et al., 2000; Haikola, 2003), overwintering survival (Nieminen et al., 2001), juvenile development time (or growth rate; Roff, 1998; Morjan et al., 1999), juvenile size (Roff, 2002), development stability (e.g., fluctuating asymmetry; Reale & Roff, 2003), female fecundity (Henter, 1993; Tanaka, 1993; Roff, 1998; van Oosterhout et al., 2000; Roff & DeRose, 2001), male fertility (Saccheri et al., 1996, 2005), adult mating behavior, sperm competition or mating success (Joron & Brakefield, 2003), adult lifespan (Henter, 1993; van Oosterhout et al., 2000), and sometimes pupal or adult size (e.g., Armbruster et al., 2000; Reale & Roff, 2003). Inbreeding can also reduce stress resistance (review in Armbruster & Reed, 2005) and increase susceptibility to parasitic infection (e.g., Stevens et al., 1997). Outbreeding depression (in which inbred individuals have, on average, better performance than outbreds) has been observed in insects, but rarely (Aspi, 2000; Peer & Taborsky, 2005).

The amount of genetic load carried by a population, and thus its susceptibility to inbreeding depression, depends on population history (Husband & Schemske, 1996). Inbreeding depression can thus vary substantially among

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populations. Because variation in inbreeding depression is both ecologically important and a major factor affecting the evolution of plant mating systems, especially selfing rate (Kelly, 2005), it has been well studied in plants. Most detailed studies of inbreeding depression in insects are limited to only a few model organism, including *Drosophila* (Miller et al., 1993; Garcia et al., 1994; Dahlgaard & Loeschke, 1997; Aspi, 2000; Bijlsma et al., 2000; Dahlgaard & Hoffmann, 2000; Radwan & Drewniak, 2001; Fowler & Whitlock, 2002; Kristensen et al., 2003; Vermeulen & Bijlsma, 2004; review in Lynch & Walsh, 1998) and *Tribolium* (Pray & Goodnight, 1995, 1997; Stevens et al., 1997) species. Inbreeding depression has been documented in many other insect species but the degree to which it varies among traits within populations, among populations within species (Morjan et al., 1999; Haikola et al., 2001), and among species is poorly understood.

Here we examine variation in inbreeding depression among three populations of the bruchine beetle, *Stator limbatus* Horn (Coleoptera: Chrysomelidae: Bruchinae). *Stator limbatus* is a widely distributed seed-feeding beetle that occurs mostly in large outbred populations (Morse, 2003). However, substantial clearing of forests and deserts for agriculture and development, and the use of many *S. limbatus* hosts as ornamental plants (resulting both in fragmented urban populations of beetles and allowing range expansion by the beetle onto translocated hosts), have increased patchiness of hosts in nature and reduced local population sizes. Here we examine how inbreeding affects egg hatch, embryonic development, larval survival, development time, and adult body mass in three populations of *S. limbatus*. We find that inbreeding depression is substantial and affects all traits except adult body size. Inbreeding depression varies among the three study populations but only inbreeding depression on development time varies among families within these populations.

Materials and methods

The biology of *Stator limbatus*

Stator limbatus is a seed-feeding beetle native to the New World and distributed through the dry forests and deserts from southwestern USA south to northern South America (Johnson & Kingsolver, 1976; Johnson et al., 1989; Morse & Farrell, 2005a,b). Its entire life cycle takes place on or near host seeds. Eggs are glued to the surface of host seeds and larvae complete development and pupation inside a single seed, emerging after pupation as an adult. Adults reproduce using larval resources; they require neither food nor water making them a very practical laboratory model. *Stator limbatus* uses >70 host species throughout its large geographic range.

Study populations

We examined inbreeding depression in three populations of *S. limbatus* from Arizona, USA. These populations were collected in September 2003 along Hwy 60 near Wenden, La Paz County (33.81°N, 113.56°W), along Mount Lemmon Hwy in Oracle, Pinal County (32.61°N, 110.77°W), and near Camp Verde in the Verde Valley, Yavapai County (34.56°N, 111.8°W). The Wenden population differs from the other two in that the host trees represent a small patch of hosts in an agricultural valley.

