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Inbreeding effects in wild populations

Lukas F. Keller and Donald M. Waller

Whether inbreeding affects the demography and persistence of natural populations has been questioned. However, new pedigree data from field populations and molecular and analytical tools for tracing patterns of relationship and inbreeding have now enhanced our ability to detect inbreeding depression within and among wild populations. This work reveals that levels of inbreeding depression vary across taxa, populations and environments, but are usually substantial enough to affect both individual and population performance. Data from bird and mammal populations suggest that inbreeding depression often significantly affects birth weight, survival, reproduction and resistance to disease, predation and environmental stress. Plant studies, based mostly on comparing populations that differ in size or levels of genetic variation, also reveal significant inbreeding effects on seed set, germination, survival and resistance to stress. Data from butterflies, birds and plants demonstrate that populations with reduced genetic diversity often experience reduced growth and increased extinction rates. Crosses between such populations often result in heterosis. Such a genetic rescue effect might reflect the masking of fixed deleterious mutations. Thus, it might be necessary to retain gene flow among increasingly fragmented habitat patches to sustain populations that are sensitive to inbreeding.

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Small and isolated populations are inherently more vulnerable to external environmental perturbations and chance fluctuations in local survival and fecundity [termed 'ENVIRONMENTAL STOCHASTICITY' and 'DEMOGRAPHIC STOCHASTICITY', respectively (see Glossary)]. Are they also threatened by inbreeding and the loss of genetic diversity? With data on the effects of inbreeding in wild populations scarce, some zoologists questioned its importance (or even its existence) in natural populations [1–3]. Recent evidence, however, now suggests that both inbreeding and inbreeding depression are more pervasive than previously realized. In addition, some studies point to inbreeding as an important threat that can directly affect population persistence, making it a key concern for conservation biologists. Here, we review this recent work, including studies of both plant and animal populations that exploit a range of traditional and molecular genetic techniques. Although we emphasize the empirical results, we also present the background theory needed to evaluate critically these approaches and place the results into context.

What genetic threats exist?

If populations remain small and isolated for many generations, they face two genetic threats. As alleles are randomly fixed or lost from the population by drift, levels of quantitative genetic variation necessary for adaptive evolution erode [4]. Simultaneously, deleterious mutations will tend to accumulate, because selection is less effective in small populations [5]. This could eventually lead to a 'mutational meltdown' for populations with an effective size (N_e) of <100. Both processes tend to be gradual, however, and thus do not threaten populations in the short term. By contrast, inbreeding can act swiftly. By restricting opportunities for mating, small populations foster inbreeding via mating among relatives. Simultaneously, small populations tend to fix an appreciable fraction of the GENETIC LOAD by drift, resulting in among-population inbreeding. Both forms of inbreeding increase the frequency of individuals that are homozygous for alleles identical by descent (Box 1). Such inbreeding results in the reduction in fitness that we term inbreeding depression. Although any of these genetic mechanisms could threaten population persistence, inbreeding poses the most immediate risk.

When should we expect inbreeding depression?

In spite of its prevalence, some researchers question whether inbreeding depression is important in wild populations, invoking two main arguments. First, if the many inbreeding avoidance mechanisms evident

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Box 1. What does 'inbreeding' mean?

'Inbreeding' is used to describe various related phenomena [a] that all refer to situations in which matings occur among relatives and to an increase in homozygosity associated with such matings. However, they differ in the reference population that is used when calculating inbreeding. Inbreeding is always a relative never an absolute measure [b]. Therefore, inbreeding estimates differ depending on the reference population to which they refer. It is this relativity that is responsible for the different meanings of the term 'inbreeding', and for some of the misunderstanding that have resulted [a]. There is a historical precedence for such misunderstandings: R.A. Fisher [c] never accepted Wright's inbreeding coefficients because they are relative.

Although the differences might appear trivial at first, it is important to be explicit about the definition of inbreeding used in a study, because the biological consequences of the various kinds of inbreeding can be quite different [d]. This is particularly true in small populations that are often the focus of inbreeding studies. We describe here three of the most commonly used definitions of inbreeding.

(a) Pedigree inbreeding

An individual is considered inbred under this definition when the parents share ancestors (Fig. I). The extent of inbreeding is related to the amount of ancestry that is shared by the parents of an inbred individual. Pedigree information is used to determine the inbreeding coefficient F, relative to the known founders of that population. Although originally derived as a correlation by Wright [e], an alternative definition based on probabilities [f] is now commonly used. F is defined as the probability of two homologous genes in an individual being identical by descent (IBD), meaning that both are derived from the same gene in a common ancestor. An individual with inbreeding coefficient F has a probability F that the two genes at a particular locus are IBD and a probability $1-F$ that they are not IBD. Note that an individual can be homozygous at a locus without the two alleles being IBD. Pedigree F only measures the inbreeding contributed by that pedigree, that is, it is always relative to a particular ancestral generation. Averaged over individuals, these values are equal to Wright's F_{it} [g].

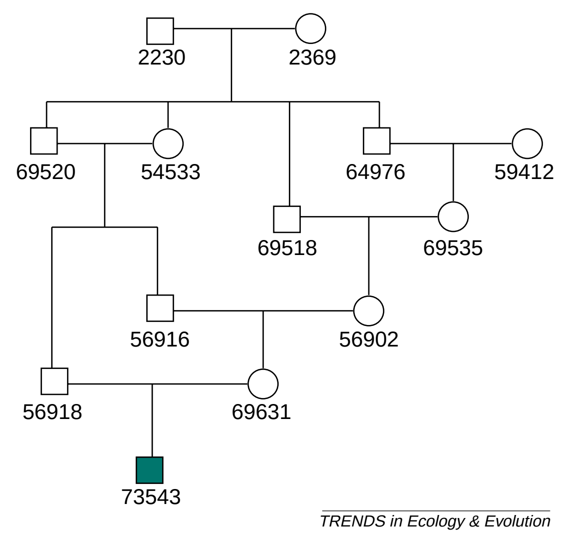

Fig. I. The pedigree of 73543, an inbred (F=0.305) male song sparrow *Melospiza melodia* born on Mandarte Island in 1992. Males are represented as squares, females as circles, illustrating the potential complexity of pedigrees in wild organisms. Note, for example, that 73543's maternal grandfather is also his paternal uncle. Pedigrees such as this allow the calculation of inbreeding coefficients.

