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Inbreeding-Stress Interactions: Evolutionary and Conservation Consequences

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Inbreeding-Stress Interactions: Evolutionary and Conservation Consequences

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Abstract

The effect of environmental stress on the magnitude of inbreeding depression has a long history of intensive study. Inbreeding-stress interactions are of great importance to the viability of populations of conservation concern and have numerous evolutionary ramifications. However, such interactions are controversial. Several meta-analyses over the last decade, combined with omic studies, have provided considerable insight into the generality of inbreeding-stress interactions, its physiological basis, and have provided the foundation for future studies. In this review, we examine the genetic and physiological mechanisms proposed to explain why inbreeding-stress interactions occur. We specifically examine whether the increase in inbreeding depression with increasing stress could be due to a concomitant increase in phenotypic variation, using a larger data set than any previous study. Phenotypic variation does usually increase with stress, and this increase can explain some of the inbreeding-stress interaction, but it cannot explain all of it. Overall, research suggests that inbreeding-stress interactions can occur via multiple independent channels, though the relative contribution of each of the mechanisms is unknown. To better understand the causes and consequences of inbreeding-

stress interactions in natural populations, future research should focus on elucidating the genetic architecture of such interactions and quantifying naturally occurring levels of stress in the wild.

Keywords: biodiversity conservation, environmental stress, evolution, omics, inbreeding

Introduction

Inbreeding and stressful environmental conditions are two major variables that influence the ecological and evolutionary dynamics of natural populations.[1–3] Inbreeding causes reduced fitness in inbred relative to outbred individuals (i.e., inbreeding depression), and exposure to abiotic and biotic stressors, by definition, also decreases fitness relative to benign environments.[4–7] Rapid changes to natural habitats that have been experienced by many plant and animal populations during the last century (e.g., due to climate change) often increase the level of stress perceived by individuals[8,9] and, at the same time, lead to a reduction in population size and increased rates of inbreeding. For the management of threatened wild and domesticated species, it is therefore crucial to understand how the combined effects of inbreeding and decreased environmental quality affect population fitness.[10] As a result, understanding the degree to which inbreeding depression changes with environmental conditions has become a central focus in evolution, ecology, conservation, and animal breeding research.

An important question that emerged in the literature is whether decreases in fitness are additive when inbreeding and stress are combined, or if fitness is decreased more (or less) than expected under the assumption that inbreeding and stress act independently. When the simultaneous effects of inbreeding and stressful environmental conditions are not additive, there is an inbreeding-stress interaction (Fig. 1). As we demonstrate in this review, inbreeding-stress interactions in which inbreeding depression increases under adverse environmental conditions are typically observed and have numerous repercussions for evolutionary biology and for the conservation of biodiversity. Nonadditive effects of these two sources of reduced population fitness, in which environmental stressors substantially increase the fitness consequences of inbreeding, can reduce thresholds for population persistence well below those predicted by models that assume that the effects of environmental stress and inbreeding are independent.[10,11]

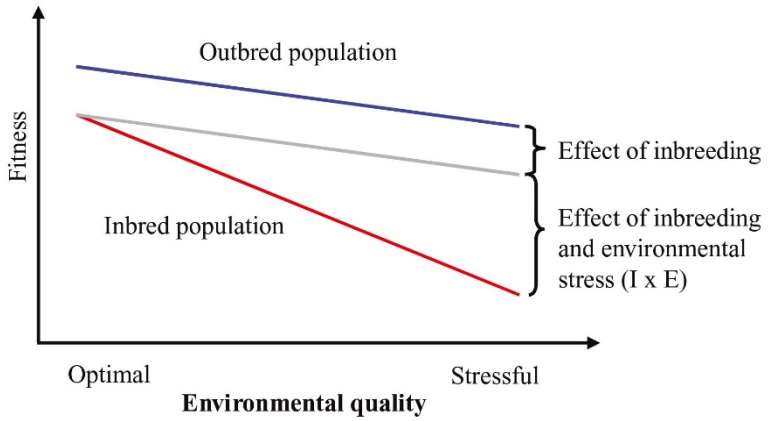

Figure 1. Fitness effects of inbreeding-environment interactions. Assuming the effect of inbreeding is independent of the environment, the reduction in fitness as a result of reduced environmental quality will be equal for outbred and inbred populations. The blue and gray lines illustrate fitness of an outbred and an inbred population, respectively, in the absence of inbreeding-environment interactions. Inbreeding depression is, however, often more severe under stressful environmental conditions. Thus, the red line illustrates fitness of an inbred population taking into account the effect of inbreeding-environment interactions (redrawn from Ref. 85).

Following the proxy that is widely used in studies of mutational effects, we here define stressfulness of an environment as a function of the mean fitness of outbred individuals in that environment relative to other environments.[4–7,12] Any environmental variable that reduces mean population fitness is thus considered a stressor. This includes ecological variables that increase physiological stress (e.g., as measured by increases in stress hormones or proteins), but only to the extent that increased physiological stress is associated with reduced fitness.

Historical development of inbreeding-stress research

Interest in inbreeding-stress interactions goes back at least 60 years. In the 1950s and 1960s, a number of papers, mostly in the agricultural literature, examined such interactions but generally lumped inbreeding-stress interactions together with the more general phenomena of genotype-environment interactions. Early papers[13–21] approached the problem from the perspective of Waddington and Lerner's ideas concerning heterozygosity, developmental stability, and maintenance of the optimum phenotype across changing environmental conditions (canalization).[22,23] Thus, the emphasis was on hybrid vigor upon crossing inbred lines of domesticated species, and not on the loss of genetic diversity and increased homozygosity due to habitat fragmentation and persistent small population size in natural environments. Surprisingly, despite increasing rates of inbreeding in many livestock breeds (which was intensified in the 1960s and 1970s with the development of reproductive technologies and advanced breeding schemes) and highly variable rearing conditions for these livestock, inbreeding-stress interactions were rarely investigated in the agricultural sciences during the last 50 years. Thus, there is a niche for developing models that incorporate

inbreeding-environment interactions into quantitative genetic models used in breeding programs, analogous to that recently attempted for genotype-environment interactions,[24] with the potential to make breeding programs more effective.

