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INBREEDING DEPRESSION INCREASES WITH ENVIRONMENTAL STRESS: AN EXPERIMENTAL STUDY AND META-ANALYSIS

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Inbreeding–environment interactions occur when inbreeding leads to differential fitness loss in different environments. Inbred individuals are often more sensitive to environmental stress than are outbred individuals, presumably because stress increases the expression of deleterious recessive alleles or cellular safeguards against stress are pushed beyond the organism's physiological limits. We examined inbreeding–environment interactions, along two environmental axes (temperature and rearing host) that differ in the amount of developmental stress they impose, in the seed-feeding beetle *Callosobruchus maculatus*. We found that inbreeding depression (inbreeding load, *L*) increased with the stressfulness of the environment, with the magnitude of stress explaining as much as 66% of the variation in inbreeding depression. This relationship between *L* and developmental stress was not explainable by an increase in phenotypic variation in more stressful environments. To examine the generality of this experimental result, we conducted a meta-analysis of the available data from published studies looking at stress and inbreeding depression. The meta-analysis confirmed that the effect of the environment on inbreeding depression scales linearly with the magnitude of stress; a population suffers one additional lethal equivalent, on average, for each 30% reduction in fitness induced by the stressful environment. Studies using less-stressful environments may lack statistical power to detect the small changes in inbreeding depression. That the magnitude of inbreeding depression scales with the magnitude of the stress applied has numerous repercussions for evolutionary and conservation genetics and may invigorate research aimed at finding the causal mechanism involved in such a relationship.

KEY WORDS: ***Callosobruchus maculatus*, fitness, inbreeding load, phenotypic variability hypothesis.**

Inbreeding increases genomic homozygosity within individuals and populations. This, in turn, commonly results in a loss of fitness termed inbreeding depression. However, the expression and magnitude of inbreeding depression can be highly sensitive to the environmental conditions under which inbreeding is being measured, because gene expression changes with environmental conditions (genotype–environment interactions; Armbruster and Reed 2005). Genotype–environment interactions have long been considered important to agriculture and animal breeding generally (e.g., Mulder and Bijma 2005), and in evolutionary ecology, because the genetic architecture for traits, and thus evolutionary dynamics, vary with environmental conditions (e.g., Sgrò and Hoffman 2004; Gutteling et al. 2007; Ouborg et al. 2010). Genotype–environment interactions are also important for their potential to maintain genetic diversity (Charlesworth and Hughes 2000). Inbreeding–environment interactions, a special form of genotype–environment interaction, will play a similarly important role whenever individuals and populations differ in their inbreeding levels; in particular inbreeding–environment interactions have been suggested to be crucial for understanding extinction risk

(e.g., Reed et al. 2007a; Liao and Reed 2009), the evolution of inbreeding avoidance (Szulkin and Sheldon 2007), and the ability of populations to purge their genetic load when the environment is changing or variable (e.g., Bijlsma et al. 1999; Fox et al. 2008).

Inbreeding–environment interactions in which inbreeding depression increases with stress are of special conservation importance. Over the past few centuries, populations of many species of plants and animals have declined and/or become highly fragmented, leading to potentially high levels of inbreeding (Sambatti et al. 2008). This inbreeding due to small population size interacts with anthropogenic environmental changes that tend to produce potentially nonadditive (i.e., worse than expected from considering each effect independently) effects on population dynamics and evolutionary potential. Although studies demonstrating that environmental conditions affect inbreeding depression are ubiquitous, and numerous researchers have posited that inbreeding depression should increase when organisms are under developmental stress, experimental results actually demonstrating that inbreeding depression increases with stress have been inconsistent (e.g., Armbruster and Reed 2005; Marr et al. 2006; Reed et al. 2007a,b; Szulkin and Sheldon 2007; Kristensen et al. 2008; Waller et al. 2008). Many studies have found some relationship between the stressfulness of an environment and inbreeding depression (review in Armbruster and Reed 2005) but many others have failed to find such a relationship (e.g., Fox et al. 2010), leading to a search for alternative explanations to explain variability in inbreeding depression among traits and environments. For example, the phenotypic variability hypothesis of Waller et al. (2008), a form of null model for the relationship between environmental conditions and inbreeding depression, predicts a positive relationship between the shift in inbreeding depression between environments and the difference in the opportunity for selection (CV^2) between those environments.

Here, we examine the effect of developmental stress, manipulated by varying temperature and diet, on the magnitude of inbreeding depression in the seed-feeding beetle, *Callosobruchus maculatus*. This beetle exhibits substantial inbreeding depression, and is a model species for previous studies of inbreeding depression and life-history evolution (e.g., Fox et al. 2006; Edvardsson et al. 2008; Bilde et al. 2009). Specifically, we ask whether inbreeding depression increases with increasing developmental stress, with stressfulness of an environment defined as the degree to which mean fitness declines in an environment relative to the best environment. We then review published experimental studies of inbreeding-stress interactions and examine the relationship between degree of stress imposed and the magnitude of inbreeding depression observed.

Materials and Methods

EXPERIMENTAL STUDY

The biology of C. maculatus

The life cycle of *C. maculatus* revolves around their host seeds. Females cement their eggs to the surface of host seeds (Messina 1991). When eggs hatch first-instar larvae burrow into the seed under the egg. Larval development and pupation are completed within a single 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.

Here, we use two populations of beetles that have been the focus of a couple of previous inbreeding and life-history studies. The South Indian (SI) population was collected in 1979 from infested pods of mung bean, *Vigna radiata* (L.) Wilczek, and the closely related black gram, *V. mungo* (L.) Hepper, in Tirunelveli, India (Mitchell 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea, *V. unguiculata* (L.) Walp., in Ouagadougou, Burkina Faso (Messina 1993). These two populations differ in body size, lifetime fecundity, patterns of egg dispersion, oviposition preference, and adult longevity (Fox et al. 2004a, 2004b; Messina 2004). Both populations were maintained in laboratory growth chambers on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at more than 1000 adults per generation for more than 100 generations (BF) or more than 200 generations (SI) prior to this experiment.

Inbreeding depression in C. maculatus

Callosobruchus maculatus suffers substantial inbreeding depression throughout development. Eggs produced from inbred matings are less likely to develop, have lower hatch rates, and larvae hatching from these eggs have reduced hatch-to-adult survival. Eggs from full-sibling matings are 17–21% less likely to produce an adult offspring than eggs from outbred matings in these two populations of *C. maculatus* (Fox et al. 2007). Inbred offspring that survive to adult develop more slowly—larval development time is extended by ∼5% (>1 day) (Tran and Credland 1995; Fox et al. 2007). Inbreeding also negatively affects female fecundity in *C. maculatus* (Tran and Credland 1995) and its congener *C. chinensis* (Tanaka 1990, 1993).

Experimental design

To measure inbreeding depression and the inbreeding load we used a "block" design (Roff 1998; Fig. 1). Blocks were created by randomly pairing two families chosen from an outbred population. From each family, we randomly chose two females and two males to become parents. We crossed these two families creating two inbred and two outbred families per block. The advantage of this design is that it assures that inbred families are created from the

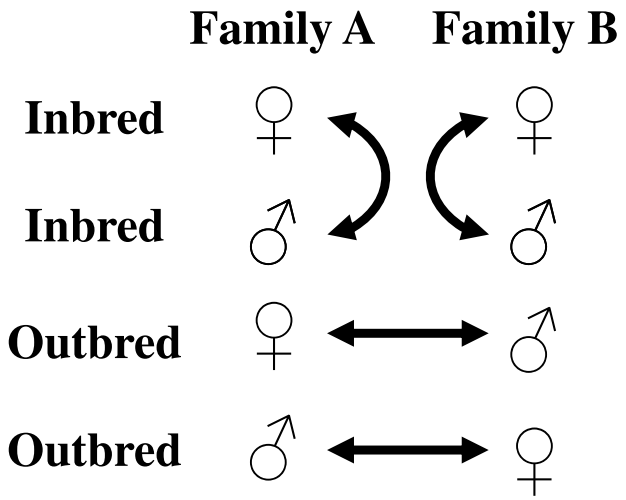

Figure 1. The block design used to measure inbreeding depression. Each block is created by crossing beetles from two unrelated families, creating two outbred matings (reciprocal crosses between the two families) and two inbred matings (crosses between full-siblings within each family). Outbreds and inbreds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

same set of alleles as are the outbred families to which they are compared (Fox 2005).

Pairs were confined in a 35-mm Petri dish with 35 seeds of either mung bean, *V. radiata*, or in a 60-mm petri dish containing 35 seeds of cowpea, *V. unguiculata*. Each block was assigned to only one host. Dishes were checked for eggs after 12 and 24 h. Eggs laid by females were evenly divided among three temperature treatments, 17°C, 27°C, and 37°C (all ± 0.5), within 12 h of being laid. These temperatures were chosen to include both a low- and high-temperature stress plus an intermediate (benign) temperature. Larval egg-to-adult survival for these two populations is highest at temperatures between 25°C and 30°C (Stillwell et al. 2007). The temperatures were picked to be extreme enough to impose substantial stress on development.