(b) Inbreeding as nonrandom mating

This use of the term 'inbreeding' refers to the degree of relatedness between mates relative to two mates chosen at random from the population. An individual is considered inbred if its parents were more closely related than two randomly chosen individuals. This type of inbreeding is relative to a random mating population of the same size. Although it can be determined from pedigree data [g,h], this type of inbreeding is typically measured by F_{is}, the deviation of the observed heterozygosity of an individual relative to the heterozygosity expected under random mating (Hardy–Weinberg equilibrium); that is: $F_{is}=1-H_o/H_e$. $F_{is}>0$ signifies more inbreeding than is expected at random, whereas $F_{is}<0$ indicates that inbreeding occurred less often than would be expected at random. Note that when a population is small, even random mating can lead to matings between relatives. Thus, average pedigree F (F_{it}) can be high, yet F_{is} will be zero [h]. Thus, statements such as 'F_{is} was zero, hence there is no evidence of inbreeding in our data' are only correct under the definition of inbreeding as nonrandom mating given here, not under definitions (a) or (c). Note that a single generation of random mating within a population will return its F_{is} to zero. This is not the case with inbreeding owing to other causes, (a) or (c).

(c) Inbreeding because of population subdivision

When populations are subdivided into more or less isolated groups, inbreeding will also occur purely because population size is restricted and genetic drift results. This occurs even if mating is random within subpopulations [d]. This third definition of inbreeding corresponds to the mean inbreeding coefficient expected in subpopulations under random mating and equals Wright's F_{st}, which measures inbreeding relative to that expected under random mating in the total population. F_{st} can be estimated from pedigree data [g,h] but is usually inferred from genetic data (Box 4). The total inbreeding in a collection of subpopulations, F_{it}, is thus a function of both within- and among-population inbreeding (Eqn I):

$$(1-F_{it})=(1-F_{is})(1-F_{st}) \qquad \text{[Eqn I]}$$

Note that experimental measures of inbreeding effects (Box 5) can measure different types of inbreeding. In addition, there are clearly circumstances where the effects of more than one definition of inbreeding are combined. For example, we might be interested in the inbreeding coefficient of an individual born to full sibs in an isolated population of 50 individuals.

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among species work [6], inbreeding, and thus inbreeding depression, should be rare in nature. Second, populations experiencing bottlenecks or other strong inbreeding should expose deleterious recessive mutations to selection, reducing inbreeding depression [7]. The evidence we review here, however, suggests that many populations cannot avoid inbreeding. In addition, the extent of purging depends on many factors and will often be inefficient (Box 2). Conservation biologists thus accept at least the potential for inbreeding effects and seek empirical data on its magnitude and effects in wild populations.

What causes inbreeding depression?

Although inbreeding does not change the frequency of alleles in a population, it does redistribute the

Box 2. Purging inbreeding depression

Simple population genetics models predict that the increased homozygosity resulting from inbreeding will expose recessive deleterious alleles to natural selection, thereby purging the genetic load [a]. Further inbreeding would then cause little or no reduction in fitness. Because many natural systems are expected to have undergone periods of small population size and therefore inbreeding (definitions (a) or (c) in Box 1), purging is often assumed to have reduced the genetic load in such populations. Conversely, the absence of purging is sometimes taken to support the overdominance mechanism for inbreeding depression. This might be unwarranted, however, as the efficiency and extent of purging depends on many genetic factors and can be quite limited [b,c]. In addition, the efficiency of purging depends on the environment in a manner similar to inbreeding depression [d].

Purging is likely to be most effective under rather restricted genetic and demographic circumstances, namely when:

- The average effect of deleterious mutations is strong relative to the genetically effective population size (N_e) such that $s > 1/2N_e$. Alleles of less selective effect will be effectively invisible to selection and thus subject to drift and fixation.
- Selective interference among loci is not too strong. Strong selection at one locus (as against a lethal or semi-lethal mutation) can interfere with selection against less deleterious alleles at nearby loci via background selection. In addition, small and inbred populations tend to generate and maintain associations among alleles at different loci (the 'Hill–Robertson' effect [e]) that reduce the independence of selection even at unlinked loci.
- Inbreeding occurs gradually and over several generations. If inbreeding is sudden and extreme, N_e is strongly reduced, more random fixation occurs, and the Hill–Robertson effect will be more important.
- The population is sufficiently isolated so that purged deleterious alleles are not reintroduced by immigration.

Highly inbred plant and animal populations must often violate the first three conditions. Evidence from *Drosophila* populations suggests that ~50% of the load is due to mildly deleterious mutations [f], suggesting that population sizes might often be too small to satisfy the first condition. However, because the other half of the load is due to mutations of large effect, partial purging often might be possible [b]. Many animal populations experience gene flow at high enough rates to reintroduce genetic load quickly via immigrants, violating the fourth condition. Even levels of gene flow that are difficult or impossible to detect in most field studies could swamp locally purged subpopulations [g]. Thus, slightly inbred plant populations and isolated animal populations with limited dispersal appear most likely to satisfy the conditions for purging.

Comparisons of inbreeding depression among more and less inbred plant species suggests that purging has some effect, particularly on traits expressed early in development that should be subject to strong selection. However, a meta-analysis of studies comparing related taxa or populations within species reveals that only ~33% provide significant evidence for purging (often for early traits) and that the declines observed are usually only 5–10% [h]. Early reports suggested that inbreeding depression declined in an inbred zoo population of Speke's gazelle, which was interpreted as being a result of purging. Reanalyses, however, suggest that purging might not have occurred [i]. In a review of purging effects in 25 captive mammal populations, Ballou [j] only found significant evidence for purging in one species, although more inbred lines in 15 of 17 species showed slight declines in inbreeding depression in neonatal survival. Even when purging does occur in experimental populations, residual inbreeding depression from mutations of minor effect can remain high [k]. Collectively, these theoretical and empirical results suggest that purging will not substantially reduce inbreeding depression for most field populations threatened by population reductions or habitat fragmentation.