All beetles were collected from seeds of *Acacia greggii* Gray (Fabaceae) trees as larvae inside of seeds. Emerging beetles were used to establish laboratory colonies (>200 beetles per population). Before this experiment the colonies were maintained in the laboratory for two generations at > 100 families per generation, at 26 °C, and L15:D9.

Experimental design

Because measurement of within-population variation in inbreeding is sensitive to the choice of parents used to create outcrossed families (Fox, 2005) we used a 'block' design (Figure 1; as in Roff, 1998). Each block was created by randomly pairing two families chosen from the laboratory colony (within populations; we did not perform between-population crosses in this study). From each family we randomly chose two female and two male beetles to become parents. We then crossed these beetles as shown in Figure 1, creating two inbred and two outbred families per block. Inbred families experienced only one generation

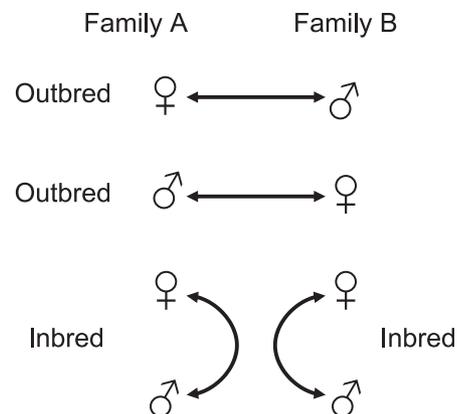


Figure 1 The block design used to measure inbreeding depression. Each block is created by crossing beetles from two unrelated families, creating two outbred matings (reciprocal crosses between the two families) and two inbred matings (crosses between full-sibs within each family). Outbreds and inbreds within each block thus have on average the same set of alleles but differ in degree of homozygosity due to the mating treatment.

of sib mating. The advantage of this design is that it assures that inbred families are created from the same set of alleles as are the outbred families to which they are compared. Thus, variation among blocks in inbreeding depression contains a component of the genetic variation in inbreeding depression in the population (Fox, 2005).

Pairs were confined in a 35 mm Petri dish with 10 seeds of *A. greggii*. These dishes were checked for eggs twice per day until females had laid 8–10 eggs. Larvae were allowed to develop at one egg per seed (excess eggs were scraped from the seed) and one seed per Petri dish inside a single temperature- and photoperiod-controlled growth chamber at 26 °C and L15:D9. Dishes were checked twice per day for adults that emerged from a seed. Half of the beetles were weighed within 12 h of emergence as an adult (these beetles were identified at the egg stage and are thus a true random sample of all emergers). We recorded egg-to-adult development time and egg/larval survival for all offspring. For egg/larval survival data, all eggs were classified to one of three fates – eggs that failed to develop, eggs that developed but did not hatch (a developing larva/embryo was visible inside the clear egg), and individuals that hatched from eggs but did not emerge as an adult. We created 107 blocks, each containing two inbred and two outbred families (36 blocks for the Oracle population, 38 for Verde Valley, and 33 for Wenden). All three populations were raised simultaneously interspersed within a single reach-in growth chamber.

Analyses

Blocks were the lowest level of independence in this design; each block contained four families, two from inbred crosses and two from outbred crosses (as in Figure 1). To produce figures we calculated treatment means first by averaging across offspring within a family and then by averaging across families within the block for each population*sex*treatment combination.

Body size and development time data fit assumptions of standard general linear models and were thus analyzed using analysis of variance (ANOVA) with the block effect (nested within population) as the denominator of all F-tests. Mortality data are proportions and do not meet the assumptions of ANOVA. We thus used a paired non-parametric analysis to test for differences in mortality between inbred and outbred beetles within each population. Each block was treated as a single data point with block means calculated as above.

We calculated a measure of the proportional reduction in traits due to inbreeding depression as $\delta = (\text{Mean}_{\text{outbred}} - \text{Mean}_{\text{inbred}}) / \text{Mean}_{\text{outbred}}$. The one exception was for development time for which δ is the proportional increase in development time, $\delta = (\text{Mean}_{\text{inbred}} - \text{Mean}_{\text{outbred}}) / \text{Mean}_{\text{outbred}}$.