Larvae were allowed to develop at one egg per seed (excess eggs were scraped from the seed), one seed per dish, inside a temperature and photoperiod (but not humidity) controlled growth chamber at light:dark 15:9. Dishes were checked twice per day for adult beetles that emerged from a seed.

We scored egg and larval survival for all offspring. All of the eggs/larvae were classified to one of four fates; those that failed to develop, developed but did not hatch (a developing larva/embryo was visible inside the clear egg), hatched but did not emerge as an adult, or emerged as an adult.

Sample sizes

In total, we created 32 blocks (BF population) or 33 blocks (SI population) on each rearing host (64 and 66 blocks total for the BF and SI populations, respectively). Each block consisted of two inbred and two outbred families. From these blocks, we collected a total of 15,664 eggs (∼15.1 eggs per block per inbreeding treatment × rearing host × rearing temperature combination) of which 13,999 developed, 12,308 hatched, and 7,719 survived to adult, from which we have development time data on 7684 offspring.

Analyses

Blocks are the lowest level of independence in this design and thus block means were used in all analyses. All block means were calculated first by averaging across offspring within a family and then by averaging across families within the block and treatment. Each block contains two means, one for each treatment (inbred and outbred). We used analysis of variance (SAS PROC MIXED; Littell et al. 1996) to test for population, treatment, and interaction effects on the block means for the four measured survival variables, and on inbreeding depression/load (δ and L; see below). We used linear contrasts (CONTRAST statement in PROC MIXED) to test for differences between specific pairs of temperatures.

Inbreeding depression, was calculated as the proportional reduction in survival,

$$\delta = \frac{Mean_{outbred} - Mean_{inbred}}{Mean_{outbred}}$$

(Lynch and Walsh 1998). δ was calculated separately for each block (i.e., each group of two inbred and two outbred families), and each estimate of δ was treated as a single independent datapoint. We also calculated the inbreeding load (genetic load, L) for genes affecting larval survival. The genetic load was estimated as

$$L_{Survival} = \frac{-[\ln(Survival_{inbred}) - \ln(Survival_{outbred})]}{F},$$

(Charlesworth and Charlesworth 1987) where $F = 0.25$ for our block design. $L_{Survival}$ was calculated separately for each block.

Although we calculated both δ and L for all four survivorship traits that we measured, the two parameters are calculated from the same two values, and are highly correlated. Thus, all statistical analyses are qualitatively identical regardless of whether we use δ or L. We present analyses of L in most places, because our main hypothesis is with regard to the relationship between stress and inbreeding load. However, we also refer to values of δ because it is often more intuitive to interpret proportional reductions in fitness than differences in inbreeding loads.

To test whether inbreeding depression was dependent on stressfulness of the environment, we used proportional reduction in survival of outbred offspring during the same period of development, relative to survival in the best temperature/host combination, as our proxy for environmental quality; i.e., $stress = 1 - Survival_{outbred(stressful)}/Survival_{outbred(benign)}$ within each period of development. This assumes that the environments in which mortality is lowest during a period of development represent the best

conditions for that period of development (Armbruster and Reed 2005). We used analysis of covariance to test for a significant relationship between mean L for each host/temperature combination versus *stress* (model: L = population + *stress*, with population as a fixed effect and *stress* as a covariate). [See also our discussion in the next section of the Methods on the intrinsic correlation between these variables].

To test the phenotypic variability hypothesis, we calculated a coefficient of variation (CV) for each survival trait among blocks within each treatment. The hypothesis predicts a positive relationship between the shift in inbreeding depression (or load) between environments and the difference in the opportunity for selection (CV^2) between those environments. This is because stress may increase phenotypic variation, enhancing the opportunity for selection and thus inbreeding depression (Waller et al. 2008). We thus tested whether inclusion of CV^2 (for each environment) into our model improved the fit of the model over inclusion of *stress* alone, or explained variation in L better than did the model including only *stress* (Burnham and Anderson 1998).

ANALYSIS OF PUBLISHED STUDIES

We then surveyed the literature for papers in which inbreeding was measured in at least two environments that differed in fitness (i.e., one could be considered the stressful environment) and in which the decrease in fitness in the outbred individuals could be ascertained as a measure of stress. The literature search found 58 datapoints, from 33 independently published studies, involving 27 species. A summary of the papers we considered is presented in Table 1, and the data extracted from these are presented in Table S4. They include studies on 11 plant, 13 invertebrate, and three vertebrate species. The stress factors include exposure to insecticides and other noxious or toxic chemicals, nutrient deprivation, temperature and desiccation stress, the effects of competition and parasitism, stressful vs. benign years, and comparisons of natural versus artificial conditions.

For each study, we computed L, the number of haploid lethal equivalents (Armbruster and Reed 2005) under benign and stressful conditions and compared the difference in lethal equivalents (L_{diff} = L in the stressful environment minus L in the benign environment) to our proxy for stress, which was the proportional decrease in fitness of outbred individuals in the stressful environment compared to the benign environment (i.e., $stress = 1 - Survival_{outbred(stressful)}/Survival_{outbred(benign)}$, as in the previous analysis). Specifically, using weighted regression, we test the hypothesis that the difference in genetic load between environments, L_{diff}, increases with *stress* (model: $L_{diff} = stress$) with estimates of L_{diff} weighted by the reciprocal of the number of parameter estimates per study. We did not weight estimates of L_{diff} by the sample sizes of the individual studies because (1) experimental units varied and were not comparable among studies (individuals, families, blocks, etc.), and (2) our variables *stress* and L_{diff} are functions of fitness and L, respectively, in multiple contexts/treatments (see above), each of which have different sample sizes. Producing a single meaningful value for the sample size or the sample variance for any specific point is not practical.

This regression analysis has one obvious problem: both L_{diff} and *stress* include the terms $Survival_{outbred(stressful)}$ and $Survival_{outbred(benign)}$, and are thus necessarily correlated. However, algebraic rearrangement shows that, ignoring the constant, L_{diff} includes the term $\ln[Survival_{outbred(stressful)}/Survival_{outbred(benign)}]$, whereas *stress* includes $-Survival_{outbred(stressful)}/Survival_{outbred(benign)}$. The intrinsic correlation is thus negative, which is in the opposite direction of our hypothesis, which predicts a positive relationship. Simulations confirm this; when L varies randomly across environments, the estimated relationship between L_{diff} and *stress* is negative, and when L is defined to increase with stress, the intrinsic correlation reduces (very slightly) the slope of the estimated relationship. When inbreeding depression (δ) is constant across stress levels (a biological possibility, but not a statistical possibility due to sampling error), L_{diff} is uncorrelated with *stress* (because L is a constant). Thus, testing for a positive correlation between L_{diff} and *stress* is a conservative test of the hypothesis that L increases with *stress;* that is, the nonindependence of the two variables reduces the magnitude of the estimated correlation, and does not inflate the correlation, and thus our estimates of the slope of the relationship underestimate the true slope by a few percent (the effect is small).

Results

Multiple previous studies have examined the effects of temperature and diet on larval development in these two beetle populations (e.g., Stillwell et al. 2007; Stillwell and Fox 2007, and references therein). Rather than repeating the details of these effects for this particular study, we have placed this information (including statistical analyses and figures) in Supporting information. Mean survival through all four periods of development, for both populations reared on both hosts at three temperatures, is presented in Table 2. Here, we summarize briefly the temperature and host effects necessary to understand our test of the hypothesis that the inbreeding load increases with developmental stress.

Rearing host had no effect on the proportion of eggs developing or egg hatch, but had a large effect on larval survival and thus on the probability that an egg gave rise to an adult offspring (Table 2; analyses in Supporting information). Mung bean was the better host for both populations of beetles. Temperature did not affect the proportion of eggs that developed, but affected larvae at all subsequent stages of development such that eggs were

Table 1. Studies used in our meta-analysis of inbreeding–stress interactions, sorted by author. Estimates of L_{diff} and *stress* used in the analyses are in Table S4 .