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frequency of genotypes, increasing the proportion of homozygotes and correspondingly decreasing the proportion of heterozygotes (Box 1). Deleterious mutations occur continuously in a population and most are at least partially recessive. Thus, increased homozygosity increases the expression of the genetic load, resulting in inbreeding depression. Alternatively, if a heterozygote is superior to both homozygotes, the reduced frequency of heterozygotes will reduce opportunities to express this overdominance. Some evidence exists to support both mechanisms, although partially recessive deleterious mutations suffice to explain most inbreeding depression observed in *Drosophila* and higher plants [8,9]. Because linked sets of recessive alleles in repulsion mimic overdominance ('associative overdominance' [10]), it is difficult to distinguish the two mechanisms [11]. Overdominance could be important in certain traits, such as sperm precedence in *Drosophila* [9] or in crosses between inbred varieties [12]. With either dominance or overdominance, fitness declines in proportion to increases in *F*, the inbreeding coefficient (Box 3).

Inbreeding depression can be dramatic. Loblolly pine *Pinus taeda*, for example, harbors an average load of at least eight LETHAL EQUIVALENTS (see Glossary) [13], implying an inbreeding depression of 98% in selfed progeny. Ironically, it might be most difficult to detect inbreeding (Box 4) when inbreeding depression is greatest. For example, lethals and sublethals expressed early in development leave only more heterozygous individuals to sample in surviving seeds and thus give low estimates of inbreeding [14]. Organisms with high fecundity and polyembryony (such as pine trees) most easily absorb (and hide) such inbreeding depression.

Inbreeding depression within and among populations

Even severe inbreeding depression will not affect population dynamics unless inbreeding occurs. Such inbreeding happens via two distinct mechanisms:

Box 3. How do we measure inbreeding depression?

In parallel with the different types of inbreeding (Box 1) there are various methods to estimate inbreeding effects within and among populations. Unfortunately, inbreeding depression is often measured in ways that make it difficult to compare across traits, taxa, or levels of inbreeding. However, such comparisons are crucial for obtaining a better understanding of the evolutionary and conservation implications of inbreeding depression. The following two methods allow such comparisons:

(a) Within-population inbreeding depression

Typically, inbreeding depression is measured by comparing the fitnesses of progeny from outcrossed (or randomly bred) individuals to those from inbred crosses (between relatives or from self-fertilization). If mutations at different loci have independent (multiplicative) effects, the logarithm of overall fitness (or a major component, such as viability or fecundity) is expected to decline linearly with increases in the inbreeding coefficient, *F* (Fig. I) [a]. Thus, the slope of this line (–*B*) serves as a convenient measure of the inbreeding load [b]. Because it is generally difficult to distinguish a few mutations of major effect (lethals and semilethals) from many mutations of small effect, the inbreeding load is expressed customarily in terms of the number of lethal equivalents (the number of segregating alleles per gamete, each of which, if made fully homozygous, would be lethal). This load is estimated by *B*.

In self-compatible populations, including many plants and some snails, inbreeding depression is usually estimated by comparing the fitness of self- and cross-fertilized progeny [c]. If a noninbred parent ($F=0$) is used, randomly outcrossed progeny will also be non-inbred ($F=0$) whereas selfed progeny will have $F=0.5$. The depression in fitness of selfed progeny relative to outcrossed progeny can then be defined as (Eqn I):

$$\delta=(W_o-W_s)/W_o=1-W_s/W_o \qquad \text{[Eqn I]}$$

where W_o is the fitness of outcrossed progeny and W_s is the fitness of selfed progeny. We can also relate δ to the inbreeding load, because the decline in log fitness between selfed and outcrossed progeny (Fig. I) will be equal to (Eqn II):

$$\log W_s-\log W_o=\log(W_s/W_o)=-B/2 \qquad \text{[Eqn II]}$$

Exponentiating both sides, δ can be expressed as (Eqn III):

$$\delta=1-W_s/W_o=1-e^{-B/2} \qquad \text{[Eqn III]}$$

allowing one to interconvert δ and B (e.g. one or four lethal equivalents per gamete should cause fitness to decline by 39% or 86%, respectively). For levels of inbreeding other than selfing, the relationship between δ and B can be written as (Eqn IV):

$$\delta=1-e^{-B*F} \qquad \text{[Eqn IV]}$$

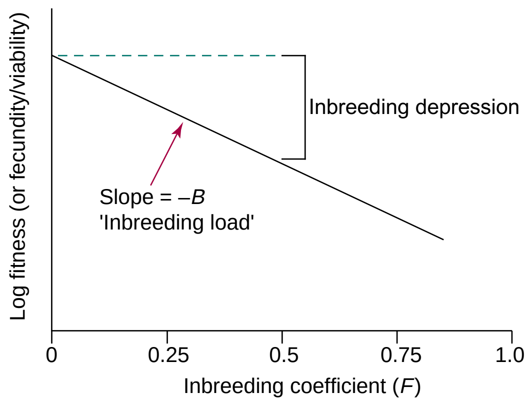

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Fig. I. The relationship between inbreeding load (the number of lethal equivalents, *B*) and inbreeding depression, δ.

(b) Among-population inbreeding depression

As noted in Box 1(c), plants or animals can be highly inbred because of population subdivision and drift even when mating is random within the subpopulation. Although crossing and inbreeding individuals within such subpopulations might produce no effect, such inbreeding can be detected by conducting crosses between subpopulations. Such crosses can produce dramatic increases in fitness (heterosis), as found, for example, in scarlet gilia *Ipomopsis aggregata* [d] and prairie chickens *Tympanuchus cupido pinnatus* [e]. Such experiments should include control crosses between large subpopulations. As these are not expected to exhibit inbreeding depression, there should be little elevation of fitness. Recent theoretical work [f] predicts that such heterosis depends on genetic parameters but can be tens of percent given reasonable values.

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within and among populations (Box 1). Here, we follow Wright's [15] broad definition of inbreeding to include the effects of both consanguineous matings within populations and random drift among them. Although the effects of inbreeding within populations are straightforward and widely appreciated, inbreeding because of population isolation and drift can be missed unless crosses are made among populations. For example, an inbred population can become fixed for several deleterious alleles. Such a population could show few or no differences among progeny of inbred and outbred matings, because both would reflect the effects of population inbreeding. Yet offspring from crosses among populations would show significantly increased fitness, a phenomenon known as HETEROSIS. This reflects the fact that different populations tend to fix different random subsets of deleterious alleles (DRIFT LOAD [16]) that mask each other when populations are crossed. Thus, paradoxically, an absence of within-population inbreeding depression cannot be used to infer the absence of inbreeding effects. For inbreeding resulting from population drift, one needs among-population comparisons of fitness and, ideally, crosses [Box 3(b)].

