

Role of inbreeding depression and purging in captive breeding and restoration programmes

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Abstract

Inbreeding depression is a major force affecting the evolution and viability of small populations in captive breeding and restoration programmes. Populations that experience small sizes may be less susceptible to future inbreeding depression because they have been purged of deleterious recessive alleles. We review issues related to purging, as they apply to the management of small populations, and discuss an experiment we conducted examining purging in populations of mosquitofish (*Gambusia affinis*). Purging is an important process in many small populations, but the literature contains a diversity of responses to purging both within and among studies. With the exception that slow inbreeding results in more purging and less threat to population viability, there seem to be few consistent trends that aid in prediction of how a purging event will affect a population. In our examination of purging on population viability in mosquitofish, single or multiple bottlenecks do not appear to have resulted in any purging of the influence of genetic load on population growth. Rather, serial bottlenecks resulted in a marked decline in population growth and an increase in extinction. Our results, taken together with those of reviewed studies, suggest that in small populations there is great uncertainty regarding the success of any single purging event in eliminating inbreeding depression, together with the high likelihood that purging will depress population viability through the fixation of deleterious alleles. In management of captive breeding and restoration programmes, the common practice of avoiding inbreeding and small population sizes should be followed whenever possible.

Keywords: bottleneck, captive breeding, genetic load, inbreeding depression, purging, restoration

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Introduction

Small numbers of individuals are a common feature of many captive breeding programmes. Even when adequate resources exist to house a large number of individuals, typically only a few individuals are available to establish a population. Likewise, once the captive population has grown sufficiently to allow for re-introduction of individuals back into the wild, often only small numbers are used in any single release. As the number of individuals in a population decrease,

matings between relatives become more common than would be expected in larger populations. Inbreeding depression, and the resulting reduction in mean population fitness, is considered a major threat to the viability of many small populations (Frankham 1995a; Saccheri *et al.* 1998; Hedrick & Kalinowski 2000; Spielman *et al.* 2004).

Inbreeding exposes deleterious, recessive alleles to selection by increasing genomic homozygosity (see Box 1 for a review of some of this terminology). In a process referred to as purging, selection decreases the frequency of deleterious recessive alleles in a population, reducing future inbreeding depression (Fu *et al.* 1998; Crnokrak & Barrett 2002). In a review of investigations evaluating purging across a wide range of taxa, Crnokrak & Barrett (2002) found a pronounced reduction in inbreeding depression. Purging has influenced discussions of conservation practices in several ways. Because a purged population would

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Box 1 Some explanations of terminology for readers unfamiliar with genetics

Inbreeding: inbreeding can be defined in a number of ways (Keller & Waller 2002; Glémin 2003). In general, we refer to inbreeding as the increasing probability of relatedness among mating individuals that occurs in small populations. This is sometimes referred to as panmictic inbreeding or inbreeding by drift (see Glémin 2003). Inbreeding can also refer to cases where the relatedness of mates is higher than the average relatedness between random members of a population. We refer to inbreeding due to such nonrandom mating as systematic inbreeding.

Inbreeding depression, fitness, and population viability: inbreeding depression typically refers to the reduced fitness of offspring of related individuals relative to the fitness of offspring of randomly mated individuals. Fitness is defined as the relative ability of different genotypes to contribute individuals to the next generation. Most empirical studies of inbreeding depression measure surrogates of fitness such as brood size or longevity. As the average level of relatedness increases among members of a population, the mean population fitness, or the average values of fitness surrogates, decline. In small populations, at risk of extinction due to stochastic factors, declines in mean fitness can result in decreased population viability. Here we define population viability as the probability that a population will persist in the face of extinction threats over a given time period.

Genetic load and purging: all populations have a genetic load, which can be defined as the relative difference between the average fitness of a population's members and the fitness of the most fit genotype. The process of purging reduces a population's genetic load by reducing the frequencies of deleterious recessive alleles. As these recessive alleles become homozygous, they are exposed to selection, reducing their frequency in the population. As the recessive alleles are eliminated from the population, they have a reduced effect on genetic load.