Citation	Species (Order or Family)	Taxonomic group	Stressor(s)	F[1]
Armbruster et al. (2000)	*Aedes geniculatus* (Diptera)	Invertebrate	Natural vs. artificial tree holes	0.25–0.375
Bijlsma et al. (1999)	*Drosophila melanogaster* (Diptera)	Invertebrate	Temperature, ethanol, DDT and crowding	0.40[2]
Bijlsma et al. (2000)	*Drosophila melanogaster* (Diptera)	Invertebrate	Temperature, ethanol	0.25–0.785
Carr et al. (2003)	*Mimulus guttatus* (Scrophulariaceae)	Plant	Viral infection	0.50
Chen (1993)	*Arianta arbustorum* (Helicidae)	Invertebrate	Laboratory vs. field	0.25
Cheptou et al. (2000a)	*Crepis sancta* (Asteraceae)	Plant	Water (drought)	0.50
Cheptou et al. (2000b)	*Crepis sancta* (Asteraceae)	Plant	Interspecific competition	0.125–0.25
Dahlgaard et al. (1995)	*Drosophila buzzatii* (Diptera)	Invertebrate	Heat shock	0.25–0.50
Dahlgaard and Hoffmann (2000)	*Drosophila melanogaster* (Diptera)	Invertebrate	Heat shock	0.375
Dahlgaard and Loeschcke (1997)	*Drosophila buzzatii* (Diptera)	Invertebrate	Heat shock	0.25–0.50
Eckert and Barrett (1994)	*Decodon verticillatus* (Lythraceae)	Plant	Intraspecific competition	0.50
Fowler and Whitlock (2002)	*Drosophila melanogaster* (Diptera)	Invertebrate	Temperature and density	0.25
Fox et al. (2010)	*Callosobruchus maculatus* (Coleoptera)	Invertebrate	Temperature	0.25
Haag et al. (2002)	*Daphnia magna* (Daphniidae)	Invertebrate	Competition	0.50
Haag et al. (2003)	*Daphnia magna* (Daphniidae)	Invertebrate	Parasitic infection	0.50
Hayes et al. (2005)	*Cucurbita pepo* (Cucurbitaceae)	Plant	Nitrogen fertilization	0.50
Henry et al. (2003)	*Physa acuta* (Physidae)	Invertebrate	Laboratory vs. field	0.50
Johnston (1992)	*Lobelia* (two species) (Lobelioideae)	Plant	Greenhouse vs. field	0.50
Koelewijn (1998)	*Plantago coronopus* (Plantaginaceae)	Plant	Greenhouse vs. field	0.25–0.875
Kristensen et al. 2003)	*Drosophila buzzatii* (Diptera)	Invertebrate	Temperature and pesticide	0.25–0.67
Kristensen et al. (2008)	*Drosophila melanogaster* (Diptera)	Invertebrate	Temperature	0.67
Markert et al. (2010)	*Americamysis bahia* (Mysidae)	Invertebrate	Diluted seawater	0.125–1.0
Marr et al. (2006)	*Melospiza melodia* (Emberizidae)	Vertebrate	Natural ecological variation	0.06–0.25[3]
Nowak et al. (2007)	*Chironomus riparius* (Diptera)	Invertebrate	Cadmium exposure	0.125–0.375
Reed and Bryant (2001)	*Musca domestica* (Diptera)	Invertebrate	Diet and temperature	0.25
Reed et al. (2002)	*Drosophila melanogaster* (Diptera)	Invertebrate	Copper sulfate and methanol	0.25–0.83
Reed et al. (2003a)	*Drosophila melanogaster* (Diptera)	Invertebrate	Copper sulfate and methanol	0.594
Reed et al. (2007b)	*Rabidosa* (two species) (Lycosidae)	Invertebrate	Natural ecological variation	0.05–0.38[3]
Rowe and Beebee (2005)	*Bufo calamita* (Bufonidae)	Vertebrate	Natural ecological variation	0.40[3]
Schemske (1983)	*Costus* (three species) (Zingiberaceae)	Plant	Light availability	0.50
Schmitt and Ehrhardt (1990)	*Impatiens capensis* (Balsaminaceae)	Plant	Intraspecific competition	0.50
Szulkin and Sheldon (2007)	*Parus major* (Paridae)	Vertebrate	Natural ecological variation	$\geq$0.125[3]
Wolfe (1993)	*Hydrophyllum appendiculatum* (Hydrophyllacea)	Plant	Intraspecific competition	0.50

[1]Estimated inbreeding coefficient (F) for inbred treatment relative to outbred treatment. F=0.25 for full-sibling matings, F=0.50 for selfing.

[2]Second chromosome completely homozygous.

[3]F based on natural matings, estimated from genetic markers or pedigrees, rather than manipulated by experimenters.

Table 2. Mean (±SEM) survival during four periods of development (egg development, egg hatch, larval survival, and cumulative probability that an egg produces an adult offspring) for outbred *Callosobruchus maculatus* reared at three temperatures on two different host species. Means were calculated separately for each family within each block, then averaged across families within blocks, then average across blocks within treatments. *N*=32 blocks per treatment for the BF population 33 blocks per treatment for the SI population.

Temperature/Trait	Burkina Faso		South India	
	Mung	Cowpea	Mung	Cowpea
17°C				
Eggs developing[1]	0.95±0.01	0.89±0.02	0.94±0.02	0.89±0.02
Eggs hatching[2]	0.94±0.01	0.93±0.01	0.95±0.02	0.91±0.01
Larval survival[3]	0.80±0.02	0.34±0.03	0.87±0.02	0.17±0.03
Egg→adult[4]	0.72±0.02	0.29±0.03	0.78±0.02	0.13±0.02
27°C				
Eggs developing[1]	0.95±0.01	0.91±0.02	0.93±0.02	0.93±0.02
Eggs hatching[2]	0.99±0.01	0.98±0.01	0.97±0.02	0.98±0.01
Larval survival[3]	0.99±0.01	0.87±0.02	0.97±0.02	0.56±0.02
Egg→adult[4]	0.93±0.01	0.78±0.03	0.88±0.02	0.52±0.02
37°C				
Eggs developing[1]	0.91±0.02	0.85±0.02	0.90±0.02	0.85±0.02
Eggs hatching[2]	0.93±0.01	0.93±0.01	0.91±0.02	0.90±0.02
Larval survival[3]	0.94±0.01	0.74±0.03	0.87±0.02	0.41±0.03
Egg→adult[4]	0.79±0.02	0.59±0.03	0.75±0.04	0.33±0.03

[1]The proportion of eggs producing a visible embryo.

[2]The proportion of developing eggs that hatch (hatching is defined to have occurred if the larvae begins digging into the seed).

[3]The proportion of hatched eggs that produce an adult offspring that successfully emerges from the seed; offspring that pupated but failed to emerge from a seed are counted as part of larval mortality.

[4]The total probability that an egg produces an adult offspring that successfully emerges from the seed.

most likely to give rise to an offspring that survived to adult when reared at 27°C, and least likely to give rise to an adult offspring at 17°C, with 37°C being intermediate ($\chi^2_1 > 13.3$, $P < 0.001$ for all linear contrasts) (Table 2).

There was no overall effect of inbreeding on the proportion of eggs that developed, and the estimate of inbreeding depression (δ) and inbreeding load (L) at this stage did not differ between rearing hosts nor vary among rearing temperatures (Table 3; Fig. 2). However, inbreeding depression and the inbreeding load were significantly >0 at all other stages of development. The clearest pattern, and an important prerequisite for a test between stress and inbreeding depression, is that the inbreeding load varied substantially among the three rearing temperatures and between the two rearing hosts for larval hatch-to adult survival. This resulted in substantial variation among environments in inbreeding depression and the inbreeding load for the proportion of eggs giving rise to an adult offspring (Table 3; Fig. 2). In general, the inbreeding load was greatest at low temperature, and lowest at intermediate temperature (Table 3). However, there were significant population × environment interactions, indicating that the degree to which the inbreeding load varied among environments differed between populations. This included a significant population × temperature × host three-way interaction, which indicated a significant interaction between stressors (temperature and host) for the SI population that did not occur in the BF populations; the effect of rearing host on the inbreeding load was especially large in SI beetles when they were reared at the two extreme temperatures, whereas the effect of host on inbreeding depression was smaller and relatively consistent across temperatures for BF beetles.

Additional details, including tables presenting statistical analyses, are included in the Supporting information.

INBREEDING DEPRESSION INCREASES WITH LARVAL STRESS IN *C. MACULATUS*

The primary objective of this study is to test whether the inbreeding load (estimated as lethal equivalents, L) is positively correlated with the degree of stressfulness experienced during each sequential period of larval mortality. We found that the inbreeding load was indeed positively correlated with stressfulness of the environment for three of the four periods of development; egg hatch (analysis of covariance, stress effect; $F_{1,8} = 7.2$, $P = 0.03$), larval survival ($F_{1,8} = 22.7$, $P = 0.001$), and the probability that an egg produces an adult (this last being cumulative across all periods; $F_{1,8} = 14.4$, $P = 0.005$), but not for the proportion of eggs that developed ($F_{1,8} = 0.07$, $P = 0.79$; Fig. 3). Neither the population nor the population-by-stress interaction was significant in any of

Table 3. The magnitude of inbreeding depression (δ±SEM) on egg development, egg hatch, larval survival, and the cumulative probability that an egg produces an adult offspring, for outbred and inbred (sib-mated) *Callosobruchus maculatus* reared on two different host species. δ is the proportional decrease in hatch/survival of inbred relative to outbred beetles. δ was calculated separately for each family within each block, then averaged across families within blocks, then average across blocks within treatments. *N*=32 blocks per treatment for the BF population, and *N*=33 blocks per treatment for the SI population. Inbreeding loads are shown in Figure 2.

Temperature/Trait	Burkina Faso		South India	
	Mung	Cowpea	Mung	Cowpea
17°C				
Eggs developing[1]	0.08±0.02	0.06±0.03	0.03±0.02	0.02±0.03
Eggs hatching[2]	0.27±0.03	0.17±0.04	0.21±0.05	0.16±0.03
Larval survival[3]	0.66±0.03	0.65±0.10	0.45±0.03	0.63±0.13
Egg→adult[4]	0.77±0.02	0.71±0.09	0.58±0.03	0.65±0.13
27°C				
Eggs developing[1]	0.02±0.01	0.03±0.02	0.00±0.03	0.05±0.02
Eggs hatching[2]	0.10±0.02	0.08±0.02	0.09±0.03	0.06±0.02
Larval survival[3]	0.09±0.02	0.24±0.03	0.11±0.03	0.20±0.05
Egg→adult[4]	0.19±0.03	0.31±0.04	0.19±0.05	0.31±0.05
37°C				
Eggs developing[1]	0.02±0.02	0.05±0.03	0.06±0.04	0.01±0.04
Eggs hatching[2]	0.18±0.03	0.15±0.03	0.14±0.06	0.17±0.41
Larval survival[3]	0.33±0.03	0.28±0.07	0.23±0.04	0.72±0.05
Egg→adult[4]	0.47±0.04	0.42±0.07	0.34±0.10	0.76±0.05

[1]The proportion of eggs producing a visible embryo.