While there was variation in the results among the early studies mentioned above, they were consistent enough forWright[25] to suggest that less heterozygous (more inbred) individuals were generally more sensitive to environmental stress. However, it took a decade after Wright's suggestion before researchers again turned their attention in earnest to the question of the magnitude of inbreeding depression in stressful or variable environments. This renewed interest developed among evolutionary and conservation geneticists who sought to understand how inbreeding depression varied with environmental conditions, for example by comparing estimates from laboratory versus field or greenhouse conditions[26–30] and wild versus captive zoo populations.[31] Particularly influential in furthering ideas and producing copious data on inbreeding-stress interactions were Volker Loeschcke, Kuke Bijlsma, and colleagues.[1,32–36] Now the question was refocused from one of heterozygote advantage to two important issues that persist in research pursuits today: (1) Are the effects of stress and inbreeding independent or are they synergistic? and (2) Are inbreeding effects general across different types of stressors or are they stress specific?

This renewed attention generated huge amounts of data on how inbreeding depression varies with environmental conditions but, due to substantial variation in results among studies, it did little to settle the basic question of whether inbreeding depression generally increases with stress. In 2005, a meta-analysis[5] confirmed Wright's intuition that environmental stress on average increased the magnitude of inbreeding depression. However, 24% of the studies showed no such increase with some even showing the opposite pattern (lower inbreeding depression in more stressful environments). Different species, populations, inbred lines, sexes, and families were highly variable in their response to inbreeding and stress. This variation helps explain why reaching any robust conclusion has been so difficult. At this point the general consensus was that while inbreeding depression often increased with stress, the specifics of the effects of stress on inbreeding depression were idiosyncratic to the genetic architecture of the population and the type of stress applied.

Despite the fact that the results from the meta-analysis by Armbruster and Reed[5] did not reveal evidence for a general mechanism underlying inbreeding-stress interactions, this paper spurred even more research investigating inbreeding-stress interactions, with more than 20 papers being published on the topic since 2005. In 2011, another meta-analysis was published, stimulated by a study of multiple levels of stress, mixing two different stressors (temperature and diet), on two populations of the seed-feeding beetle *Callosobruchus maculates*.[6] This meta-analysis found that much of the variation in the environmental impact on levels of inbreeding depression among studies could be explained by the amount of stress imposed; studies imposing very little stress tended to find no effect on inbreeding depression (Fig. 2A), whereas studies imposing severe stress found large effects of stress on inbreeding depression.[6] In this study, the inbreeding load, *L*, increased by about one lethal equivalent for each 30% difference in outbred fitness between environments. These results suggest that the effects of stress on inbreeding depression are more homogeneous than formerly thought, and not so idiosyncratic regarding the genetic archi-

tecture of the population or the type of environmental variable causing stressful conditions, with greater levels of stress consistently leading to more inbreeding depression. This result has been confirmed by an independent meta-analysis using *Drosophila* and experiments in both the laboratory and field.[37] Just as was found by Fox and Reed,[6] Enders and Nunney[37] found a strong linear relationship between inbreeding depression and the magnitude of multiple stressors, with inbreeding depression increasing linearly as the level of stress increases (Fig. 2B). This review examines the hypotheses proposed to explain how inbreeding-stress interactions occur, current evidence to support these hypotheses, and what they mean for the evolution of small populations and conservation of biodiversity. We also make numerous suggestions concerning where future research in this field should be directed.

Why does inbreeding depression increase with stress?

A number of hypotheses have been proposed to explain the mechanisms by which stress can amplify levels of inbreeding depression.[2,38] In general, inbreeding-stress interactions can be viewed as resulting from (1) the effects of exposure to stress on the expression of deleterious alleles (focus is at the genetic level) and/or (2) the phenotypic effects caused by the expression of genetic load that affects resistance to stress (focus is at the phenotypic level). Here, we outline three major hypotheses proposed to explain inbreeding-stress interactions as well as current evidence to support each. It is important to note that these hypotheses are nonmutually exclusive and the relative contribution of each to inbreeding-stress interactions is currently unknown and should be the subject of future research. Moreover, because it is ultimately the expression of genetic load that will lead to the physiological and phenotypic changes that reduce the fitness of inbred individuals, the hypotheses presented are not entirely distinct (see discussion below) but have been organized according to whether the focus is the genetic (hypothesis 1) or phenotypic level (hypotheses 2 and 3).

Hypothesis 1: ***Exposure to stressful environments alters the genetic architecture underlying inbreeding depression (i.e., the expression of genetic load).***

The level of inbreeding depression is dependent, at least in part, on the expression of recessive deleterious alleles (genetic load)—specifically, the overall number of deleterious alleles expressed and the relative fitness effect of each expressed recessive allele.[2,35,38,39] Exposure to stressful environments can therefore lead to increased levels of inbreeding depression by affecting the expression of genetic load in two ways: (1) increasing fitness costs associated with particular deleterious alleles and/or (2) increasing the number of deleterious alleles expressed relative to those expressed in benign environments.