δ was calculated separately for each block then averaged across blocks to obtain population means.

To test for variation among populations in the effects of inbreeding depression on development time and body mass, we examined the treatment*population effect in the ANOVA. To test for variation among populations in inbreeding depression on mortality estimates, we tested for variance in δ among populations using non-parametric ANOVA, with each block treated as a single data point.

To test for genetic variation in inbreeding depression, we tested for the presence of a treatment*block interaction. This analysis specifically tests for variance among blocks in the linear difference between fitness of inbred beetles vs. outbred beetles (i.e., tests for variance among blocks in $\text{Mean}_{\text{outbred}} - \text{Mean}_{\text{inbred}}$). For this to be an appropriate test for variance in δ , we divided all trait values by the mean trait value of outbreds in that block ($\text{Mean}_{\text{outbred}}$), such that the interaction term in the ANOVA tests for variation among blocks in $\text{Mean}_{\text{outbred}} / \text{Mean}_{\text{outbred}} - \text{Mean}_{\text{inbred}} / \text{Mean}_{\text{outbred}} = 1 - \text{Mean}_{\text{inbred}} / \text{Mean}_{\text{outbred}}$, i.e., variation in δ .

Results

Beetles suffered inbreeding depression at all stages of the life cycle. Eggs from sib matings were less likely to develop (Figure 2A), less likely to hatch (given they developed; Figure 2B), and larvae were less likely to survive to adult (given the egg hatched; Figure 2C). Overall, inbreeding resulted in a substantial reduction in the proportion of eggs that produced an adult offspring from >80% for outbred offspring in all three populations to an average of only 54% for inbred offspring (Figure 2D). Most of the inbreeding effect on mortality occurred during post-hatch larval development (i.e., highest δ), but the relative degree to which different developmental stages were affected varied among populations (Table 1). There was no evidence that larval mortality was non-random with respect to larval gender – the sex ratio of emerging beetles did not differ from 1 : 1 for either treatment in any population (sign tests, $P > 0.17$ for all treatment-by-population combinations) and there was no evidence that sex ratio differed between the inbred and outbred treatments for any population ($\chi^2 < 0.89$, d.f. = 1, $P > 0.82$ for each).

Of those larvae that survived to adult, inbred beetles took ~1.5 days (>5%) longer to reach adult ($F_{1,104} = 115$, $P < 0.001$; Figure 3), but there was no effect of inbreeding on adult body mass ($F_{1,104} = 0.35$, $P = 0.56$; Figure 3). The degree to which inbreeding increased mortality varied among these three populations (Figure 2; statistics in Table 1), although the population effect was marginally non-significant for egg hatch. Post hoc tests indicate that total inbreeding depression on survival was lower in the

