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Annual Review of Ecology and Systematics, Vol. 30. (1999), pp. 479-513.

Annu. Rev. Ecol. Syst. 1999. 30:479–513

DO PLANT POPULATIONS PURGE THEIR GENETIC LOAD? EFFECTS OF POPULATION SIZE AND MATING HISTORY ON INBREEDING DEPRESSION

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Key Words mating system, genetic load, purging, self-fertilization, genetic effects of small population size

■ **Abstract** Inbreeding depression critically influences both mating system evolution and the persistence of small populations prone to accumulate mutations. Under some circumstances, however, inbreeding will tend to purge populations of enough deleterious recessive mutations to reduce inbreeding depression (ID). The extent of purging depends on many population and genetic factors, making it impossible to make universal predictions. We review 52 studies that compare levels of ID among species, populations, and lineages inferred to differ in inbreeding history. Fourteen of 34 studies comparing ID among populations and species found significant evidence for purging. Within populations, many studies report among-family variation in ID, and 6 of 18 studies found evidence for purging among lineages. Regression analyses suggest that purging is most likely to ameliorate ID for early traits (6 studies), but these declines are typically modest (5–10%). Meta-analyses of results from 45 populations in 11 studies reveal no significant overall evidence for purging, but rather the opposite tendency, for more selfing populations to experience higher ID for early traits. The likelihood of finding purging does not vary systematically with experimental design or whether early or late traits are considered. Perennials are somewhat less likely to show purging than annuals (2 of 10 vs. 7 of 14). We conclude that although these results doubtless reflect variation in population and genetic parameters, they also suggest that purging is an inconsistent force within populations. Such results also imply that attempts to deliberately reduce the load via inbreeding in captive rearing programs may be misguided. Future studies should examine male and female fitness traits over the entire life cycle, estimate mating histories at all levels (i.e. population and families within populations), report data necessary for meta-analysis, and statistically test for purging of genetic loads.

0066-4162/99/1120-0479$08.00

INTRODUCTION

> It often occurred to me that it would be advisable to try whether seedlings from cross-fertilised flowers were in any way superior to those from self-fertilised flowers.
>
> C. Darwin (36, p. 8)

In the course of working on inheritance, Darwin grew self- and cross-fertilized seedlings of *Linaria vulgaris* side by side in beds and was surprised to observe a clear difference between them. This led him to began systematic investigations into the effects of close inbreeding that extended over 52 taxa and 11 years (36). He was careful to pair selfed and outcrossed seeds for simultaneous germination and grew pairs side by side in the same pots. He soon realized progeny from related plants suffered constitutional changes and concluded "cross-fertilisation is generally beneficial, and self-fertilisation injurious." He further noted that selfed progeny suffered reductions in performance at almost every stage of growth even in taxa that were self-fertile. In *Ipomea purpurea*, Darwin found fewer seed capsules per self-pollination (69%), fewer seeds in selfed capsules (93%), and selfed seedlings that were shorter (76%). In the sixth generation of selfing, he found a plant with surprising vigor he christened Hero. Hero's descendants maintained unusual vigor, leading Darwin to note that the deleterious changes produced by selfing could be ameliorated under some circumstances. He concluded (36, p. 442) that "the advantages which follow from cross-fertilisation differ much in different plants" and "There does not seem to exist any close correspondence between the degree to which the flowers of species are adapted for cross-fertilisation and the degree to which their offspring profit by this process." Remarkably, he did all this work with no notion of Mendelian genetics.

Today, we refer to the reduced fitness we observe in progeny from matings among relatives as inbreeding depression. Inbreeding depression (ID) is indeed widespread among species and has been investigated thoroughly in domesticated plants and animals in this century (Ch. 2 in 182). We now interpret the occasional superior performance of selfed individuals like Hero and reductions in ID in inbred lines to be the result of purging, the preferential elimination of recessive deleterious alleles in inbred lines (26, 29, 31, 102, 151, 156, 182). While Darwin failed to find any correspondence between mating system and the degree of ID, several authors report reductions in ID in more inbred populations in both plants (7, 41, 72) and animals (12, 109, 113, 137). If purging is an effective process, we might expect it to progressively favor selfing in more inbred lines. This might account both for plant species that persistently self-fertilize (37, 77) and for the apparent tendency for plant species to either mostly self or mostly outcross (143). More selfing populations also tend to show reduced ID for early components of fitness as would be expected if selection preferentially purges lethal and semi-lethal mutations that act early (76). Thus, the evolutionary dynamics of inbreeding have become central in attempts to account for the remarkable diversity of plant mating systems (26, 65, 163).

Interest in ID dynamics has also expanded to include conservation biologists who wish to understand how inbreeding affects the persistence of small and/or inbred populations. Some researchers, impressed by the potential for purging, recommend intentional inbreeding as a strategy to reduce ID in captively bred populations (135, 152, 160). This faith in purging, however, may be misplaced if selection does not consistently reduce the load. Recent theoretical work suggests that purging may be ineffective in small populations in which mutations may accumulate to the point that they threaten population persistence (e.g., 110). Because theoretical predictions regarding ID dynamics hinge on assumptions and parameter values, there is now considerable interest in assessing how real populations respond to a history of inbreeding. This has spawned a renaissance of empirical work. Much of this work focuses on plants for the same reasons Darwin chose them for experimental work: Many are capable of the closest possible inbreeding (self-fertilization); they are relatively easy to control and cross; and they can be propagated in sufficient number to generate statistically meaningful results.

In this review, we first summarize the theory pertaining to ID and the accumulation of mutations in small populations. After considering mechanisms of ID, we explore how populations accumulate and reduce genetic load via mutation and selection. Theoretical work reveals that the efficiency of purging is strongly affected by the degree of dominance, the distribution of mutational effects, interactions among loci, and the breeding system. In addition, selection in small and/or inbred populations is often compromised, further affecting load dynamics. We next consider studies aimed at assessing how ID responds to varying histories of inbreeding. We concentrate on 52 empirical plant studies that compare ID among taxa, populations, or lineages that differ in their inferred histories of inbreeding. This burst of activity, mostly in the last 10 years, stands in contrast to the century after Darwin's initial work which produced few studies of ID outside agricultural settings. Because these studies differ considerably in design, scale, and execution, we first enumerate how frequently purging has been observed in various types of study. We then explore how the extent of purging varies in magnitude over studies, using regression and meta-analyses to assess how the type of experiment, plant characteristics, and the traits measured affect whether purging is observed. We conclude that although much evidence for purging exists, it is not consistent or substantial enough in most cases to favor fully inbred forms of mating or intentionally inbred schemes of mating for conserving small populations. To better understand purging and the genetic basis for ID, we recommend that future work of this kind incorporate a consistent set of methodological and analytical features.

THEORETICAL BACKGROUND

Mechanisms of ID

Deleterious alleles are often both recessive (38) and rare, meaning that their effects will usually be masked in heterozygous form in outbred populations. Thus, dominance is sufficient to account for the decline in fitness observed upon inbreeding

(35, 47, 182). Overdominance could also account for ID, however, in that fitness will also decline upon inbreeding as complementary alleles segregate as homozygotes (31, 32, 63, 184). Both dominance and overdominance predict similar declines in inbred fitness in proportion to the inbreeding coefficient, F (180a). If fitness effects interact independently across loci, the logarithm of fitness (or fitness components) should decline linearly with increased progeny inbreeding with a slope that measures the extent of the mutational load in terms of the number of lethal equivalents (104, 123, 180). Epistatic interactions among loci could also account for some ID (13, 90). Declines in log fitness that are less than linear (positive F^2 term) indicate diminishing epistasis, while fitness declines that are more than linear reflect synergistic or reinforcing epistasis (p. 79, 35).

Controversy over whether ID primarily reflects dominance or overdominance has persisted for much of this century (34). Much of the difficulty in resolving the question of mechanism stems from the fact that beneficial and dominant alleles may be linked to detrimental recessive alleles, causing pairs of such linked loci to mimic single overdominant loci (85, 130). Such associative (pseudo) overdominance could account for the positive correlations we often observe between fitness and heterozygosity (reviewed in 120) and empirical estimates of overdominance in natural populations (e.g., 53). While work in maize initially implicated overdominance, later work in maize and *Drosophila* observed declines in apparent dominance levels after further recombination (26, 33, 34, 79, 153). Associations between heterozygosity and fitness in *Drosophila* and pines appear attributable to associative overdominance (140, 159). Simulations confirm that associative overdominance arises in inbred lineages and that mutation to partially recessive deleterious alleles produces biologically realistic levels of ID (25, 29). A recent, well-designed study of two species of *Mimulus* concluded inbreeding depression was attributable to greater expression of recessive alleles (43).

Selection Against the Load

Classic population genetic models illustrate how recurrent mutation to deleterious alleles is balanced by selection against these alleles, leading to an equilibrium that depends on mutation rates, selective effects, the mating system and levels of dominance (35). If deleterious mutations occur at a locus at rate u and are selected against so that relative fitness is $1-s$ in homozygotes and $1-hs$ in heterozygotes, the equilibrium frequency of completely recessive mutations will be $\surd(u/s)$ in an infinite panmictic population. These alleles become much rarer (u/s) in a completely inbred population as these alleles are exposed more often to selection. Similarly, selection in heterozygotes becomes far more significant with even partial dominance, decreasing equilibrium frequency to approximately u/hs (or $2u/s$ with no dominance). In the absence of inbreeding ($F = 0$) and dominance ($h = 0.5$), the reduction in average fitness due to mutation–the mutational load (125) per locus–is independent of the selective effect and $L = 2u/(1 + u)$ (35). With partial dominance, $L \approx 2u$. If the mutant is completely recessive or if inbreeding causes most of them to be eliminated as homozygotes, the load is reduced ($L = u$). This implies that with complete recessivity, the maximum per locus decrease in the load due to purging is u. Over all loci, the load is equal to the sum of the mutation rates at each locus ($U = \Sigma u$).

The distribution of selective effects strongly affects the extent of purging. While lethal or semilethal mutations are efficiently purged (102), purging is far less efficient against mutations of minor effect, particularly if they affect heterozygote performance (26, 61). Classic work in *Drosophila* suggests that dominance of the wild type is rarely complete and most mutations have minor effects (89, 124). Assuming that inbreeding populations retain a substantial fraction of their load, the architecture of the load will shift with inbreeding to include fewer lethals and semilethals relative to mildly deleterious mutations. For example, five generations of selfing in *Mimulus guttatus* exposed many major mutations, leading to the loss of almost half of the lines, yet this did not appreciably diminish subsequent levels of ID in many of the surviving lines (44).

What mutation rates and levels of dominance characterize real populations? Total mutation rates are difficult to estimate, but it is clear that lethal mutations occur regularly in long-lived plants like ferns and mangroves (92, 93). Mukai's (124) classic experiments with chromosome II in *Drosophila* suggest a haploid genome mutation rate (U) of at least 0.17 with an average dominance (h) of 0.21 for minor viability genes at equilibrium (vs. 2–3% for lethals within a natural population; 33). However, recent work in Drosophila use improved methods have found lower mutation rates of 0.02 and suggest mutations have small effects (52a). Using these estimates of dominance and existing data on ID, Charlesworth (28) estimated the mutation rate to be $U = 1.3$–1.7 for *Leavenworthia crassa* and $U = 0.7$–0.9 in *L. uniflora*. In the diploid *Amsinckia spectabilis*, Johnston & Schoen (83) estimated U at 0.24–0.4 with h at 0.07–0.14, while the tetraploid congener, *A. gloriosa*, appeared to have roughly twice the mutation rate and load and higher levels of dominance. Other experiments suggest a genomic mutation rate of at least 0.25 (74), or 0.57 (112) or perhaps 1.0 or above (46, 97).