Box 4. Genetic methods to estimate inbreeding and inbreeding depression

Genetic markers can be used to infer levels of inbreeding and inbreeding depression directly from sampled individuals. These approaches present the distinct advantage of not interfering with natural patterns of mating, dispersal and survival. Disadvantages include the fact that they often require screening of a high number of loci and/or alleles and that they are often imprecise, because of high sampling variance. These techniques are likely to become increasingly important in studies of wild populations, and typically rely on co-dominant markers, such as isozymes or microsatellites.

(a) Correlations of individual fitness with heterozygosity

In many species, more heterozygous individuals grow, survive, or reproduce better than more homozygous individuals [a]. Blue mussels grow longer shells, oysters grow larger, coot clams grow faster, grasses grow more panicles, and the variance in these parameters often declines in more heterozygous individuals. Although these observations have been interpreted as evidence of overdominance, the increase of fitness with heterozygosity could also reflect reduced homozygosity or associative overdominance because of the masking of deleterious recessive mutations. This approach has been used only occasionally to estimate the magnitude of inbreeding depression explicitly.

(b) Shifts in the inbreeding coefficient

Just as mating between related individuals increases F in a population, selection against more inbred individuals tends to reduce F. Ritland [b] introduced a method that exploits these dynamic shifts in F to estimate inbreeding depression. The original method assumes that inbreeding reflects partial self-fertilization rather than biparental inbreeding, limiting its application to plants and snails. However, it could be extended to incorporate less severe forms of inbreeding provided identity disequilibria result. The method requires either three estimates of F over two generations or two estimates of F and one of the selfing rate. However, the technique relies on assumptions that might cause estimates to vary considerably from those obtained using more conventional techniques, as Jarne *et al.* [c] found in a self-fertile snail species.

(c) Correlation of individual fitness with estimates of (pairwise) relatedness

Just as the similarity in state between the two alleles at a locus can be used to estimate F, marker alleles shared between two individuals can be used to estimate their degree of relatedness in natural populations (relative, as always, to a base population). For example, an index of bandsharing (e.g. from multilocus DNA fingerprinting) can be empirically calibrated against pairs of individuals with known degrees of relatedness. This index can then be evaluated via regression against the fitness of the resulting offspring to infer inbreeding depression [d].

These techniques have been extended to directly estimate relatedness from isozyme or microsatellite markers without calibration against related pairs of individuals, and to estimate the degree of inbreeding of an individual. Several estimators are currently in use [e] and new ones are appearing [f]. However, sampling variance remains high for all these methods due to both the nature of Mendelian segregation and the need to let single locus estimates extend beyond the 0–1 range to avoid bias [g]. Generally, at least ten variable loci are needed with the standard error scaling with the inverse of the number of variable loci and the number of variable alleles per locus. Some of these approaches have been used to estimate the occurrence of inbreeding in natural populations [h] and one study used them to infer inbreeding depression [i].

(d) Correlation of individual fitness with mean d^2

The alleles at microsatellite loci are expected to mutate via a stepwise process. This implies that we can infer the time since coalescence from the difference in repeat numbers between two alleles. Coulson *et al.* [j] used this reasoning to propose the squared difference in length of the two alleles at a locus (averaged over several loci) as a measure of the genetic distance between the gametes that formed that individual, and thus a measure of inbreeding. Regressing fitness measures against mean d^2 provides estimates of inbreeding (or outbreeding) depression. However, a study of captive wolves shows that mean d^2 is not more predictive of pedigree F than heterozygosity [k], and recent theoretical work [l] (see Goudet and Keller, this issue) suggests that heterozygosity outperforms mean d^2 in estimating relationships between genetic variability and fitness in all but a few circumstances. Thus, d^2 might be of limited use.

(e) Paternity analysis

Before estimating relatedness between mates to infer inbreeding, it is often necessary to determine who the parents are. Both dominant and co-dominant markers can be used to estimate the probability that various individuals father particular offspring and thus patterns of matings in a population (e.g. [m]). If repeated over several generations, such paternity assignment could be used to construct pedigrees. These techniques are particularly useful for plants and those animals where behavioral observations of mating are difficult or misleading.

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Deleterious and at least partly recessive alleles contribute to both inbreeding depression within populations and heterosis in crosses among populations. Nevertheless, heterosis is not simply the reverse of inbreeding depression within populations. As was postulated half a century ago [17] but formally examined only recently [16], heterosis results more from the accumulation of different alleles of small effect, whereas inbreeding depression is influenced more by alleles of large effect. Inbreeding within populations will quickly expose recessive lethal mutations to selection, causing them to be purged even in small and isolated populations. Deleterious alleles with smaller selective effects ($<1/2N_e$), however, will be invisible to selection and thus will often drift to fixation. Even limited gene flow ($N_m<1$) will not prevent drift load from accumulating, implying that heterosis should not be taken as evidence of complete population isolation [16].

Populations of various size and history are likely to differ strongly in drift load. We suggest using F_{st} as an index of the susceptibility of a population to the deleterious effects of drift load. Estimates of F_{st} within a particular population could be derived either from long-term pedigrees within a population [18] or, perhaps more practically, from its average pairwise F_{st} with all other sampled populations using molecular markers [19]. In practice, biologists have tended to use population size to indicate which populations are most inbred. Although this has proved effective in many instances, it relies on assuming that census sizes are autocorrelated through time and that such counts reflect N_e, and thus the magnitude of the drift load. Other studies compare populations that differ in overall levels of genetic variation, involving a similar assumption. Although these measures often will be correlated with one another, we urge researchers to apply multiple methods for internal comparison.

If the genetic load in the ancestral population was high, small isolated subpopulations will experience considerable drift load, even in the short term. Such populations will also be at risk of accumulating new mutations via mutational meltdown [5] but this will take longer. Although small and semi-isolated populations can often experience a GENETIC RESCUE effect [20] when gene flow is re-established, gene flow can also re-introduce genetic load.

