

The effect of inbreeding on natural selection in a seed-feeding beetle

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Abstract

Little is known about how inbreeding alters selection on ecologically relevant traits. Inbreeding could affect selection by changing the distribution of traits and/or fitness, or by changing the causal effect of traits on fitness. Here, I test whether selection on egg size varies with the degree of inbreeding in the seed-feeding beetle, *Stator limbatus*. There was strong directional selection favoring large eggs for both inbred and outbred beetles; offspring from smaller eggs had lower survivorship on a resistant host. Inbreeding treatment had no effect on the magnitude of selection on egg size; all selection coefficients were between ~ 0.078 and 0.096 , regardless of treatment. However, inbreeding depression declined with egg size; this is because the difference in fitness between inbreds and outbreds did not change, but average fitness increased, with egg size. A consequence of this is that populations that differ in mean egg size should experience different magnitudes of inbreeding depression (all else being equal) and thus should differ in the magnitude of selection on traits that affect mating, simply as a consequence of variation in egg size. Also, maternal traits (such as egg size) that mediate stressfulness of the environment for offspring can mediate the severity of inbreeding depression.

Introduction

Inbreeding typically changes the distribution of phenotypes and genotypes within a population (Demontis *et al.*, 2009) and generally reduces mean fitness (Charlesworth & Willis, 2009). Inbreeding generally decreases genetic variation within populations (though this effect is highly variable among traits and populations; Fowler & Whitlock, 1999) and reduces effective population sizes (Ingvarsson, 2002), and thus can affect evolutionary responses to selection and the balance between selection and other evolutionary processes (e.g. the selection-drift balance or the rate of migration needed to swamp selection, Hu, 2011). The fitness consequences of inbreeding influence the evolution of plant and animal mating systems and associated traits (e.g. Barrett, 2002; Escobar *et al.*, 2011; Ozimec & Husband, 2011; Puurtinen, 2011), and mating system

evolution in turn affects natural selection and the evolution of a wide diversity of life-history and morphological traits (Wright *et al.*, 2008; Sicard & Lenhard, 2011; Karron *et al.*, 2012).

Less is known, however, about how inbreeding alters the form of selection, that is, the shape of selection gradients, on ecologically relevant traits, especially outside the context of mating system evolution. Inbreeding could alter selection gradients because it commonly changes both the phenotypic distribution of traits that are under selection and the distribution of fitness, or because it changes the causal effect of traits on fitness. Because effects of inbreeding generally vary with the degree of stress experienced by individuals (Cheptou & Donohue, 2011; Fox & Reed, 2011; Bijlsma & Loeschke, 2012; Reed *et al.*, 2012), selection on traits that mediate responses to stressful environments may be expected to be particularly sensitive to inbreeding (Reed *et al.*, 2012). Because habitat destruction and fragmentation can reduce population sizes and increase homozygosity, predicting adaptive evolution in such populations will require understanding how inbreeding-mediated changes in fitness and trait distributions alter natural selection.

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In this study, I examine how inbreeding affects the magnitude of selection on egg size in the seed-feeding beetle, *Stator limbatus* (Horn). The size of eggs laid by *S. limbatus* is under strong directional selection; large eggs are favored on seeds of the well-defended host plant, blue paloverde (*Parkinsonia florida*) (Fox & Mousseau, 1996; Fox *et al.*, 2001). Larvae hatching from small eggs are unable to penetrate the seed coat and thus suffer high mortality immediately after hatching. Selection favoring large eggs is balanced by selection favoring high fecundity; egg size and fecundity trade-off genetically, generating an intermediate optimal egg size that changes as the relationship between egg size and offspring survival changes (Fox *et al.*, 1997; Roff, 2002). The beetle also suffers substantial inbreeding depression for a large number of fitness traits, which suggests that *Stator* populations routinely harbour high genetic loads of segregating deleterious recessive alleles (Fox & Scheibly, 2006; Fox *et al.*, 2006). Larval survivorship is particularly susceptible to inbreeding depression; inbreds experience a 22–41% reduction in larval survival relative to outbreds (Fox & Scheibly, 2006; Fox *et al.*, 2007). Using this model system, I test whether the effect of egg size on larval survival is influenced by inbreeding, and thus test whether selection on egg size varies with the degree of inbreeding.