[2]The proportion of developing eggs that hatch (hatching is defined to have occurred if the larvae begins digging into the seed).

[3]The proportion of hatched eggs that produce an adult offspring that successfully emerges from the seed; offspring that pupated but failed to emerge from a seed are counted as part of larval mortality.

[4]The total probability that an egg produces an adult offspring that successfully emerges from the seed.

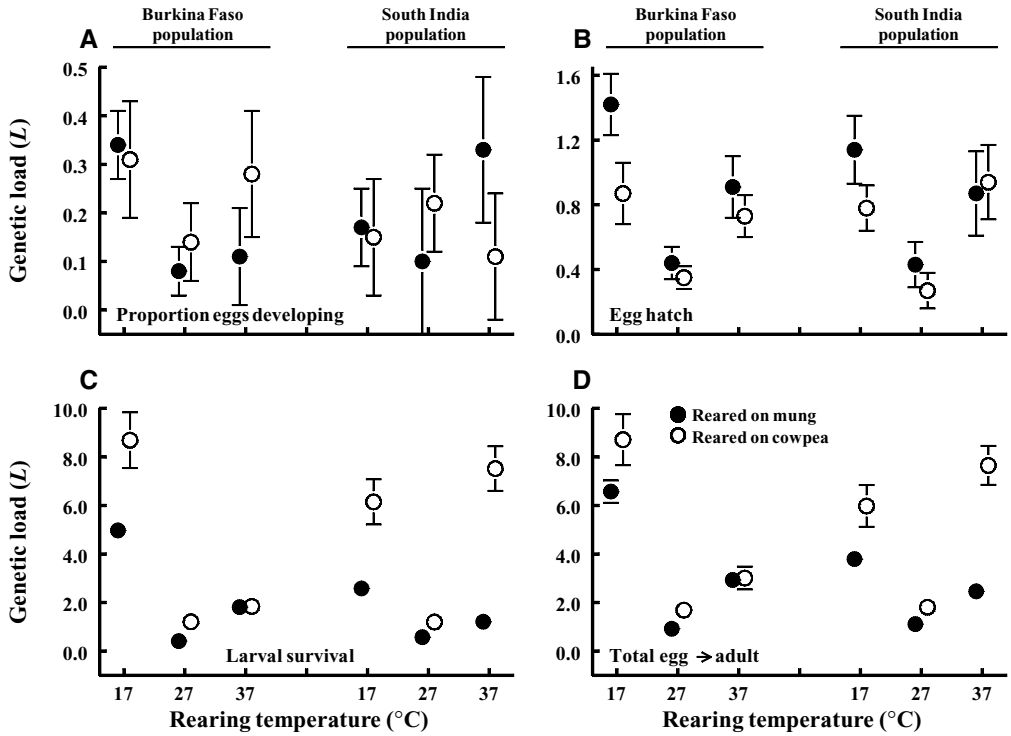

Figure 2. The genetic load (inbreeding load, *L*) for *Callosobruchus maculatus* reared at three temperatures on seeds of either mung (•) or cowpea (○). Estimates of inbreeding depression (δ) are presented in Table 3.

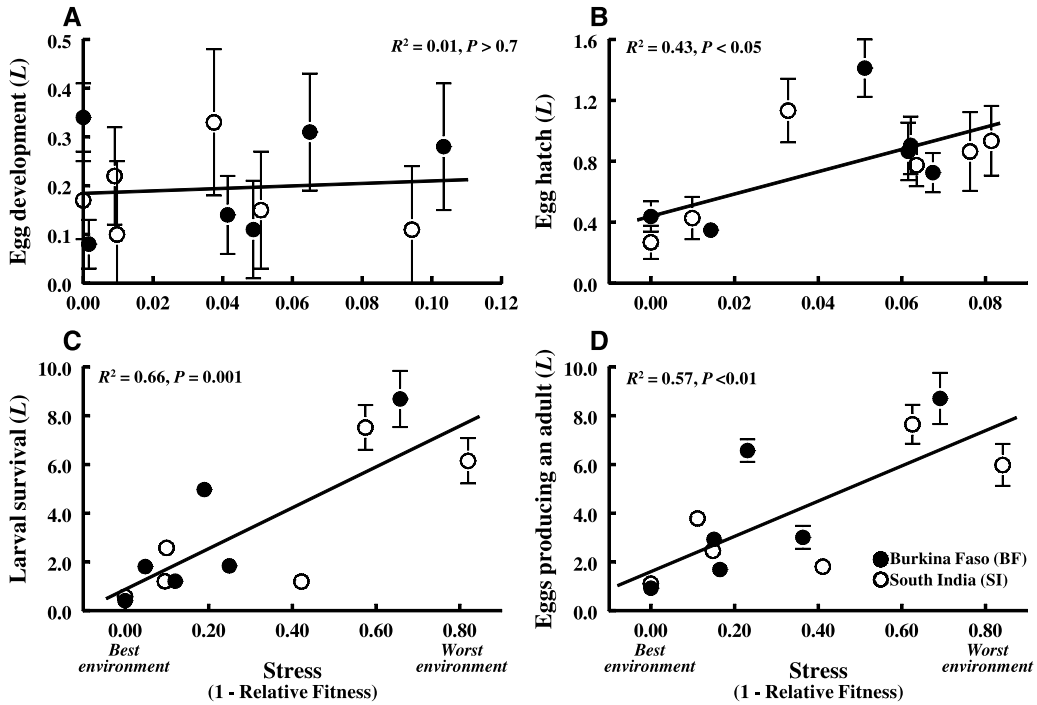

Figure 3. The relationship between inbreeding load (*L*) and *stress* for *C. maculatus* reared at three temperatures on two host species. *Stress* is calculated as 1 – $Survival_{outbred(stressful)}/Survival_{outbred(benign)}$, calculated separately for each sequential period, and separately for each beetle population. Note that stress is by definition 0 in the most benign environment (the temperature-seed combination on which survival is highest through that period of development).

the models ($F_{1,8} < 2.2$, $P > 0.18$ in all analyses). The inbreeding load for egg hatch (Fig. 3B) increased by 0.78 lethal equivalents for each 10% reduction in survival, through this stage of development, relative to the best environment. The inbreeding load for larval survival (Fig. 3C) increased by 0.84 lethal equivalents per 10% reduction in survival. Cumulative across all stages of development, the estimate of lethal equivalents increases by 0.73 per 10% reduction in the relative proportion of eggs producing an adult.

The results of this analysis did not change when we incorporated the magnitude of the opportunity for selection (CV^2) in each environment into the statistical model. CV^2 was correlated with the estimated inbreeding loads for all traits except egg development, but for none of these traits did the model including just CV^2, or CV^2 plus *stress*, explain the variation in *L* better than the model including just *stress*; the model containing just *stress* always had the lowest Akaike's information criterion value (Burnham and Anderson 1998). The relationship between *L* and developmental stress was thus not explainable by an increase in phenotypic variation in more stressful environments.

Thus, the expression of the inbreeding load was lowest in treatment combinations for which outbred larval survival was highest (during that specific period of development) and which were presumably less stressful for larval development. The results of this analysis are qualitatively identical if we use the proportion of outbred eggs producing an adult as our proxy for developmental stress for all four analyses (rather than using survival through each respective developmental period), and if we use inbreeding depression (δ) as our dependent variable.