Inbreeding depression within animal populations

Increased interest in inbreeding has now yielded enough data to suggest that inbreeding depression in wild animals occurs regularly and can be severe (Table 1). A few points emerge from Table 1. First, the skepticism voiced by some about the effects of inbreeding in the wild stemmed, in part, from the observation that few deaths observed in wild populations 'could have been attributable to genetic defects' [2]. However, several studies suggest that mortalities associated with genetic factors are rarely detectable as such. Rather, inbreeding makes individuals more susceptible to environmentally inflicted mortality. For example, Coltman *et al.* [21] report significant inbreeding depression in survival of Soay sheep *Ovis aries* on the island of Hirta in the St Kilda archipelago (UK). Intestinal nematodes mediated the inbreeding effect because more homozygous Soay sheep suffered higher rates of parasitism and, in turn, lower overwinter survival than did heterozygous sheep. Sheep relieved temporarily of their parasite burden with anthelminthic drugs showed no association between heterozygosity and survival. Inbreeding effects thus appear as parasite-induced deaths in that population. Another example involves song sparrows *Melospiza melodia* living on Mandarte Island in western Canada, where inbred birds [pedigree F; Box 1(a)] died at a much higher rate during a severe storm than did outbred birds [22]. There is no doubt that severe weather caused this mortality, yet inbreeding determined, in part, who survived that storm [22]. This is clear evidence that inbreeding makes individuals more susceptible to environmentally inflicted mortality.

Inbreeding depression not only reduces traits associated with early fitness (e.g. juvenile survival) but also reduces overall fitness in natural populations [23,24]. It has also been documented in endangered species, such as the red-cockaded woodpecker *Picoides borealis* living in the southeastern USA. Daniels and Walters [25] found that inbreeding [pedigree F; Box 1(a)] resulted in significantly reduced hatching rates, fledgling survival and recruitment to the breeding population.

Most recent studies of inbreeding depression in wild animals use genetic markers to estimate inbreeding and inbreeding depression. One drawback of several genetic methods to infer inbreeding (e.g. mean d^2) is that the relationship between these measures and the traditional inbreeding coefficients has not been examined theoretically. This, and the fact that these methods provide relative measures that vary among populations (Box 5), makes it difficult to compare these studies with others. Pedigree F, however, [Box 1(a)] allows easy comparisons among studies. Using pedigree analyses to quantify inbreeding depression allowed Keller [23] to infer that inbred song sparrows on Mandarte Island carry about five lethal equivalents per zygote (2^*B), corresponding to an inbreeding depression (δ) of 49% in progeny from a mating between first-degree relatives (F_i=0.25). Because of maternal effects, eggs produced by inbred females in this population have lower hatching rates, increasing δ measured over two generations to 79%. Even higher numbers of lethal equivalents (B=6.3) occur in inbred white-footed mice *Peromyscus leucopus* released into the wild [26] as well as in several zoo populations. Such high estimates exceed those found in captive Japanese quails *Coturnix coturnix* (B=1.7) and those reported in laboratory studies [23].

Inbreeding depression among animal populations

We know, from laboratory experiments with *Drosophila*, that inbreeding can at least potentially affect the persistence of populations [27,28]. Several field studies also provide evidence that inbreeding depression can affect the dynamics and extinction risk of small animal populations. In their study of the Glanville fritillary butterfly *Melitaea cinxia* in Finland, Saccheri *et al.* [29] found that inbreeding (measured by heterozygosity) significantly increased the extinction risk of populations via effects on larval survival, adult longevity and egg-hatching rates. Furthermore, these effects remained significant even after accounting for the effects of population size. Thus, inbreeding represents a significant risk for these populations beyond that owing to demographic factors.

Additional evidence that inbreeding can affect population dynamics comes from studies where new individuals from genetically diverse (i.e. outbred) populations were introduced into apparently inbred populations characterized by low reproductive success [20]. An isolated population of greater prairie chicken *Tympanuchus cupido pinnatus* in Illinois, USA that had

Table 1. Studies that report within- or among-population inbreeding[a,b]

Species	Method	N	No. of inbred matings	No. with $F \geq 0.25$	Population size	Trait showing i.d.	Refs
Animals							
Great tit	PED	397	7	5	120–341	Recruitment	[69]
	PED	885	13	12	120–341	Nestling mortality	[70]
	PED	1297	245	13	76–188	Hatch rate	[71]
Blue tit	PED; BAND SH	469 pairs	–/4	4	80–120	Hatch rate	[72]
Medium ground finch	PED	583	27	3	28–200 females	None	[73]
Large ground finch	PED and HET	31	11	6	2–60	Longevity; survival to breeding	[74]
Cooper's hawk	PED	43	1	0	Unknown	Hatching success	[75]
Great reed warbler	BAND SH	80	--/2 from pedigree	--/0	36–64	Hatch rate, no. of fledged young	[76]
	HET and d^2	100	--	--	*c.* 120 following founder event	Recruitment	[77]
Moorhen	PED	12	6	6	*c.* 200	Hatch rate	[78]
Mexican jay	PED	657	32	4	100	Brood size, first year survival	[79]
Song sparrow	PED	671	297	21	11–144	Survival, reproductive success, etc.	[23]
Greater prairie chicken	Pop. size	278 clutches	--	--	Declining from 2000 to 50	Fertility and egg-hatching rates	[30]
Taita thrush	Ritland '96	237	--	14–152	N_e=30–750	Fluctuating asymmetry	[80][c]
Takahe	PED	37 pairs	14	--	8–19	Juveniles raised (but NS)	[81][d]
Red-cockaded woodpecker	PED	845	52	21	>500	Hatch rate, first year survival	[25]
Bighorn sheep	N_e estimates	--	Mean F=0.02–0.21	--	49–51	Lamb mortality	[82][e]
Olive baboon	PED	46	8	--	3 troops, 26–51 individuals each	Juvenile survival to 30 days	[83]
Chacma baboon	PED	101	20	--	72 females	None	[84]
Yellow baboon	PED	164	3	0	>500	Survival: conception to 30 days	[85]
Black-tailed prairie dog	PED	553	198	6	125	None	[86]
Common shrew	BAND SH	51	(31)	(9)	Unknown	Survival to maturity, body length	[87]
Golden lion tamarin	PED	165	14	14	274	Survival to weaning	[88]
White-footed mouse	PED*	786	--	367	--	Survival, mass gain in males	[26]
Dwarf mongoose	PED; BAND SH	$\leq$81	18	4–18	*c.* 94 000	None	[89]
Muskox	Sim PED	49	Unknown	>50%	12–30	Reproductive success; longevity	[90]
Red deer	d^2	670	–/14 from pedigrees	--/0	1175–1724	Birth weight, neonatal survival	[91]
	d^2	549–633	--	--	1175–1724	First winter survival.	[92]
	HET	209	--	--	1175–1724	Lifetime breeding success	[24]
Harbor seal	d^2	36; 68	--	--	700–1200	Birth weight, neonatal survival	[93]
Black-footed rock wallaby	Pop. HET	25	--	--	150	Fecundity	[94][f]
Soay sheep	HET	398; 657	--/19 from pedigrees	--/0	694–1826	Winter survival; no. fecal eggs	[21]
Horseshoe bat	d^2	140	--	--	68	Male survival to third summer	[95]
Adders	Isolation and pop. size	2 pop., 81 individuals	--	--	38 (N_e=10)	Brood size, % viability	[31, 96]
Glanville fritillary	Pop. HET	42 pop.	--	--	Variable	Larval survival, adult longevity, egg hatching	[29][g]
Natterjack toad	Pop. HET	6 pop.	--	--	--	Larval growth rate	[97]
Plants							
Silene regia	Pop. size	23			3–1000	Germ. success	[33]
S. alba	PED*	55 pairs	40	25	Variable	Germ. success	[40][h]
	Isolation	12 pop.	60	20	Variable	Germ. success	[40][i]
Mimulus guttatus	Shift in F	98–115 fam. in each of 3 yrs	--	57–70% **	500	Seedling to adult viability	[98]
M. platycalyx	Shift in F	84–93 fam. in each of 3 yrs	--	80–92% **	2000	Seedling to adult viability	[98]
Ipomopsis aggregata	Pop. size*	10 pop.	--	--	Variable	Seed size; germ. success	[39]
Gentiana pneumonanthe	Pop. size	19 pop.	--	--	5–50 000	Seed and seedling size; survival	[34]
Clarkia pulchella	Var N_e	32 pop.	--	--	12 at start of exp.	Seed germ. and survival; pop. survival	[41]
Gentianella germanica	Pop. size	23 pop.	--	--	40–5000	Seed no.; pop. growth	[36]
	RAPD var	11 pop.	--	--	40–5000	Seed no.; flowers per plant	[37]
Pedicularis palustris	AFLP var	13 pop.	--	--	3–28 500	Seed no.; seedling no.	[38]