Recessive alleles, dominant alleles, and dominance: fully recessive alleles are those that only have an influence on phenotypes when present as homozygotes. Fully dominant alleles have a strong influence on phenotype and mask the presence of recessive

alleles. Partial dominance occurs when the effects of the recessive allele on the phenotype are only partially masked by the effects of the dominant allele. Inbreeding depression is due to dominance or partial dominance when reduced fitness in offspring of relatives is the result of an increase in the homozygosity of deleterious recessive alleles. When homozygotes of recessive alleles are only slightly less fit than other genotypes, we refer to the alleles as being slightly or mildly deleterious. Homozygotes for lethal recessive alleles have no fitness relative to other genotypes.

Overdominance: when heterozygous genotypes have a higher fitness than homozygous genotypes, it is referred to as overdominance. When overdominance is influencing phenotypes, inbreeding depression is caused by a reduction in the frequency of heterozygous genotypes as the result of inbreeding.

Small populations and brief bottlenecks: small populations are populations of a finite size in which matings between related individuals are likely to occur. It is impossible to provide a threshold between large and small populations, as the relationship between population size and accumulation of homozygosity due to mating between relatives is continuous. Furthermore, several factors in addition to population size affect the accumulation of homozygosity due to inbreeding. For example, an unequal sex ratio or a high variance in reproductive success among members, might result in a population accumulating homozygosity faster than would be expected for a given population size (see Leberg 2005 for a review). The population size of an ideal, randomly mating population that would accumulate homozygosity at the same rate as the population of interest is sometimes referred to as the inbreeding effective population size. This distinction between effective and actual population sizes is not important to our discussion as it is the occurrence of a finite number of individuals in a population that creates the possibility for the accumulation of homozygosity and the opportunity for purging by drift. The smaller the number of individuals, the greater the likelihood that mating between related individuals will affect mean fitness. When we refer to brief bottlenecks, we are referring to temporary reductions of populations to very small numbers of individuals (often between 2 and 20 individuals in the studies we reviewed). These bottlenecks were brief in that these small population sizes were typically maintained for only one to three generations.

experience reduced inbreeding depression, purging has been suggested as one possible tool in the management of endangered species in captive breeding programmes (Templeton & Read 1983; Laikre *et al.* 1993; Fu *et al.* 1998; Swindell & Bouzat 2006a). A history of small population sizes and associated inbreeding has been used to explain a lack of inbreeding depression in some populations (Zschokke & Baur 2002; Duarte *et al.* 2003; Windig *et al.* 2004). Such populations might be less susceptible to future reductions in population size than populations with higher levels of deleterious alleles, reducing the need for managers to focus on genetic considerations. Purged populations might also be good choices for re-introduction programmes, as newly established populations would be unlikely to suffer from inbreeding depression (Leberg 1990a; Craig *et al.* 2000). However, other findings suggest purging may be an ineffective means of eliminating inbreeding depression and might result in adverse consequences for small populations (Hedrick 1994; Ballou 1997; Fu *et al.* 1998; Kalinowski *et al.* 2000; Wang 2000).

There is uncertainty about how purging might influence inbreeding depression and viability of small populations found in many captive breeding and restoration programmes. Our first objective is to briefly review several issues that could affect how small populations, resulting from captive breeding and wildlife releases, respond to purging. There have been other reviews of purging-related issues (see Crnokrak & Barrett 2002 and Keller & Waller 2002 for an introduction to this literature); we focus on general issues that should help guide our understanding of how inbreeding depression in animal populations could be altered by management practices. Our second objective is to present some results from an experiment assessing whether past bottlenecks reduce the susceptibility of fish populations to additional close inbreeding. While such experiments have been conducted with insect models, few have assessed the influence of small population size on subsequent viability of vertebrate populations. This study builds on our limited knowledge of the effect of purging on the viability of vertebrate populations in complex environments similar to those often encountered during re-introduction attempts.

