Effect of Inbreeding on Host Discrimination and Other Fitness Components in a Seed Beetle

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ABSTRACT   Inbreeding is well known to have adverse effects on fitness-related traits in insects, but less is known about its effect on behavior, particularly outside the context of mating success. We used the seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) to determine the effect of moderate inbreeding (inbreeding coefficient $F = 0.25$) on host discrimination, that is, the ability of ovipositing females to avoid occupied hosts and thereby reduce competition among larval offspring. Inbreeding depression was simultaneously estimated for morphological (body mass), physiological (egg hatch), and life-history (realized fecundity) traits in the same population. Compared with outbred females, inbred females exhibited an 11% reduction in mass, a 9% reduction in fecundity, and a small (4%) but significant reduction in egg hatching success. Nearly all of the decline in fecundity among inbred females could be explained by the decline in body mass. In contrast to the other three traits, host discrimination was unaffected by inbreeding. Inbred females actually distributed their eggs slightly more uniformly among seeds than did outbred females, but this difference disappeared after we corrected for inbreeding effects on fecundity. An assay of doubly inbred females ($F = 0.375$) confirmed that the tendency to avoid occupied hosts is immune to moderate inbreeding. The lack of inbreeding depression for host discrimination suggests that heritable variation for the trait within populations (as detected in previous studies) is largely caused by alleles with additive rather than dominant effects, and contrasts with predictions based on population crosses.

KEY WORDS   *Callosobruchus maculatus*, fecundity, genetic load, inbreeding depression, oviposition

Inbreeding, or mating between close relatives, increases homozygosity beyond that expected by random mating (Lynch and Walsh 1998). Greater homozygosity can lead to a decline in fitness (inbreeding depression) through either an increase in the expression of deleterious recessive alleles (the directional dominance hypothesis) or a loss of heterozygote advantage (the overdominance hypothesis) (Roff 2002, Charlesworth and Willis 2009; but see also Vergeer et al. 2012). The former mechanism appears to be more common in natural populations, which inevitably harbor rare deleterious alleles that are masked from selection in heterozygotes (Deng et al. 1998, Carr and Dudash 2003). The widespread occurrence of inbreeding depression in nature is reflected in the frequent evolution of specific inbreeding-avoidance mechanisms (Pusey and Wolf 1996). Inbreeding costs can also shape the evolution of plant and animal mating systems (e.g., by favoring polyandry when mating between relatives is likely; Kelly 2005, Michalczyk et al. 2011, Harano and Katsuki 2012) and increase extinction rates in small populations (Saccheri et al.

1998, Bijlsma et al. 2000, Nieminen et al. 2001, Fauvergue et al. 2012, Mattila et al. 2012).

Although inbreeding depression is common (Crnokrak and Roff 1999, Keller and Waller 2000), its magnitude can vary considerably among traits within the same population or among populations within a single species (Fox and Scheibly 2006). Such variability is due in part to the different numbers and kinds of deleterious recessive alleles that underlie each trait or are present in any particular population (Haikola et al. 2001, Gerloff and Schmid–Hempel 2005, Facon et al. 2011). Inbreeding depression has been well documented for a variety of morphological, physiological, and life-history traits in insects, particularly in *Drosophila* and *Tribolium* (e.g., Hughes 1995, Pray and Goodnight 1997, DeRose and Roff 1999, Vermeulen and Bijlsma 2004), but effects of inbreeding on behavior are not as well known. Most behavioral studies have focused on mate choice or mating success (Meffert and Bryant 1991, Miller et al. 1993, Joron and Brakefield 2003). In this study, we examined the effect of moderate inbreeding on an egg-laying behavior, host discrimination.

Many insects lay eggs in small, discrete hosts, such as seeds, fruits, or other insects (as in the case of parasitoids). Not surprisingly, females in some of these species can detect and avoid occupied hosts and thereby reduce the degree of competition experi-

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enced by their sedentary offspring (Nufio and Papaj 2001, Messina 2002). This behavior has been well studied in the seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae). Eggs are laid singly on the surface of legume seeds, and hatching larvae burrow into the seed directly beneath the oviposition site. Larvae cannot move between seeds and so must complete development within a single seed. Ovipositing females can discriminate between seeds bearing different egg densities as well as between seeds with or without eggs (Messina and Renwick 1985a, Wilson 1988, Horng 1997). Avoidance of occupied seeds produces a nonrandom dispersion of eggs that is uniform or nearly uniform (Messina and Mitchell 1989). Fitness benefits associated with host discrimination are substantial; larval competition within seeds decreases survival, prolongs development, and reduces the size, fecundity, and longevity of *C. maculatus* adults (e.g., Mitchell 1975; Credland et al. 1986; Messina 1991, 2004; Toquenaga 1993). Nevertheless, there is considerable genetic variation for the trait both within and between populations (Messina 1993; Messina and Karren 2003; Fox et al. 2004a, 2009). Line crosses between populations differing in host discrimination have indicated directional dominance toward more uniform egg-laying (Messina 1989; Fox et al. 2004a, 2009). If alleles that reduce host discrimination are predominantly recessive, they would be exposed by the greater homozygosity after inbreeding.