[a] For within-population inbreeding, only studies that measured inbreeding depression directly and in the wild were included. For among-population inbreeding, studies that used common garden experiments were also included. Studies superseded by a more up-to-date analysis of the same data were omitted.

[b] Key: BAND SH, DNA bandsharing; d^2, mean distance based on SSR lengths; F, inbreeding coefficient; HET, individual heterozygosity; Isolation, populations with varying degree of isolation; N, individuals unless noted otherwise; Ritland '96, estimation of F from polymorphic markers; PED, pedigree; Pop. size, populations with varying sizes; Pop. HET, population heterozygosity; Shift in F, Ritland's method based on changes in F; Sim PED, simulated pedigrees; Var N_e, synthetic populations; Variable, variable but unknown; *, experimentally inbred or crossed; **, selfing rate; --, data not available.

[c] Analysed i.d. in three different populations; effects only found in one.

[d] Populations translocated to small islands: population fitness lower than in population of origin.

[e] Longitudinal study, correlating increase in lamb mortality with increasing average F.

[f] Comparison between 2 population averages.

[g] Demonstrates effects of heterozygosity on extinction risk.

[h] 4 crosses x 10–15 families.

[i] 12 populations x 2 families x 3 crossing treatments.

Box 5. Measuring inbreeding and inbreeding depression

Studies of inbreeding depression in natural populations use one of several approaches:

- Pedigree analysis. Pedigree information from long-term studies of marked animals is used to determine the inbreeding coefficient (F_i) of individuals (Box 1, Fig. I). Measures of individual fitness, such as survival probability and reproductive success, are then correlated with these F_i values across individuals [a]).
- Genetic analysis. Genetic markers can also be used to estimate individual F_i values, which are then correlated with measures of fitness (Box 4). Genetic markers can also be used to track changes in levels of population inbreeding (F) in cohorts as they mature and reproduce [b,c]. Any initial inbreeding will tend to inflate F, whereas subsequent differential selection against inbred individuals will tend to reduce it, allowing inbreeding depression to be estimated.
- Experimental inbred matings. Wild-caught animals or plants are brought into the laboratory and inbred for one generation (typically via full-sib matings or selfing). Their progeny are released into the wild (or a greenhouse) and the fitness of inbred progeny is compared to that of outbred progeny.

Each of these approaches present particular advantages and disadvantages (Table I).

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Table I. Advantages and disadvantages of approaches for measuring inbreeding and inbreeding depression

Method	Advantages	Disadvantages
Pedigree analysis	Natural mating patterns Natural levels of environmental variation Accurate estimates of individual inbreeding and its effects Easy comparisons among studies	Requires accurate information on paternity and long-term monitoring to establish pedigrees
Genetic analysis	Natural mating patterns Natural levels of environmental variation Parentage assignment using genetic markers	Tracking of changes in F so far limited to partially selfing plants and animals Many techniques do not allow comparisons of results among studies
Experimentally inbred matings	Most precise control of inbreeding and potentially confounding variables True replication providing rigorous and accurate estimates of inbreeding depression	Levels of inbreeding studied usually exceed those present in natural populations [a], thus the results might not show how much inbreeding depression occurs in nature, if high levels of inbreeding bring synergistic effects [d,e].

been dwindling for 22 years rebounded markedly with higher fertility rates once birds from large populations 600 km away were introduced [30]. Translocating 20 males restored fitness and population size to a population of adders *Vipera berus* in southern Sweden that had previously experienced many deformed and stillborn offspring, which could reflect inbreeding [31]. Similarly, introduction of cougars from Texas, USA into the last remaining population of Florida panthers *Puma concolor coryi* has greatly reduced the occurrence of detrimental traits (e.g. cowlick, kinked tail and cryptochordism) in the Florida panthers [32].

Inbreeding depression within and among plant populations

Evolutionists have also begun to explore how inbreeding in wild plant populations depresses fitness (Table 1). Most of these studies examine differences among populations of varying size or isolation [Box 1(c)] rather than differences among individuals within a population, making them sensitive to drift load. This work began with the observation by Menges [33] that seed germination declines dramatically when isolated Midwestern USA prairie populations of *Silene regia* fall below 150 plants. Such declines cannot be explained simply by poor pollination but could, conceivably, reflect maternal effects (e.g. poor soil could reduce both population size and germinability). Common garden experiments could be used to test for such effects.