GREATER INBREEDING LOAD IS GENERALLY ASSOCIATED WITH INCREASED STRESS

In our analysis of studies from the literature, the proportional difference in fitness experienced by an outbred population between two environments (our proxy for the difference in degree of stress between two environments) is significantly and linearly related to the difference between those environments in the number of lethal equivalents expressed by inbreeding (the inbreeding load, *L*) ($R^2 = 0.41$, $P < 0.001$) (Fig. 4). Adding a nonlinear term does not improve the model fit. Specifically, the change in the number of lethal equivalents when comparing two environments (L_{diff}) is

$$L_{diff} = -0.07 + 3.35stress,$$

where *stress* is the proportional reduction in fitness of outbred individuals in the stressful environment relative to the benign environment (SEM for slope = 0.53). The linear regression provides an intercept that is not significantly different from zero, which is expected theoretically if one environment is, in fact, not stressful compared to the other (i.e., *stress* = 0). The equation predicts that

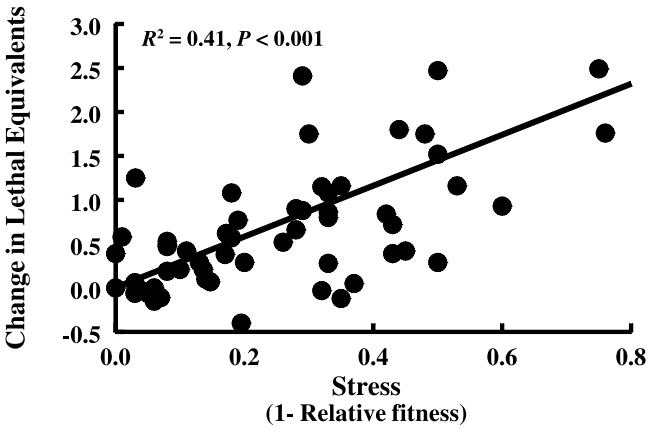

Figure 4. The relationship between L_{diff} (the difference in the number of lethal equivalents expressed in the stressful versus benign environment) and the magnitude of the stress. *Stress* is calculated as 1 – $Survival_{outbred(stressful)}/Survival_{outbred(benign)}$, where the most benign environment is the environment in which fitness, or the measured fitness trait, is greatest. L_{diff} is positively and linearly related to the degree to which outbred populations find the environment stressful ($R^2 = 0.41$, $P < 0.001$). The data represent 60 datapoints from 33 different peer-reviewed studies. The regression predicts an increase of one lethal equivalent upon inbreeding, for every 30% reduction in fitness imposed on the outbred population by the stress.

a stress that lowers fitness to 30% in outbred individuals will have one additional lethal equivalent expressed with inbreeding compared to the benign environment, and a stressful environment that reduces fitness to 60% normally will have two additional lethal equivalents with inbreeding compared with the benign environment. Addition of the *C. maculatus* study presented above does not change these results (R^2 [with the current *C. maculatus* study added] = 0.40, $P < 0.001$; slope = 3.69 ± SEM 0.56).

There was no difference in L_{diff} between studies of plants, vertebrate animals, and invertebrate animals (analysis of covariance [weighted] with taxonomic category as a fixed effect, $F_{5,52} = 0.89$, $P = 0.42$), nor was there any evidence that the relationship between L_{diff} and *stress* differs between these broad taxonomic categories (taxon-by-stress interaction, $F_{2,52} = 0.17$, $P = 0.58$).

Discussion

The key result of this study is that the magnitude of inbreeding depression was positively correlated with the stressfulness of the environment. The relative fitness of the outbred populations in the different environments was able to explain 57% of the variation in inbreeding depression (the inbreeding load) for the proportion of *C. maculatus* eggs giving rise to an adult beetle (Fig. 3D), and as much as 66% of the variation in a specific development stage (larval hatch-to-adult survival; Fig. 3C). This highly significant relationship was not due to variation in the phenotypic variance (CV^2), and thus the opportunity for selection, among environments. This relationship between the magnitude of the stress (decrease in fitness) and the magnitude of inbreeding load observed for *C. maculatus* is in agreement with results of our meta-analysis, but is at odds with the results of a previous study on these same populations of beetles that manipulated just temperature to impose stress. We elaborate on our experimental findings, and reconcile the results of this study with our previous research, in the following paragraphs.

INBREEDING-STRESS INTERACTIONS IN *C. MACULATUS*

Overall, we observed large effects of both temperature stress (high and low extreme temperature) and rearing host on fitness of *C. maculatus*. More importantly, the rearing environment also impacted inbreeding depression, with δ and *L* consistently greater in the more stressful environments (i.e., the environments in which outbred larval mortality was greatest) than a less stressful one. At 37°C, the magnitude of inbreeding depression (δ) was twice as large (averaged across populations and hosts) as at 27°C, and at 17°C inbreeding depression was 2.7 times as severe. However, the degree to which environments were stressful differed between the two beetle populations; SI beetles had much lower outbred survival, and exhibited much greater inbreeding depression, when reared on cowpea. Thus, consistent with our previous results (Fox et al. 2010), there was an inbreeding–environment interaction, where environmental conditions that were mildly stressful to outbred individuals were perceived as highly stressful by inbred individuals. The degree to which environments were stressful, and which environments were most stressful, was specific not only to the inbreeding level but also to the different populations. Future research should be aimed at mapping specific loci involved in inbreeding–stress interactions or determining if they are in fact due to a loss of general homeostasis in more inbred individuals.

However, the results of our current study contrast with our previous study (Fox et al. 2010) in that, although we found that inbreeding depression varied among rearing temperatures, we previously found no relationship between the stressfulness of the rearing temperature and the magnitude of inbreeding depression. However, our meta-analysis provides insight into why our results contrast between studies; the degree of stress imposed differed substantially between studies. Specifically, in our previous study we manipulated temperature along a much smaller range than in the current study, and did not manipulate rearing host. This created only a weak and narrow range of stress compared to the current study. The current study used more stressful temperatures, at both the high and low end, and combined stressors (using also two hosts that differ in suitability), and produced a very strong relationship between stress and inbreeding depression. There are two likely

explanations for how differences in the range of stress imposed can generate different relationships between stress and inbreeding depression between studies. First, the small range of stress used in the previous study would have small effects on inbreeding depression, giving us little power to detect a stress-inbreeding relationship in that study. Alternatively, there may be some threshold level of stress below which stress has little effect on inbreeding depression but above which inbreeding–environment interactions become significant. Unfortunately, to test this hypothesis we need a study that examined a large enough range of stressors (within a single study) and tests for nonlinear relationships between stress and inbreeding depression.

Callosobruchus maculatus is an excellent model system for examining the combined effects of inbreeding and environmental stress because laboratory conditions mimic very well the conditions experienced by larvae in nature; beetles develop inside seeds and have no contact with the environment outside their seed until they emerge as adults. Larval density is easily controlled—all beetles can be reared at one beetle per seed, one seed per dish (larvae cannot move among seeds and cannot interact with larvae in different seeds)—avoiding confounding effects of density on inbreeding depression. However, because trade-offs between fitness components may be common, controlled studies such as ours may significantly oversimplify the natural conditions in which beetles will experience inbreeding depression. The small number of studies that have used crowding or competition as a primary stress have often shown little or no increase in inbreeding depression over the benign environment ($+0.71 \pm 0.42$ lethal equivalents); however, the crowding or competitive conditions used often imposed little stress (reduction in fitness averaged $22 \pm 8\%$) (Schmitt and Ehrhardt 1990; Wolfe 1993; Cheptou et al. 2001; Fowler and Whitlock 2002; Haag et al. 2003; Rowe and Beebee 2005). Thus, it is not yet possible to generalize or make predictions about how larval competition would have changed our results. It is not yet clear that interspecific competition does not magnify the effects of inbreeding as stated by Willi et al. (2007). In fact, the data from studies on competition show the same trend of increasing lethal equivalents with increasing stress levels (least-squares linear regression; $R^2 = 0.92$, $P < 0.003$).

As in the previous study using these populations and temperature as a stress, correlations among blocks for inbreeding depression for fitness (proportion of eggs producing an adult) were small but consistently positive ($r = 0.27 \pm 0.05$). The correlation coefficient among blocks, using the same populations but less-severe temperatures stresses in another study, was 0.37 ± 0.05 (Fox et al. 2010). This agrees with the general trend that the more disparate the sources of the stresses are, the weaker the correlations become, as suggested by Fox et al. (2010). Thus, although at least some of the loci contributing to inbreeding depression are shared at the different temperatures, the number of loci shared or the homogeneity of their impacts seems to lessen the further apart the rearing temperatures become.

THE GENERALITY OF INBREEDING–STRESS INTERACTIONS

Our meta-analysis of the available data (58 datasets) on inbreeding–stress interactions (or lack thereof) suggests that the phenomenon witnessed in our studies of *C. maculates*—that inbreeding–stress interactions should be most significant when stress is greater—is a general one. That is, when organisms are subjected to a mild form of stress, there is little or no effect on the magnitude of inbreeding depression, whereas when the stress is greater a positive relationship between the magnitude of the stress and the magnitude of inbreeding depression becomes apparent. Inbreeding–stress interactions are weak and not consistently positive until the stressful environment imposes approximately a 25% loss in fitness on the outbred population. Thus, small increases in the magnitude in inbreeding depression can easily be missed in experiments in which the stress is weak; especially given limits on sample size and the typically large among family variance in inbreeding depression (Armbruster and Reed 2005).

In our meta-analysis, a linear regression of the change in lethal equivalents as a function of the magnitude of the stress (defined as the proportional decrease in fitness, or measured fitness component, of the outbred population reared in the stressful relative to the environment in which they have the highest fitness), indicates that the difference in stressfulness of environments between the stressful and benign environments explains 41% of the variation in inbreeding depression among studies. Given the inherent noise in such a meta-analysis (e.g., different taxonomic groups), combined with the standard errors surrounding each point estimate, it is almost certain that the true relationship is much stronger than suggested by the regression. It is also reassuring that the intercept of the slope is not significantly different from zero. Thus, the equation predicts that two different environments that provide identical fitness to the outbred populations will also have almost identical levels of inbreeding depression in those environments, whereas increasingly stressful environments will produce increasingly greater magnitudes of inbreeding depression.