Selection is far less efficient in small and inbred populations as selection can act effectively only against mutations with selective effects greater than $1/(2\ N_e)$ (182). As N_e declines due to demographic factors or inbreeding, an increasing proportion of deleterious alleles becomes effectively neutral and invisible to selection, reducing the rate at which they are eliminated. Ultimately, we expect drift to result in these alleles being fixed at a probability equal to their starting frequencies. Once fixed, these mutations add to the load in a difficult-to-reverse process known as Muller's ratchet (62). In addition, strong selection against lethals and semilethals can incidentally fix mildly deleterious mutations via background selection or selective sweeps (24). This will cause many slightly deleterious mutations to be fixed in inbreeding populations even as genes with greater effects are being purged. Ironically, once fixed, these mutations decrease the fitness of both selfed and outcrossed progeny, reducing the difference between them and thus between any estimates of ID based on relative performance. Thus, studies that simply compare levels of

ID before and after inbreeding could observe reduced ID even in the absence of purging (see Discussion).

Mating System Evolution

Classically, ID has been considered the primary impediment to the evolution of increased selfing, with various flowering mechanisms (e.g., dioecy, gynodioecy, and monoecy) being interpreted as specifically evolved to avoid or reduce selfing (36). Fisher (51) first enunciated the substantial transmission advantage enjoyed by selfing and other forms of uniparental reproduction that avoid what Williams (176) termed the "cost of meiosis." Since then, the evolution of self-fertilization has usually been viewed as a balance between its transmission advantage (often assumed to be 50%) and the disadvantage of ID among selfed progeny (77, 173). If, however, increased selfing reduces opportunities to donate pollen, selfing may not enjoy the full transmission advantage commonly assumed (21, 69, 127). Selfing also brings the advantage of reproductive assurance following colonization (Baker's Law) (5, 36, 156), which may be more important than hitherto appreciated (147). Clearly, the mechanism by which selfing occurs also affects how selection can act on the mating system (i.e., prior, competing, or delayed selfing; 108). While most models place particular emphasis on genetic factors in mating system evolution, reproductive assurance, pollen discounting, and other ecological factors deserve similar emphasis in models of mating system evolution (65, 163).

Classic models of mating system evolution considered ID to be a property of the population and assumed its level to be fixed. ID is usually estimated as $\delta = 1 - W_s / W_o$, where W_s and W_o refer to the fitnesses of selfed and outcrossed progeny, respectively. This estimation of the parameter ID δ will be bounded from -1 to 1. Such models typically predict that selection (due to the transmission advantage) favors decreases in the rate of selfing if δ exceeds 0.5. In some situations, it may take two or more generations of selfing for the decline of fitness with F to exceed this threshold (116, 176). This might increasingly favor outcrossing in progressively more inbred populations, allowing a stable mixed (selfed and outcrossed) mating system (176). If δ is always less (or greater) than 0.5, however, simple models predict that plants should either completely self (or outcross) (102, 143).

Recent models that consider dynamic processes in mating system evolution make somewhat different predictions (163, 173). The most influential such model incorporated the capacity for populations to purge themselves of deleterious mutations and hence evolve lower rates of ID after a history of inbreeding (102). This model allowed ID and the selfing rate to coevolve by alternating selection on the mating system with selection against the load (due to recurrent major mutations at unlinked loci). As expected, reduced ID in more inbred lines can favor runaway selection for ever increasing levels of selfing in such models, reinforcing the binary prediction of classical static models. In contrast, models incorporating associations among load and mating system loci make a richer set of predictions.

In these simultaneous coevolutionary models, ID alone does not predict whether increased levels of selfing will evolve because genetic associations between load and modifier loci complicate selection (67, 68, 164). In addition, mixed mating systems can be evolutionarily stable under some circumstances (165). These models also predict that populations will retain among-family variation in ID and mating system characters as well as associations between these (23, 27, 29, 166). While some empirical studies detected such associations (169; N Takebayashi, LF Delph, in review; S-M Chang, MD Rausher, in review), others have not.

Our view of mating system dynamics is thus being extended to explore how levels of selfing, population structure, and ID all coevolve (173). This picture is complicated further by load dynamics in small inbred populations.

Genetic Hazards in Small and Inbred Populations

While conservation biologists have traditionally worried most immediately about demographic and environmental hazards, it is now clear that these are compounded by, and interact with, the genetic hazards faced by small populations (48, 52, 55). Lande (100) identifies three classes of genetic risk to small populations: immediate ID, the loss of possibly adaptive genetic variability, and the fixation of new deleterious mutations (accentuating ID). Early predictions based on the attrition of genetic variance in small populations suggested little genetic danger to populations above a few hundred—the so-called 50/500 rule (155). Recent theoretical work suggests that populations of several thousand may be necessary to maintain quantitative genetic variation and slow the accumulation of deleterious mutations (14, 101, 110). Empirical support is also emerging (e.g., small populations of *Silene regia* suffer reduced seed viability; 118).

Lynch and colleagues (54, 111) linked demographic and genetic models to demonstrate how the accumulation of deleterious mutations may reduce population persistence via a runaway process they term "mutational meltdown." These models have now been extended to include sexual populations; they conclude that populations with $N_e < 100$ are highly vulnerable to extinction on time scales of about 100 generations (110). In such populations, purging is "at best transient, as intentional inbreeding can only enhance the probability of fixation of deleterious alleles, and those alleles that are purged are rapidly replaced with new mutations." Mostly selfing plant populations appear to be at particular risk of mutational meltdown as they have small N_e and associations among alleles at different loci. Lande's (100) similar models led him to conclude that mildly deleterious mutations are "far more important in causing loss of fitness and eventual extinction than are lethal and semilethal mutations in populations with effective sizes, N_e, larger than a few individuals." Inbred populations also suffer from decreases in quantitative genetic variation that could further increase their risk of extinction (101).

Variable mutational effects, synergistic epistasis, and recurrent beneficial mutations all reduce the accumulation of mutations, but purging becomes impeded in small or inbred populations with N_e less than about 100 (148). Although linkage can increase the efficiency of selection in some circumstances (30), small and/or fluctuating population size also restricts selection for favorable mutations (131).

The strong selection that accompanies the purging of strongly deleterious alleles also causes allele frequencies at linked loci to change via background selection (24). This tends to increase population differentiation while further decreasing within-population genetic variation.

Paradoxes of Purging

If purging eliminates much of the load, we should expect inbred populations to show reduced levels of ID. The Charlesworth's earlier review (26) noted that several inbred plant and animal populations express relatively low levels of ID, but others retain substantial amounts. A more recent review of data from 79 populations in 54 species found that ID tends to decline with increases in the estimated selfing rate (rank correlation $r_s = -0.42$) (76). These authors found average δ levels of 0.23 in the selfers vs. 0.53 in predominantly outcrossed plants. This significant difference supports the purging hypothesis, but ID could also be depressed in selfing populations via fixation as noted above. Perhaps more tellingly, the ID remaining in most of the selfing species occurred later in life as expected if selection had effectively purged early-acting mutations of major effect.

Because purging appears effective, some suggest using intentional inbreeding as a conservation strategy in particular situations (135, 152, 160). In their work with Speke's Gazelle, Templeton & Read (161) reported that the mutational load decreased from 3.09 to 1.35 lethal equivalents for 30-day viability after a period of enforced inbreeding. These results, however, may reflect a statistical artifact (6, 178). The several genetic complications reviewed above suggest that purging will be relatively slow and constrained in its effects on ID for mutations of mild effect and partial dominance. At the same time, the inbred conditions necessary for purging to occur tend to accelerate the fixation of mildly deleterious alleles. Such equivocal results suggest that we should be cautious in promoting purging as a tactic to reduce the load.

Plants with persistently mixed mating systems pose an evolutionary enigma in that a history of inbreeding within a population is expected to purge populations of their genetic load and reduce ID. Thus, we expect species to undergo accelerating, or disruptive, selection for either complete outcrossing or ever-increasing levels of self-fertilization (102). While many plant species do appear to be either mostly selfing or mostly outcrossing (143), this partly reflects dominant modes of pollination (2). Exclusively selfing species are rare, and the many species with mixed mating systems do not appear to be undergoing strong directional selection (172).

Thus, theory leaves unresolved how effective purging will be in real populations. As both purging and mutation fixation plausibly occur in inbred populations, it is important to assess empirically how ID responds to inbreeding history.

APPROACH

Here, we review studies that test how levels of ID respond to a history of inbreeding. Our goal was to find studies that directly compare levels of ID among groups with divergent inbreeding histories. Most of this work is recent. In choosing studies for our analyses, we specifically sought studies that compare taxa, populations, or lineages that differ in their inferred levels of inbreeding. The studies differ considerably, however, in the taxa chosen, in how differences in inbreeding history were inferred (or manipulated), and in methods for estimating ID. After categorizing the studies according to their scale of comparison (taxon, population or lineage comparisons), we further categorize them according to how inbreeding history was inferred and how ID was measured. These divisions allow us to judge whether the estimated extent of purging varies across these groups.

We looked for studies via online literature searches and surveys of particular journals (*Evolution, Heredity*, and the *American Journal of Botany* since 1985), and we used personal contacts to find studies not yet in print. We chose not to include studies that only compare levels of ID in selfed lines to the 0.5 threshold used in classical models, as measurements of ID are sensitive to the trait chosen and assay conditions (42, 76). The comparisons presented here provide stronger and more quantitative assessments of the extent of purging.

Fifty-two studies provided usable results spanning 29 families and 52 species (full data online: www.bio.ilstu.edu/BEES/byers/). Ten studies involved *Mimulus*, reflecting its utility as a model genus due to its diverse mating systems and short life cycle. All studies found significant ID in at least one trait, but many did not explicitly test for purging.

Scale of Comparison

The studies we review address the degree of purging on one or more of the following scales:

1. *Among taxa*—In these 14 studies, authors compared levels of ID among related species within a genus that differ in flower morphology, self-compatibility, or some other indicator of the extent of inbreeding. The best such studies use molecular systematics methods to confirm that the species chosen are closely related (e.g., *Linanthus*—C Goodwillie, in review).
2. *Among populations within species*—These 21 studies compare the relative expression of ID among populations known or inferred to differ in their mating history (e.g., 72).
3. *Among lineages within a population*—In these 18 studies, levels of ID are compared among lines known or thought to differ with respect to their amount of inbreeding (e.g., 7). In some cases, inbreeding history is inferred

(e.g., from floral characters in Mimulus; 20) while in other cases it is experimentally manipulated (e.g., 19).

Selection has had progressively less time to act over these nested scales, suggesting that comparisons among them could indicate how effective purging is over various scales. Comparisons among related taxa are likely to reflect average differences in the extent of purging that occur over long periods (since divergence) and among several populations. Such comparisons lack strong controls, however, in that taxa could differ in many aspects of their inbreeding and ecological history. In contrast, studies that experimentally cross plants to compare lineages gain maximum control while losing the opportunity to assess how inbreeding affects purging over longer time periods and variable population circumstances. Comparisons among populations are somewhere in between. Comparisons at multiple levels in the same taxa might provide the most comprehensive information on how purging occurs in different contexts and its effects on mating system evolution.

Inferring Inbreeding History

Reliably inferring inbreeding history is critical for evaluating whether more inbred populations experience reduced levels of ID in accord with the purging hypothesis. Several distinct methods are used to infer the degree to which various taxa, populations, or lineages have experienced inbreeding in the past. Like Darwin (36), some use flower morphology or estimates of the degree of self-compatibility as measures of population inbreeding (e.g., 105). Others rely on population size, outcrossing rates, or inbreeding coefficients to infer inbreeding history. Each of these approaches has particular advantages and disadvantages summarized here.