The genetic basis of inbreeding depression

The genetic basis for inbreeding depression is of critical importance in determining whether purging will occur in small populations (Hedrick 1994; Wang *et al.* 1999). The general assumption is that inbreeding depression is due to some combination of recessive alleles with a range of mild to lethal effects, and overdominance, where heterozygote genotypes have the highest fitness (Keller & Waller 2002). More rarely discussed is the role of gene interactions in inbreeding depression, although epistasis is thought to

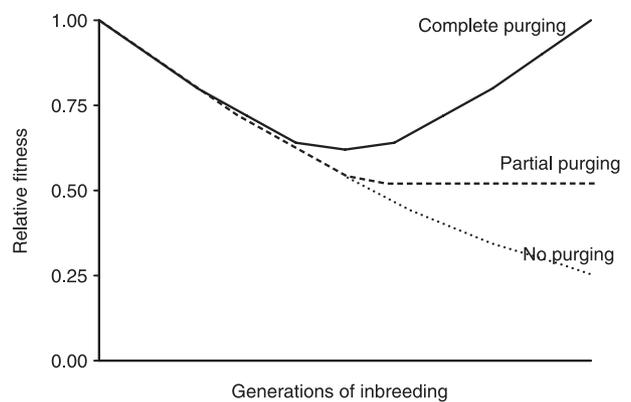


Fig. 1 Possible effects of purging on fitness. Complete purging of genetic load, as might be expected with highly deleterious alleles, results in a recovery of fitness after a period of reduced fitness. Partially effective purging might occur in small populations when highly deleterious alleles are successfully purged but slightly deleterious alleles become fixed through drift. Ineffective purging would be expected in cases where inbreeding depression was due to overdominance or many slightly deleterious recessives (modified from Crnokrak & Barrett 2002).

influence the efficiency of purging (Templeton & Read 1984; Wang *et al.* 1999).

Theory and models predict that recessive alleles that are lethal or strongly deleterious should be rapidly purged from small populations (Hedrick 1994; Fu *et al.* 1998; Wang *et al.* 1999). In such cases, fitness of inbreeding populations is expected to recover after an initial decline (Fig. 1). If, however, inbreeding depression is due to many mildly deleterious recessives or overdominance, purging will be inefficient or impossible, because the frequencies of mildly deleterious alleles will be more strongly affected by drift than by selection (Hedrick 1994). Strong selection against a lethal allele at one locus can also interfere with selection against less deleterious alleles at nearby loci (Keller & Waller 2002). Furthermore, in small populations, drift can generate associations between unlinked loci that also make selection inefficient (Wang *et al.* 1999). Clearly, predicting how small populations are going to respond to inbreeding in captive and restoration programmes requires knowledge of the genetic basis of inbreeding depression.

Unfortunately, we know little about the genetic basis of inbreeding depression in most natural populations; however, there appears to be a general consensus that deleterious recessives are more important than overdominance (Crnokrak & Barrett 2002; Keller & Waller 2002; Kristensen & Sørensen 2005). In laboratory experiments, populations often exhibit responses to purging that suggest most inbreeding depression is due to highly deleterious recessives (Bryant *et al.* 1990; Ribble & Millar 1991; García *et al.* 1994; Saccheri *et al.* 1996; Swindell & Bouzat 2006a). However, other laboratory studies indicate purging is at best only

partially effective at eliminating inbreeding depression (Frankham *et al.* 1993; Fowler & Whitlock 1999; van Oosterhout *et al.* 2000; Radwan 2003; Pederson *et al.* 2005), suggesting that inbreeding depression is due to other genetic mechanisms such as overdominance or mildly deleterious recessives. Often a striking amount of variation occurs in response to inbreeding among populations within the same study or experimental treatment (Lacy *et al.* 1996; Saccheri *et al.* 1996; Fowler & Whitlock 1999; Reed *et al.* 2003; Armbruster & Reed 2005). Because we know almost nothing about the genetic basis of inbreeding depression differs widely (see Hedrick & Kalinowski 2000), and because responses vary so much among replicates in laboratory investigations of purging, predicting the extent of purging in small captive and wild populations is difficult.