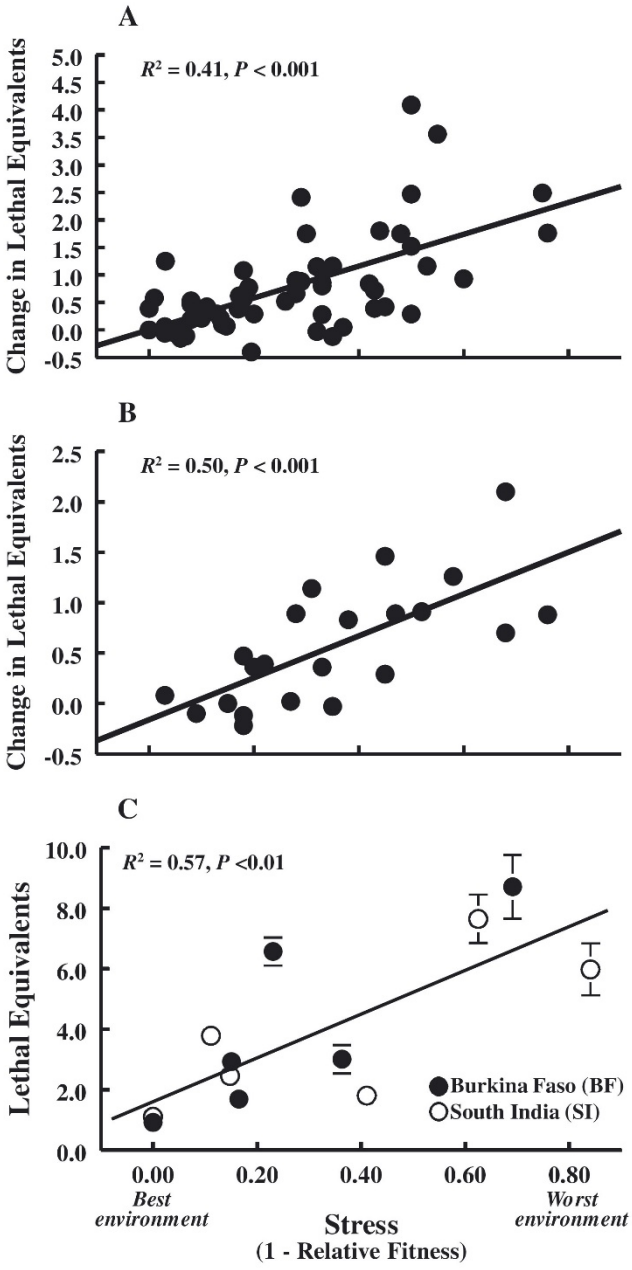

Figure 2. The relationship between inbreeding load (L) or L_{diff} (the difference in the number of lethal equivalents expressed in the stressful vs. benign environment) and the magnitude of stress in (A) a meta-analysis of published studies,[6] (B) a meta-analysis of *Drosophila* laboratory studies,[37] and (C) an experimental study of the beetle *Callosobruchus maculatus* reared at three temperatures on two host species.[6] Stress is calculated as 1 – $Survival_{outbred(stressful)}$/$Survival_{outbred(benign)}$, and thus is by definition 0 in the most benign environment.

Inbreeding-stress interactions can occur when stress magnifies the average negative effect of deleterious recessive alleles, leading to increases in the selection coefficients against these alleles in stressful environments.[40–43] Environmental differences in natural selection (i.e., environment-dependent selection) are recognized as important sources of inbreeding-stress interactions, distinct from those mechanisms that contribute to environment-dependent phenotypic expression.[2] It is not necessary to assume genotype-by-environment interactions when changes in the intensity of selection against deleterious alleles contribute to increased levels of inbreeding depression, simply because the mortality of inbred individuals relative to outbred individuals increases as selection increases.[38]

Numerous studies demonstrate that different loci often affect the same trait in different environments (e.g., in QTL studies), and that the degree to which specific loci affect a trait varies with environmental conditions.[44,45] For such changes in effects of individual loci to generate an increase in inbreeding depression under stressful conditions requires that deleterious mutations—those that are generally recessive and thus exposed to selection by increased inbreeding—be, on average, disproportionately affected by stressful conditions. This is, however, inconsistent with the results of some models that predict that environmental conditions primarily change the variance of mutational fitness effects rather than their average effect or their net expression level. Stress should affect which alleles are expressed, and the variance in effect size among alleles, but not the average effect size of deleterious alleles, and thus not the average effect of the genetic load.[12]

Inbreeding-stress interactions may also result from specific genotype-by-environment interactions that arise through the expression of condition-dependent deleterious alleles that are neutral or beneficial under benign environments but become deleterious under stress.[35,38] This explanation is distinct from the above hypothesis (where inbreeding-stress interactions magnify the average negative effect of deleterious recessive alleles) in that alleles that are neutral or beneficial in benign environments become detrimental under stressful environmental conditions; that is, there is a change in the sign of the selection coefficient on these recessive alleles. For example, Vermeulen and Bijlsma[46,47] demonstrated temperature-specific adult mortality in inbred *Drosophila melanogaster* lines caused by the expression of temperature-sensitive lethal alleles, alleles that were neutral or even beneficial at some temperatures but lethal at other temperatures. Condition-dependent deleterious alleles can be maintained in a population when purging is ineffective due to the infrequency with which organisms encounter stressful conditions, such as novel or particularly extreme conditions that may be typically avoided due to habitat selection. Environmental-dependent deleterious alleles may contribute to the significant lineage effects observed under stress in many studies,[5,46,47] explaining in part why independent but equally inbred lines can behave very differently under stress. However, some evidence indicates that the effects of new mutations are highly and positively correlated across environmental conditions[48–52] (but see Refs. 53–55), and that inbreeding depression of genotypes is generally positively correlated across environmental conditions (e.g., Ref. 56 and references therein), suggesting that increased expression of condition-dependent deleterious alleles may not be the only mechanism causing an increase in inbreeding depression with stress. This is, however, in contrast to the finding of condition-dependent deleterious alleles being very important for explaining levels of inbreeding depression in lifespan and

thermal tolerance in *D. melanogaster*.[46,47] Mutations segregating within populations appear to be far less positively correlated in their effects across environments,[5,57,58] as would be expected since selection against mutations that are deleterious in all environments should remove such mutations rapidly. Furthermore, Hillenmeyer et al.[59] found that 97% of genes that are required for optimal growth were environment-specific in yeast. In summary, there is support for the hypothesis that conditionally expressed recessive deleterious alleles partly explain inbreeding-environment interactions.