1. Flower morphology—In many species, outcrossing rates increase with increasing flower size or stigma-anther distance, allowing one to infer historical patterns of mating from flower form (e.g., 133). These inferences are likely to be reliable if flower form is genetically based and stable, and if flower form and mating system are highly correlated. Such appears to be the case in recently derived selfing *Amsinckia* (146). Anther-stigma separation is also associated with outcrossing in populations of *Clarkia tembloriensis* (73), *Mimulus guttatus* (16a, 41), *Mimulus ringens* (88), *Turnera ulmifolia* (8), and *Ipomoea purpurea* (S-M Chang, MD Rausher, in review). Herkogamy and dichogamy are both correlated with the outcrossing rate (t) in *Aquilegia caerulea* (11). Flower form can also respond to microhabitat variation and seasonal changes, however (73).
2. Self-compatibility—Like flower form, levels of self-compatibility (SC) are often genetically based and remain stable long enough for populations with differing levels of SC to differ reliably in their inbreeding history. However, the expression of self-incompatibility may also vary with environmental conditions (157). We include only one study that used this approach in *Campanula rapunculoides* (169).

3. Population size—A few studies compare populations that differ in size, implicitly assuming population size to be stable and smaller populations to have experienced greater amounts of inbreeding (e.g., 168). Decreases in population size do increase selfing rates in some outcrossing species (e.g., 3). Such assumptions are unwarranted, however, in colonizing or other species where populations rapidly fluctuate in size. In addition, population substructuring influences mating (181) in that localized mating may cause biparental inbreeding even in large populations (15, 49, 50, 173). Few purging studies quantify local gene flow or the influence of population structure.
4. Outcrossing rate—Many studies use empirical estimates of the outcrossing rate (t) to infer historical levels of inbreeding in populations (e.g., 143, 174). Outcrossing rates are typically estimated using isozyme or microsatellite markers. Like SC and population size, the utility of t as an indicator of inbreeding history hinges on how stable it is over multiple generations. If outcrossing rates vary significantly among years in response to fluctuations in the availability of pollinators or other environmental conditions, t may be a poor predictor of inbreeding history. Such often appears to be the case (e.g., 8). Those who use this approach are advised to use multilocus estimators (e.g., 138) averaged over several years (e.g., 177).
5. Inbreeding coefficient—The inbreeding coefficient F estimates the probability of identity by descent between alleles at a locus and thus reflects inbreeding not only in the current generation but also in previous generations. This cumulative aspect of F makes it superior to t for inferring population history (172). Nevertheless, because a single generation of random mating resets F to 0, this approach could mischaracterize some historically inbred populations.

Ideally, studies should employ combinations of these approaches, and some do (see data online). Jain (78) compared several methods (population size, outcrossing rate, percent polymorphic loci, and percent heterozygosity) to determine their relationship to expression of inbreeding depression in seven populations. He did not find any relationship leading him to suggest the use of several methods to estimate genetic structure. Studies should also report how selfing is achieved in SC taxa (prior, competing, or delayed; 108).

Estimating Inbreeding Depression

Various techniques are used to estimate ID. Comparisons among taxa and populations typically rely on levels of ID as measured by comparing fitness traits between experimentally produced selfed and outcrossed progeny ($\delta = 1 - 6W_s / W_o$). We term these multiple comparisons (abbr.: M). Because ostensibly outcrossed progeny may actually be somewhat inbred, it behooves researchers to ensure that $F = 0$ in their parental group (26). Alternatively, when levels of inbreeding exceed

0 for outcrossed progeny or 0.5 for inbred progeny, one should plot log fitness directly against the inbreeding coefficient (173). The slope of this line (-b) reflects the number of lethal equivalents per gamete present in the population (123). Values as high as 16 have been reported in trees (154).

In some comparisons among populations, levels of ID are inferred indirectly from shifts in inbreeding levels over different life history stages and generations. We term these Ritland comparisons (abbr.: R) after K Ritland, who developed and applied this technique (139). If families differ in inbreeding history, however, they may also differ in levels of ID, adding to the variance and reducing the precision of comparisons among populations. In such cases, it may be preferable to experimentally manipulate the genetic background and levels of inbreeding. Studies that compare ID among lineages within populations often generate progeny at several levels of inbreeding via multiple generations of selfing (e.g., 7, 115). We term these S studies. Such studies clearly reduce the amount of unknown among-family variance, particularly if they start with a randomly outcrossed parental generation.

Measures of ID obtained from different environments do not always agree (see, e.g., 107, 119, 144, 170). Assays under greenhouse conditions may be less stressful than those in natural habitats, perhaps resulting in a decrease in the expression of ID (42, 122, 136, 142). Similarly, reducing opportunities for competition in greenhouse studies (e.g., by growing plants individually in pots) may reduce the expression of ID (179). Higher fertilizer levels increased apparent ID in *Schiedea* (129). In *Impatiens capensis*, high density enhanced ID in one experiment (144) but had little consistent effect in another (170). Field experiments, on the other hand, are prone to both disasters and environmental noise that may obscure even appreciable fitness differences (119, 170). Field experiments could also underestimate ID if field conditions restrict growth. Greenhouse studies that incorporate competition could provide reasonable estimates of ID.

The expression of ID may also differ among traits. Traits expressed early in the life cycle such as seed traits may be strongly influenced by maternal effects, clouding the effects of progeny inbreeding (15, 171). As a population's mating history may influence when ID is expressed (75), it is important for studies to report estimates of ID for multiple traits over the life cycle. Similarly, while most researchers report information on female components of fitness, male components are also sensitive to inbreeding (18, 19, 36, 80, 115) and should be reported.

Methods of Analysis and Meta-Analysis

We first tally the number of studies that find evidence of purging in relation to the type of study (multiple comparisons–M, successive selfing–S, Ritland's method–R) and level of comparison (taxa, population, or line; Table 1). Most studies involve multiple comparisons of populations or species (M-studies–see online Table (www.bio.ilstu.edu/BEES/byers/) for a fuller description). We consider these M studies in more detail as they appear to be the most straightforward and powerful for detecting evidence for purging. Our tallies of which studies find purging or not

TABLE 1 Number of studies that have or have not found evidence for purging of mutational load. Note study by Holtsford and Ellstrand (72) is listed twice since it consists of a greenhouse study and a separate field study.

Study type	Level of comparison	Evidence for purging (citations)		
		Yes	No	Maybe
Multiple Comparisons (M)	Taxa	4 (18, 105, 133, A)	6 (1, 28, 84, 87, 103, 141)	1 (81)
	Population	6 (60, 70, 72, 86, 115, 177)	10 (8, 45, 58, 72, 78, 114, 132, 168, 175, B)	2 (17, 91)
	Lineage	4 (129, 169, C, D)	3 (4, 20, 126)	—
	Total	14	19	3
Ritland Method (R)	Taxa	1 (41)	1 (139)	—
	Population	—	—	—
	Lineage	—	—	1 (96)
	Total	1	1	1
Successive inbreeding (S)	Taxa	1 (7)	—	—
	Population	2 (19, 117)	1 (107)	—
	Lineage	2 (44, 145)	8 (40, 59, 94, 106, 121, 128, 179, E)	—
	Total	5	9	0
TOTALS	Taxa	6	7	1
	Population	8	11	2
	Lineage	6	11	1
	Grand Total	20	29	4

A. C Goodwillie, in review.

B. DM Waller, unpub. data.

C. S-M Chang and MD Rausher, in review.

D. N Takebayashi and LF Delph, in review.

E. JH Willis, in review.

are generally based on the authors' judgement. For further analyses, we consider M type studies that compare lineages within a population separately, but exclude successive selfing to avoid bias as studies employing single seed descent allow only near lethal mutations to be purged (see Theory section). There were only three Ritland-type studies, making it difficult to further assess them as a group.

For studies that present results for a number of populations that differ in t or F, we plotted δ versus these predictors of population inbreeding and applied regression analysis (Figure 1, Table 2). This figure and table allow ready comparison among populations that naturally span a continuous range of inbreeding histories.

We also applied meta-analysis on a different but overlapping subset of studies to assess how evidence for purging varies with respect to plant life history, how inbreeding was inferred, and which traits were measured. This potentially powerful method weights each study for magnitude of effect and variance in estimates and sample size (which the tallies and regressions do not). Unfortunately, only a few studies (11) had sufficient information to be included in these analyses (a mean for each trait value, for sample sizes and standard deviations). Because this method requires assignment of populations to a category (either selfing or outcrossing), we included only studies with clearly distinguishable populations. If populations varied appreciably among years in F or t, the study was not included (e.g. 177). In one case, the populations spanned a wide range of outcrossing rates, so we included only the seven most selfed and outcrossed populations (8). These constraints limited our meta-analyses to 10 studies for early traits (4 among-taxa and 6 among-population comparisons), 9 for late traits (2 among-taxa and 7 among-populations), and 2 for cumulative fitness (both among taxa; see data online). We used the mixed model of Gurevitch & Hedges (56) to compare the magnitude of cumulative effects (of selfing relative to outcrossing) for early traits (seed weight or germination), late traits (number of flowers or fruits, seeds/fruit or plant, or biomass), and cumulative fitness (when provided). For each comparison, we present the cumulative effect size for populations (or taxa) with and without a history of inbreeding. The cumulative effect size is essentially the difference in means (selfed individuals–outcrossed) for a particular trait divided by a pooled standard deviation for the two groups. These values are then pooled as a weighted sum across studies with similar mating histories. The cumulative effect size values are evaluated for homogeneity within groups (Q_W, same mating history) and between groups (Q_B). Since we do not expect a particular value for either the self or outcross groups, we used the mixed model, and therefore the cumulative effect size was corrected by a constant (for further details see 56).

RESULTS

General Patterns

While many studies found evidence for purging, others did not (Table 1). Overall, 20 studies observed a significant decrease in ID (δ) with a greater history of selfing; 29 found no such decrease; and 4 were inconclusive. The type of study