Materials and methods

The biology of *S. limbatus*

Female *S. limbatus* cement their eggs to the surface of host seeds. When eggs hatch, first-instar larvae burrow into the seed under the egg. Larval development and pupation are completed within the seed; larvae do not move among seeds and are thus restricted to the seed chosen by their mother. Beetles emerge as reproductively mature adults and require neither food nor water as adults before mating and laying eggs.

The population used in this study was collected from seeds of *Acacia greggii* (Oracle, Pinal Co., AZ, USA, 32° 36'39"N; 110° 46'13"W). Beetles were reared in the laboratory for ~12 generations on seeds of *A. greggii* before use in this experiment. To provide seeds for the experiment, mature pods were collected from *A. greggii* and *P. florida* trees. Undamaged pods were shipped to the laboratory where seeds were frozen until used in experiments. Within a species, seeds from all trees were thoroughly mixed to control for variation among trees in seed-coat resistance and its effects on larval survival (Fox *et al.*, 2001). Note that selection varies substantially among trees (Fox *et al.*, 2001), but it is not practical to consider this variation in our current study due to the large number of families that are needed to measure selection. I thus average across this variation in seed-coat resistance by mixing seeds from a large number (> 20) of different trees.

Experimental design

Our experimental design was to mate outbred females to a single randomly chosen nonsibling or sibling outbred male, after which I allowed females to lay eggs on seeds of *P. florida* and recorded offspring survival. I used seeds of *A. greggii* for laboratory rearing because larval survival is very high, and selection favoring large eggs weak or absent, when offspring develop on this host (Fox & Mousseau, 1996; Fox *et al.*, 2001). However, selection was measured on seeds of *P. florida*, on which directional selection favours large eggs in nature (Fox, 2000).

In the first generation, full-sib families of beetles were created by randomly pairing nonsib beetles from our laboratory colony. When beetles from this first generation emerged from their *A. greggii* seed as adults, I created a new set of families (second generation) derived from crosses either between individuals within families (full-sib matings) or between individuals that were unrelated to produce offspring that were inbred ($f = 0.25$) or outbred ($f = 0$), respectively. Mated pairs were placed in a 35-mm Petri dish containing 20 seeds of *P. florida*. Dishes were checked for eggs twice daily, and females were allowed to lay eggs until 24 h after the first egg was detected. Larvae were allowed to develop at one egg per seed (excess eggs were scraped from the seed), inside a growth chamber at light/dark 15:9, 29–30 °C. Dishes were checked once per day for adult beetles that emerged from a seed.

Two or three eggs were measured per dish (i.e. per female) using an optical micrometer on a 55× dissecting scope (0.005 mm precision). Egg length correlates highly with egg mass ($r = 0.94$). For each dish, I thus have an average length of the eggs laid by that female (the mean of 2–3 eggs per female) and average survival of offspring hatching from the eggs laid by that female during an ~24 h period.

In total, I collected eggs from 460 females mated to nonsibs and 512 females mated to sibs, averaging 8.5 and 8.2 offspring (3912 and 4217 total outbred and inbred offspring, respectively), per family.

Analyses

To describe the relationship between offspring survival and egg size, I used nonparametric regression with a cubic smoothing spline for both the inbred and outbred treatments using the generalized additive models (GAM) procedure in SAS (SAS Institute Inc., 2010; spline d.f. = 3) (Fig. 1). The main question of interest here is whether the relationship between egg size and fitness varies with inbreeding treatment. Unfortunately, testing for a treatment vs. egg size interaction in a logistic regression does not test this hypothesis. This is because the interpretation of the interaction term in logit and probit models (which are analyses of odds