Hypothesis 2: ***The expression of genetic load increases the sensitivity of inbred individuals to the physiological effects of environmental stress.***

The expression of deleterious alleles is predicted to render inbred individuals more susceptible to the effects of environmental stress by causing overall physiological weakening and thus greater fitness costs relative to outbred individuals.[23,60,61] Increased sensitivity can result from changes in the expression of genetic load (as described in hypothesis 1) that adversely affect basic cellular functioning and ultimately influence an individual's phenotype and overall fitness. In general, disruption of the stress response system caused by the expression of genetic load is predicted to reduce or eliminate the ability of organisms to buffer their physiology and repair or reduce tissue and genomic damage experienced during exposure to stress. A growing body of literature suggests that deleterious mutations commonly decanalize the phenotype against random environmental perturbations, and thus increase the sensitivity of most traits to environmental perturbations.[52] The hypothesis that deleterious mutations decanalize the phenotype against environmental stress is indirectly supported by observations that inbreeding depression often increases with age[60,62] (but see Ref. 63). Assuming that inbreeding depression is caused by recessive deleterious alleles and that natural selection acts more weakly against late-acting deleterious alleles (such that the expression of deleterious mutations increases with age, as predicted by mutation accumulation models of senescence), then inbreeding depression should increase with age. In this case, deleterious recessive alleles are expressed and thus decanalize the phenotype only at old age. Thus, inbreeding-age interactions share characteristics with inbreeding-environment interactions where the deleterious effect of certain recessive alleles is observed only under harsh environmental conditions. In vertebrates (and to a lesser degree other groups), inbreeding also directly increases expression of mutations that disrupt the generalized immune and stress response system. This has given rise to the hypothesis that inbreeding reduces an individual's ability to resist parasites and pathogens.[39]

Another proposed explanation for increased sensitivity of inbred individuals to stressful environments is that inbreeding hinders adaptive phenotypic plasticity.[38] Plasticity can be defined as the ability of a genotype to produce varied phenotypic outcomes depending on the environment. If plastic responses provide a short-term and partly "emergency" solution to cope with sudden changes in the environment, then a reduced ability of more homozygous individuals to exhibit plasticity in response to changes in the environment, especially if this occurs at the physiological level, may provide a general explanation for why environmental conditions that are harmless to outbred individuals could be perceived as highly stressful by inbred individuals. This hypothesis is supported by work in *Drosophila* demonstrating that inbreeding can reduce the capacity to maintain high fitness across

environments[64,65] as well as recent data showing that inbreeding reduces the expression of predator-induced adaptive plasticity in shell thickness in a hermaphroditic snail species (*Physa acuta*).[66] However, studies across plants and animals examining the effect of inbreeding on plastic responses have shown varied results.[67] In general, a better understanding of the ability to react to environmental changes via adaptive phenotypic plasticity in small and fragmented natural populations exposed to ecologically relevant environmental variation is needed to verify the generality of the "inbreeding depression for plasticity" hypothesis. Future work is needed to determine how sensitive inbred populations in nature are to the effects of stress and to what extent inbreeding may impede the ability of such populations to cope with environmental change via plasticity and/or evolutionary adaptation.

Hypothesis 3: ***Inbreeding depression under stress is the consequence of increased phenotypic variation.***

It has been proposed that inbreeding depression itself is a form of selection and therefore predicted to increase under stressful conditions that accentuate phenotypic variance.[61] The amount of phenotypic variance in a population sets a limit on the degree to which the fitness of distinct groups of individuals can differ. Crow[68] showed this for natural selection by demonstrating that the index of total selection (CV^2, the squared phenotypic coefficient of variation) sets a limit to how much selection can occur in a population (though Downhower et al.[69] caution that this index can be misleading when CV^2 and the mean are not independent of each other). Waller et al.[61] pointed out that CV^2 also constrains the magnitude of inbreeding depression that can occur in a population. If the amount of phenotypic variation present increases with the stressfulness of the environment, then the opportunity for fitness to differ between inbred and outbred individuals similarly increases, and so we might expect inbreeding depression to covary with the degree of stress. Thus, an increase in phenotypic variance with stress (Waller's hypothesis) is a mechanism by which stress can increase inbreeding depression; stress often increases phenotypic variation, and thus the slope of the relationship between stress and inbreeding depression, which is constrained by the relationship between stress and phenotypic variation, will increase with stress.

There are several mechanisms that could contribute to increased phenotypic variation under stressful conditions. As previously discussed, exposure to stress can decanalize growth and development, which has been shown to reveal cryptic genetic variation and give rise to the appearance of new phenotypes.[70,71] In *Drosophila* (flies) and *Danio* (zebra fishes), a reduced ability to buffer against the cellular effects of stress have been shown to cause increased morphological asymmetries and even lead to changes in the frequencies of novel phenotypes in laboratory populations.[72,73] Stressful conditions may therefore alter the expression of genetic load by revealing underlying mutations that are otherwise hidden by normal physiological buffering, thus increasing the variance in fitness of both inbred and outbred individuals. Increased phenotypic variation could also result from the effects of stress on the regulation of gene expression, for example, by increasing transcriptional errors and introducing noise in expression.[74] There is some evidence suggesting that gene expression is more variable when individuals are exposed to stress and that stress-

related genes exhibit high levels of noise relative to housekeeping genes,[75] increasing phenotypic variation in the population. However, it remains unclear if inbred individuals are more susceptible to the effects of stress on phenotypic variation and whether this may influence levels of inbreeding depression.