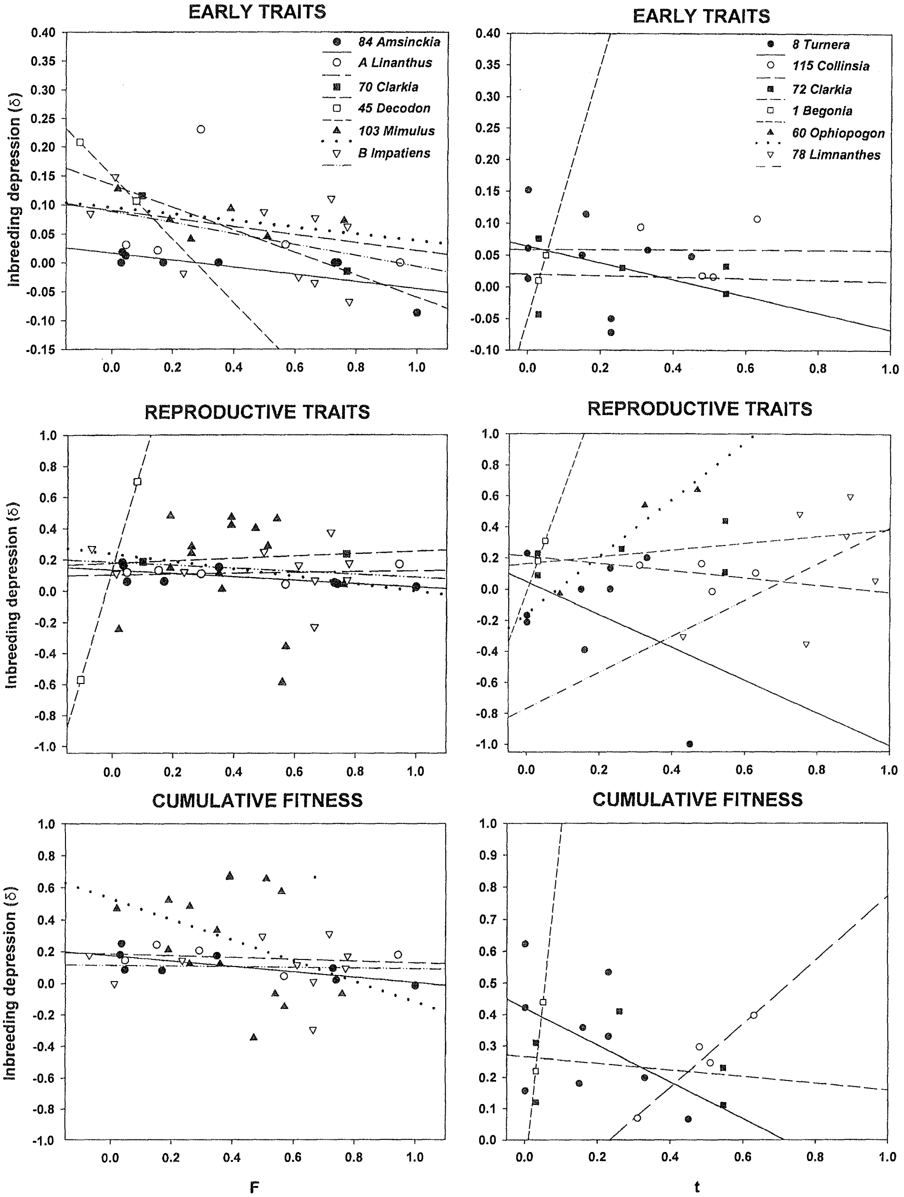

Figure 1 This series of graphs corresponds to the regression analysis in Table 2. Symbols represent populations within each study. Regression lines are shown for the individual studies. A negative slope with increasing values of *F* (left panels) indicates purging of mutational load, while a positive slope with increasing values of *t* (right panels) indicates purging. Legend indicates reference # except A = C Goodwillie, in review; B = DM Waller, unpub. data.

TABLE 2 Regression analyses: How the magnitude of inbreeding depression (ID) responds to mating history. Results are shown for each of the three ways to infer mating history. Regressions were performed on the $\log_{10}$ of population size. n = number of populations.

Reference number	Genes	Early traits			Survivorship			Reproductive traits			Cumulative fitness		
		n	slope	r^2	n	slope	r^2	n	Slope	r^2	n	slope	r^2
F *studies:*													
(45)	*Decodon*	2	−0.548	1.000	2	0.446	1.000	2	6.833	1.000			
(70)	*Clarkia*	2	−0.194	1.000	2	−0.187	1.000	2	0.075	1.000			
(84)	*Amsinckia*	8	-0.062^{*}	0.515				8	$-0.111^{0.06}$	0.466	8	-0.174^{*}	0.563
(103)	*Mimulus*	6	-0.057^{ns}	0.216				15	-0.240^{ns}	0.019	15	-0.653^{ns}	0.137
A	*Linanthus*	5	-0.069^{ns}	0.070	5	0.009^{ns}	0.054	5	0.023^{ns}	0.032	5	-0.060^{ns}	0.081
B	*Impatiens*	10	-0.095^{ns}	0.169				10	-0.099^{ns}	0.037	10	-0.025^{ns}	0.002
Overall		33	-0.102^{**}	0.222	9	-0.049^{ns}	0.097	42	-0.030^{ns}	0.001	38	-0.211^{ns}	0.063
t studies:													
(1)	*Begonia*	2	2.000	1.000	2	2.000	1.000	2	6.500	1.000	2	11.000	1.000
(8)	*Turnera*	9	-0.131^{ns}	0.084	9	-0.119^{ns}	0.322	9	-1.052^{ns}	0.186	9	-0.587^{ns}	0.247
(60)	*Ophiopogon*							3	1.840^{ns}	0.939			
(72)	*Clarkia*	5	-0.011^{ns}	0.004	5	-0.296^{ns}	0.223	5	0.219^{ns}	0.162	5	-0.105^{ns}	0.045
(78)	*Limnanthes*							5	1.161^{ns}	0.293			
(115)	*Collinsia*	4	-0.001^{ns}	0.000	2	−0.013	1.000	4	-0.230^{ns}	0.137	4	1.011^{*}	0.940
Overall		20	-0.013^{ns}	0.003	18	-0.074^{ns}	0.029	28	0.194^{ns}	0.029	20	-0.193^{ns}	0.073
Population size studies:													
(132)	*Salvia*	6	0.054^{ns}	0.159				6	0.011^{ns}	0.007			
(168)	*Scabiosa*				6	-0.013^{ns}	0.005	6	-0.073^{ns}	0.217	6	0.069^{ns}	0.092
Overall								12	-0.020^{ns}	0.014			

A. C Goodwillie, in review.

B. DM Waller, unpub. data.

only slightly affects the chance of finding declines in δ. Among the 36 multiple comparison studies (M), 14 produced evidence for purging, 19 did not, and 3 were inconclusive. Of the three studies that inferred ID from changes in F (R), one found a decline in genetic load, one did not, and one was inconclusive. The least frequent evidence for purging (5/14) occurred in studies involving successive generations of selfing within populations (as expected if small N_e limits purging in selfed lines). Those S studies with five generations of selfing (e.g., 7) usually found declines in δ, whereas similarly designed studies with 2–3 generations of selfing rarely did (e.g., 107, 121, 179).

The scale at which purging is examined only slightly affects the outcome. Of the 14 studies comparing species, 6 found support for purging, 7 did not, and 1 was inconclusive. Of the 21 among-population comparisons, 8 found reduced load in more inbred populations, 11 did not, and 2 were inconclusive. The least frequent evidence for purging (6/18) occurred in comparisons among lineages within a population, perhaps again reflecting diminished opportunities for purging nonlethal mutations in selfing lines.

Does Method of Inferring Mating History Matter?

Does the chance of finding evidence for purging vary with respect to what method is used to infer inbreeding history? The tallies here only include the M and R studies. One study used only population estimates of F, 6 studies used both F and outcrossing rate (t), 4 studies used just t, and 3 used Ritland's (139) method. Of these 14 studies, 9 found purging, 3 did not, and 2 were inconclusive. Studies that used both t and floral morphology or population size to determine mating history also did not find consistent purging (3 vs. 7). Those studies that used variation in floral morphology or breeding system to infer history also showed no particular pattern (5 vs. 4 and 1 inconclusive). None of the 5 studies that used population size to infer inbreeding history found evidence for purging. This may reflect inaccuracies in estimating N, variation in N over time, the inefficiency of purging, fixation of the load in small populations, or that N is simply a poor predictor of inbreeding.

Comparisons Among Taxa

Only 4 of 11 M studies comparing more and less inbred taxa found evidence for purging (18, 105, 133; C Goodwillie, in review). In *Phlox* and *Epilobium*, the cross × species interaction (or correlation of δ with mating history) was significant for early traits, indicating purging (105, 133). Similar studies of *Amphicarpaea* and *Linanthus* found evidence for the purging of mutations affecting later traits (reproduction or biomass) (134; C Goodwillie, in review). Three of the four studies that noted declines in δ for cumulative fitness in more inbred species found a significant cross × species effect (84; C Goodwillie, in review; 134). In one of these (84), however, the correlation of δ (for cumulative fitness) vs. $(1 - t)$ was not significant, leading them to conclude that purging was not significant. Our regression analysis of these data (δ vs. F) found a significant relationship, however (Table 2). ID for biomass declined in more selfing taxa of *Mimulus*, although this was not statistically analyzed (18). In a similar study, δ for five fitness traits tended to decline in the more inbred of 15 populations of four species in the *Mimulus guttatus* complex, but this relationship was inconstant and only significant for height (103). The other studies did not directly analyze the relationship between ID and mating history and did not show any evidence of purging.

Comparisons Among Populations

In the first study of this kind, Jain (78) compared seven populations of *Limnanthes alba* that differed widely in outcrossing rate (0.43–0.97), heterozygosity (0.12–0.27), and percentage of polymorphic loci (0.29–0.57). Although ID was significant in 4 of the 7 populations for several traits, he observed no association between ID and these indicators of inbreeding history among populations. In our overall comparison, 6 of 18 M studies comparing populations found evidence for purging (Table 1). Of the 17 that examined early traits, two studies found a significant decrease in δ in populations with a greater history of selfing (70, 86). Several reported nonsignificant cross × population interactions or correlations of δ with mating history for early traits (8, 45, 57, 60, 114, 132, 175). The other studies provided no support for purging (but no inbreeding depression for early traits was observed in 5 studies).

Of 12 M studies comparing ID for late (reproductive or biomass) traits among populations, seven tested directly for purging and none were significant. Two studies found significant variation for δ among populations, but no clear association with mating history (8) or population size (168). In four populations of *Collinsia heterophylla* with significant ID, regression analysis showed ID to decrease in more selfing populations (115). ID for reproductive traits (seed weight, seed set, and fruit set) declined in more selfing populations, although this was not tested statistically (*Clarkia*; 72).

Regression Results

Fourteen of the 27 M studies of taxa and populations provided sufficient information to analyze linear relationships between δ and F, t, or estimated population size (Figure 1, Table 2). If purging is effective, we expect δ to decline in more inbred populations (higher F) and to increase in populations with higher outcrossing rates (t) or larger populations. Overall, these trends are modest, with only one F and one t study showing individual significance (Table 2). In *Amsinckia*, δ's for both early and cumulative fitness traits decline in more inbred species and populations (84). While this was the only F study to be individually significant, enough studies showed declines in δ for early traits for the overall trend to be significant ($p < 0.01$; Table 2). This result suggests that purging may be more effective against mutations affecting early traits (75). In contrast, decreases in δ observed across studies were not large or consistent enough to be significant for survivorship, reproductive traits, or cumulative fitness (Figure 1; Table 2).

Evidence for purging is less evident in studies exploiting variation in outcrossing rates. Only about half the individual regressions find δ to increase with t as expected under the purging hypothesis, and most overall regressions were in the wrong direction (Table 2). The only significant individual regression (for cumulative fitness in *Collinsia heterophylla*) was positive (115) supporting the purging hypothesis. Only two studies used population size to infer mating history, and they did not show any pattern.

Meta-Analysis Results

For the 11 studies that provided sufficient data (M studies of taxa and populations), we applied meta-analyses aimed at testing whether ID regularly declines in more selfing/inbred groups, as reflected by a significant value of Q_B^* (Table 3). Overall tests revealed no consistent difference between the more and less inbred groups ("Over all studies" lines; Table 3). In fact, there appeared to be more ID in the more inbred groups for early and late traits, contrary to the prediction of purging. The variation within groups, however, is too great to demonstrate significance. Likewise, with only 7 populations from 2 studies, we cannot draw conclusions regarding cumulative fitness.

Meta-analyses also reveal little effect of life history (Table 3) despite the fact that tallies suggest that herbaceous perennials are less likely to purge mutational load than annuals (2 of 10 vs. 7 of 14 studies, respectively). One of 4 studies involving woody perennials found purging.

Of the three methods used to infer inbreeding history (t, F, or Other), only studies comparing populations that differ in t showed a significant difference between selfing and outcrossing populations (Table 3). This difference, however, again showed inbreeding populations to have greater ID than outcrossing populations, contrary to predictions of the purging hypothesis. Tallies of the M (taxa and population) studies revealed no pattern except that studies using population size to infer mating history found no evidence of purging.