Callosobruchus beetles have also served as model organisms for estimating effects of inbreeding on fitness-related traits (Tanaka 1990, Edvardsson et al. 2008, Bilde et al. 2009). Periods of inbreeding may be common in these stored-product pests, because populations in stores of grain legumes are often founded by small numbers of individuals that emerge from field-infested or transported seeds (Tran and Credland 1995, Semeao et al. 2012). Beetle populations in both the field and laboratory exhibit substantial inbreeding depression for such traits as larval survival and development time, adult body size and lifespan, and male and female reproduction (Tran and Credland 1995; Fox et al. 2006, 2007, 2012). Inbreeding depression for some beetle traits (e.g., survival) increases with the stressfulness of the environment (Fox and Reed 2010, Fox et al. 2011).

Here, we quantified the degree of inbreeding depression with respect to how females distribute their eggs among available seeds. We compared the effect of inbreeding on oviposition behavior with its effect on morphological (body size), physiological (egg hatch), and life-history (fecundity) traits in the same population. Our experimental design also allowed us to investigate whether among-family variation in inbreeding depression is correlated between traits, that is, whether families especially susceptible to inbreeding for one trait are also highly susceptible for another (Fox 2005).
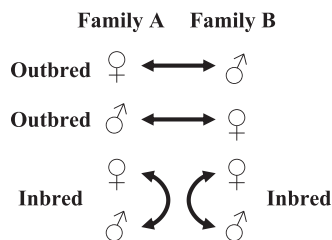

Fig. 1. Block design for estimating inbreeding depression. Each block is created by crossing two unrelated families, with two outbred matings (reciprocal crosses between families) and two inbred matings (crosses between full siblings) per block. Outbred and inbred females from the same block will on average possess the same set of alleles but differ in homozygosity.

Materials and Methods

Source Population. We used a laboratory population of *C. maculatus* that was established from infested mung beans, *Vigna radiata* (L.) Wilczek, in southern India (Messina 1991, Mitchell 1991). It had been maintained in the laboratory on its ancestral host for >200 generations before the start of the experiments and was thus likely to have reached genetic equilibrium with respect to laboratory conditions. Despite long-term rearing in the laboratory, this population exhibits levels of inbreeding depression similar to those in wild seed-beetle populations (Fox et al. 2007). Females in this Indian population show especially strong host discrimination, perhaps because the population is also characterized by unusually strong "contest" competition between larvae within seeds; on mung bean, nearly all seeds receiving multiple larvae yield only a single emerging adult (Messina 1991, 2004). Unlike females from populations with weaker host discrimination, Indian females usually cease ovipositing if all available seeds bear two eggs (Messina and Mitchell 1989, Messina and Karren 2003). Stock cultures were formed by adding 1,500–2,500 newly emerged adults (estimated by volume) to a 2-liter jar containing ≈750 g mung beans in each generation. We maintained beetle cultures and conducted the experiments in a growth chamber at 24°C and constant light.

Experimental Design. In the main experiment, we used a "block" design to estimate the magnitude of inbreeding depression (Fig. 1; Roff 1998). We first obtained unmated females and males by isolating 1,200 infested seeds from a stock culture in individual 4-ml vials. Newly emerged beetles were collected to form 200 parental pairs (these beetles would serve as the grandparents of the inbred and outbred test females). Each pair was placed in a 60-mm petri dish containing ≈100 mung beans. From each dish, we then isolated 12 seeds bearing a single hatched egg (i.e., a single offspring larva) in 4-ml vials. Seeds isolated from the same dish thus bore full siblings.

When the offspring generation began to emerge, we created blocks by pairing two randomly chosen families per block. Two females and two males were collected from each family for either reciprocal crosses

between families (to produce outbred offspring) or for crosses between full-sibs within a family (to produce inbred offspring) (Fig. 1). The advantage of this design is that inbred offspring are created from the same set of alleles as the outbred offspring to which they are directly compared; the two groups within a block differ only in their levels of homozygosity (Fox 2005). Variation among blocks in the magnitude of inbreeding depression contains a component of the population-wide genetic variation for inbreeding depression.

Pairs used for between- or within-family crosses were placed in petri dishes with ≈100 mung beans. We then isolated 14 seeds bearing a single offspring larva for each family. Vials were checked daily. Newly emerged females (up to eight inbred or outbred females per family) were weighed on an electronic balance (Mettler Instrument Corp., Hightstown, NJ). Each female was paired with an unrelated, outbred male, and both beetles were placed in a petri dish with 30 mung beans. Females produced from crosses between full-siblings had an inbreeding coefficient (F) of 0.25; those produced from between-family crosses in the same block had a coefficient of zero (Fig. 1).

After females died and eggs had ample time to hatch, dishes were frozen to stop larval development. We recorded for each dish the number of eggs, the proportion of eggs that hatched, and the dispersion of eggs among the 30 available seeds. An egg was considered hatched if the larva successfully burrowed into the seed. Thus, unhatched eggs included both eggs showing no embryogenesis and those in which a larva died before fully entering the seed (all or part of the dead larva could be observed beneath the transparent egg cover). A comparison of mean fecundity between inbred and outbred females did not allow us to detect whether the two groups differed in the ability to lay any eggs, which could even result from differences in mating success. We therefore calculated for each block the proportion of females laying at least one egg, and included this trait in the analyses described below.