In a similar study, Oostermeijer *et al.* [34,35] found that seed and seedling size and survival declined in smaller populations of a rare perennial gentian *Gentiana pneumonanthe*. Phenotypic variation also increased in the smaller populations. Interestingly, adult size and flowering decreased in populations with less isozyme variation even though isozyme variation was only weakly correlated with population size. Work on 23 populations of another rare European gentian *Gentianella germanica* (a biennial) by Fischer and Matthies [36] found fewer seeds per fruit and per plant and steeper population declines in smaller populations. A survey of random amplified polymorphic DNA variation in 11 of these populations [37] further demonstrated that molecular variation increases in the larger populations and that seed set and plant performance increases in populations with more genetic variation.

These studies demonstrate how genetic markers (Box 5) can be used to infer how variable inbreeding among populations contributes to declines in plant fitness and population persistence. Such markers also allow us to confirm whether population size and genetic variation covary. Schmidt and Jensen [38] used amplified fragment length polymorphism markers to explore genetic variation in 13 populations of the central European fen biennial *Pedicularis palustris*. This short-lived plant lacks a seed bank, making it vulnerable to fluctuating site conditions, possibly accentuating inbreeding. The authors found that both fecundity and the seedlings:flowering plants ratio increased significantly in populations with more genetic variation and that these effects exceeded those owing to population size alone. As in the perennial gentian [34,35], genetic variability was independent of population size. Such results suggest that historical population dynamics can affect inbreeding more than does the current population size (as with the song sparrows of Mandarte Island [23]).

Correlative studies based on population size and genetic variation are now being complemented by experimental approaches that strengthen the evidence for the threat posed by population inbreeding. Seed size, germination success, and resistance to stress all decline in populations of scarlet gilia *Ipomopsis aggregata* with <100 flowering plants [39]. To test for population inbreeding effects, Heschel and Paige transferred pollen from a distant population into small and large populations. Seed mass and germinability increased in the small populations but not in the large population, demonstrating the presence of population inbreeding and the corresponding potential for genetic rescue.

Richards [40] also demonstrated the potential for genetic rescue in experiments with *Silene alba*. Isolated populations of this colonizing weed showed reduced (and similar) fitnesses following both sib-crosses and outcrosses within patches but elevated fitness in between-patch crosses. By contrast, central populations showed no differences in within- versus between-patch crosses. In addition, experiments demonstrated that patches of full sibs are more likely to gain pollen from distant sources than patches with unrelated plants. Thus, pollen-mediated gene flow is both genetically important in 'rescuing' inbred populations and responds to local population genetic structure.

Newman and Pilson [41] also used an experimental approach to examine how the numbers of founders affects population persistence. They created artificial populations of the annual *Clarkia pulchella* with the same census population size but varying effective population sizes (N_e) by adjusting the number and relatedness of founders. After three generations, the low N_e (inbred) populations suffered reduced seed germination and survival with a fitness of only 21% of that found in the large N_e populations. Only 31% of low N_e populations survived compared with 75% of the large N_e populations, demonstrating that the effects of inbreeding on individual performance can strongly affect population persistence (as seen in the butterfly study [29]).

Statistical issues

Inbreeding depression is not always high and will not always affect population dynamics. In fact, we expect populations to differ in their amounts of inbreeding depression on theoretical grounds [42]. Given that only a few studies exist and that they give only approximate estimates of inbreeding depression, we remain short of an accurate and comprehensive understanding of inbreeding effects in wild populations. Only ~50% of the studies reviewed by Crnokrak and Roff [43] reported significant levels of inbreeding depression. Negative results could reflect a true lack of inbreeding depression, fixation of the load within sub-populations and a failure to test for among-population inbreeding depression, or simply a lack of sufficient statistical power to detect inbreeding depression.

To avoid biasing our overall picture of inbreeding depression in the wild, it is important to report negative results [44]. However, care should also be taken to examine whether the data support the notion of no (or low) inbreeding depression or whether the data lack the power to detect inbreeding depression of the expected magnitude. The issue of statistical power is often neglected in studies of inbreeding depression, yet detecting inbreeding depression generally requires large sample sizes [45]. A rule of thumb derived from agricultural systems is that an increase in inbreeding by 10% leads to a reduction in fitness components of ~5–10% [46]. Thus, mating among first-degree relatives (F_i=0.25) is only expected to reduce fitness components by 15–25%. Given that environmental sources can lead to variation in fitness components of several 100%, a 25% decline in fitness components could be difficult to detect. In fact, several studies that found no statistically significant inbreeding depression noted declines in fitness components of the order of 20% in individuals with F_i of 0.25. We find it noteworthy that all studies that we surveyed that used 20 or more full-sib matings detected significant inbreeding depression (Table 1). We therefore urge researchers to determine whether they have the statistical power to detect expected levels of inbreeding depression. Calculating confidence intervals for δ provides perhaps the best means to do so, in that values within the confidence interval represent hypotheses that cannot be rejected [47]. It is also important to evaluate as many fitness components as possible so that these can be combined to estimate total fitness, which is often the most sensitive and statistically significant indicator of inbreeding depression.

When are populations purged of their load?

If inbreeding depression reflects the presence of partially recessive deleterious alleles, selection against inbred individuals should reduce their frequency and so purge a population of at least part of its load. Such purging will be efficient against lethals, but is unlikely to be as effective against the mildly deleterious mutations that might make up much of the load. The efficiency of purging declines in response to several demographic and genetic factors, which appear particularly likely to occur in small and isolated populations (Box 2). In the absence of efficient purging, however, deleterious alleles will be particularly prone to

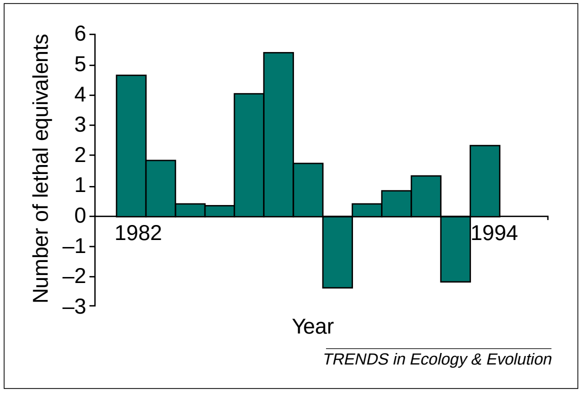

Fig. 1. Variation in the number of lethal equivalents expressed in juvenile song sparrows *Melospiza melodia*, derived independently for juvenile survival of birds born in different years. Based on data in [23].

fix in such populations, contributing to the drift load and thus the population component of inbreeding depression. For this reason, we should avoid inferring that purging has been effective in reducing the load when we find reduced differences between inbred and outbred individuals within small populations. Such reductions could just as well reflect declines in fitness in 'outbred' progeny (because of fixation) as increases in inbred fitness (because of purging).