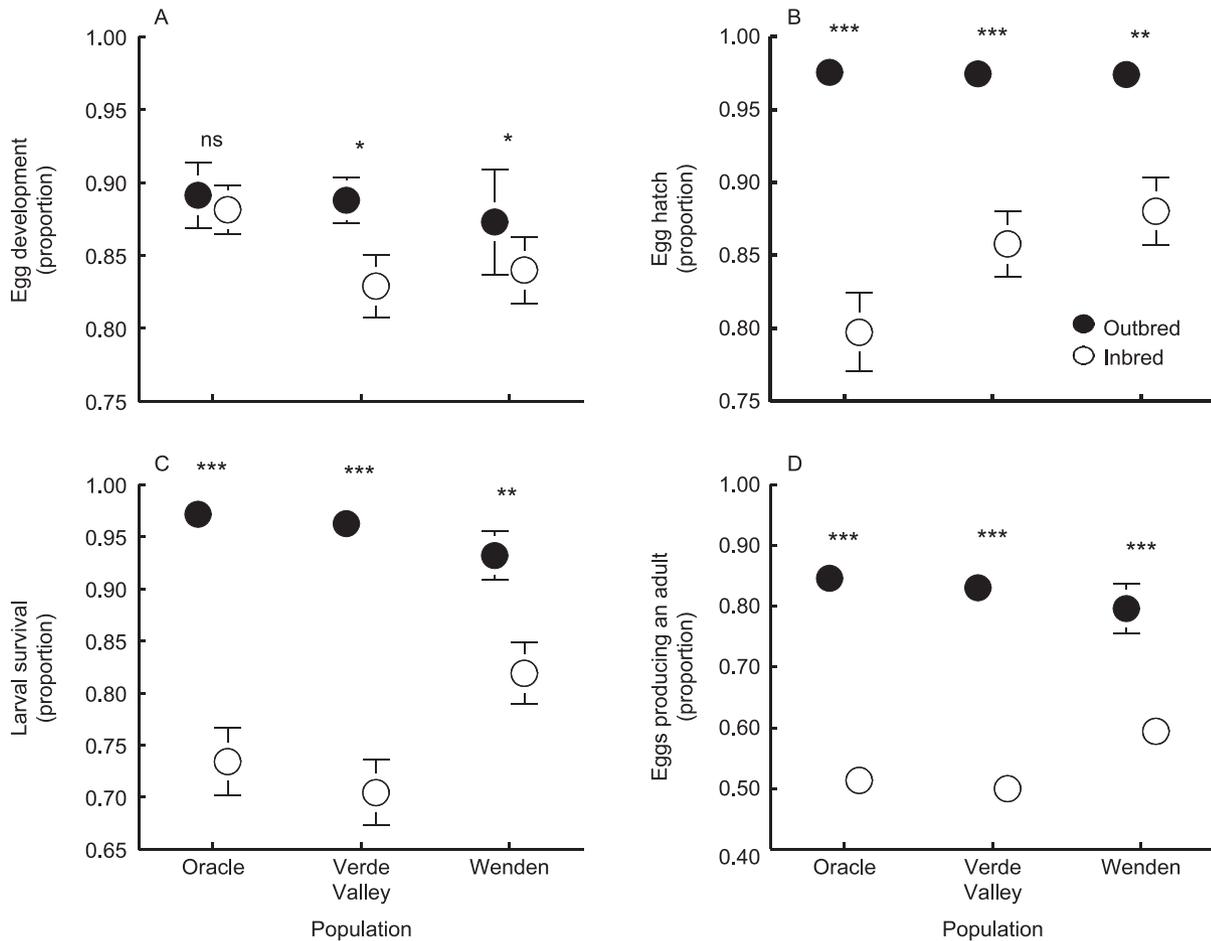


Figure 2 The effect of inbreeding (sib mating) on (A) the proportion of eggs failing to develop, (B) the proportion of developed eggs failing to hatch, (C) the proportion of hatched eggs failing to produce an adult offspring, and (D) the proportion of eggs that produced an adult offspring (after all sources of egg and larval mortality) in three populations of the seed beetle, *Stator limbatus*. Means are calculated first by averaging across families in a block, then across blocks within a replicate. Significance levels are from a Wilcoxon signed-rank test comparing inbred vs. outbred means, with each block treated as one independent data point (each block produces one mean for inbred and one mean for outbred offspring); ns, non-significant ($P > 0.05$); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Wenden population than in either the Oracle or Verde Valley populations, although all three populations experienced substantial inbreeding depression relative to the magnitude of δ commonly observed in most other organisms.

Mean development time varied among the populations (highest in the Oracle population and lowest for the Wenden population: $F_{2,104} = 5.6$, $P = 0.005$), but the magnitude of inbreeding depression on development time did not (no population*treatment interaction, Figure 4; statistics in Table 1). Neither mean development time nor the effect of inbreeding on development time differed between the sexes ($F_{1,104} < 2.3$, $P > 0.15$ for both). Body mass also varied among populations ($F_{2,104} = 3.72$, $P = 0.03$; Figure 3) and differed between the sexes (males are larger: $F_{1,104} = 12.8$,

$P < 0.001$) but there was no evidence that the effect of inbreeding treatment varied among populations nor differed between sexes (no sex*treatment or population*treatment interaction: $F < 0.47$, $P > 0.55$ for each).

We found no evidence that inbreeding depression on survivorship varied among blocks within populations ($F < 0.96$, $P > 0.52$ for all stages of development). However, the effect of inbreeding on development time did vary significantly among blocks (nested within populations; $F_{104,559} = 2.02$, $P < 0.001$). The magnitude of inbreeding depression on female development time was positively correlated (among blocks) to the magnitude of inbreeding depression on male development time ($F_{1,79} = 6.4$, $P = 0.01$).