Lineages Within Populations

Most within-population studies compared lineages that differ in mating system characters known or expected to affect the selfing rate. Four of these seven studies found evidence for purging. Only one of three studies that compared δ between females and hermaphrodites within gynodioecious species found a reduction in the load in hermaphrodites as expected if purging occurs swiftly in response to selfing in these lines. Del Castillo (39) found higher ID for fruit set and seed production in females in *Phacelia dubia*. Mutikainen & Delph (126), however, examined maternal family by cross effects for four fitness traits and found δ was approximately twice as high (0.3) in hermaphrodites as in females, contrary to the purging hypothesis. Ashman (4) found no relation. Two of three studies that used

TABLE 3 Results of meta-analyses, n = number of populations and 'cumulative effect size' refers to estimated level of inbreeding depression (δ)/pooled standard deviation.

			For Selfing Populations		For Outcrossing Populations		Significant evidence of mating history on purging?		
		n	Cumulative effect size	95% conf. int	Cumulative effect size	95% conf. int	Q_w	Q^*_B	
Early Fitness Traits:									
OVER ALL STUDIES		45	−0.486	−0.899 to −0.072	−0.350	−0.753 to 0.053	436.70	0.213	n.s.
Broken down by groups according to:	Trait[a]								
Life History	Ann	24	−0.696	−1.232 to −0.160	−0.538	−1.123 to 0.046	404.28	0.151	n.s.
	Per	21	−0.153	−1.295 to 0.989	−0.161	−1.145 to 0.823	22.94	0.0001	n.s.
Method of inferring population history	*t*	13	−1.457	−2.212 to −0.702	−0.469	−1.162 to 0.224	308.66	3.57	P = 0.059
	F	18	−0.189	−1.205 to 0.827	−0.331	−1.471 to 0.810	10.52	1.52	n.s.
	Other	14	−0.067	−0.339 to 0.205	−0.261	−0.443 to −0.078	19.44	0.53[b]	n.s.
Late Fitness Traits:									
OVER ALL STUDIES		45	−0.808	−1.188 to −0.428	−0.540	−0.911 to −0.169	345.28	0.974	n.s.
Broken down by groups according to:	Trait								
Life History	Ann	24	−0.700	−1.202 to −0.198	−0.312	−0.855 to 0.230	183.15	1.057	n.s.
	Per	21	−0.987	−1.609 to −0.365	−0.764	−1.301 to −0.228	141.19	0.283	n.s.
Method of inferring population history	*t*	19	−1.607	−2.276 to −0.938	−0.869	−1.425 to −0.313	188.42	2.76	n.s.
	F	18	−0.455	−1.115 to 0.205	−0.196	−0.938 to 0.546	91.05	0.351	n.s.
	Other	8	−0.664	−1.242 to −0.087	−0.596	−1.176 to −0.016	9.61	0.027	n.s.
Cumulative Fitness:									
OVER ALL STUDIES		7	−0.550	−1.000 to −0.101	−0.615	−1.021 to −0.208	3.40	0.04[b]	n.s.

[a] Traits Ann = Annual; Per = Perennial.

[b] Not corrected for mixed model analysis due to small uncorrected Q_B value.

[c] Studies included: Early traits-Overall (8, 18, 87, C Goodwillie, in review, DM Waller unpub. data, 103, 115, 132, 133, 175), Life history–annuals (18, 103, 115, 133, C Goodwillie in review, DM Waller unpub. data) and perennials (8, 87, 132, 133, 175), Method of inferring population history-*t* (8, 18, 115), *F* (103, C Goodwillie in review, DM Waller unpub. data) and other (87, 132, 133, 175). Reproductive traits-Overall (8, 18, 103, 115, 132, C Goodwillie in review, DM Waller unpub. data, 133, 168), Life history-annuals (18, 103, C Goodwillie in review, DM Waller unpub. data, 115) and perennials (8, 132, 133, 168), Method on inferring population history-*t* (8, 18, 115, 168), *F* (103, C Goodwillie in review, DM Waller unpub. data), and other (132, 133). Cumulative fitness studies-Overall (133, C Goodwillie in review).

anther-stigma distance to infer inbreeding history found evidence for purging (e.g., *Ipomoea purpurea*. where δ values for late traits and cumulative fitness decrease in lineages with less anther-stigma separation; S-M Chang, MD Rausher, in review). Interestingly, male fitness responds more than female fitness to inbreeding in this species. Unfortunately, male traits are only rarely measured in other studies.

One study compared the variable expression of self-incompatibility with variation in ID. In *Campanula rapunculoides*, different *S* genotypes vary in their expression of self-incompatibility, causing variable rates of selfing that parallel slight decreases in δ for early traits (169).

Effects of lineage are also evident in the many taxa and population studies where maternal family by cross interactions are significant (1, 70, 132, 133, 141). Such results reflect the potential for greater selfing rates to coevolve with purging within populations. Unfortunately, such interactions are not often quantified or investigated in relation to known differences in the mating history of maternal families.

Experimental Conditions

We found no relationship between the conditions under which experiments were conducted and the likelihood of finding evidence for purging. Fewer than half (7 of 19) of the greenhouse studies found decreased ID in more inbred populations or lineages. Of the few studies that used common garden (3) or field (2) assays, two showed reduced load, two did not, and the fifth was inconclusive. Of the five studies that used greenhouse and field or garden assays, 1 found decreased ID, 3 did not, and one was inconclusive. Johnston's (81) study, for example, found limited ID in two species of *Lobelia*, but not any systematic difference in the expression of ID in the field vs. the greenhouse. Holtsford & Ellstrand (72) found greater expression of ID in the greenhouse, but Eckert & Barrett (45) found more ID in the field.

Several greenhouse studies varied growing conditions in order to examine how ID varied with respect to conditions. Three of 8 studies found ID to depend on conditions, but none of these found conditions to affect the detection of purging (8, 17; C Goodwillie, in review). Thus, while the greenhouse conditions may better control environmental variance, they do not appear to increase the probability of finding purging.

DISCUSSION

How Major and Consistent a Force Is Purging?

While some studies indicate that while purging of the mutational load occurs in individual cases, purging does not appear to act consistently as a major force in natural populations (Table 1, Figure 1). We chose and categorized studies according to their experimental design, favoring those that attempted to directly test how a history of inbreeding affects the expression of inbreeding depression. The strongest studies compared many populations or lineages that varied in estimated rates of selfing or inbreeding (e.g. 8, 20, 73, 84, 91, 103; C Goodwillie, in review; DM Waller unpublished data). However, only one of these (C Goodwillie, in review) found significant evidence for purging. In other studies, ID sometimes appears to decline in more selfing populations, but the relationship is not significant (e.g. 84). In an extensive study involving several *Mimulus* spp., δ appeared to decline with greater selfing (higher F) across species and populations, but the relationship was not significant for reproductive traits or cumulative fitness (103). These inconsistent results parallel Darwin's (36) conclusion cited in the Introduction and appear to undermine some recent assertions that purging can be a powerful force in inbred populations.

Parallel studies in animal populations also reveal variable results (e.g. 98, 99, 150, 167). Ballou (6) recently assessed the extent of purging in 25 captive mammalian populations by comparing levels of ID in the inbred progeny of parents with and without ancestral inbreeding. Inbreeding depression for neonatal survival declined in 15 of 17 species, but the decline was only statistically significant for the Sumatran tiger. While the combined data strongly support the existence of purging, the median effect was small (2% at $F = 0.25$) and similar to the slope observed in the F studies for early traits (=0.102) (Table 2). These data also suggest that purging affects early survival more than later traits.

What Limits Purging?

Why should reductions in the genetic load in more inbred groups be difficult to detect? Authors who did not find evidence for purging often volunteer explanations. For example, some suggest that experimental designs lack sensitivity for detecting changes in ID. Others suggest that their populations may be too similar in mating history or population size to provide good evidence for purging (e.g. 115, 132, 168). Others argue that long-lived species may be unlikely to shed their genetic load (e.g. 45). Finally, many argue that purging may be limited by its genetic basis (mildly deleterious or partially penetrant alleles, epistasis, etc—106, 126; JH Willis, in review; 128). Let us consider these in turn.

Experimental design—Does experimental design affect the likelihood of finding purging? Studies clearly differed greatly in design and setting in ways that could affect the detection of purging. Holtsford & Ellstrand (72), for example, found evidence that more selfed populations in the lathhouse purged some of their mutational load but did not in similar experiments in the field. We found no consistent patterns over all the studies, however.

The accuracy with which we can infer mating history may also influence our ability to test its effect on purging. Because outcrossing rates can vary widely among years in response to fluctuating ecological conditions (e.g. 45), we expected t to be a poor predictor of purging. Likewise, N may fluctuate widely and unpredictably over time, particularly in short-lived plants. The inbreeding coefficient

(F) appears better suited to comparative studies in that it often reflects the integrated effects of inbreeding history due to both small N and the mating system. This may explain why only the F studies provided overall evidence for purging (of early traits—Table 2). Such effects were not evident in the meta-analyses, however, suggesting few clear differences in the likelihood of finding purging among methods. Studies involving limited contrasts among mating histories are constrained in their ability to detect purging (e.g. 115), but even studies comparing populations across a broad range of mating histories did not consistently find evidence for purging. Of the studies that used t to infer inbreeding history, only one showed significant increases in δ in more outcrossed populations (Figure 1). While variable selfing rates likely affect individual studies, the lack of any overall pattern across studies suggests that purging is an inconstant force.

Life history traits influence the extent of purging—Perennials were less likely to decrease their genetic load than annuals (17% vs. 50%). If the purging of mildly deleterious mutations occurs slowly in mixed mating populations (22, 102), such results are not surprising.

Effectiveness of purging on early vs. later traits—Husband & Schemske (76) compared levels of ID in selfing and outcrossing species and found lower ID for early traits in the selfers. We therefore expected to find more purging for early traits. While the regression analyses reveal some purging of ID for early traits in the F studies (Table 2), no such pattern emerged from the meta-analysis. In some cases, purging appeared to occur for later traits (e.g. 8, 20, 72). Whereas species characteristics and experimental design obviously influence our ability to detect ID and thus purging, collectively these results suggest that purging may be limited in its extent and consistency.

The genetic basis of inbreeding depression—The extent to which purging occurs in more inbred populations strongly depends on genetic details including the distribution of selective effects, the degree of dominance, and interactions among loci (see Theoretical Background). Mutations that are even slightly penetrant in the heterozygote are far more likely to be eliminated as heterozygotes, greatly reducing the degree to which inbreeding can reduce the load (26). Similarly, although selection can act swiftly to eliminate recessive lethal mutations exposed upon inbreeding, selection acts far more slowly against mildly deleterious recessive mutations. Such selection also becomes ineffective in small populations for mutations below a threshold effect, limiting the extent of purging. Limitations may also exist in the form of selective interference among loci segregating for deleterious mutations.

Thus, variability in the extent of purging found among studies may reflect variation in dominance, mutational effects, and the history of selection. Recent empirical studies suggest that slightly deleterious mutations with intermediate degrees of dominance may contribute the most to ID (43, 45, 83; JH Willis, in review; 103). Many authors now explain failures to find purging as the likely consequence of slightly deleterious mutations contributing most of the genetic load (94; JH Willis; in review, 128). Mutation accumulation studies to document rates of mutation and the distribution of mutational effects are needed to bolster this conclusion.