Environment and purging

Environmental factors that decrease the relative fitness of homozygotes of deleterious recessives should make purging more efficient. Inbreeding depression is often more severe in stressful than in nonstressful environments (see Hedrick & Kalinowski 2000; Armbruster & Reed 2005), but not always (Keller & Waller 2002). Experiments that have examined purging in different environments have often found that purging might be more successful under stressful or competitive conditions (Latter *et al.* 1985; Bijlsma *et al.* 1999, 2000; Swindell & Bouzat 2006b). More importantly, in terms of conservation, purging of some alleles may only be effective in the environment in which purging occurred (Bijlsma *et al.* 1999, 2000; Reed & Bryant 2001; Armbruster & Reed 2005). Alleles that become fixed in one environment during purging might have deleterious effects in other environments. Thus, purging that occurs in small captive populations might diminish individual viability if individuals are released in a restoration programme.

Environmental variation can play another role in assessing the efficiency of purging by giving the appearance of purging when little loss of deleterious recessives may have occurred. If populations are becoming adapted to captive conditions, or if husbandry is being improved, fitness might improve in spite of, rather than because of, additional inbreeding (Kalinowski *et al.* 2000). Crnokrak & Barrett (2002) point out that a similar problem might occur in some laboratory investigations of purging, but doubt that such adaptation was responsible for much of the reduction in inbreeding observed in their review.

Response to different levels of inbreeding

Theory and models predict that slow rates of inbreeding should result in more efficient selection against deleterious

recessives than strong inbreeding (Hedrick 1994; Fu *et al.* 1998). Small populations experiencing high rates of inbreeding, also experience high rates of drift that interferes with selective changes in allele frequencies and could result in the fixation of deleterious alleles (Hedrick 1994). As population sizes become very small, only highly deleterious or lethal recessives are likely to be purged (Fu *et al.* 1998; Wang *et al.* 1999). Most experimental investigations confirm that slow rates of inbreeding, as found in larger populations, purge genetic load more efficiently than rapid inbreeding (Latter *et al.* 1985; Ehiobu *et al.* 1989; Bijlsma *et al.* 2000; Day *et al.* 2003; Pederson *et al.* 2005; Swindell & Bouzat 2006c), although the difference between fast and slow inbreeding may depend on environment (Swindell & Bouzat 2006b). Day *et al.* (2003) found that slow inbreeding was more likely to allow populations to retain environmental responsiveness than more intense inbreeding.

Whether inbreeding occurs because of reduced population size or through systematic inbreeding makes a difference in the efficiency of purging to reduce inbreeding depression. In the first case, purging is due to drift; matings occur between relatives based on chance alone (Glémin 2003). This purging by drift is effective under a much narrower range of conditions than under a systematic inbreeding scheme. Swindell & Bouzat (2006a) used a systematic mating scheme, maximizing ancestral inbreeding over current inbreeding, and observed effective purging in *Drosophila*. Under both systematic inbreeding and drift forms of purging, recessive alleles can be purged; however, partially recessive alleles can only be purged under systematic inbreeding (Glémin 2003). Purging under systematic inbreeding was more effective in large populations than small ones, unless alleles were highly deleterious. For the more common case of purging by drift, purging is only effective for lethal or highly deleterious alleles that are fully recessive. Purging mildly deleterious recessives by drift would be ineffective at very large or small populations; highest effectiveness was observed at populations containing several hundred individuals in the examples provided by Glémin (2003). The increased efficiency of purging with slow inbreeding and relatively large population sizes poses a paradox, as the smallest captive populations, often proposed as the subject of intentional purging, are those in which purging is least likely to be successful.

Purging and brief bottlenecks

Brief bottlenecks of only a few generations, followed by population expansions, provide another potential opportunity for purging. Short bottlenecks are probably more common in captive breeding and restoration programmes than are prolonged periods of small population size. Short periods of inbreeding should be sufficient to purge highly deleterious recessives, while minimizing the fixation of

minor deleterious alleles through drift (Fu *et al.* 1998). Bryant (1990) reported recovery of fitness-related traits to prebottleneck levels in laboratory populations subjected to serial bottlenecks with intervening periods of population expansion. Saccheri *et al.* (1996) demonstrated similar recoveries in populations following single generation bottlenecks.