Table 1 The magnitude of inbreeding depression in three populations of *Stator limbatus*. $\delta = (\text{Mean}_{\text{outbred}} - \text{Mean}_{\text{inbred}}) / \text{Mean}_{\text{outbred}}$ except for development time, for which an increase reflects lower fitness and thus $\delta = (\text{Mean}_{\text{inbred}} - \text{Mean}_{\text{outbred}}) / \text{Mean}_{\text{inbred}}$. δ is calculated separately for each block and then averaged across blocks. The χ^2 is for a non-parametric analysis of variance testing for a significant variance among populations, with each block treated as a single data point per line. The F-test is the test for a significant treatment*population interaction for development time and adult body mass. δ has an expectation of 0 when there is no inbreeding depression, and can vary from positive to negative depending on whether inbreds have lower or higher performance than outbreds, respectively

Trait	Population			Variance among populations	Post hoc comparisons ¹
	Oracle	Verde Valley	Wenden		
Egg development	-0.01 ± 0.05	0.07 ± 0.03	0.08 ± 0.03	$\chi^2 = 6.1$, d.f. = 2 (P < 0.05)	O = VV = W
Larval hatch	0.21 ± 0.03	0.11 ± 0.03	0.10 ± 0.03	$\chi^2 = 5.4$, d.f. = 2 (P = 0.07)	O > VV = W
Larval survival	0.25 ± 0.04	0.27 ± 0.03	0.11 ± 0.05	$\chi^2 = 10.2$, d.f. = 2 (P < 0.01)	O = VV > W
Eggs producing an adult ²	0.41 ± 0.03	0.35 ± 0.04	0.22 ± 0.04	$\chi^2 = 5.6$, d.f. = 2 (P < 0.05)	O = VV > W
Development time					
Females	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	$F_{2,104} = 0.39$ (P = 0.68)	O = VV = W
Males	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	$F_{2,102} = 1.12$ (P = 0.33)	O = VV = W
Adult body mass					
Females	0.02 ± 0.02	-0.01 ± 0.02	-0.03 ± 0.02	$F_{2,104} = 1.05$ (P = 0.35)	O = VV = W
Males	0.02 ± 0.02	-0.00 ± 0.02	-0.00 ± 0.03	$F_{2,102} = 0.14$ (P = 0.87)	O = VV = W

¹O, Oracle; VV, Verde Valley; W, Wenden.

²The proportion of eggs laid that produced an emerging adult offspring.

Discussion

Stator limbatus exhibited substantial inbreeding depression at all stages of development. Eggs from sib matings were less likely to develop and hatch, and larval mortality was higher and development time longer for offspring of sib matings. The only measured trait not affected by inbreeding was adult body mass.

Stator limbatus occur in large populations, and are highly mobile insects, such that inbreeding is likely rare in nature. Indeed, molecular data indicate that populations are highly outbred (Morse, 2003). A low frequency of inbreeding in nature means that recessive alleles are rarely homozygous, and thus rarely expressed, allowing them to increase to moderate frequency in populations. In a companion study to this (CW Fox & KL Scheibly, unpubl.) we created replicate lines that were sequentially inbred for three to four generations ('purge' lines). We measured the rate at which population mean fitness increased, and inbreeding depression decreased, relative to control (outbred) lines. Our objective was to test whether recessive deleterious alleles of large effect could be responsible for the substantial inbreeding depression observed in this current study. We found that inbreeding depression decreased substantially in the inbred lines and even approached $\delta = 0$ for two traits in one of the replicates. This indicates the presence of recessive deleterious alleles of large effect that can be purged from the population in just a couple

generations of sib mating (Crnokrak & Barrett, 2002). Although we only used the Oracle population for the purge study, we expect deleterious alleles of large effect to be common in all populations.