Implications for Mating System Evolution

Initial dynamic models of mating system evolution suggested that periodic bottlenecks or pollinator failures could enforce selfing in a way that could purge mutational load due to recessive alleles of major effect (102). We expect such purging to cause runaway selection for ever-higher rates of selfing. Exclusively selfed populations and species are quite rare, however (36, 77, 172), suggesting that purging may not regularly act as an efficient force or that it may be countered in real populations by mutation accumulation in selfed or bottlenecked lines. This review reveals only limited evidence for purging and, in a few cases, evidence that load may even increase in more inbred populations or lines. If small or more inbred populations do not efficiently and regularly purge their mutational load, the potential for runaway selection for increased selfing is correspondingly limited. The low fraction of purging observed in woody perennials (1 of 4 studies) lends some support to the conjecture that there may not be sufficient time for purging to occur under some circumstances (102). However, the many populations that retain intermediate selfing rates (e.g. 71, 158) suggest evolutionary stability.

Ecological factors can also favor selfing for reasons unrelated to reduced ID (e.g., lower resource costs of selfing, pollinator limitation, etc–67, 163). Pollen discounting (reduced availability of pollen for outcrossing due to selfing) can favor mixed mating as well as pollen limitation (64, 163). Limited pollen and seed dispersal will also tend to structure local populations (49, 182) in a way that increases biparental inbreeding (66). Biparental inbreeding mimics selfing in its effects on both parent-offspring relatedness and purging and could help maintain mixed mating systems (162, 183).

These models indicate that the evolution of mixed mating systems depends on more than levels of ID. Johnston (82) and Schoen examined the joint effects of pollen discounting and ID on the mating system. Mixed mating is favored when an increase in selfing causes declines in the proportion of outcrossed ovules fertilized or a decrease in male fitness via pollen export. They also found evolutionarily stable intermediate selfing rates under conditions of low mutation rates and moderate dominance coefficients. Pollen discounting is apparently sufficient in *Ipomoea purpurea* to prevent more selfing lineages from becoming fixed in populations (S-M Chang, MD Rausher, in review). Fixation of deleterious mutations over successive generations of selfing will also counter ever-higher selfing rates (112).

Most studies that checked found evidence for significant family (or lineage) by cross-type interactions (e.g. 40, 129), indicating variation in how lines respond to inbreeding. Such variation could be maternal effects. Alternatively, any of several direct genetic effects could occur. First, the mutation of major effect will increase variation among lines; this could be increased further by drift. Second, variation in the amount of inbreeding among lineages implies that the recovery of fitness upon wide outcrossing will be greater for more inbred lines. This variation also contributes to the differences in heterozygosity among lines referred to as identity disequilibrium (10, 95). Third, variation in inbreeding history may cause purging in more inbred lines. Thus, the increased variance for δ among lines observed by Dudash et al (44) after five generations of selfing in *Mimulus guttatus* could reflect both differential purging and mutation fixation among lines. A recent model by Schultz & Willis (149) predicts that random genetic variation of mutations will contribute more to variation in the load among lines than mating history. This latter source of variation was predicted to be too small to reliably detect.

Note that these mechanisms make different predictions regarding possible associations between breeding history and ID. If random mutation and drift cause lines to differ, we expect little consistent relationship between ID and breeding history among lineages. If differential recovery of fitness is responsible for the differences among lines, we expect estimated levels of ID to be greater for more inbred lines (assuming parents are not randomly mated before testing ID). And if purging accounts for differences among lines, we expect ID to decrease in more inbred lines. Associations between load and mating system loci also influence mating system evolution (16, 163). ID declined significantly in more inbred lines in 6 of 18 lineage comparisons (Table 1), including two studies that inferred mating history using anther-stigma separation (S-M Chang, MD Rausher, in review; N Takebayashi, LF Delph, in review). This seems surprising in light of the Schultz and Willis prediction.

Implication for Small Population Conservation

Some have advocated deliberate inbreeding to purge the mutational load in captive populations (e.g. 161). Such programs appear risky, however, in that some mutations will be fixed as purging is occurring, particularly in small populations (110). The tendency for inbreeding to fix deleterious mutations has been noted in some empirical work (e.g. 44). Computer simulations of ID in large vs. small populations further indicate that there is increased variance in the expression of ID in small populations (57). Not detecting inbreeding depression in a small or inbred population does not necessarily demonstrate that purging has occurred. Fixation of the load can also cause ID to decline (by depressing outcrossed fitness rather than by raising selfed fitness). Similarly, selection occurring in multigeneration experiments can raise the fitness of both inbred and outbred progeny to similar extents, causing δ to decline even though no purging of mutations has occurred (as in corn—see 9). Thus, to accurately determine the extent to which small or inbred populations have shed or fixed mutations, one must compare absolute fitnesses (and, ideally, evaluate the progeny from crosses between populations). The studies reviewed here suggest that intentional inbreeding to promote purging could result primarily in temporary reductions in lethal and semilethal mutations at the cost of fixing many mildly deleterious mutations that could eventually erode population fitness.

Recommendations for Future Research

Future studies designed to assess purging would be most useful if they routinely incorporated a number of design and reporting features:

1. Studies of ID should examine multiple fitness traits that extend over the full life-cycle of the plant. This will ensure that one can judge the relative degree to which ID for both early and late traits may be purged.
2. Estimates of ID based on comparisons of progeny fitness should statistically control for maternal effects (e.g., differences in seed size) that can appreciably affect progeny performance and cloud genetic effects of inbreeding between populations.
3. Successive inbreeding studies should pay particular attention to male fitness components such as pollen number and viability. Most studies only quantify ID for female fitness, although male fitness can be significantly influenced by inbreeding (17, 80; JH Willis, in review). This lack of information is particularly critical if ID effects differ between the sexes (S-M Chang, MD Rausher, in review).
4. Comparative studies should incorporate appropriate controls and report more than simply the levels of inbreeding depression observed. In particular, we urge authors to report absolute selfed and outcrossed fitnesses and inbreeding load (the number of lethal equivalents).
5. Studies should routinely and clearly report sample sizes, variances (or S.D.), and means. Such data are needed to compile meta-analyses of the data.
6. Inbreeding studies should routinely test for family or line by cross-type interactions and whenever possible test for relations between levels of ID and any known differences in the breeding history of those lines.

CONCLUSIONS

Like Darwin, late twentieth century researchers usually take steps to make controlled and unbiased comparisons, measure several successive components of fitness, combine these to calculate overall estimates of inbreeding decline, and apply state-of-the-art statistical analyses. Some also follow Darwin in considering the effects of growing conditions on the expression of ID and documenting the cumulative effects of multiple generations of selfing. In doing such work, we should also attempt to emulate Darwin's work in terms of its careful execution, concern for male fitness traits, and attention to potentially confounding variables.

The efficiency with which selection can purge populations of their genetic load depends on the size of mutational effects, the degree of dominance, interactions among loci, the breeding system, and population size. The many theoretical impediments to efficient purging suggest that we should not expect it to occur universally in natural or captive populations. Thus, we should not be surprised that comparisons like Darwin's (36), Ballou's (6), and these 52 plant studies reveal only limited support for purging. Whereas these studies vary considerably in species examined, how inbreeding history was inferred (F, outcrossing rate, etc), how ID was evaluated, and the particular traits measured, these factors were of only limited utility in predicting when purging would occur. While other factors may yet better predict patterns of purging, these results suggest that purging may not occur even in consistently inbred lineages.

The variation we observe among studies probably reflects both variation in experimental design and conditions and real variation among populations and taxa in the extent to which purging occurs. Thus, although populations are capable of purging under some circumstances, purging appears neither consistent nor effective enough to reliably reduce ID in small and inbred populations.

ACKNOWLEDGMENTS

We are indebted to C Bristow for assistance in graphics, reading many versions of this paper and chasing down references in the library. We are grateful to the following authors that provided us with copies of unpublished manuscripts and papers: S Chang, L Delph, C Goodwillie, A Stephenson, and J Willis. We particularly wish to thank J Crow, J Dole, M Dudash, C Fenster, and D Charlesworth for their insightful comments on an earlier version of this paper. DB thanks D Andow for his encouragement to undertake this project.

LITERATURE CITED

1. Ågren J, Schemske DW. 1993. Outcrossing rate and inbreeding depression in two annual monoecious herbs, *Begonia hirsuta* and *B. semiovata*. *Evolution* 47:125–35
2. Aide TM. 1986. The influence of wind and animal pollination on variation in outcrossing rates. *Evolution* 40:434–35
3. Aldrich PR, Hamrick JL. 1998. Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science* 281: 103–5
4. Ashman TL. 1992. The relative importance of inbreeding and maternal sex in determining progeny fitness in *Sidalcea oregana* ssp. *spicata*, a gynodioecious plant. *Evolution* 46:1862–74
5. Baker HG. 1955. Self-compatibility and establishment after 'long-distance' dispersal. *Evolution* 9:347–48
6. Ballou JD. 1997. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *J. Hered.* 88: 169–78
7. Barrett SCH, Charlesworth D. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* 352:522–24
8. Belaoussoff S, Shore JS. 1995. Floral correlates and fitness consequences of mating-system variation in *Turnera ulmifolia*. *Evolution* 49:545–56
9. Benson DL, Hallauer AR. 1994. Inbreeding depression rates in maize populations before and after recurrent selection. *J. Hered.* 85:122–28

10. Brown AHD, Feldman MW. 1981. Population structure of multilocus associations. *Proc. Natl. Acad. Sci. USA* 78: 5913–16
11. Brunet J, Eckert C. 1998. Effects of floral morphology and display on outcrossing in blue columbine, *Aquilegia caerulea* (Ranunculaceae). *Func. Ecol.* 12:596–606
12. Bryant EH, Meffert LM. 1990. Multivariate phenotypic differentiation among bottleneck lines of housefly. *Evolution* 44: 660–68
13. Bulmer MG. 1980. *The Mathematical Theory of Quantitative Genetics.* New York: Oxford Univ. Press. 254 pp.
14. Burger R, Lynch M. 1995. Evolution and extinction in a changing environment: a quantitative-genetic analysis. *Evolution* 49:151–63
15. Byers DL. 1998. Effect of cross proximity on progeny fitness in a rare and a common species of *Eupatorium* (Asteraceae). *Am. J. Bot.* 85:644–53
16. Campbell RB. 1986. The interdependence of mating structure and inbreeding depression. *Theor. Pop. Biol.* 30:232–44
16a. Carr DE, Fenster CB. 1994. Levels of genetic variation and covariation for *Mimulus* (Scrophulariaceae) floral traits. *Heredity* 72:606–18
17. Carr DE, Dudash MR. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* 75:437–45
18. Carr DE, Dudash MR. 1996. Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. *Am. J. Bot.* 83:586–93
19. Carr DE, Dudash MR. 1997. The effects of five generations of enforced selfing on potential male and female function in *Mimulus guttatus. Evolution* 51:1797–807
20. Carr DE, Fenster CB, Dudash MR. 1997. The relationship between mating-system characters and inbreeding depression in *Mimulus guttatus. Evolution* 51:363–72
21. Charlesworth B. 1980. The cost of sex in relation to mating system. *J. Theor. Biol.* 84:655–72
22. Charlesworth B, Charlesworth D. 1998. Some evolutionary consequences of deleterious mutations. *Genetica* 102/103:3–19
23. Charlesworth B, Morgan MT, Charlesworth D. 1991. Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genet. Res. (Camb.)* 57:177–94
24. Charlesworth B, Nordborg M, Charlesworth D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genet. Res.* 70:155–74
25. Charlesworth D. 1991. The apparent selection on neutral gene marker loci in partially inbreeding populations. *Genet. Res.* 57:159–75
26. Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237–68
27. Charlesworth D, Charlesworth B. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* 44:870–88
28. Charlesworth D, Lyons EE, Litchfield LB. 1994. Inbreeding depression in two highly inbreeding populations of *Leavenworthia. Proc. R. Soc. Lond. Ser. B* 258: 209–14
29. Charlesworth D, Morgan MT, Charlesworth B. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44:1469–89
30. Charlesworth D, Morgan MT, Charlesworth B. 1992. The effect of linkage and population size on inbreeding depression