The duration and size of bottlenecks influence purging. Substantial inbreeding depression, and thus purging, is unlikely in short bottlenecks with effective population sizes > 10 (Kirkpatrick & Jarne 2000). Miller & Hedrick (2001) report more efficient purging when populations are allowed to recover to larger sizes between bottlenecks than when populations remain small. Comparing populations that had passed through a single bottleneck and continuously small populations experiencing the same level of inbreeding, Reed & Bryant (2001) found more recovery of fitness in lines that had experienced a single bottleneck. Day *et al.* (2003) found that inbreeding depression resulting from brief, serial bottlenecks was more similar to that resulting from continuous rapid inbreeding than the more effective continuous, slow inbreeding. These results support the suggestion that lethal recessives can be purged with only a short period of inbreeding and that prolonging inbreeding does not increase the effectiveness of purging.

Although purging is observed after some bottlenecks, in many studies, fitness measures do not recover to prebottleneck levels (Miller & Hedrick 2001; Reed & Bryant 2001; Day *et al.* 2003; Meffert *et al.* 2006). Bryant *et al.* (1999) and Reed & Bryant (2000) found that while populations experiencing serial bottlenecks did not recover fitness lost during the first bottleneck, subsequent bottlenecks had little additional effects on fitness. Failure to recover fitness completely is most likely due to deleterious alleles becoming fixed in the population during bottlenecks (Hedrick 1994; Kirkpatrick & Jarne 2000). Variance in purging success among replicate populations from the same bottleneck treatments is often large, suggesting that the stochastic changes in the genetic composition associated with bottlenecks are important in influencing purging efficiency (Saccheri *et al.* 1996; Miller & Hedrick 2001). This uncertainty created by the bottleneck process will complicate any attempt to predict how any managed population might respond to a severe reduction in size.

If past bottlenecks are important to purging, natural populations that were isolated and exposed to previous bottlenecks might be good candidates for captive breeding or restoration programmes (Leberg 1990a; Craig *et al.* 2000). However, because effective purging is only likely under a relatively small set of conditions (an isolated population, with slow inbreeding, and with inbreeding depression due to relatively few highly deleterious recessives), the influence of purging is probably quite limited in many natural populations (Keller & Waller 2002). In some

cases, isolated or partially isolated populations that may have experienced past bottlenecks exhibit reduced inbreeding depression (Visscher *et al.* 2001; Zschokke & Baur 2002; Duarte *et al.* 2003; Windig *et al.* 2004). Still, many small, isolated populations have significant levels of inbreeding, suggesting that little purging has occurred (Keller & Waller 2002; Keller *et al.* 2002). For example, Brewer *et al.* (1990) and Lacy *et al.* (1996) found little difference in inbreeding depression between island and mainland populations of mice *Peromyscus polionotus*, suggesting that the expected purging had not occurred in the insular population. One of two mainland populations did experience a fitness rebound, but the insular lineage, with a greater history of bottlenecks, appeared to lack response to further efforts to purge inbreeding depression (Lacy & Ballou 1998). In this lineage, fitness measures declined with additional inbreeding, raising the possibility that the components of genetic load remaining after bottlenecks had purged deleterious recessives of large effect and were not subject to additional purging. Although the generality of this finding is unclear, some populations that experienced past bottlenecks might be more rather than less sensitive to future inbreeding.

Purging and population viability

Usually conservation biologists are more concerned about the implications of purging for population viability rather than how inbreeding depresses individual fitness. In this regard, problems occur with using inbreeding as a management tool. Populations may go extinct during the purging process (Hedrick 1994; Fu *et al.* 1998; Wang *et al.* 1999; Wang 2000), especially in very small populations undergoing intensive inbreeding or experiencing stressful conditions (Frankham 1995b; Bijlsma *et al.* 2000; Radwan 2003). Reed *et al.* (2003) concluded that reducing the rate of inbreeding might improve the efficiency of purging slightly, but not enough to prevent compromising population viability. Even if a population survives purging, its future viability may be compromised. Deleterious but nonlethal alleles can become fixed in inbred populations (Hedrick 1994; Fu *et al.* 1998). Using simulation modelling, O'Grady *et al.* (2006) found that inbreeding, despite the possibility of purging, could strongly affect the extinction risk of wild populations.