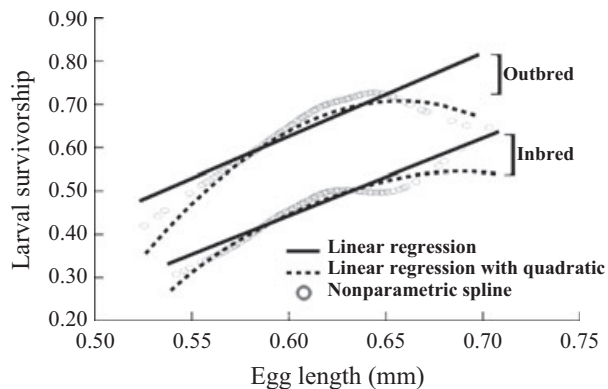


Fig. 1 The relationship between offspring survival (family means) and maternal egg size (length) in the seed-feeding beetle, *Stator limbatus*. Outbred offspring are those produced by matings between unrelated parents, whereas inbred offspring are those produced by sib mating (inbreeding coefficient, $f = 0.25$). For comparison of models, I present both the nonparametric regression (with cubic spline smoothing) and the general linear model both with and without the quadratic term. The figure shows the relationship between absolute trait values (egg length) and fitness, but estimates of selection were based on standardized egg length (egg length converted to a normal[0,1] distribution). The actual data are not presented because there are too many points (8129 offspring from 972 families of beetles).

ratios, not probabilities) is not analogous to the interpretation of interaction terms in linear models (the interaction effect is conditional on the independent variables, unlike the interaction effect in general linear models) (Ganzach *et al.*, 2000; Ai & Norton, 2003; Norton *et al.*, 2004; Mood, 2010). Thus, statistical inferences regarding selection cannot be drawn from the magnitude or significance of the interaction term, and alternative techniques are not yet available that yield an interpretation analogous to the interaction in general linear models (Berry *et al.*, 2010). I thus used a linear probability model (SAS GENMOD; SAS Institute Inc., 2010) to test whether the estimated selection coefficients differed between inbreeding treatments, first testing for an effect of egg size on survival separately for each treatment (model: Survival = Intercept + Egg Length + Egg Length²) and then testing for treatment effects (model: Survival = Intercept + Treatment + Egg Length + Egg Length² + Treatment*Egg Length + Treatment*Egg Length²). Linear probability models differ slightly from logistic regression models in the coefficients estimated for independent variables (Denk & Finkel, 1992), with logistic regression generally providing more accurate estimates of the dependent variable coefficients (Pohlmann & Leitner, 2003). However, significance tests for the main effects are comparable between both methods (Pohlmann & Leitner, 2003) with the interpretation of the interaction terms in linear probability models being more analogous to those in ordinary

linear models (Ganzach *et al.*, 2000). Linear probability models are sensitive to heteroscedasticity, but this problem can be minimized by using weighted regression [weighting by the predicted probabilities, $\hat{y}(1 - \hat{y})$, SAS Institute Inc., 2010].

Selection gradients were estimated from logistic regression coefficients and using standardized egg length (normal[0,1]) following the method of Janzen & Stern (1998). For comparison, linear and quadratic selection gradients were calculated (SAS REG, Lande & Arnold, 1983); parametric selection coefficients are more directly comparable to selection values presented in previous studies of selection (Fox, 2000; Fox *et al.*, 2001; Kingsolver *et al.*, 2001). Note that estimating selection coefficients from parametric models does not require that the response variable (fitness) be normally distributed (Janzen & Stern, 1998).

Results

The distribution of egg size did not differ between females mated to a sibling male and females mated to a nonsibling male [mean \pm standard deviation (SD) in egg size = 0.609 ± 0.024 and 0.610 ± 0.024 for outbred and inbred matings, respectively]. This indicates that females do not adjust egg size in response to whether their mate is a sibling or not (although I cannot exclude the possibility that females adjust egg content in response to the relatedness of their mate). However, inbreeding did have a large effect on offspring survival; average egg-to-adult survival was $0.66 \pm \text{SEM}$ 0.01 for offspring from outbred matings, but only 0.47 ± 0.01 for offspring of inbred matings, which is a 29.5% reduction in survival due to inbreeding. Inbreeding also increased the variance in offspring survival among families from 0.066 to 0.083 ($F_{5,11,459} = 1.27$, $P = 0.005$).

There was strong directional selection on egg size; offspring hatching from large eggs had greater survivorship than did offspring hatching from smaller eggs (linear probability model, $\chi^2_1 = 19.53$, $P < 0.001$ and $\chi^2_1 = 11.24$, $P < 0.001$ for outbred and inbred beetles, respectively; Table 1, Fig. 1). Including a quadratic term did not significantly improve the fit of either selection model (likelihood ratio test comparing models with and without the quadratic term, $\chi^2_1 = 0.22$ and $\chi^2_1 = 0.01$, $P > 0.64$, for both outbreds and inbreds).