due to mutational load. *Genet. Res.* 59: 49–61
31. Crow JF. 1948. Alternative hypotheses of hybrid vigor. *Genetics* 33:477–87
32. Crow JF. 1987. Muller, Dobzhansky and overdominance. *J. Hist. Biol.* 20:351–80
33. Crow JF. 1993. Mutation, mean fitness, and genetic load. *Oxford Ser. in Evol. Biol.* 9:3–42
34. Crow JF. 1999. The rise and fall of overdominance. *Plant Breed. Rev.* 17:225–57
35. Crow JF, Kimura M. 1970. *An Introduction to Population Genetics Theory*. Minneapolis, MN: Burgess. 591 pp.
36. Darwin C. 1876. *The Effects of Cross and Self Fertilization in the Vegetable Kingdom.* London: J. Murray
37. Darwin C. 1877. *Different Forms of Flowers of Plants of the Same Species*. London: J. Murray. 352 pp.
38. Davenport CB. 1908. Degeneration, albinism and inbreeding. *Science* 28:454–55
39. del Castillo RF. 1993. Consequences of male sterility in *Phacelia dubia. Evol. Trends Plants* 7:15–22
40. del Castillo RF. 1998. Fitness consequences of maternal and non-maternal components of inbreeding in the gynodioecious *Phacelia dubia. Evolution* 52:44–60
41. Dole J, Ritland K. 1993. Inbreeding depression in two *Mimulus* taxa measured by multigenerational changes in the inbreeding coefficient. *Evolution* 47:361–73
42. Dudash MR. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44:1129–39
43. Dudash MR, Carr DE. 1998. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* 393:682–84
44. Dudash MR, Carr DE, Fenster CB. 1997. Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. *Evolution* 51:54–65
45. Eckert CG, Barrett SCH. 1994. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution* 48:952–64
46. Eyre-Walker A, Keightley PD. 1999. High genomic deleterious mutation rates in hominids. *Nature* 397:344–47
47. Falconer DS, Mackay TFC. 1996. *Introduction to Quantitative Genetics*,. Essex, England: Longman Group. 464 pp. 4th ed
48. Falk DA, Holsinger KE, eds. 1991. *Genetics and Conservation of Rare Plants*, New York: Oxford Univ. Press. 283 pp.
49. Fenster CB. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) I. Gene dispersal. *Evolution* 45:398–409
50. Fenster CB. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae) II. Gene establishment. *Evolution* 45:410–22
51. Fisher RA. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.* 11:53–63
52. Franklin IR. 1980. Evolutionary change in small populations. In *Conservation Biology: An Evolutionary-Ecological Perspective*, ed. ME Soule, BA Wilcox, pp. 135-49. Sunderland, MA: Sinauer
52a. Fry JD, Keightley PD, Heinsohn SL, Nuzhdin SV. 1999. New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA* 96:574–79
53. Fu YB, Ritland K. 1994. Marker-based inferences about fecundity genes contributing to inbreeding depression in *Mimulus guttatus. Genome* 37:1005–10
54. Gabriel W, Lynch M, Bürger R. 1993. Muller's ratchet and mutational meltdowns. *Evolution* 47:1744–57
55. Gilpin M, Hanski I, eds. 1991. *Metapopulation Dynamics: Empirical and Theoretical Investigations*, New York: Academic Press. 336 pp.
56. Gurevitch J, Hedges LV. 1993. Meta-

analysis: combining the results of independent experiments. In *Design and Analysis of Ecological Experiments*, eds. SM Scheiner, J Gurevitch, pp. 378–98. New York: Chapman & Hall

57. Hauser TP, Damgaard C, Loeschcke V. 1994. Effects of inbreeding in small plant populations: expectations and implications for conservation. In *Conservation Genetics*, eds. V Loeschcke, J Tomiuk, SK Jain, pp. 115–29. Basel, Switzerland: Birkhäuser Verlag
58. Hauser TP, Loeschcke V. 1994. Inbreeding depression and mating-distance dependent offspring fitness in large and small populations of *Lychnis flos-cuculi* (Caryophyllaceae). *J. Evol. Biol.* 7:609–22
59. Hauser TP, Loeschcke V. 1995. Inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae): effects of different levels of inbreeding. *J. Evol. Biol.* 8:589–600
60. He TH, Rao GY, You RL, Ge S. 1998. Mating system of *Ophiopogon xylorrhizus* (Liliaceae), an endangered species in southwest China. *Int. J. Plant Sci.* 159: 440–45
61. Hedrick PW. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73:363–72
62. Heller R, Smith JM. 1978. Does Muller's ratchet work with selfing? *Genet. Res. (Camb.)* 32:289–93
63. Hill WG, Robertson A. 1968. The effects of inbreeding at loci with heterozygote advantage. *Genetics* 60:615–28
64. Holsinger K. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat.* 138:606–22
65. Holsinger K. 1992. Ecological models of plant mating systems and the evolutionary stability of mixed mating systems. In *Ecology and Evolution of Plant Reproduction*, ed. R Wyatt, pp. 169–91. New York: Chapman & Hall
66. Holsinger KE. 1986. Dispersal and plant mating systems: the evolution of self-fertilization in subdivided populations. *Evolution* 40:405–13
67. Holsinger KE. 1988. Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. *Evolution* 42: 1235–44
68. Holsinger KE. 1991. Inbreeding depression and the evolution of plant mating systems. *Trends Evol. Ecol.* 6:307–8
69. Holsinger KE, Feldman MW, Christainsen FB. 1984. The evolution of self-fertilization in plants: a population genetic model. *Am. Nat.* 124:446–53
70. Holtsford TP. 1996. Variation in inbreeding depression among families and populations of *Clarkia tembloriensis* (Onagraceae). *Heredity* 76:83–91
71. Holtsford TP, Ellstrand NC. 1989. Variation in outcrossing rate and population genetic structure of *Clarkia tembloriensis* (Onagraceae). *Theoret. Appl. Genet.* 78: 480–88
72. Holtsford TP, Ellstrand NC. 1990. Inbreeding effects in *Clarkia tembloriensis* (Onagraceae) populations with different natural outcrossing rates. *Evolution* 44:2031–46
73. Holtsford TP, Ellstrand NC. 1992. Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* 46:216–25
74. Houle D, Hoffmaster DK, Assimacopoulos S, Charlesworth B. 1992. The genomic mutation rate for fitness in *Drosophila. Nature* 359:58–60
75. Husband BC, Schemske DW. 1995. Magnitude and timing of inbreeding depression in a diploid population of *Epilobium angustifolium* (Onagraceae). *Heredity* 75:206–15
76. Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70
77. Jain SK. 1976. The evolution of inbreeding in plants. *Annu. Rev. Ecol. Syst.* 7:469–95
78. Jain SK. 1978. Breeding system in *Lim-*

nanthes alba: several alternative measures. *Am. J. Bot.* 65:272–75

79. Jinks JL. 1983. Biometrical genetics of heterosis. In *Heterosis, Reappraisal of Theory and Practice*, ed. R Frankel. New York: Springer Verlag
80. Jóhannsson MH, Gates MJ, Stephenson AG. 1998. Inbreeding depression affects pollen performance in *Cucurbita texana*. *J. Evol. Biol.* 11:579–88
81. Johnston MO. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. *Evolution* 46:688–702
82. Johnston MO. 1998. Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* 102/103:267–78
83. Johnston MO, Schoen DJ. 1995. Mutation rates and dominance levels of genes affecting total fitness in two angiosperm species. *Science* 267:226–29
84. Johnston MO, Schoen DJ. 1996. Correlated evolution of self-fertilization and inbreeding depression: an experimental study of nine populations of *Amsinckia* (Boraginaceae). *Evolution* 50:1478–91
85. Jones DF. 1917. Dominance of linked factors as a means of accounting for heterosis. *Genetics* 2:466–79
86. Kärkkäinen K, Koski V, Savolainen O. 1996. Geographical variation in the inbreeding depression of scots pine. *Evolution* 50:111–19
87. Karron JD. 1989. Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of *Astragalus* (Fabaceae). *Am. J. Bot.* 76:331–40
88. Karron JD, Jackson RT, Thumser NN, Schlicht SL. 1997. Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther-stigma separation. *Heredity* 79:365–70
89. Keightley PD. 1994. The distribution of mutation effects on viability in *Drosophila melanogaster*. *Genetics* 138:1315–22
90. Kempthorne O. 1957. *An Introduction to Genetic Statistics*. New York: John Wiley
91. Kennington WJ, James SH. 1997. The effect of small population size on the mating system of a rare clonal mallee, *Eucalyptus argutifolia* (Myrtaceae). *Heredity* 78:252–60
92. Klekowski EJ. 1988. *Mutation, Developmental Selection, and Plant Evolution*. New York: Columbia Univ. Press. 373 pp.
93. Klekowski EJ, Lowenfeld R, Hepler PK. 1994. Mangrove genetics. II. Outcrossing and lower spontaneous mutation rates in Puerto Rican rhizophora. *Int. J. Plant Sci.* 155:373–81
94. Koelewijn HP. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution* 52:692–702
95. Kohane I, Kidwell JF. 1983. Effect of selection, mutation, and linkage on the equilibrium structure of selfing systems. *J. Hered.* 74:175–80
96. Kohn JR, Biard JE. 1995. Outcrossing rates and inferred levels of inbreeding depression in gynodioecious *Cucurbita foetidissima* (Cucurbitaceae). *Heredity* 75:77–83
97. Kondrashov AS, Houle D. 1994. Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. *Proc. R. Soc. London Ser. B* 258:221–27
98. Lacy RC, Alaks G, Walsh A. 1996. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution* 50:2187–2200
99. Lacy RC, Ballou JD. 1998. Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution* 52:900–9
100. Lande R. 1994. Risk of population extinction from fixation of new deleterious mutations. *Evolution* 48:1460–69
101. Lande R. 1995. Mutation and conservation. *Conserv. Biol.* 9:782–91