In fact, many selfing plants still harbor considerable genetic load [48] as do insular vertebrate populations [21,23] (Box 2). This suggests that purging is often rather limited in extent. Alternatively, some interpret the persistence of inbreeding depression in inbred populations as evidence for the overdominance mechanism of inbreeding depression. It is true that symmetric overdominance will block selection and thus maintain variation and inbreeding depression. However, asymmetric overdominance appears as likely to occur and is prone to selection in inbred populations. As persistent inbreeding depression could reflect either symmetric overdominance or inefficient purging (Box 2), we should use careful tests rather than an absence of purging to infer the mechanism of inbreeding depression [11].

Is inbreeding depression constant?

Some studies report higher inbreeding depression in the wild than in captivity e.g. [26,43], whereas others [49] show no such effect. Clearly, before reaching any general conclusion, we need more estimates of inbreeding depression in the wild, and particularly comparisons of the same species in captivity and in the wild [49]. Crnokrak and Roff [43] attempted to summarize quantitatively data like those in Table 1. However, these data included values obtained in captivity and did not exploit meta-analysis to test significance levels, leading to potentially biased results [50]. A meta-analysis that also accounts for publication bias would be worthwhile.

The amount of inbreeding depression measured often varies according to life-history stage, trait measured, experimental habitat, environmental conditions, or year of study, as seen in Mandarte song sparrows (Fig. 1). Such variation reflects the interaction of genetic with environmental factors and could reflect either increased expression of deleterious mutations in some environments or the expression of more such loci [51]. These two mechanisms differ in how they might affect selective purging of the mutational load (Box 2). For example, if the deleterious effects of mutations are enhanced under harsh conditions, purging might be more efficient than under benign conditions. If, however, mutations are conditionally neutral, purging might be harder.

Inbreeding depression reflects the cumulative effects of directional dominance variation across loci of both major and minor effect. Because we expect major genes to be expressed early in development, dramatic inbreeding effects are often observed early in embryos. However, such genes are also the part of the load most likely to be purged in inbred populations (Box 2), suggesting that outbred populations should exhibit more inbreeding depression for early-acting traits than should inbred populations. This statistical trend is observed among plant studies [52].

Some traits also appear to show higher levels of inbreeding depression than do others. For example, *Drosophila* consistently experience more inbreeding depression in fertility than in viability [53]. Fitness traits exhibit more inbreeding depression than do morphological traits among animal species, perhaps reflecting larger directional dominance in fitness-related traits [54]. Fitness-related traits might also be influenced by more loci [55].

Inbred individuals often appear more likely to succumb to stressful conditions than are outbreds, implying that harsh environmental conditions could magnify inbreeding depression [56]. Increased competition, disease or harsher field conditions can all magnify inbreeding depression [57–64]. This could put small populations exposed to both inbreeding and heightened stress at particular risk [65]. However, inbreeding depression is not universally higher in wild or more stressed populations [48,49,66], suggesting that we should avoid generalizations. Further data are needed to quantify how environmental and genetic factors interact to affect inbreeding depression.

Conclusions

Historically, much of the interest in inbreeding depression has come from either animal and plant breeders or researchers investigating the evolution of mating systems, local adaptation, or how inbreeding contributes to selection among sub-populations. At the same time, the historical focus of conservation biologists has been demographic and ecological events. The studies reviewed here make it clear that the demography, ecology, and genetics of small populations all interact to affect population persistence. None should be ignored.

Inbreeding within and among populations can strongly affect both individual and population viability. Whereas our understanding of how inbreeding affects individuals has improved, we still know little about the effects of inbreeding on population dynamics. In the presence of strong

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Glossary

Demographic stochasticity: random variation in the reproduction and mortality of individuals. Individuals are affected independently.

Drift load: the reduction in mean fitness that results from local genetic drift [a]. Termed finite population load by Crow and Kimura [b].

Environmental stochasticity: unpredictable variation in population growth imposed by environmental factors (e.g. weather, food supply, and populations of predators, competitors or parasites). The entire population is affected similarly.

F_{st}: the probability that two genes, chosen at random from the subpopulation, are both descended from a gene in the subpopulation. Equivalently, it is the correlation between randomly chosen genes in the subpopulation relative to the total population.

Genetic load: the fraction by which the population mean fitness is changed as a consequence of mutation, segregation, recombination, etc. in comparison with a population, assumed otherwise identical, in which that factor is missing [b].

Genetic rescue: the increase in mean fitness of a population resulting from the immigration of genes from another population.

Heterosis: the increase in fitness of F1 progeny relative to the average of the two parental lines. Also termed hybrid vigor.

Inbreeding depression: the decline in fitness observed in inbred progeny, usually defined as the decline relative to outbred progeny: $\delta=1-W_s/W_o$

Lethal equivalent: the number of deleterious genes per haploid genome whose cumulative effect is the equivalent of one lethal gene.

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density-dependent population regulation, inbreeding depression might eliminate inbred individuals without affecting population dynamics (so-called 'soft selection'). In such circumstances, the magnitude of inbreeding depression would not necessarily directly affect population dynamics. When selection is 'hard', however, inbreeding could substantially affect population dynamics. Results from butterfly and plant populations suggest that inbreeding might often affect population dynamics.

Inbreeding and inbreeding depression do occur commonly in nature and can be severe enough to affect the viability of small and isolated populations. Thus, the argument over the occurrence of inbreeding depression in the wild can be put to rest. Nevertheless, the studies reviewed here, by Crnokrak and Roff [43], and by Hedrick and Kalinowski [67] leave open the search for general patterns in how inbreeding depression varies among taxa, environments, and populations with contrasting demographic and genetic histories. Although it is often asserted that inbreeding depression is greater under stress or field conditions, this pattern is neither universal nor theoretically resolved. It also would be of interest to know how variance in the degree of inbreeding among individuals within a population [68] affects the expression of inbreeding depression and subsequent population dynamics. We need to learn more about how genetics and metapopulation dynamics interact [40] if we are to understand just when and how inbreeding contributes to the 'extinction vortex' of fragmented populations.

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