The main objective of this study was to test the hypothesis that inbreeding changes the relationship between egg size and offspring survival. I found no evidence that selection differed between treatments; the treatment (inbred or outbred) vs. egg size interaction was nonsignificant, regardless of whether a quadratic term was included ($\chi^2_1 = 0.23$, $P = 0.63$ in the model without the quadratic term; $\chi^2_1 = 0.14$, $P = 0.71$ in the model with the quadratic term). The lack of a treatment effect on selection is also obvious from the estimated selection coefficients. The logistic selection

Table 1 Estimated quadratic (B) and parametric (linear, β , and quadratic, γ) selection on egg size for inbred (sib-mated) and outbred populations of beetles. Estimates of selection are based on standardized egg length (egg length converted to a normal[0,1] distribution) and relative fitness, whereas Fig. 1 presents the actual (nonstandardized) egg size and fitness.

	Linear selection coefficient	Quadratic selection coefficient
Logistic model		
Outbred	0.091 ± 0.012***	–
Inbred	0.084 ± 0.017***	–
Linear model		
Outbred	0.078 ± 0.018***	–
Inbred	0.092 ± 0.027***	–
Linear model with quadratic		
Outbred	0.078 ± 0.018***	–0.020 ± 0.011 ns
Inbred	0.096 ± 0.028***	–0.017 ± 0.018 ns

*** $P < 0.001$; ns, $P > 0.10$.

coefficients were very similar in each treatment (0.091 and 0.084 for outbred and inbred populations, respectively; Table 1), as were the parametric selection gradients ($\beta = 0.078$ and 0.092 for the outbred and inbred treatments, respectively; Table 1). The selection intensities (i , the standardized difference in size between all eggs and those producing a surviving larva) closely match the estimated parametric selection gradients, with $i = 0.079$ for outbreds and 0.090 for inbreds.

The logistic model, presented above, failed to detect any evidence that the magnitude of selection decreased with increasing egg length. The quadratic selection coefficients (γ) from the parametric model, although negative for both outbreds and inbreds (as would be expected if selection is declining with increasing egg size), were likewise nonsignificant (Table 1), but only marginally so ($F_{1,966} = 3.4$, $P = 0.065$).

Discussion

In the seed beetle *S. limbatus*, inbreeding changes the distribution of offspring fitness by substantially increasing offspring mortality. I tested whether this effect of inbreeding on the distribution of offspring fitness affects selection on egg size. Despite having large effects on the distribution of offspring survival, inbreeding had no effect on the magnitude of selection on egg size. Selection coefficients (from the logistic regression), selection gradients (from linear regression) and univariate selection intensities are consistently between ~0.078 and 0.096, regardless of inbreeding treatment (Table 1). This small range of the estimates of selection, and the corresponding statistical analyses, indicates that inbreeding treatment had little or no effect on the magnitude of selection on egg size, despite the very large effect on fitness itself. The primary effect of inbreeding was to

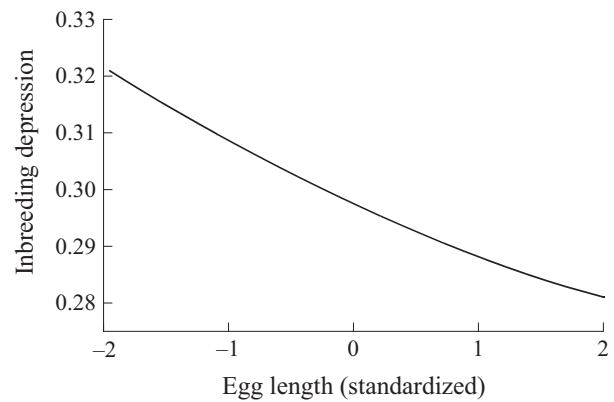


Fig. 2 The relationship between inbreeding depression in offspring survival ($\delta = [\text{Survival}_{\text{Outbred}} - \text{Survival}_{\text{Inbred}}]/\text{Survival}_{\text{Outbred}}$) and maternal egg length (standardized to a normal[0,1]). The curve was calculated from the best fit linear models (Table 1).

depress mean fitness and not to change the relationship between egg size and fitness.