Due to the inconsistency of the evidence for inbreeding–environment interactions, it would appear that such interactions are idiosyncratic to the genetic background of the population and the particulars of the environment (Armbruster and Reed 2005). Data from this model system (*C. maculatus*) and our meta-analysis suggest that not only are inbreeding–environment interactions ubiquitous, but that they may be inevitable and directional. They are directional in the sense that more stressful environments consistently lead to increased inbreeding depression. Further, the increase in inbreeding depression (lethal equivalents) scales, at

least among studies, linearly with the stress. This suggests that some fundamental and widespread principles underlie these interactions. One underlying causal mechanism might be that inbreeding itself is a stress and that the resulting instability of gene expression for networks involved in stress response or metabolism generally might release cryptic deleterious mutations or simply overwhelm the organism's ability to respond to further external stresses (Kristensen et al. 2006; Tomala and Korona 2008). Another, not mutually exclusive, explanation is that the deleterious mutations on average have smaller selection coefficients in the more benign environment and as the organism approaches its physiological limits the detrimental effects of those alleles are magnified. The first explanation may be more compatible with a gradual and linear increase in inbreeding depression with environmental stress whereas the second might imply a threshold effect where previously neutral (or even beneficial) mutations suddenly become deleterious under a certain environment, giving rise to the case in which an environment that is perceived as nonstressful to outbred individuals is perceived as highly stressful by inbred individuals. The increase in the number of lethal equivalents in the meta-analysis is linear and there does not appear to be a threshold effect. However, this should not be taken as evidence against threshold effects in individuals, because individual variation in inbreeding levels and the specific sensitivity of different genotypes within a population can give rise to a linear increase in population-level inbreeding depression even if the effects are threshold effects on individual fitness.

The generality of inbreeding–stress interactions has important implications for evolutionary and conservation biology, impacting the behavior of organisms (e.g., dispersal tendencies, self-fertilization), the maintenance of genetic variation, and the genetic structure of populations, as well as population dynamics and viability. Our finding may be particularly important for biodiversity conservation. Liao and Reed (2009) found that predicted extinction times decreased 23% on average across a range of realistic parameter space when the inbreeding–stress interaction was included as compared to disregarding it. Vulnerability to extinction is often driven by severe short-term downturns in environmental quality or long-term directional changes in the environment (Reed et al. 2003b). Anything that exacerbates the extent of negative population growth during environmental perturbations can greatly impact extinction risk. Thus, Liao and Reed (2009) may have significantly underestimated the impact of inbreeding stress interactions, because in their model the interaction was held constant rather than increasing with the increasing stress levels. The findings of this study also emphasize the need for continuing studies on purging of genetic load in stressful environments, particularly as it relates to the ability of repeated bouts of low levels of a stress to precondition populations for survival during higher levels of the same stress. Further, more work is needed to examine the correlation between purging of the genetic load under one stress and whether it is effective in other stressful environments.

Future studies of inbreeding–environment interactions should focus on measuring inbreeding depression at multiple degrees of stress, and using multiple stressors, at differing levels of inbreeding. Using a range of stress levels will be necessary for detecting nonlinear relationships between stress and inbreeding depression, which is necessary to test whether a threshold effect exists. Ultimately, identifying the mechanism responsible for inbreeding–environment interactions will require mapping of the genes responsible for inbreeding depression across multiple stressful environments. If the same genes are responsible across multiple environments (and species), then this would suggest that general stress-coping mechanisms are being overwhelmed and leading to a loss of fitness homeostasis. More idiosyncratic groupings of a small or large number of genes across environments (and species) would suggest a prominent role for small increases in the deleterious effects for most alleles affecting the trait or large increases in the detrimental effects of one or a few such loci.

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Supporting Information

The following supporting information is available for this article:

Figure S1. The proportion of eggs that develop, egg hatch, hatch-to-emergence survival, and the proportion of all eggs that gave rise to an emerged adult offspring for outbred (•) and inbred (○) beetles from two populations of *Callosobruchus maculatus* raised at three temperatures and on two hosts.

Table S1. Logistic regression analysis for the effects of population (SI or BF), rearing temperature (17°C, 27°C, or 37°C), and rearing host (mung or azuki) on egg development, egg hatch, larval survival, and cumulative probability that an egg produces an adult offspring, for outbred *Callosobruchus maculatus*.

Table S2. Analysis of variance testing for the effects of population (SI or BF), rearing temperature (17°C, 27°C, or 37°C), and rearing host (mung or azuki) on the inbreeding load (*L*) for egg development, egg hatch, larval survival, and cumulative probability that an egg produces an adult offspring, for *Callosobruchus maculatus*.

Table S3. The Pearson moment correlations for inbreeding depression (δ) between pairs of temperatures (correlations are among blocks).

Table S4. Studies used in our meta-analysis of inbreeding–stress interactions.

Supporting Information may be found in the online version of this article.

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Online Supplemental Material

Inbreeding depression increases with environmental stress: An experimental study and meta-analysis

Charles W. Fox and David H. Reed

Host and temperature effects on survival and development of outbred offspring

The probability that an *outbred* egg gave rise to an adult offspring varied substantially among the three rearing temperatures (Table S1; Figure S1); eggs were most likely to give rise to an offspring that survived to adult when reared at 27°C, and least likely to give rise to an adult offspring at 17°C, with 37°C being intermediate ($\chi_1^2 > 2.1$, $P < 0.023$ for all linear contrasts). This was because larval survival and egg hatch both varied among the three rearing temperatures (Table S1). Larval survival was lowest at 17°C, intermediate at 37°C, and highest at 27°C ($\chi_1^2 > 5.22$, $P < 0.025$ for all pair-wise contrasts) (Figure S1). Egg hatch was lowest when beetles were reared at the highest temperature (37°C; $P < 0.05$ for 17x37°C and 27x37°C post-hoc contrasts), but egg hatch did not differ between 17 and 27°C.

Rearing host had no effect on egg hatch, as expected since larvae do not interact with their host seed until after hatching (Table S1). However, larvae survived from hatch to adult much worse on cowpea seeds than on mung (Table S1). This effect of rearing host on larval survival led to eggs laid on cowpea being much less likely to produce an adult offspring than were eggs laid on mung. In previous studies (e.g., Stillwell et al. 2007) the magnitude of the host effect of larval survival differed between these two *C. maculates* populations; such as interaction was suggested in the results presented in Table 1 but the population-x-host interaction was not statistically significant (Table S1).

Effect of inbreeding on survival and development

Inbreeding had no detectable effect on the proportion of eggs that developed (logistic regression, effect of inbreeding; $\chi_1^2 = 3.76$, $P = 0.05$). Though all estimates of δ (Table 2) and L (Figure 2) were positive, they were all small ($\delta < 0.08$, $L < 0.35$). In contrast, and as observed in previous studies, eggs from sib-matings had significantly reduced egg hatch ($\chi_1^2 = 41.1$, $P < 0.001$), and those eggs had lower larval survival ($\chi_1^2 = 69.7$, $P < 0.001$), than did eggs from outbred matings. Thus, eggs from inbred matings were substantially less likely to produce an adult offspring than were eggs from outbred matings ($\delta > 0.19$, $L > 1.1$ for all treatment-

population combinations; $\chi_1^2 = 81.7$, $P < 0.001$). There was no detectable difference between populations in the magnitude of inbreeding depression for any of the periods of mortality ($P > 0.58$ for all four variables; Table S2).

Rearing host and temperature affect inbreeding depression

The egg hatch and larval survival data (above) clearly indicate that the two extreme temperatures represent stressful environments for outbred *C. maculatus* larvae (relative to the intermediate 27°C), with 37°C more stressful (greater effect on survival) than 17°C. Likewise, cowpea is the least suitable of the two hosts. We thus expected inbreeding depression to vary among rearing temperatures and between hosts, with inbreeding depression greatest at the two extreme temperatures (especially 37°C) and when beetles were reared on cowpea.

There was no evidence that the inbreeding load for the proportion of eggs that developed differed between rearing hosts or varied among rearing temperatures (analysis of variance comparing estimates of L; $P > 0.3$). This was not surprising since the main effect of inbreeding was not significant for this stage of development. The inbreeding load for egg hatch did not differ significantly between rearing hosts but did vary among temperatures (Table S2). The inbreeding for larval hatch-to adult survival varied substantially both among the rearing temperatures and between rearing hosts (Table S2).