Evaluating the role of phenotypic variance in inbreeding–stress interactions

In general, it is unknown to what extent increased phenotypic variation under stress contributes to inbreeding-stress interactions. Waller and colleagues[61] found that CV^2 was a poor predictor of inbreeding depression for a given trait across abiotic and biotic stress treatments, but that levels of inbreeding depression were positively correlated with levels of phenotypic variability (CV^2) when considered across nine fitness-related traits measured in *Brassica rapa*. However, this study did not evaluate the role of stress level in the expression of inbreeding depression, which may explain why inbreeding depression was found to be positive, negative, or zero depending on the trait and stressor applied. Currently, there are no studies examining the role of both phenotypic variation and stress levels in determining the outcome of inbreeding-stress interactions. To test the relative importance of increasing phenotypic variance in generating observed inbreeding-stress interactions, we performed a multiple regression analysis on nine data sets (Tables 1 and S1). For each data set, the dependent variable is the number of lethal equivalents for each inbred line in each of the environments, which differed in stress levels. The independent variables are CV^2, degree of stress (decrement in relative fitness of the outbred population in each environment), and the interaction between the two.

Table 1. The relative importance of stress,CV^2, and their interaction, in explaining variation in the inbreeding load across environments.

Variable	Importance value (weight)
CV^2	0.630
Stress	0.862
CV^2∗ stress	0.226

Note: See Table S1 for details of the nine analyses that were included using model averaging to estimate importance values.

We found that that stress increased CV^2 in eight of nine data sets, but the correlation between the degree of stress and the increase in CV^2 was weak (mean correlation coefficient, $r = 0.34 \pm 0.12$) for all except one study.[6] The best-fit multiple regression model consistently explained significant amounts of the variation among inbred lines in their number of lethal equivalents ($P < 0.01$ in all cases, mean $R^2 = 0.53 \pm 0.03$). We then used an information-theoretic approach to select the best-fit model (Table S1) and used model averaging to weight the relative importance of CV^2, stress, and their interaction in determining inbreeding depression (Table 1).

The model including an effect of stress, but not CV^2, was the best-fit model in four of nine data sets, whereas the model including just CV^2 alone was the best fit in only one data set (Table S1). In the remaining data sets, both CV^2 and stress were important; for two data

sets the best-fit model included stress and CV^2 and in two others the best-fit model included CV^2, stress, and the interaction between them. Stress effects independent of increases in CV^2 were the single most important variable, across models, determining the level of inbreeding depression (Table 1). However, CV^2 was similar in importance to stress level. Thus, the consensus strongly suggests that stress often increases inbreeding depression by increasing CV^2, but that the increase in CV^2 explains only part of the variance in inbreeding depression; there are also other independent mechanisms by which stress increases inbreeding depression. The interaction between CV^2 and the independent effects of stress is clearly not as important as the main effects (Table 1). However, the interaction term seems important in three of the nine data sets and is consistently negative.

These analyses were performed on a very limited subset of published data and on only a few study species. They are projects that included at least one of the authors of this paper as a coauthor and to which we had unfettered access to the data. It is worth noting, however, that there are no consistent patterns among authors or organisms. For two species of spiders within the same genus, stress alone was the best-fit model for one species and the worst-fit model for the other species. One study, using a single population of *D. melanogaster*, found very different results depending on whether fecundity or egg-to-adult survival was used as a fitness surrogate. Thus, we expect these results to be fairly general.[76]

These findings differ from those of Waller et al.[61] where mixed support for CV^2 and no support for independent effects of stress were found. Differences in results may be due to the strength of the stress used in the studies and the amount of inbreeding depression the populations actually experience.

The physiological basis of inbreeding depression and inbreeding-environment interactions

Inbreeding itself can mimic environmental stress at the cellular level. Kristensen et al.[77] and Pedersen et al.[78] found increased expression levels of the stress-induced heat shock protein 70 in replicate inbred lines as compared with outbred lines of *D. melanogaster* and *D. buzzatii*. An increase in levels of heat shock proteins in inbred individuals may be a general phenomenon that is involved in buffering the effects of deleterious mutations on protein instability and misfolding; inbreeding increases expression of deleterious alleles that reduce protein stability and increase protein misfolding, which, in turn, induces upregulation of heat shock proteins.[79–81]

Consistent with the results showing upregulation of heat shock proteins in inbred lines, it has been found in full genome transcriptomics studies that inbreeding leaves a directional fingerprint on gene regulation across lineages of *D. melanogaster*.[82,83] Genes that respond transcriptionally to inbreeding are primarily involved in stress resistance, immunity, and fundamental metabolic processes. The transcriptomic analyses of inbred lines show that although the genetic causation of inbreeding depression is unique for every population, a general response can be identified that is likely to be explained by stress mechanisms being induced by inbreeding and not due to disruption of specific gene products (which would be lineage specific). This view is supported by metabolite profiling, which also reveals a clear separation of inbred and outbred lines.[84,85] In summary, the available

data from transcriptomic and metabolomic investigations of inbreeding effects demonstrate that inbreeding imposes physiological changes, as expected, given the clear reduction in fitness often observed in response to inbreeding. More unexpectedly, the data show that expression of the genetic load induces directional molecular responses, such as differential expression of major metabolic pathways and protein quality control systems that may counteract the deleterious effects of inbreeding. Most notable for our understanding of inbreeding-stress interactions is that many of the genes whose transcription responds to inbreeding are those involved in a variety of stress responses, including heat shock proteins and genes involved in immune processes, indicating that physiologically organisms respond to inbreeding as if they are being exposed to multisimultaneous environmental stressors.