Although I found no evidence that inbreeding changes selection on egg size, these results do suggest that egg size changes the fitness consequences of inbreeding; specifically, inbreeding depression, a measure of the relative degree to which survival of inbreds is lower than outbreds, declines with increasing egg size (Fig. 2). This decline in inbreeding depression with egg size is an inevitable consequence of the absence of an egg size effect on selection. It occurs because the absolute difference in survival between inbreds and outbreds is unaffected by egg size, whereas the average outbred survival increases with egg size. Thus, the ratio of these two variables $[(\text{survival}_{\text{outbreds}} - \text{survival}_{\text{inbreds}})/\text{survival}_{\text{outbreds}}]$ necessarily declines with egg size (for a discussion of how relative vs. absolute differences in fitness affect inbreeding depression, see Cheptou & Donohue, 2011). This observation has two interesting conceptual implications. First, it is the fitness of inbreds relative to that of outbreds that determines the penalty associated with mating between relatives. It is thus the relative difference in fitness between inbreds and outbreds that affects the evolution of traits that mediate inbreeding (the mating system) (Cheptou & Donohue, 2011). The result for *S. limbatus* – that inbreeding depression varies with egg size – suggests that selection against inbreds, and thus selection on traits that mediate mating decisions, can vary as a consequence of variation in traits (e.g. egg size) unrelated to mating *per se*. In *S. limbatus*, populations that vary in mean egg size should experience different magnitudes of inbreeding depression (all else being equal) and thus should differ in the magnitude of selection on traits that affect mating simply as a consequence of variation in egg size.

Second, inbreeding depression generally increases with stressfulness of the environment (Armbruster &

Reed, 2005; Fox & Reed, 2010; Mood, 2010; Cheptou & Donohue, 2011; Reed *et al.*, 2012), leading to the prediction that traits that mediate the fitness consequences of ecological stressors should also mediate inbreeding depression (Reed *et al.*, 2012). The current study demonstrates that a maternal trait (egg size) that mediates stressfulness of the seed environment for offspring also mediates the severity of inbreeding depression; egg size mediates stress-related larval mortality (i.e. mortality caused by plant defenses in seeds) and thus effectively mediates the degree to which an environment is stressful for larvae. An alternate way to think about this is to consider egg size as the maternally mediated environmental condition in which offspring start life; offspring that start with a poor-quality maternally produced environment (small egg size and few starting resources) experience greater inbreeding depression than do offspring that start with a higher-quality maternally produced environment (large egg size and more starting resources). The similarity in shape of survival relationships in the two treatments (Fig. 1) suggests that increased allocation to eggs may indeed offset much of the inbreeding effect on larval survival; an increase in egg size of ~0.1 mm, and the associated increase in resources, would compensate for much of the survival deficit experienced by inbred larvae.

My estimates of selection were lower than observed in previous studies of selection on egg size in this beetle. Previous studies in both the laboratory and field have shown that selection on egg size varies with the degree of seed-coat resistance to penetration by larvae (Fox, 2000; Fox *et al.*, 2001); seeds that are very resistant to beetles impose substantial selection on egg size, whereas seeds on which larval survivorship is high impose little selection on egg size. For the level of larval mortality observed in the current study (~35% for outbred beetles), previous studies had observed selection intensities of between ~0.15 (Fox, 2000) and ~0.5 (Fox *et al.*, 2001). However, selection varies among populations of beetles, and egg sizes were ~0.02–0.03 mm larger in the current study than in Fox *et al.* (2001). If the decline in selection with increasing egg size (i.e. the quadratic effect, γ , in Table 1) is real then we would expect the evolution of eggs that are ~0.02–0.03 mm larger to reduce the amount of directional selection on egg size. Laboratory selection experiments demonstrate this; the rate of increase in larval survivorship on *P. florida* declines and ultimately reaches a plateau with the evolution of larger eggs (unpublished data from Czesak & Fox, 2003).

The current study is a laboratory-based study of selection on egg size, which necessarily eliminates many sources of selection that occur in the field. However, a previous study has compared the distribution of selection intensities for egg size, which vary among trees according to their level of seed-coat resistance, between the field and the laboratory (Fox, 2000). That

study found that the treatment effects observed in the laboratory matched those observed in the field, and that selection intensities calculated in the laboratory, although slightly lower than those in the field, correlate well (among trees) with those calculated in a laboratory experiment ($r = 0.50$; Fox, 2000). I thus expect that the results of the current study to underestimate selection on egg size that would be observed in nature, but also expect the inbreeding treatment effect to be similar between laboratory and nature.

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