Numerous studies have shown that environmental conditions affect the magnitude of inbreeding depression and that inbreeding reduces resistance to stress (Armbruster et al., 2000; Kristensen et al., 2003). Our study was performed in the laboratory under somewhat ideal conditions for *S. limbatus*. Some authors have argued that captivity masks inbreeding depression (Joron & Brakefield, 2003). It is thus likely that the fitness consequences of inbreeding for *S. limbatus* would be even greater in the field than we observed here in the laboratory (Keller & Waller, 2000) although the frequency of inbreeding is likely low in the field because populations of *S. limbatus* are large. It is possible that the large effect of inbreeding on *S. limbatus* survival and development is the reason that dispersal is so high in nature (Morse, 2003); inbreeding depression produces a large fitness advantage for outbred animals (due to heterosis), which selects for increased dispersal and leads to high gene flow among populations (Saccheri & Brakefield, 2002).

We found that the effect of inbreeding on eggs and larvae varied among these three *S. limbatus* populations indicating that these populations likely differ in their frequency of recessive deleterious alleles affecting egg development, egg hatch, and larval survival. The Wenden

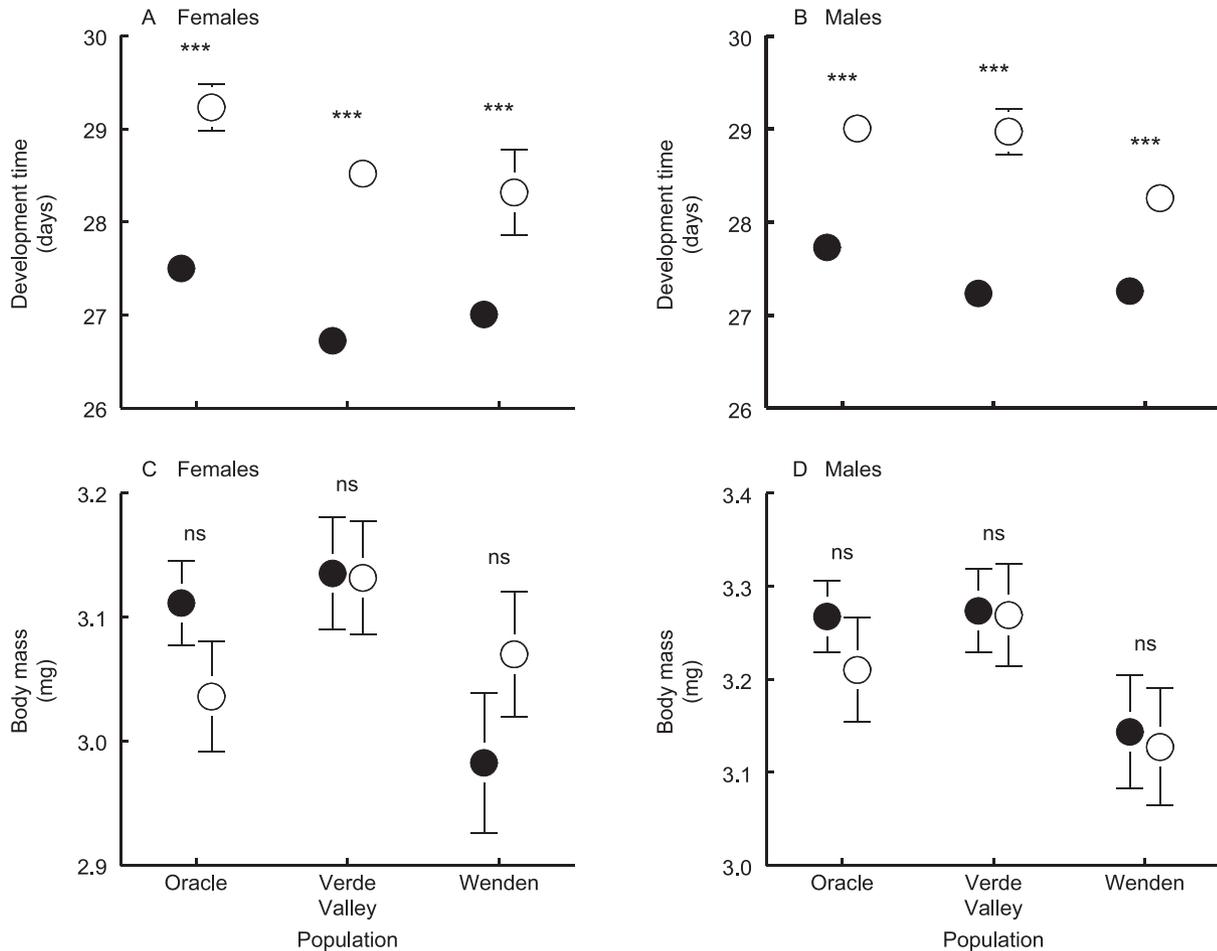


Figure 3 The effect of inbreeding (sib mating) on egg-to-adult development time and adult body mass at emergence (i.e., at maturity) for three populations of the seed beetle, *Stator limbatus*. Means are calculated first by averaging both families in a block, then across blocks within a replicate. Note the ~1.5-day increase in development time due to inbreeding. There is no detectable effect of inbreeding on adult body mass at emergence; ns, non-significant ($P > 0.05$); *** $P < 0.001$.

population is the most isolated of these three *S. limbatus* populations (it represents a small number of trees along a highway in a largely agricultural part of central Arizona). We thus expect this population to be the smallest and thus most likely to experience inbreeding in nature. The Wenden population did indeed exhibit the least inbreeding depression on the proportion of eggs producing an adult offspring, a variable that includes all sources of larval mortality (Figure 2D). It is unclear if this reduced inbreeding depression in Wenden beetles is directly related to habitat fragmentation, but it is consistent with the expectation that more isolated populations experience greater inbreeding such that deleterious recessive alleles are frequently expressed and thus selected against in these more isolated populations (Keller & Waller, 2000).