We quantified egg dispersion using the U index of Messina and Mitchell (1989). When egg distributions are more uniform than expected by chance, this index is preferable to other dispersion indices (e.g., the variance-to-mean ratio), because it is less affected by the mean itself (Messina and Mitchell 1989). A female's U score is obtained by first estimating her number of "mistakes," that is, the number of eggs that would have to be shifted among the 30 seeds to transform her observed dispersion into the most uniform one possible (e.g., one in which no seed receives two eggs unless all seeds already bear an egg). This number is then compared with the expected number of mistakes, that is, the number of eggs that would have to be shifted if a female distributed the same number of eggs randomly (according to a Poisson distribution). $U = (\textit{Expected mistakes} - \textit{Observed mistakes})/\textit{Expected mistakes}$, so that a female distributing eggs randomly obtains a score of zero, and a female that distributes eggs in the most uniform way possible obtains a score of one. A Python-language program was written to

calculate the observed number of mistakes and generate the corresponding Poisson distributions and expected numbers of mistakes for a female laying any number of eggs on any number of seeds.

In total, we measured mass at adult emergence, realized fecundity, egg hatch, and egg dispersion for 1,451 females (689 inbred and 753 outbred) from 61 blocks, with a mean ($\pm$ SD) of 23.8 ($\pm$3.3) females per block.

Analyses. Blocks are the lowest level of independence in this experimental design. Thus, we used block means in our analyses. Block means were calculated first by averaging across offspring within a family and then by averaging across the two families per treatment within the block (i.e., between the two inbred families or two outbred families per block, Fig. 1). Inbreeding depression (δ) was calculated for each block separately as $\delta = (\textit{Mean}_{outbred} - \textit{Mean}_{inbred})/\textit{Mean}_{outbred}$ (Lynch and Walsh 1998). The magnitude of δ is thus a proportional measure of the degree to which outbred females have higher trait values than inbred females. Values of δ were then averaged across blocks to obtain a population mean and SE.

Not all traits were normally distributed. Therefore, we used nonparametric tests for the analyses. To test whether inbreeding depression, δ, differed from 0, we used a Wilcoxon signed-rank test, with each block providing a single data point for the analysis. Because we obtained a significant effect of inbreeding for both female mass and fecundity, and mass is usually positively correlated with fecundity among *C. maculatus* females, we tested whether the effect of inbreeding on fecundity persists even after we accounted for the effect of reduced mass. We removed the effect of mass by calculating least-squares means for fecundity for each block-by-treatment combination using SAS GLM (SAS Institute Inc., Cary, NC), so that the resulting least-squares means for fecundity were independent of mass.

Doubly Inbred Females. A second small-scale experiment was performed to estimate mean values for the four traits after two generations of full-sib mating instead of one. Twenty of the original 200 families were not incorporated into the block design described above, but we nevertheless performed two full-sib crosses within each family. Emerging inbred offspring ($F = 0.25$) were then paired with a full sibling and placed in dishes containing ≈100 mung beans. We later isolated 20 one-larva seeds per family in 4-ml vials and collected up to 10 test females per family. As the product of two generations of full-sib mating, these females had an inbreeding coefficient, F, of 0.375. Each female was again weighed, paired with an unrelated, outbred male, and placed in a dish with 30 mung beans. Body mass, fecundity, egg hatch, and egg dispersion were estimated for 163 test females (8.2 females per family). Mean trait values for doubly inbred females were not statistically compared with those of singly inbred females, because the two groups were not assayed simultaneously (and we did not generate a corresponding group of outbred females for the doubly

Table 1. Mean trait values (± SE) and mean inbreeding depression (δ ± SE) for four traits measured in outbred and inbred females of *C. maculatus*

Trait	Outbred	Inbred	δ	P^a
Female mass (mg)	6.28 ± 0.06	5.60 ± 0.06	0.11 ± 0.01	<0.001
Proportion laying eggs	0.991 ± 0.003	0.981 ± 0.006	0.01 ± 0.01	0.22
Fecundity	42.3 ± 0.6	37.9 ± 0.6	0.09 ± 0.02	<0.001
Egg hatch (proportion)	0.92 ± 0.01	0.88 ± 0.01	0.04 ± 0.01	<0.001
Egg dispersion (*U* score)	0.879 ± 0.008	0.912 ± 0.006	−0.04 ± 0.01	<0.001

Trait means were calculated by first averaging across females within families, then between two families per treatment within a block, and finally across 61 blocks. δ was also calculated separately for each block, and then averaged among blocks.
[a] Wilcoxon signed-rank test of the hypothesis that δ differs from zero, with each block providing a single data point.

inbred females). Means for singly and doubly inbred females were merely compared qualitatively.