Genome-wide transcriptome studies have also been used to describe how inbreeding-environment interactions manifest at the biochemical and physiological levels.[81] Kristensen et al.[81] showed that more genes were differentially expressed with inbreeding in *D. melanogaster* after exposure to temperature stress relative to benign conditions, signifying inbreeding-environment interactions. Transcripts involved in major metabolic pathways, in particular, were affected by the interaction. Thus, the sparse documentation of inbreeding-environment interactions on the transcript level suggests that at this molecular level inbreeding and the environment do not influence organisms additively.

Future perspectives using omics tools

The ability to investigate molecular phenotypes using omics technologies has been influential in expanding our knowledge about the effects of inbreeding and inbreeding-environment interactions. Nevertheless, the underlying molecular and biochemical mechanistic details of inbreeding effects are still unclear and there is a need for more hypothesis-driven investigations (e.g., using genetically modified organisms) in which the roles of specific genes, transcripts, proteins, and metabolites in inbred and outbred individuals are tested at different environmental conditions. Results of studies at the transcript level should be followed up by mechanistic studies that pinpoint the importance of candidate genes and biochemical pathways for explaining inbreeding-environment interactions.

Genomic tools enabling the establishment of complete genome sequences, not only for model organisms but also for species of conservation interest, will enable researchers to perform genotyping at low cost for thousands of single-nucleotide polymorphism (SNP) markers.[86] Information on genome-wide SNPs can, for example, be useful in pinpointing the genetic basis of variation in inbreeding effects across environments, species, populations, and families. Potentially, information from genomic studies revealing recessive deleterious alleles of importance for inbreeding-environment interactions can be used to control recessive defects in captive populations by using this molecular information to select parents for the next generation.

Genomic information is currently being used intensively to guide selection decisions in animal and plant breeding, as it is expected that this will lead to faster rates of genetic improvement than does the use of traditional methods.[87] For example, methods are being developed that allow estimation of the level of inbreeding based on genomic information

(personal communication, Louise Dybdahl Pedersen). This will enable a much more accurate estimate of inbreeding compared to estimates obtained based on pedigree information. Genome-wide SNP genotyping can therefore be used to precisely monitor and efficiently control the rate of inbreeding[88] in domesticated or managed wild populations. Genomic tools have the potential to allow control of inbreeding rates and heterozygosity at loci of crucial importance for fitness, which will allow fixation of favorable alleles in traits of importance for fitness while maintaining genetic variation in other parts of the genome. However, this field is in its infancy and the method described is obviously only of practical use in domesticated animals, zoo populations, plants in botanical garden, or otherwise heavily managed populations. Furthermore, for it to be efficient in relation to minimizing detrimental effects of inbreeding-environment interactions, genes/SNPs that govern inbreeding depression across environmental conditions should be identified.

Genomic approaches can potentially also be used to address basic questions about the molecular basis and genetic architecture of inbreeding depression.[89] For instance, is inbreeding depression caused by a few or many loci? And, how much of the inbreeding depression results from dominance, overdominance, or epistasis? Such knowledge is important for predicting the potential efficacy of purging, genomic selection, and assisted migration between populations.[38,86,90] If inbreeding depression is covered by a few loci of large effect,[91] and if inbreeding depression in benign and stressful environments is covered by some of the same genes, genomic selection might be effective in purging the genetic load.

Despite the fascinating prospect of employing genomic technologies in research to identify mechanisms responsible for inbreeding depression and the environmental dependency of inbreeding depression, it is also important to keep in mind challenges and limitations. First, the genetic architecture of inbreeding depression and inbreeding-environment interactions is likely complex and varies among species populations and individuals within populations.[38,92] Second, loci of importance for inbreeding depression will probably not be the same across environments.[5,57,58] Therefore, for genomic selection to be efficient in populations kept in zoos, botanical gardens, or in semicaptive environments, management practices should be developed that minimize adaptation to captivity and resample environmental conditions that the populations are likely to experience if translocated back to nature.[93] Third, threatened inbred populations will be small by definition. This will reduce power and thereby accuracy of the results and reduce the potential to select effectively against recessive deleterious alleles. Fourth, today only a few species have genomes that have been sequenced, and reference genomes are available for an even smaller number of species. This means that for almost all species of conservation concern, we are still far from being able to do what we have suggested above. However, this is likely to change within the next 10 years with further developments in molecular biology, noninvasive sampling methods, and in bioinformatics.

The importance of inbreeding-stress interactions for conservation and evolutionary biology

We have defined a stressor as any environmental factor that reduces the fitness of an individual or population.[4–7,12] Populations in nature are constantly exposed to various forms of

stress, such as pathogens and parasites, hunger and thirst, extreme heat or cold, toxic substances, and the risk of predation. Stress is likely particularly high in organisms of conservation interest because of anthropogenic activities that create novel or suboptimal conditions (e.g., global climate change, introduced species, pathogens, and pollution). For example, a growing body of literature demonstrates that individuals in fragmented or poor-quality habitats,[94,95] and those exposed to novel predators[96] or parasites,[97] express higher levels of stress hormones, indicating that they experience greater levels of physiological stress. Environmental stressors can induce physiological stress, such as changes in hormone levels, which can in turn lead to increased susceptibility to disease and predation, and/or generally reduce fitness.