102. Lande R, Schemske DW. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic model. *Evolution* 39:24–40
103. Latta R, Ritland K. 1994. The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution* 48:806–17
104. Latter BDH, Robertson A. 1962. The effects of inbreeding and artificial selection on reproductive fitness. *Genet. Res.* 3:110–38
105. Levin DA. 1989. Inbreeding depression in partially self-fertilizing *Phlox*. *Evol.* 43: 1417–23
106. Levin DA. 1991. The effect of inbreeding on seed survivorship in *Phlox*. *Evol.* 45:1047–49
107. Levin DA, Bulinska-Radomska Z. 1988. Effects of hybridization and inbreeding on fitness in *Phlox*. *Am. J. Bot.* 75:1632–39
108. Lloyd DG. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* 113:67–79
109. Lorenc E. 1980. Analysis of fertility in inbred lines of mice derived from populations differing in their genetic load. *Zeierzeta Lab* 17:3–16
110. Lynch M, Conery J, Burger R. 1995. Mutation accumulation and the extinction of small populations. *Am. Nat.* 146:489–518
111. Lynch M, Gabriel W. 1990. Mutation load and the survival of small populations. *Evolution* 44:1725–37
112. Lynch M, Walsh B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Assoc. 980 pp.
113. MacNeil MD, Kress DD, Flower AE, Blackwell RL. 1984. Effects of mating system in Japanese quail 2. Genetic parameters, response and correlated response to selection. *Theor. Appl. Genet.* 67:407–12
114. Mahy G, Jacquemart AL. 1998. Mating system of *Calluna vulgaris*: self-sterility and outcrossing estimates. *Can. J. Bot.* 76: 37–42
115. Mayer SS, Charlesworth D, Meyers B. 1996. Inbreeding depression in four populations of *Collinsia heterophylla* Nutt (Scrophulariaceae). *Evolution* 50:879–91
116. Maynard Smith J. 1978. *The Evolution of Sex*. New York: Cambridge Univ. Press
117. McCall C, Waller DM, Mitchell-Olds T. 1994. Effects of serial inbreeding on fitness components in *Impatiens capensis*. *Evolution* 48:818–27
118. Menges E. 1991. Seed germination percentage increases with population size in a fragmented prairie species. *Conserv. Biol.* 5:158–64
119. Mitchell-Olds ST, Waller DM. 1985. Relative performance of selfed and outcrossed progeny in *Impatiens capensis*. *Evolution* 39:533–44
120. Mitton JB, Grant MC. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annu. Rev. Ecol. Syst.* 15:479–99
121. Molina-Freaner F, Jain SK. 1993. Inbreeding effects in a gynodioecious population of the colonizing species *Trifolium hirtum* All. *Evolution* 47:1472–79
122. Montalvo AM. 1994. Inbreeding depression and maternal effects in *Aquilegia caerulea*, a partially selfing plant. *Ecology* 75:2395–2409
123. Morton NE, Crow J.F. Muller H.J. 1956. An estimate of the mutational damage in man from data on consanguineous marriages. *Proc. Natl. Acad. Sci. USA* 42: 855–63
124. Mukai T, Chigusa SI, Mettler LE, Crow JF. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* 72:335–55
125. Muller HJ. 1950. Our load of mutations. *Am. J. Human Genet.* 2:111–76
126. Mutikainen P, Delph LF. 1998. Inbreeding depression in gynodioecious

Lobelia siphilitica: among-family differences override between-morph differences. *Evolution* 52:1572–82

127. Nagylaki T. 1976. A model for the evolution of self fertilization and vegetative reproduction. *J. Theor. Biol.* 58:55–58
128. Nason JD, Ellstrand NC. 1995. Lifetime estimates of biparental inbreeding depression in the self-incompatible annual plant *Raphanus sativus*. *Evolution* 49:307–16
129. Norman JK, Sakai AK, Weller SG, Dawson TE. 1995. Inbreeding depression in morphological and physiological traits of *Schiedea lydgatei* (Caryophyllaceae) in two environments. *Evolution* 49:297–306
130. Ohta T. 1971. Associative overdominance caused by linked detrimental mutations. *Genet. Res.* 18:277–86
131. Otto SP, Whitlock MC. 1997. The probability of fixation in populations of changing size. *Genetics* 146:723–33
132. Ouborg NJ, van Treuren R. 1994. The significance of genetic erosion in the process of extinction. IV. Inbreeding load and heterosis in relation to population size in the mint *Salvia pratensis*. *Evolution* 1994: 996–1008
133. Parker IM, Nakamura RR, Schemske DW. 1995. Reproductive allocation and the fitness consequences of selfing in two sympatric species of *Epilobium* (Onagraceae) with contrasting mating systems. *Am. J. Bot.* 82:1007–16
134. Parker MA. 1985. Local population differentiation for compatibility in an annual legume and its host specific fungal/pathogen. *Evolution* 39:713–23
135. Ralls K, Ballou JD. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. *Conserv. Biol.* 2:185–93
136. Ramsey M, Vaughton G. 1998. Effect of environment on the magnitude of inbreeding depression in seed germination in a partially self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae). *Int. J. Plant Sci.* 159:98–104
137. Ribble DO, Miller JS. 1992. Inbreeding effects among inbred and outbred laboratory colonies of *Peromyscus maniculatus*. *Can. J. Zool.* 70:820–24
138. Ritland K, S. Jain. 1981. A model for the estimation of outcrossing rate and gene frequencies based on independent loci. *Heredity* 47:35–52
139. Ritland K. 1990. Inferences about inbreeding depression based on changes of the inbreeding coefficient. *Evolution* 44: 1230–41
140. Rumball W, Franklin IR, Frankham R, Sheldon BL. 1994. Decline in heterozygosity under full-sib and double first-cousin inbreeding in *Drosophila melanogaster*. *Genetics* 136:1039–49
141. Sakai AK, Karoly KK, Weller SG. 1989. Inbreeding depression in *Schiedea globosa* and *S. salicaria* (Caryophyllaceae), subdioecious and gynodioecious Hawaiian species. *Am. J. Bot.* 76:437–44
142. Schemske D. 1983. Breeding system and habitat effects on fitness components in three neotropical *Costus* (Zingiberaceae). *Evolution* 37:523–39
143. Schemske DW, Lande R. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52
144. Schmitt J, Ehrhardt DW. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. *Evolution* 44:269–78
145. Schoen DJ. 1983. Relative fitnesses of selfed and outcrossed progeny in *Gilia achilleifolia* (Polemoniaceae). *Evolution* 37:292–301
146. Schoen DJ, Johnston MO, L'Heureux AM, Marsolais JV. 1997. Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). *Evolution* 51:1090–99
147. Schoen DJ, Morgan MT, Bataillon T. 1996. How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Philos. Trans. R. Soc. Lond. Ser. B.* 351:1281–90
148. Schultz ST, Lynch M. 1997. Mutation

and extinction: the role of variable mutational effects, synergistic epistasis, beneficial mutations, and degree of outcrossing. *Evolution* 51:1363–71

149. Schultz ST, Willis JH. 1995. Individual variation in inbreeding depression: the roles of inbreeding history and mutation. *Genetics* 141:1209–23
150. Sharp PM. 1984. The effect of inbreeding on competitive male-mating ability in *Drosophila melanogaster*. *Genetics* 106:601–12
151. Shields WM. 1982. *Philopatry, Inbreeding, and the Evolution of Sex*. Albany, NY: State Univ. of New York Press
152. Simberloff D. 1988. The contribution of population and community biology to conservation science. *Annu. Rev. Ecol. Syst.* 19:473–511
153. Simmons MJ, Crow JF. 1977. Mutations affecting fitness in *Drosophila* populations. *Annu. Rev. Genetics* 11:49–78
154. Sorensen FC. 1969. Embryonic genetic load in coastal Douglas-fir, *Pseudotsuga menziesii*. *Am. Nat.* 103:389–98
155. Soulé ME. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. In *Conservation Biology*, ed. ME Soulé, BA Wilcox, pp. 151–69. Sunderland, MA: Sinauer
156. Stebbins GL. 1957. Self fertilization and population variability in the higher plants. *Am. Midl. Nat.* 91:337–54
157. Stephenson AG, Winsor JA, Richardson TE, Singh A, Kao T-H. 1992. Effects of style age on the performance of self and cross pollen in *Campanula rapunculoides*. In *Angiosperm Pollen and Ovules: Basic and Applied Aspects*, eds. D Mulchay, G Bergamini-Mulchay, E Ottaviano, pp. 1-6. New York: Springer-Verlag
158. Stewart SC. 1994. Genetic constraints on mating system evolution in the cleistogamous annual *Impatiens pallida*: inbreeding in chasmogamous flowers. *Heredity* 73:265–74
159. Strauss SH. 1986. Heterosis at allozyme loci under inbreeding and crossbreeding in *Pinus attenuatus*. *Genetics* 113:115–34
160. Templeton AR, Hemmer H, Mace G, Seal US, Shields WM, et al. 1986. Local adaptation, coadaptation and population boundaries. *Zoo Biol.* 5:115–25
161. Templeton AR, Read B. 1984. Factors eliminating inbreeding depression in a captive herd of Speke's gazelle (*Gazella Spekei*). *Zoo Biol.* 3:177–99
162. Uyenoyama MK. 1986. Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. *Evolution* 40:388–404
163. Uyenoyama MK, Holsinger KE, Waller DM. 1993. Ecological and genetic factors directing the evolution of self-fertilization. *Oxford Surv. Evol. Biol.* 9: 327–81
164. Uyenoyama MK, Waller DM. 1991. Coevolution of self-fertilization and inbreeding depression I. Mutation-selection balance in haploids and diploids. *Theor. Pop. Biol.* 40:14–46
165. Uyenoyama MK, Waller DM. 1991. Coevolution of self-fertilization and inbreeding depression II. Symmetric overdominance in viability. *Theor. Pop. Biol.* 40: 47–77
166. Uyenoyama MK, Waller DM. 1991. Coevolution of self-fertilization and inbreeding depression III. Homozygous lethal mutations at multiple loci. *Theor. Pop. Biol.* 40:173–210
167. Van Noordwijk AJ, Scharloo W. 1981. Inbreeding in an island population of the great tit. *Evolution* 35:674–88
168. van Treuren R, Bijlsma R, Ouborg NJ, van Delden W. 1993. The significance of genetic erosion in the process of extinction. IV. Inbreeding depression and heterosis effects caused by selfing and outcrossing *Scabiosa columbaria*. *Evolution* 47:1669–80
169. Vogler DW, Filmore K, Stephenson AG. Inbreeding depression in *Campan-*

ula rapunculoides L. I: A comparison of inbreeding depression in plants derived from strong and weak self-incompatibility phenotypes. *J. Evol. Biol.* 12:483–94

170. Waller DM. 1984. Differences in fitness between seedlings derived from cleistogamous and chasmogamous flowers in *Impatiens capensis*. *Evolution* 38:427–40
171. Waller DM. 1985. The genesis of size hierarchies in seedling populations of *Impatiens capensis*. *New Phytol.* 100:243–60
172. Waller DM. 1986. Is there disruptive selection for self-fertilization? *Am. Nat.* 128:421–26
173. Waller DM. 1993. The statics and dynamics of mating system evolution. In *The Natural History of Inbreeding and Outbreeding*, ed. N Thornhill, pp. 97–117. Chicago, IL: Univ. Chicago Press
174. Whitlock MC, Fowler K. 1996. The distribution among populations in phenotypic variance with inbreeding. *Evolution* 50:1919–26
175. Widén B. 1993. Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius* (Asteraceae). *Biol. J. Linn. Soc.* 50:179–95
176. Williams GC. 1975. *Sex and Evolution*. Princeton, N.J.: Princeton Univ. Press
177. Willis JH. 1993. Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus*. *Heredity* 71: 145–54
178. Willis K, Wiese RJ. 1997. Elimination of inbreeding depression from captive populations: Speke's gazelle revisited. *Zoo Biol.* 16:9–16
179. Wolfe LM. 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. *Evolution* 47:374–86
180. Wright S. 1922. Coefficients of inbreeding and relationship. *Am. Nat.* 56:330–38

180a. Wright S. 1922. The effects of inbreeding and crossbreeding on guines pigs. III. Crosses between highly inbred families. Bull. 1121 US Dep. Agric., pp. 1–60

181. Wright S. 1969. *Evolution the Genetics of Populations. The Theory of Gene Frequencies*. Chicago: Univ. Chicago Press
182. Wright S. 1977. *Evolution and the Genetics of Populations* Vol. 3: *Experimental Results and Evolutionary Deductions*. Chicago: Univ. Chicago Press. 611 pp.
183. Yahara T, Maki M. 1993. Effects of biparental inbreeding on the evolution of gynodioecy: a model and a case study in *Chionographis japonica* var. *kurohimensis*. *J. Plant Res.* 106:279–81
184. Ziehe M, Roberds JH. 1989. Inbreeding depression due to overdominance in partially self-fertilizing plant populations. *Genetics* 121:861–68