We detected a large effect of inbreeding on egg hatch (Figure 2B). Many previous studies have likewise shown large effects of inbreeding on egg hatch in insects (Dahlgard & Loeschke, 1997; Higashiura et al., 1999; Morjan et al., 1999; Armbruster et al., 2000; van Oosterhout et al., 2000; Nieminen et al., 2001; Haikola, 2003). However, few of these studies distinguish effects of inbreeding on the proportion of eggs fertilized, embryonic development, and the proportion of developing eggs that hatch. This is because most inbreeding studies manipulate the inbreeding coefficient across multiple generations of sib mating. Although this allows researchers to study the consequences of variation in the inbreeding coefficient, it confounds effects of inbreeding on parental traits (such as male fertility) with effects on offspring traits (such as embryonic mortality).

Our experimental design does not confound parental vs. offspring inbreeding coefficients (because all parents are outbred). Also, because we scored eggs individually our estimates of inbreeding depression on egg hatch include only eggs that began cell differentiation before death. Thus, the inbreeding depression in egg hatch observed here must necessarily be due to post-fertilization developmental problems. However, our design does not allow us to examine the effect on inbreeding on fertility (because all parents are outbred; only their offspring vary in degree of inbreeding), although effects of inbreeding on fertility can be large (Saccheri et al., 2005).

Inbreeding depression varied substantially among the traits that we measured, being relatively large for egg hatch and larval survival ($\delta > 0.10$ and as high as 0.27), smaller for development time ($\delta \sim 0.05$), and absent for adult body size. The lack of an effect of inbreeding on body size is consistent with most studies of inbreeding depression; primary fitness traits, such as viability, fertility, fecundity, and age at maturation (e.g., development time) tend to show larger effects of inbreeding than do traits less directly associated with fitness (e.g., morphological traits) (Falconer, 1989; Lynch & Walsh, 1998; Roff, 1998; DeRose & Roff, 1999, but see Rao et al., 2002; Reale & Roff, 2003). Experimental studies have demonstrated that mutations affecting life history traits often show significant dominance/overdominance (e.g., Houle et al., 1997; Peters et al., 2003), that natural variation in life history traits is often affected by substantial dominance (e.g., Roff & DeRose, 2001), and that morphological traits tend to exhibit less dominance genetic variation than do life history traits (Roff, 1997). It is likely that these differences in dominance between life history and morphological traits causes the observed difference in inbreeding depression (Lynch & Walsh, 1998; DeRose & Roff, 1999) but this hypothesis has not been directly tested.

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