Consequences of inbreeding-stress interactions for small populations

As environments continue to rapidly change worldwide, populations are not only subjected to progressively higher levels of stress in the form of industrial pollution, pesticides, and changes in ambient temperatures but are also becoming increasingly smaller, more fragmented, and less genetically diverse. The increased risk of extinction due to the negative impacts of random genetic drift and inbreeding on disease resistance, evolutionary potential, and overall fitness are well established;[3,98–104] genetically depauperate populations have lower fitness, lowered disease resistance, and less evolutionary potential.[102,105] However, there is added risk for small populations when the deleterious effects of stress are amplified in inbred individuals. Simultaneous increases in stress and inbreeding rates and levels are thus expected to rapidly ratchet up extinction rates.[1,10,106] Extinction risk is going to be determined primarily by the extreme downturns in population size[102,107] and these will become more extreme than predicted by Liao and Reed[10] under the assumption that the interaction becomes stronger as stress becomes greater.[6,37]

Liao and Reed[10] determined that including reasonable estimates of the inbreeding-environment interaction reduces persistence times by 17.5–28.5% for a wide range of realistic assumptions about population dynamics and genetics and Robert[11] concluded that unbiased assessments of the viabilities of species is only obtained by identifying and integrating the most important processes governing persistence times (i.e., demography and genetics). Liao and Reed[10] also identified some counterintuitive patterns; for example, the influence of the inbreeding-stress interaction on the median time to population extinction was greatest for larger populations. This is because populations currently viewed as relatively safe from extinction can more quickly cross the threshold into the extinction vortex when large inbreeding-stress interactions occur. Of course, this does not mean that inbreeding-environment interactions are insignificant for small populations. In contrast, although the proportional effect of the inbreeding-environment interaction may be less for small populations, such populations are already in crisis, and already experiencing the inbreeding conditions for which the interaction is important. The consequences of the interaction in increasing the risk of extinction are thus more imminent for smaller populations. Consideration of inbreeding-environment interactions in models of population persistence and conservation efforts should therefore be a priority.

Despite evidence from simulation studies and studies on organisms in the laboratory, it still remains to be shown in nature whether inbreeding-stress interactions do speed up

extinction rates to the degree predicted based on studies that do not take into account all specific genetic details, such as the specifics of selection (purging, and balancing and directional selection). In addition, inbreeding is known to have multigenerational effects on fitness, and the same is certainly true for some types of stress,[108] but it is unclear whether the effects of inbreeding-stress interactions persist across generations. Low reproductive values persisting beyond the period of actual stress could prolong population recovery and increase the probability of entering an extinction vortex. Future studies are needed that examine the role of inbreeding-stress interactions under natural conditions, particularly in small and fragmented populations, with focus on the potential for multigenerational effects. Most laboratory studies can be criticized for not being ecologically relevant as they often investigate rather extreme levels of inbreeding and only one stressor (but see Ref. 6). This is problematic as the importance of inbreeding-stress interactions are depending on the level of inbreeding and expected to be more severe with exposure to multiple stresses that can interact in their effect on the phenotype.[109–112] We have only limited knowledge on such inbreeding-stress interactions, and future studies should also focus on natural populations or in laboratory studies investigating multiple environmental stresses.

Relevance of inbreeding-stress interactions for purging genetic load

Although environmental stress is commonly viewed as increasing inbreeding depression,[5,61] stress has also been proposed to increase selection against recessive deleterious alleles expressed in homozygous individuals, thus purging genetic load.[35,113] Exposure to stress over multiple generations is predicted to reduce inbreeding depression by decreasing the frequency of deleterious alleles in the population over multiple generations,[114–116] but can also have an effect within generations (intragenerational) if fitness correlations exist across multiple life history stages.[60] Purging of genetic load has been heavily studied,[98] yet we still have little idea whether the effects of purging are general versus environment specific or if different type of stress vary in their ability to purge genetic load.[35,117]

Inbreeding-stress interactions could, in theory, lead to very rapid purging of the deleterious alleles responsible for such interactions. However, specific stresses can increase, decrease, or have no effect on the magnitude of selection against mutations,[118] thus contributing to differences in the degree of purging across stress types. In addition, understanding the contribution of stress-specific versus stress-general genes or pathways to inbreeding-stress interactions is imperative to understanding the dynamics of purging in natural populations. The genomics work cited above for *Drosophila* suggests that many of the deleterious alleles affecting inbreeding depression do so through genes affecting generalized stress responses, but we have far too little data to generalize. The answer will have particularly significant consequences for our ability to extrapolate from results of laboratory studies to nature, and for predicting responses of populations bred and studied in captivity that are intended for reintroduction into natural, and generally more stressful, conditions. For example, we might predict that the consequences of inbreeding depression will be greatest in novel environmental conditions—those to which the organism is not adapted and in which they have not had an opportunity to purge their genetic load. Limiting adaptation to the captive environment, such as for *ex situ* populations intended for reintroduction, may warrant explicit attempts to limit inbreeding-stress interactions.

Future inbreeding-stress research

Much remains to be understood about the impacts of inbreeding-stress interactions for biodiversity conservation. There is still much unexplained variation in the magnitude of inbreeding depression expressed under stressful conditions, suggesting that additional factors may be important in explaining how stress and inbreeding interact in populations of conservation interest.[5,6,37,61] Identifying the types of stress that are more or less likely to induce such interactions will have direct application to species management. Identifying categories of stressors that do or do not trigger inbreeding-stress interactions may also help us to understand the genomic and proteomic underpinnings of such interactions. It is thus particularly important that more research be done on the effects of inbreeding and environmental stress in wild populations. Laboratory experiments can only go so far in mimicking the complex variety of stressors and stress levels faced by organisms and it is also important to impose realistic levels of inbreeding. How inbreeding-stress interactions affect population dynamics has rarely been studied in natural populations. Few studies have looked at temporal variation in levels of inbreeding depression in the wild[119–125] and only one has correlated seasonal changes in inbreeding depression with concurrent changes in levels of stress.[37] Studies on natural populations, in the field, are therefore crucial for extrapolating from the wide diversity of studies on model laboratory systems to natural systems of conservation importance.