Results

Moderate inbreeding caused a significant reduction in female mass; inbred females were ≈11% smaller than outbred females (Table 1). Inbreeding had no effect the probability of oviposition; >98% of both inbred and outbred females laid at least one egg (Table 1). However, among females that did lay eggs, inbred females laid ≈9% fewer eggs than did outbred females, and this difference was highly significant (Table 1). A regression analysis confirmed that fecundity was positively correlated with female mass ($F = 42.1$, df = 1, 744, $P < 0.001$ for outbred females; $F = 126.0$, df = 1, 682, $P < 0.001$ for inbred females) and that mass covaried with fecundity among blocks ($F > 4.2$, df = 1, 59, $P < 0.05$ for both inbred and outbred females). Therefore, we tested whether the effect of inbreeding on mass could fully account for the effect of inbreeding on fecundity. Analysis of mass-corrected fecundity (using least-squares means for fecundity, which removed the effect of body size) indicated that the inbreeding depression on fecundity was indeed mainly because of inbreeding depression in body size: δ values for mass-corrected fecundity were significantly lower than those for total fecundity ($δ = 0.03$ versus $δ = 0.09$; Wilcoxon signed-rank test; $P < 0.001$). Although the magnitude of inbreeding depression for mass-corrected fecundity was quite small ($δ = 0.028 ± 0.026$), it remained significantly greater than zero (Wilcoxon signed-rank test; $P = 0.005$).

Like mass and fecundity, egg hatch was also reduced significantly by inbreeding. Because all females were mated to unrelated and outbred males, sperm quality of mates would have been similar between females from the inbred and outbred treatments. Nevertheless, eggs laid by inbred females were ≈4% less likely to hatch than eggs laid by outbred females (Table 1).

As expected from previous studies, females were adept at detecting and avoiding occupied seeds. Most individuals spread their eggs among the 30 available seeds in a perfect or nearly perfect uniform distribution, so that nearly all *U* scores were one or close to one (Table 1). Because females frequently laid ≈40 eggs on the 30 available seeds, a score of one corresponds to depositing exactly one egg on 20 of the seeds and two eggs on the remaining 10 seeds. Contrary to expectation, inbred females showed no reduction in the ability to discriminate among seeds with varying egg densities. In fact, inbred females laid eggs slightly but significantly *more* uniformly than outbred females; *U* scores were ≈4% lower among outbred females than among inbred females (Table 1).

This effect of inbreeding status on egg dispersion was largely because of its concomitant effect on fecundity. Although the *U* score is constructed to eliminate a purely statistical dependence of egg dispersion on mean egg number, females that laid more eggs nevertheless tended to lay them less uniformly. For both outbred and inbred females, there was a negative correlation among blocks between mean fecundity and mean *U* scores (Spearman rank correlations, $r_s = -0.71$ and -0.58, respectively; $P < 0.001$ for each group). Similarly, the degree of inbreeding depression (δ) for egg dispersion was negatively correlated among blocks with the degree of inbreeding depression for fecundity ($r_s = -0.61$; Fig. 2). This was the only significant Spearman-rank correlation between pairs of traits for level of inbreeding depression. To test whether the small difference in egg dispersion between outbred and inbred females was because of differences in fecundity, we recalculated *U* values as least-squares means that removed the effect of fecundity (SAS GLM was again used as described in the Materials and Methods) that removed the effect of fecundity. After this correction, mean *U* scores became very similar between outbred and inbred females ($U = 0.90 ± 0.01$ and $0.91 ± 0.01$, respectively), and the level of inbreeding depression for fecundity-corrected *U* did not differ significantly from zero (δ =
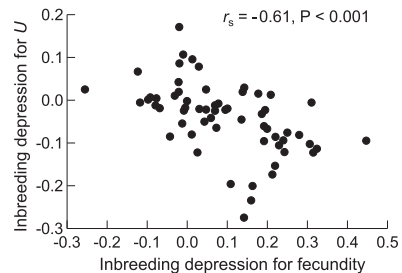

Fig. 2. Relationship between the degree of inbreeding depression (δ) for *U* scores (host discrimination) and realized fecundity. Each point represents a block mean (N = 61 blocks).

-0.025 ± 0.013; Wilcoxon signed-rank test; $P = 0.091$). Thus, with or without the correction for fecundity, we obtained no evidence that inbreeding reduces a female's ability to recognize and avoid occupied seeds.

The behavior of the 163 doubly inbred females ($F = 0.375$) confirmed that host discrimination in *C. maculatus* is largely immune to at least moderate inbreeding. The mean U score ($\pm$SE) of this group was 0.92 ± 0.01 and was thus just as high as the mean U score of females produced by only one generation of full-sib mating (Table 1). Doubly inbred females had an average mass at adult emergence of 5.28 ± 0.05 mg, laid an average of 36.4 ± 0.9 eggs, and average hatching success of their eggs was 0.87 ± 0.01. Means for these three traits were thus close to those observed among singly inbred females and lower than those observed among outbred females in the block-design experiment (Table 1).

Discussion

Following a single generation of full-sib mating, inbreeding depression was observed for three of the four traits measured in this study: body mass, fecundity, and egg hatch showed an effect of inbreeding, but host discrimination did not. Previous studies of *C. maculatus* have revealed inbreeding effects for several other traits, including egg size, larval survival, and development time, and adult lifespan (Fox et al. 2007, Fox and Stillwell 2009). Thus, host discrimination may be unusual in its apparent resistance to the effects of moderate inbreeding. Host discrimination is best characterized as a composite behavior; variation in the tendency to avoid occupied seeds could be generated by underlying variation in search effort (e.g., the thoroughness by which a female finds and inspects potential hosts), in the strength or composition of the putative marking pheromone, or in the sensitivity of egg-laying females to pheromone on previously deposited eggs (Messina and Renwick 1985b, Credland and Wright 1990, Messina et al. 1991, Messina and Dickinson 1993, Parr et al. 1996). Our results suggest that none of these component traits is altered by inbreeding in a manner that translates into less uniform oviposition.