Because the statistics are qualitatively the same for both larval survival and the probability that an egg produced a surviving adult offspring – all significant main effects and interactions are the same for both periods of mortality – we focus here on larval survival because it does not confound the three periods of mortality. For larval hatch-to-adult survival, δ was lowest at 27°C (the temperature at which survival was highest) averaging (across hosts and populations) only 0.16; i.e., inbred beetles had only 16% lower larval hatch-to-adult survival than did outbred beetles when reared at 27°C, equal to L=1.38 lethal equivalents. In contrast, δ (averaged across hosts) was 0.39 at 37°C and 0.60 at 37°C (all linear contrasts between temperatures were significant, $P < 0.05$). This is a difference of expression of 4.9 lethal equivalents between the least and most stressful temperatures (Figure 2). Thus, while the two extreme rearing temperatures were clearly stressful for all beetles, the consequences of this stress (the decrease in survival) were much more dramatic for inbred than outbred larvae. Inbreeding depression was also greater when beetles were reared on cowpea ($\delta = 0.45$, $L = 4.81$, averaged across

populations and temperatures) than when they were reared on mung ($\delta = 0.31$, $L = 2.96$). All two way interactions were significant except the temperature-x-host interaction, and there was a highly significant three-way interaction between population, rearing host and rearing temperature (Table S2). These interactions can be summarized as follows: (1) Though inbreeding depression was greatest at the two extreme temperatures (relative to the intermediate temperature) for both populations, the magnitude of the temperature effect differed between the two populations (the temperature effect was much greater for BF than for SI beetles; population-x-temperature interaction, $F_{2,378} = 20.5$, $P < 0.001$); (2) Inbreeding depression was greater on cowpea for both populations, but the rearing host effect on inbreeding depression was, on average (across temperatures), much smaller for BF beetles than for SI beetles (population-x-host interaction; $F_{1,378} = 9.74$, $P = 0.002$); (3) However, this difference between populations in the magnitude of the host effects depended on temperature (significant 3-way interaction; $F_{2,378} = 11.3$, $P < 0.001$) – whereas inbreeding depression was greater for SI beetles when they were reared on cowpea (relative to mung) at all temperatures, inbreeding depression was greater for BF beetles reared on cowpea (relative to mung) only at 27°C; when BF beetles were reared at 17°C or 37°C, there was no difference in inbreeding depression between the two rearing hosts.

Correlation between inbreeding depression across treatments

For the four periods of larval mortality, 41 of the 48 correlations between δ for pairs of temperatures were positive (sign test, $P < 0.001$), and 15 of 48 were significantly greater than 0 ($P < 0.001$ against the expected 5% frequency of false positives) (Table S3). The mean correlation among blocks between pairs of temperatures for the probability an egg produces an adult offspring was significantly greater than 0 but a rather modest, 0.16 ± 0.05.

Table S1. Logistic regression analysis for the effects of population (SI or BF), rearing temperature (17, 27 or 37°C), and rearing host (mung or azuki) on egg development, egg hatch, larval survival, and cumulative probability that an egg produces an adult offspring, for outbred *Callosobruchus maculatus*.

Trait/Source	DF	χ^2	*P*
Egg development [a]			
Population	1	0.02	0.89
Temperature	2	2.26	0.32
Population*Temperature	2	0.01	0.99
Host	1	1.99	0.16
Population*Host	1	0.15	0.70
Temperature*Host	2	0.23	0.89
Pop*Temp*Host	2	0.12	0.94
Egg hatch [b]			
Population	1	0.40	0.52
Temperature	2	6.57	**0.04**
Population*Temperature	2	0.21	0.90
Host	1	0.20	0.65
Population*Host	1	0.15	0.70
Temperature*Host	2	0.09	0.94
Pop*Temp*Host	2	0.37	0.83
Larval survival [c]			
Population	1	5.57	**0.02**
Temperature	2	28.42	**< 0.001**
Population*Temperature	2	2.50	0.29
Host	1	63.95	**< 0.001**
Population*Host	1	0.82	0.36
Temperature*Host	2	1.26	0.53
Pop*Temp*Host	2	0.40	0.82
Egg → adult [d]			
Population	1	6.23	**0.01**
Temperature	2	26.55	**< 0.001**
Population*Temperature	2	0.97	0.62
Host	1	56.60	**< 0.001**
Population*Host	1	2.68	0.10
Temperature*Host	2	3.71	0.16
Pop*Temp*Host	2	0.30	0.86

[a] The proportion of eggs producing a visible embryo; [b] The proportion of developing eggs that hatch (hatching is defined to have occurred if the larvae begins digging into the seed); [c] The proportion of hatched eggs that produce an adult offspring that successfully emerges from the seed; offspring that pupated but failed to emerge from a seed are counted as part of larval mortality; [d] The total probability that an egg produces an adult offspring that successfully emerges from the seed.

Table S2. Analysis of variance testing for the effects of population (SI or BF), rearing temperature (17, 27 or 37°C), and rearing host (mung or azuki) on the inbreeding load (*L*) for egg development, egg hatch, larval survival, and cumulative probability that an egg produces an adult offspring, for *Callosobruchus maculatus*. Data are presented in Figure 2.

Trait/Source	DF	χ^2	*P*
Egg development [a]			
Population	1	0.20	0.65
Temperature	2	0.97	0.38
Population*Temperature	2	1.12	0.33
Host	1	0.04	0.84
Population*Host	1	0.59	0.44
Temperature*Host	2	0.34	0.71
Pop*Temp*Host	2	1.27	0.28
Egg hatch [b]			
Population	1	0.23	0.63
Temperature	2	16.83	**< 0.001**
Population*Temperature	2	0.61	0.54
Host	1	4.41	**0.04**
Population*Host	1	0.38	0.54
Temperature*Host	2	1.52	0.22
Pop*Temp*Host	2	0.24	0.79
Larval survival [c]			
Population	1	0.02	0.88
Temperature	2	73.97	**< 0.001**
Population*Temperature	2	20.54	**< 0.001**
Host	1	61.88	**< 0.001**
Population*Host	1	9.74	**0.002**
Temperature*Host	2	8.07	**< 0.001**
Pop*Temp*Host	2	11.32	**< 0.001**
Egg → adult [d]			
Population	1	0.31	0.58
Temperature	2	81.44	**< 0.001**
Population*Temperature	2	20.25	**< 0.001**
Host	1	35.02	**< 0.001**
Population*Host	1	7.36	**< 0.01**
Temperature*Host	2	3.34	**0.04**
Pop*Temp*Host	2	7.44	**< 0.001**

[a] The proportion of eggs producing a visible embryo; [b] The proportion of developing eggs that hatch (hatching is defined to have occurred if the larvae begins digging into the seed); [c] The proportion of hatched eggs that produce an adult offspring that successfully emerges from the seed; offspring that pupated but failed to emerge from a seed are counted as part of larval mortality; [d] The total probability that an egg produces an adult offspring that successfully emerges from the seed.

Table S3. The Pearson moment correlations for inbreeding depression (δ) between pairs of temperatures (correlations are among blocks).

	BF		SI	
	Mung	Cowpea	Mung	Cowpea
Eggs developing [a]				
17 v 27° C	0.32	0.35*	0.32	0.34
17 v 37° C	-0.06	0.20	0.31	0.36*
27 v 37° C	-0.34	0.16	0.09	0.42*
Eggs Hatching [b]				
17 v 27° C	0.46**	0.16	0.52**	0.40*
17 v 37° C	0.40*	0.28	-0.16	0.04
27 v 37° C	0.33	0.50*	0.04	0.29
Larval survival [c]				
17 v 27° C	0.19	0.06	0.36*	0.42*
17 v 37° C	-0.09	0.35*	-0.05	0.08
27 v 37° C	-0.06	0.00	-0.04	0.15
Probability of an adult [d]				
17 v 27° C	0.38*	0.06	0.52**	0.50**
17 v 37° C	0.25	0.39*	0.32	0.06
27 v 37° C	0.25	0.04	0.17	0.30

[a] The proportion of eggs producing a visible embryo; [b] The proportion of developing eggs that hatch (hatching is defined to have occurred if the larvae begins digging into the seed); [c] The proportion of hatched eggs that produce an adult offspring that successfully emerges from the seed; offspring that pupated but failed to emerge from a seed are counted as part of larval mortality; [d] The total probability that an egg produces an adult offspring that successfully emerges from the seed.

* $P < 0.05$; ** $P < 0.01$. Note that 41 of the 48 estimates were positive, far greater than the frequency of positive estimates expected by chance ($P < 0.001$). Likewise, 15 of the 48 estimates we significantly greater than 0, far greater than the frequency expected by chance ($P < 0.001$).

Table S4. Studies used in our meta-analysis of inbreeding-stress interactions.