Among the more important aspects of natural environments that we poorly understand is the frequency and magnitude of various stressors. Stress can come in the form of fluctuations in temperature, humidity, food availability, mating opportunities, and risk of predation. However, the extent to which stressors, such as these contribute to inbreeding-stress interactions is relatively unexplored in natural populations. In addition, it is unknown to what degree various stressors might be similar in plants, invertebrate animals, and vertebrate animals. If common stressors can be identified, it will allow us to examine whether negative genetic correlations generally exist between them. Negative genetic correlations to different stressors can severely limit evolutionary potential and curtail population growth.[44,126–128] Genetic correlations for resistance to commonly encountered stressors with moderately strong selection should be mostly or entirely positive, as selection should strongly favor mutations with positive effects across several stressors. This will be particularly the case if a small set of generalized stress responses mediates fitness across a range of most commonly encountered stressors. However, many things might limit or prevent these positive correlations from evolving. Populations may be too small to generate and effectively fix such mutations, there may be physiological reasons for the negative genetic correlation, or there may be a negative temporal correlation between heritability for a trait and the strength of selection against that trait.[129] Under these conditions, inbreeding-stress interactions will likely lead to inefficient purging of the genetic load even in the environment the purging occurred in and lead to rapid fixation of potentially deleterious alleles for other forms of stress.

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Supporting Information

Table S1. Comparisons of AIC_c values for statistical models testing whether stress or CV^2 is a better predictor of the inbreeding load for multiple species studied by the authors. Models are ranked from the best (lowest AIC_c) to worst (highest AIC_c). Δ_i is the difference between the best-fit model and the model being compared to the best. w_i is the weight or likelihood of a model relative to other models.

Callosobruchus maculatus (Fox & Reed 2011); manipulation of temperature and host plant.

Model[1]	**AIC_c**	**Δ_i**	**w_i**
Stress	42.8	0.0	0.998
CV^2 + Stress	55.5	12.7	0.002
CV^2	62.8	20.0	0.000
CV^2 + Stress + Interaction	64.6	21.8	0.000

$R^2 = 0.64$; $r_{CV,S} = 0.92$

1. Model includes two populations of beetles, with population treated as a fixed effect in analysis of covariance

Drosophila melanogaster (Kristensen et al. 2003); manipulation of temperature and chemical stress.

Model	**AIC_c**	**Δ_i**	**w_i**
CV^2	–21.3	0.0	0.661
CV^2 + Stress	–19.3	2.05	0.238
CV^2 + Stress + Interaction	–17.5	3.81	0.099
Stress	–10.2	11.16	0.002

$R^2 = 0.46$; $r_{CV,S} = 0.24$

Drosophila melanogaster (Kristensen et al. 2011) fecundity, manipulation of temperature.

Model	**AIC_c**	**Δ_i**	**w_i**
Stress	88.9	0.00	0.807
CV^2	84.6	4.35	0.092
CV^2 + Stress + Interaction	84.1	4.80	0.073
CV^2 + Stress	82.2	6.70	0.028

$R^2 = 0.41$; $r_{CV,S} = 0.55$

Drosophila melanogaster (Kristensen et al. 2011); egg-to-adult survival, manipulation of temperature.

Model	**AIC_c**	**Δ_i**	**w_i**
CV^2 + Stress + Interaction	–49.7	0.00	0.563
CV^2 + Stress	–48.6	1.11	0.324
Stress	–45.8	3.96	0.078
CV^2	–44.2	5.54	0.035

$R^2 = 0.48$; $r_{CV,S} = 0.41$

Drosophila melanogaster (Reed et al. 2003); manipulation of chemical stressors.

Model	**AIC_c**	**Δ_i**	**w_i**
Stress	–1667.3	0.00	0.620
CV^2 + Stress	–1665.0	2.27	0.199
CV^2 + Stress + Interaction	–1664.8	2.47	0.180
CV^2	–1653.5	13.76	0.001

$R^2 = 0.49$; $r_{CV,S} = 0.38$

Drosophila melanogaster (Kristensen et al. 2008); manipulation of temperature.

Model	**AIC_c**	**Δ_i**	**w_i**
CV^2 + Stress + Interaction	55.7	0.00	0.461
CV^2	56.0	0.31	0.395
CV^2 + Stress	58.1	3.34	0.144
Stress	94.7	38.99	0.000

$R^2 = 0.68$; $r_{CV,S} = 0.33$

Musca domestica (Reed & Bryant 2000, 2001); manipulation of diet and temperature.

Model	**AIC_c**	**Δ_i**	**w_i**
CV^2 + Stress	–40.0	0.00	0.587
CV^2 + Stress + Interaction	–39.0	0.97	0.361
Stress	–35.0	5.00	0.048
CV^2	–30.0	9.93	0.004

$R^2 = 0.51$; $r_{CV,S} = 0.28$

Rabidosa punctulata (Reed et al. 2007 and Reed & Nicholas 2008); natural environmental variation.

Model	**AIC_c**	**Δ_i**	**w_i**
CV^2 + Stress	13.7	0.00	0.763
CV^2 + Stress + Interaction	17.0	3.30	0.147
CV^2	18.9	5.23	0.055
Stress	19.9	6.19	0.035

$R^2 = 0.58$; $r_{CV,S} = 0.36$

Rabidosa rabida (Reed et al. 2007 and Reed & Nicholas 2008); natural environmental variation.

Model	**AIC_c**	**Δ_i**	**w_i**
Stress	5.8	0.00	0.744
CV^2 + Stress + Interaction	9.0	3.25	0.147
CV^2 + Stress	9.6	3.88	0.107
CV^2	17.2	11.43	0.002

$R^2 = 0.51$; $r_{CV,S} = -0.42$