This study was limited to a single population, which makes it difficult to generalize about inbreeding effects on oviposition at the scale of an entire species. However, our results are consistent with those of Tran and Credland (1995), who used a different approach by performing >20 generations of serial inbreeding in a Brazilian-origin population of *C. maculatus.* They found no consistent decline in egg dispersion among four inbred lines, although there was considerable variation among lines. Their experimental design could not rule out a confounding effect of purging, however, in which the serial inbreeding used to generate inbred lines selectively eliminates deleterious recessive alleles from the population (see Nepoux et al. 2010). As a consequence, the genetic load (and thus the estimate of inbreeding depression) in the test generation may have substantially underestimated the genetic load present in the initial outbred population. Interestingly, Tran and Credland (1995) used a population with relatively poor host discrimination; females in their experiments distributed eggs nonrandomly, but egg dispersions were not nearly as uniform as those produced in this study (Table 1; Messina and Mitchell 1989). The likelihood of detecting inbreeding depression therefore does not appear to be related to the average level of host discrimination observed among outbred females.

We expected to observe an adverse effect of inbreeding on host discrimination in part because of the mode of inheritance of this trait in crosses between divergent geographic populations or laboratory selection-lines. These studies consistently indicated that there is directional dominance toward more uniform egg-laying, with an additional contribution of epistasis (Messina 1989; Fox et al. 2004a, 2009). Therefore, we might assume that alleles that reduce host discrimination would be predominantly recessive, and would be exposed by the greater homozygosity after inbreeding. However, this study and that of Tran and Credland (1995) suggest that genetic variation in host discrimination within populations, as demonstrated by both breeding designs (Messina 1993) and selection experiments (Messina and Karren 2003), is instead largely because of alleles with additive effects. Reconciling the contrasting pattern of genetic variation between versus within populations may require identifying specific candidate genes influencing host discrimination. For another fitness-related trait, lifespan, inbreeding experiments and population crosses were consistent in demonstrating that there is directional dominance in *C. maculatus* populations (Fox et al. 2004b, 2006).

More studies are needed before we can generalize about inbreeding loads associated with behavioral traits in insects. Inbreeding is well-known to affect male courtship (e.g., Joron and Brakefield 2003), but effects on oviposition choices remain relatively unexplored. One might predict that a complex behavior would be especially susceptible to inbreeding depression because phenotypic variation in behavior is commonly determined by nonadditive genetic effects, with strong dominance and/or epistasis (Meffert et al. 2002, Mackay 2009). However, Nepoux et al. (2010) found that moderate inbreeding ($F = 0.375$) in *Drosophila* had no effect on two behavioral traits, aversive learning or odor responsiveness, even though it was sufficient to reduce egg-to-adult viability. They speculated that the absence of inbreeding depression for learning was a consequence of mostly additive-genetic variation and stabilizing selection. If a trait is under stabilizing selection rather than directional selection, alleles that increase the value of a trait may be as deleterious as those that decrease it, and standing genetic variation is not as likely to show directional dominance. Such a scenario could apply to host discrimination in *C. maculatus* if there is a trade-off associated with the highest level of discrimination (e.g., if careful inspection of a large number of seeds delays oviposition or reduces lifetime fecundity). Additive-

genetic variation for host discrimination could also be maintained as a pleiotropic effect of selection on other traits (Messina 1993, Messina and Fox 2011). Like aversive learning in *Drosophila,* host discrimination in *C. maculatus* may also involve a modification of behavior with experience. A role of learning has been suggested because females in some populations respond to egg densities quantitatively; once all available seeds bear eggs, they are able to detect and prefer seeds with below-average egg densities (Messina and Renwick 1985a, Wilson 1988, Mitchell 1990, Horng 1997).

The significant effects of inbreeding on mass, fecundity, and egg hatch observed in this study confirm that even long-term laboratory populations of *Callosobruchus* species maintain substantial genetic loads for fitness-related traits (Fox et al. 2007). Typical culturing methods and population sizes in the laboratory thus do not appear to cause efficient purging of deleterious alleles (Lynch and Walsh 1998). Previous studies have similarly detected effects of inbreeding on body mass and hatching rate in *C. maculatus* (Fox et al. 2007, 2011), but it does not appear that these results can be extrapolated to other seed beetle species (Fox and Scheibly 2006, Harano 2011). In our study, reduced fecundity among inbred females was mainly a consequence of lower body mass, but there may be additional, direct effects of inbreeding on egg production (Tran and Credland 1995). Using a different *Callosobruchus* species and a similar protocol, Harano (2011) found that one generation of full-sib mating decreased fecundity by 11% without reducing female size (measured as length instead of mass). Other environmental stresses, such as low host quality and larval competition, can reduce egg number in *C. maculatus* in a size-independent manner (Timms 1998, Vamosi and Lesack 2007).