Citation	Species (Family)	Stress[1]	L_{diff}[2]	Weight[3]
Armbruster et al. 2000	*Aedes geniculatus* (Diptera)	0.2	0.29	1
Bijlsma et al. 1999	*Drosophila melanogaster* (Diptera)	0.37	0.05	0.5
Bijlsma et al. 1999	*Drosophila melanogaster* (Diptera)	0.45	0.42	0.5
Bijlsma et al. 2000	*Drosophila melanogaster* (Diptera)	0.5	1.52	0.5
Bijlsma et al. 2000	*Drosophila melanogaster* (Diptera)	0.75	2.49	0.5
Carr et al. 2003	*Mimulus guttatus* (Scrophulariaceae)	0.11	0.42	1
Chen 1993	*Arianta arbustorum* (Helicidae)	0.07	-0.11	1
Cheptou et al. 2000a	*Crepis sancta* (Asteraceae)	0.05	-0.06	1
Cheptou et al. 2000b	*Crepis sancta* (Asteraceae)	0	0.39	0.333
Cheptou et al. 2000b	*Crepis sancta* (Asteraceae)	0.18	0.57	0.333
Cheptou et al. 2000b	*Crepis sancta* (Asteraceae)	0.08	0.53	0.333
Dahlgaard et al. 1995	*Drosophila buzzatii* (Diptera)	0.14	0.1	1
Dahlgaard & Hoffman 2000	*Drosophila melanogaster* (Diptera)	0.29	0.88	1
Dahlgaard & Loeschcke 1997	*Drosophila buzzatii* (Diptera)	0.32	-0.03	1
Eckert & Barrett 1994	*Decodon verticillatus* (Lythraceae)	0.17	0.38	0.5
Eckert & Barrett 1994	*Decodon verticillatus* (Lythraceae)	0.18	1.08	0.5
Fowler & Whitlock 2002	*Drosophila melanogaster* (Diptera)	0.48	1.75	0.5
Fowler & Whitlock 2002	*Drosophila melanogaster* (Diptera)	0.03	0.06	0.5
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.03	1.25	0.17
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.19	-0.4	0.17
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.14	0.21	0.17
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.01	0.58	0.17
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.17	0.62	0.17
Fox et al. 2010	*Callosobruchus maculatus* (Coleoptera)	0.15	0.07	0.17
Haag et al. 2002	*Daphnia magna* (Daphniidae)	0.33	0.8	1
Haag et al. 2003	*Daphnia magna* (Daphniidae)	0.33	0.28	0.5
Haag et al. 2003	*Daphnia magna* (Daphniidae)	0.03	-0.06	0.5
Hayes et al. 2005	*Cucurbita pepo* (Cucurbitaceae)	0	0	1
Henry et al. 2003	*Physa acuta* (Physidae)	0.04	-0.03	1
Johnston 1992	*Lobelia* (2 species) (Lobelioideae)	0.08	0.49	1
Johnston 1992	*Lobelia* (2 species) (Lobelioideae)	0.76	1.76	0.5
Johnston 1992	*Lobelia* (2 species) (Lobelioideae)	0.35	-0.12	0.5
Koelwijn 1998	*Plantago coronopus* (Plantaginaceae)	0.53	1.16	1
Kristensen et al. 2003	*Drosophila buzzatii* (Diptera)	0.43	0.39	0.333
Kristensen et al. 2003	*Drosophila buzzatii* (Diptera)	0.26	0.52	0.333

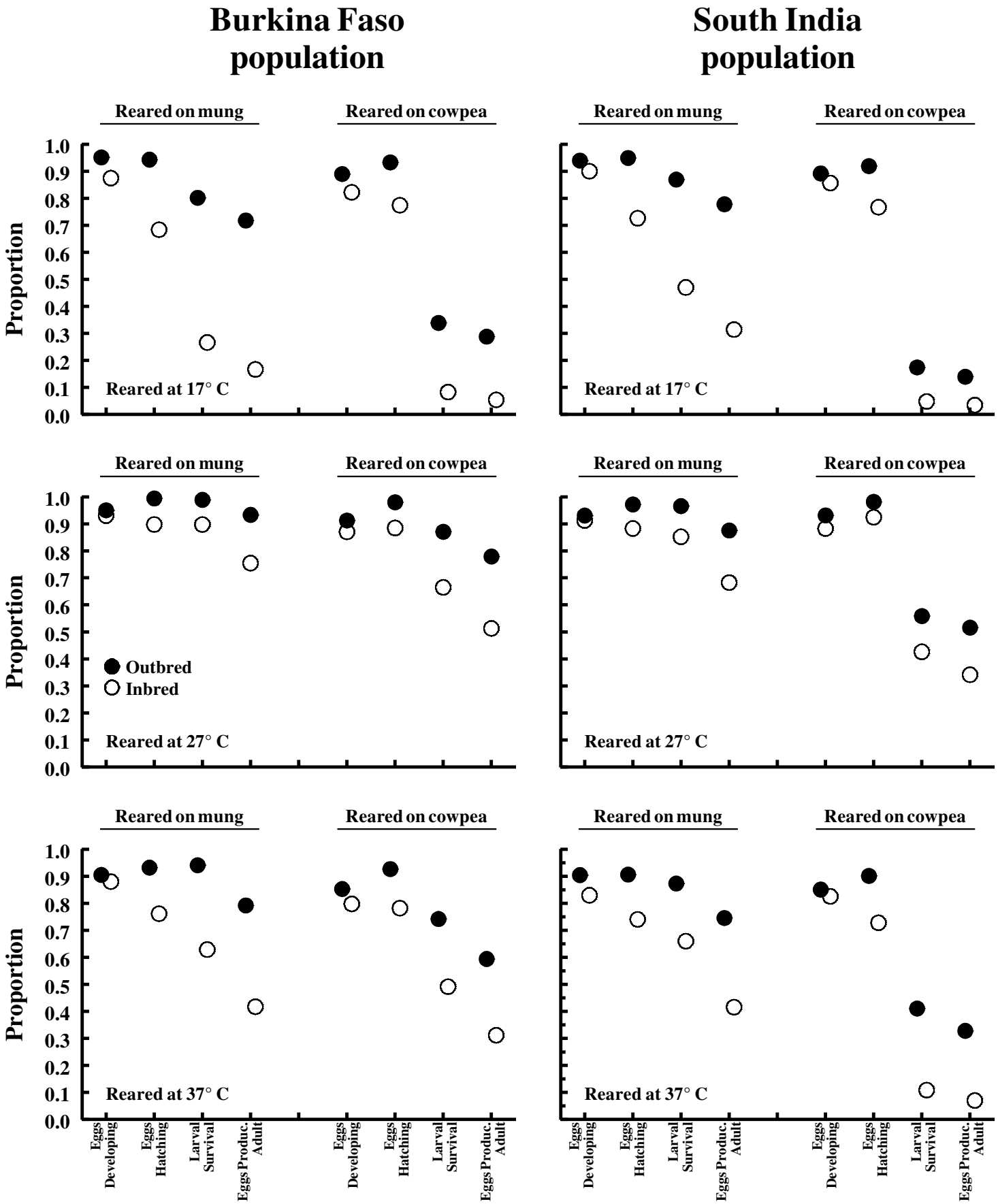
Kristensen et al. 2003	*Drosophila buzzatii* (Diptera)	0.08	0.19	0.333
Kristensen et al. 2008	*Drosophila melanogaster* (Diptera)	0.1	0.21	0.2
Kristensen et al. 2008	*Drosophila melanogaster* (Diptera)	0.43	0.72	0.2
Kristensen et al. 2008	*Drosophila melanogaster* (Diptera)	0.13	0.29	0.2
Kristensen et al. 2008	*Drosophila melanogaster* (Diptera)	0.32	1.15	0.2
Kristensen et al. 2008	*Drosophila melanogaster* (Diptera)	0.33	1.08	0.2
Markert et al. 2010	*Americamysis bahia* (Mysidae)	0.5	4.09	1
Marr et al. 2006	*Melospiza melodia* (Emberizidae)	0.3	1.75	1
Nowak et al. 2007	*Chironomus riparius* (Chironomidae)	0.6	0.93	1
Reed & Bryant 2001	*Musca domestica* (Diptera)	0.33	0.86	0.5
Reed & Bryant 2001	*Musca domestica* (Diptera)	0.28	0.9	0.5
Reed et al. 2002	*Drosophila melanogaster* (Diptera)	0.28	0.66	0.5
Reed et al. 2002	*Drosophila melanogaster* (Diptera)	0.35	1.16	0.5
Reed et al. 2003	*Drosophila melanogaster* (Diptera)	0.42	0.84	1
Reed et al. 2007	*Rabidosa* (2 species) (Lycosidae)	0.44	1.8	1
Reed et al. 2007	*Rabidosa* (2 species) (Lycosidae)	0.5	2.47	1
Rowe & Beebee 2005	*Bufo calamita* (Bufonidae)	0.5	0.29	1
Schemske 1983	*Costus* (3 species) (Zingiberaceae)	0.06	-0.15	1
Schemske 1983	*Costus* (3 species) (Zingiberaceae)	0.29	2.41	1
Schemske 1983	*Costus* (3 species) (Zingiberaceae)	0.08	0.47	1
Schmitt & Ehrhardt 1990	*Impatiens capensis* (Balsaminaceae)	0.19	0.77	1
Szulkin & Sheldon 2007	*Parus major* (Paridae)	0.55	3.56	1
Wolfe 1992	*Hydrophyllum appendiculatum* (Hydrophyllacea)	0.06	0	1

[1] *Stress* is the proportional decrease in fitness in the stressful versus the relatively benign environment: $1 - (W_S / W_B)$, where W is the value of the fitness measure.

[2] L_{diff} is a measure of the difference in the number of lethal equivalents, between benign and stressful environments, expressed with inbreeding: $L_{Benign} - L_{Stressful}$. A negative number means there were a larger number of lethal equivalents estimated in the benign than in the stressful environment.

[3] Weight is the inverse of the number of estimates of *L* from a single study.

Figure S1. The proportion of eggs that develop, egg hatch, hatch-to-emergence survival, and the proportion of all eggs that gave rise to an emerged adult offspring for outbred (●) and inbred (○) beetles from two populations of *Callosobruchus maculatus* raised at three temperatures and on two hosts. Means are calculated first by averaging across families in a block, then across blocks, for each population-temperature-host combination.


Burkina Faso population
South India population
Reared on mung
Reared on cowpea
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Reared at 17° C
Reared at 27° C
Reared at 37° C
Outbred
Inbred
Eggs Developing
Eggs Hatching
Larval Survival
Eggs Produc. Adult