Our experiment demonstrated that eggs laid by inbred females are less likely to hatch than eggs laid by outbred females, even when inbred females mate with unrelated, outbred males. Such a transgenerational effect of inbreeding may be expected because early development is controlled by maternally derived proteins and mRNA transcripts in the fertilized egg, which are in turn dependent on maternal genotype and thus maternal inbreeding status. Eggs produced by inbred females may also be inferior at the stage of egg formation and provisioning. A previous study of *C. maculatus* found that inbred males produce fewer sperm (Fox et al. 2012), so it is possible that inbred females experience similar problems during oogenesis, which could reduce both egg number and quality. Given our broad definition of egg hatch, more detailed studies can reveal whether eggs laid by inbred females exhibit reduced embryogenesis, greater larval mortality before seed entry, or both (Fox et al. 2007).

This study illustrates the uncertainty in predicting the magnitude of inbreeding depression for different traits within a population (Roff 1998, Fox et al. 2007). Theoretically, traits more closely related to fitness are expected to show greater inbreeding depression because they are underlain by greater non-additive genetic variance, specifically directional dominance variance (Lynch and Walsh 1998). This generalization has received statistical support in comparisons of life-history traits versus morphological traits (DeRose and Roff 1999). However, the cost of inbreeding is also highly sensitive to the conditions under which it is measured (Armbruster and Reed 2005, Kristensen et al. 2006), with greater inbreeding depression exhibited in more stressful environments (Fox and Reed 2010, Enders and Nunney 2012). Our estimates of δ were obtained in the same benign environment that the experimental population had experienced for >200 generations. Inbreeding depression would likely have been greater if inbred and outbred females had been compared after larval development in a poor-quality host or under conditions of strong larval competition. Pest populations of *C. maculatus* that colonize a novel, low-quality host will simultaneously encounter both poorer nutrition and elevated levels of inbreeding associated with population bottlenecks (Messina et al. 2009). Additional studies under a range of environmental conditions, from benign to stressful, are necessary before we can conclude that oviposition behavior in *C. maculatus* or other insects is relatively insensitive to the negative effects of inbreeding.

Acknowledgments

References Cited

Armbruster, P., and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity 95: 235–242.

Bilde, T. A., A. Maklakov, K. Meisner, L. la Guardia, and U. Friberg. 2009. Sex differences in the genetic architecture of lifespan in a seed beetle: extreme inbreeding extends male lifespan. BMC Evol. Biol. 9: 33.

Bijlsma, R., J. Bundgaard, and A. C. Boerema. 2000. Does inbreeding affect the extinction risk of small populations? Predictions from *Drosophila.* J. Evol. Biol. 13: 502–514.

Carr, D. E., and M. R. Dudash. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. Phil. Trans. Roy. Soc. Lond. B. 358: 1071–1084.

Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. Nature Rev. Genet 10: 783–796.

Credland, P. F., and A. W. Wright. 1990. Oviposition deterrents of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Physiol. Entomol. 15: 285–298.

Credland, P. F., K. M. Dick, and A. W. Wright. 1986. Relationships between larval density, adult size and egg production in the cowpea seed beetle, *Callosobruchus maculatus.* Ecol. Entomol. 11: 41–50.

Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity 83: 260–270.

Deng, H. W., Y. X. Fu, and M. Lynch. 1998. Inferring the major genomic mode of dominance and overdominance. Genetica 102–103: 559–567.

DeRose, M. A., and D. A. Roff. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. Evolution 53: 1288–1292.

Edvardsson, M., R. Rodriguez–Munoz, and T. Tregenza. 2008. No evidence that female bruchid beetles, Callosobruchus maculatus, use remating to reduce the costs of inbreeding. Anim. Behav. 75: 1519–1524.

Enders, L. S., and L. Nunney. 2012. Seasonal stress drives predictable changes in inbreeding depression in field-tested captive populations of Drosophila melanogaster. Proc. Roy. Soc. B. 279: 3756–3764.

Facon, B., R. A. Hufbauer, A. Tayeh, A. Loiseau, E. Lombaert, R. Vitalis, T. Guillemaud, J. G. Lundgren, and A. Estoup. 2011. Inbreeding depression is purged in the invasive insect Harmonia axyridis. Curr. Biol. 21: 424–427.

Fauvergue, X., E. Vercken, T. Malausa, and R. A. Hufbauer. 2012. The biology of small, introduced populations, with special reference to biological control. Evol. Applic. 5: 424–443.

Fox, C. W. 2005. Problems in measuring among-family variation in inbreeding depression. Am. J. Bot. 92: 1929–1932.

Fox, C. W., and D. H. Reed. 2010. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65: 246–258.

Fox, C. W., and K. L. Scheibly. 2006. Variation in inbreeding depression among populations of the seed beetle, Stator limbatus. Entomol. Exp. Appl. 121: 137–144.

Fox, C. W., and R. C. Stillwell. 2009. Environmental effects on sex differences in the genetic load for adult lifespan in a seed-feeding beetle. Heredity 103: 62–72.

Fox, C. W., R. C. Stillwell, A. R. Amarillo-S, M. E. Czesak, and F. J. Messina. 2004a. Genetic architecture of population differences in oviposition behaviour of the seed beetle Callosobruchus maculatus. J. Evol. Biol. 17: 1141–1151.

Fox, C. W., M. E. Czesak, and W. G. Wallin. 2004b. Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. J. Evol. Biol. 17: 1007–1017.

Fox, C. W., K. L. Scheibly, W. G. Wallin, L. J. Hitchcock, R. C. Stillwell, and B. P. Smith. 2006. The genetic architecture of life span and mortality rates: gender and species differences in inbreeding load of two seed-feeding beetles. Genetics 174: 763–773.

Fox, C. W., K. L. Scheibly, B. P. Smith, and W. G. Wallin. 2007. Inbreeding depression in two seed-feeding beetles, Callosobruchus maculatus and Stator limbatus (Coleoptera: Chrysomelidae: Bruchinae). Bull. Entomol. Res. 97: 49–54.

Fox, C. W., J. D. Wagner, S. Cline, F. A. Thomas, and F. J. Messina. 2009. Genetic architecture underlying convergent evolution of egg-laying behavior in a seed-feeding beetle. Genetica 136: 179–187.

Fox, C. W., R. C. Stillwell, W. G. Wallin, C. L. Curtis, and D. H. Reed. 2011. Inbreeding-environment interactions for fitness: complex relationships between inbreeding depression and temperature stress in a seed-feeding beetle. Evol. Ecol. 25: 25–43.

Fox, C. W., J. Xu, W. G. Wallin, and C. L. Curtis. 2012. Male inbreeding status affects female fitness in a seed-feeding beetle. J. Evol. Biol. 25: 29–37.

Gerloff, C. U., and P. Schmid–Hempel. 2005. Inbreeding depression and family variation in a social insect, Bombus terrestris (Hymenoptera: Apidae). Oikos 111: 67–80.

Haikola, S., W. Fortelius, R. B. O'Hara, M. Kuussaari, N. Wahlberg, I. J. Saccheri, M. C. Singer, and I. Hanski. 2001. Inbreeding depression and the maintenance of genetic load in Melitaea cinxia metapopulations. Conserv. Genet. 2: 325–335.

Harano, T. 2011. Inbreeding depression in development, survival and reproduction in the adzuki bean beetle (Callosobruchus chinensis). Ecol. Res. 26: 327–332.

Harano, T., and M. Katsuki. 2012. Female seed beetles, Callosobruchus chinensis, remate more readily after mating with relatives. Anim. Behav. 83: 1007–1010.

Horng, S. 1997. Larval competition and egg-laying decisions by the bean weevil Callosobruchus maculatus. Anim. Behav. 53: 1–12.

Hughes, K. A. 1995. The inbreeding decline and average dominance of genes affecting male life-history characters in Drosophila melanogaster. Genet. Res. 65: 41–52.

Joron, M., and P. M. Brakefield. 2003. Captivity masks inbreeding effects on male mating success in butterflies. Nature 424: 191–194.

Keller, L. F., and D. M. Waller. 2000. Inbreeding effects in wild populations. Trends Ecol. Evol. 17: 230–241.

Kelly, J. K. 2005. Family level inbreeding depression and the evolution of plant mating systems. New Phytol. 165: 55–62.

Kristensen, T. N., P. Sørenson, K. S. Pedersen, M. Kruhøffer, and V. Loeschcke. 2006. Inbreeding by environmental interactions affect gene expression in Drosophila melanogaster. Genetics 173: 1329–1336.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Mackay, T.F.C. 2009. The genetic architecture of complex behaviors: lessons from Drosophila. Genetica 136: 295–302.

Mattila, A.L.K., A. Duplouy, M. Kirjokangas, R. Lehtonen, P. Rastas, and I. Hanski. 2012. High genetic load in an old isolated butterfly population. Proc. Natl. Acad. Sci. U.S.A. 109: E2496–E2502.

Meffert, L., and E. Bryant. 1991. Mating propensity and courtship behavior in serially bottlenecked lines of the housefly. Evolution 45: 293–306.

Meffert, L. M., S. K. Hicks, and J. L. Regan. 2002. Nonadditive genetic effects in animal behavior. Am. Natur. 160: S198–S213.

Messina, F. J. 1989. Genetic basis of variable oviposition behavior in Callosobruchus maculatus (Coleoptera: Bruchidae). Ann. Entomol. Soc. Am. 82: 792–796.

Messina, F. J. 1991. Life history variation in a seed beetle: adult egg-laying vs. larval competitive ability. Oecologia (Berl.) 85: 447–455.

Messina, F. J. 1993. Heritability and 'evolvability' of fitness components in Callosobruchus maculatus. Heredity 71: 623–629.

Messina, F. J. 2002. Host discrimination by seed parasites, pp. 65–87. In E. E. Lewis, J. F. Campbell, and M.V.K. Sukhdeo (eds.), The Behavioural Ecology of Parasites. CAB International, Oxon, United Kingdom.

Messina, F. J. 2004. Predictable modification of body size and competitive ability following a host shift by a seed beetle. Evolution 58: 2788–2797.

Messina, F. J., and J. A. Dickinson. 1993. Egg-laying behavior in divergent strains of the cowpea weevil (Coleoptera: Bruchidae): time budgets and transition matrices. Ann. Entomol. Soc. Am. 86: 207–214.

Messina, F. J., and C. W. Fox. 2011. Egg-dumping behavior is not correlated with wider host acceptance in the seed beetle Callosobruchus maculatus (Coleptera: Chyrsomelidae: Bruchinae). Ann. Entomol. Soc. Am. 104: 850–856.

Messina, F. J., and M. E. Karren. 2003. Adaptation to a novel host modifies host discrimination by the seed beetle *Callosobruchus maculatus.* Anim. Behav. 65: 501–507.

Messina, F. J., and R. Mitchell. 1989. Intraspecific variation in the egg-spacing behavior of the seed beetle *Callosobruchus maculatus.* J. Insect Behav. 2: 727–742.

Messina, F. J., and J.A.A. Renwick. 1985a. Ability of ovipositing seed beetles to discriminate between seeds with differing egg loads. Ecol. Entomol. 10: 225–230.

Messina, F. J., and J.A.A. Renwick. 1985b. Mechanism of egg recognition by the cowpea weevil *Callosobruchus maculatus.* Entomol. Exp. Appl. 37: 241–245.

Messina, F. J., S. L. Gardner, and G. E. Morse. 1991. Host discrimination by egg-laying seed beetles: causes of population differences. Anim. Behav. 41: 773–780.

Messina, F. J., M. Mendenhall, and J. C. Jones. 2009. An experimentally induced host shift in a seed beetle. Entomol. Exp. Appl. 132: 39–49.

Michalczyk, L., A. L. Millard, O. Y. Martin, A. J. Lumley, B. C. Emerson, T. Chapman, and M.J.G. Gage. 2011. Inbreeding promotes female promiscuity. Science 333: 1739–1742.

Miller, P. S., J. Glasner, and P. W. Hedrick. 1993. Inbreeding depression and male-mating behavior in *Drosophila melanogaster.* Genetica 88: 29–36.

Mitchell, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* (F.). Ecology 56: 696–702.

Mitchell, R. 1990. Behavioral ecology of *Callosobruchus maculatus,* pp. 317–330. *In* K. Fujii, K., A.M.R. Gatehouse, C. D. Johnson, R, Mitchell, and T. Yoshida (eds.), Bruchids and Legumes: Economics, Ecology and Coevolution. Kluwer Academic, Dordrecht, The Netherlands.

Mitchell, R. 1991. The traits of a biotype of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) from South India. J. Stored Prod. Res. 27: 221–224.

Nepoux, V., C. R. Haag, and T. J. Kawecki. 2010. Effects of inbreeding on aversive learning in *Drosophila.* J. Evol. Biol. 23: 2333–2345.

Nieminen, M., M. C. Singer, W. Fortelius, K. Schöps, and I. Hanski. 2001. Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. Am. Natur. 157: 237–244.

Nufio, C. R., and D. R. Papaj. 2001. Host marking behavior in phytophagous insects and parasitoids. Entomol. Exp. Appl. 99: 273–293.

Parr, M. J., B.M.D. Tran, M.S.J. Simmonds, and P. F. Credland. 1996. Oviposition behaviour of the cowpea seed beetle, *Callosobruchus maculatus.* Physiol. Entomol. 21: 107–117.

Pray, L. A., and C. J. Goodnight. 1997. Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum.* Evolution 51: 308: 313.

Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. Trends Ecol. Evol. 11: 201–206.

Roff, D. A. 1998. Effects of inbreeding on morphological and life-history traits of the sand cricket, *Gryllus firmus.* Heredity 81: 28–37.

Roff, D. A. 2002. Inbreeding depression: tests of the overdominance and partial dominance hypotheses. Evolution 56: 768–775.

Saccheri, I., M. Kuusaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392: 491–494.

Semeao, A. A., J. F. Campbell, R. W. Beeman, M. D. Lorenzen, R. J. Whitworth, and P. E. Sloderbeck. 2012. Genetic structure of *Tribolium castaneum* (Coleoptera: Tenebrionidae) populations in mills. Environ. Entomol. 41: 188–199.

Tanaka, Y. 1990. Age specificity of inbreeding depression during a life cycle of *Callosobruchus chinensis* (Coleoptera: Bruchidae). Res. Popul. Ecol. 32: 318–321.

Timms, R. 1998. Size-independent effects of larval host on adult fitness in *Callosobruchus maculatus.* Ecol. Entomol. 23: 480–483.

Toquenaga, Y. 1993. Contest and scramble competitions in *Callosobruchus maculatus* (Coleoptera: Bruchidae) II. Larval competition and interference mechanisms. Res. Popul. Ecol. 35: 57–68.

Tran, B.M.D., and P. F. Credland. 1995. Consequences of inbreeding for the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Biol. J. Linn. Soc. 56: 483–503.

Vamosi, S. M., and T. L. Lesack. 2007. Direct effects of larval competition on development time and fecundity in seed beetles. Evol. Ecol. Res. 9: 1131–1143.

Vergeer, P., N. Wagemaker, and N. J. Ouborg. 2012. Evidence for an epigenetic role in inbreeding depression. Biol. Lett. 8: 798–801.

Vermeulen, C. J., and R. Bijlsma. 2004. Changes in mortality patterns and temperature dependence of lifespan in *Drosophila melanogaster* caused by inbreeding. Heredity 92: 275–281.

Wilson, K. 1988. Egg laying decisions by the bean weevil *Callosobruchus maculatus.* Ecol. Entomol. 13: 107–118.