Loss of overall levels of genetic variation due to drift during periods of inbreeding may reduce a population's potential to adapt to changing environmental conditions. If inbreeding depression in a small population is due to many mildly deleterious alleles, loss of variation at other loci due to drift might be reduced (see Wang *et al.* 1999). However, if inbreeding depression is due to a few highly deleterious alleles, losses of variation at other loci will be increased with purging (Wang *et al.* 1999). Experiments suggest that purged populations may experience reduced ability to

adapt to new conditions (Bijlsma *et al.* 2000; Reed & Bryant 2000). Reductions in the potential of populations to adapt to new conditions should be of special concern to managers of captive populations.

Purging and the recovery of fish populations

Several studies using insect models have suggested that bottlenecks might reduce the effects of inbreeding on population viability (e.g. Bryant *et al.* 1990; Saccheri *et al.* 1996), but this is not always the case (Day *et al.* 2003). The extent to which laboratory cultures of insects provide a reasonable means for examining the effects of purging in vertebrate species is unclear because large brood sizes might increase the viability of populations during purging (Hedrick 1994; Wang *et al.* 1999). This concern has prompted several investigations using insect models to limit brood sizes to better mimic purging that might occur in vertebrates with small brood sizes (e.g. Backus *et al.* 1995; Bryant *et al.* 1999; Reed & Bryant 2000). However, because the majority of captive breeding and restoration programmes involve vertebrates, there is a need to explore the influence of purging on the viability of vertebrate populations.

We have been investigating responses of populations of western mosquitofish (*Gambusia affinis*) to reductions in population size and environmental challenges. While this species has a high reproductive capacity, median brood sizes (≈ 7) in our mesocosms are closer to other vertebrates than to laboratory insects. Here, we summarize results from part of an experiment examining whether purging, resulting from severe bottlenecks, affects the growth and viability of populations. We know that recovery from bottlenecks of populations of the eastern mosquitofish (*Gambusia holbrooki*), a sibling species of *G. affinis*, is strongly affected by the relatedness of individuals at the time of the bottleneck, and that this response is seen in complex mesocosm environments (Leberg 1990b). We examined differences in the success of populations founded with siblings and nonsiblings among populations with different histories of bottlenecks. If purging that occurred during bottlenecks reduced inbreeding depression, differences in the growth and reproduction of populations founded with siblings and nonsiblings would be smallest in lineages that had experienced a history of severe bottlenecks.

Methods

One advantage of using *Gambusia* in investigations of population recovery is that studies can be conducted in large (2-m dia.), outdoor mesocosms. These mesocosms represent replicated aquatic environments with natural predators and prey; they are not covered and no supplemental feeding is necessary (Leberg 1990b, 1993; Spencer

et al. 2000). Mesocosms are best considered to be quasi-natural environments, as fish are exposed to a more complex environment than organisms in most laboratory studies; however, mesocosms lack many components of natural habitats. This experimental arena provides less control and replicability than laboratory studies using insects; however, it offers an opportunity to examine responses of vertebrate populations under conditions more akin to those experienced in re-introduction programmes.

Stocks used in this experiment originated from a common source (Spencer *et al.* 2000). Virgin offspring were utilized to establish all experimental populations. Founders for experimental populations, as well as populations experiencing past bottlenecks, were generally selected at random from available adults; however, we did not use individuals with large morphological deformities. Six populations were established with the offspring of fish maintained in a large outbred stock that had experienced no bottleneck. Six populations experienced a bottleneck of a single pair of individuals. A serial bottleneck treatment consisted of 12 initial populations that experienced four bottlenecks before our experiment: 1 female and 2 male siblings, 1 male and 2 females (nonsibs), 4 females and 1 male (nonsibs), and 1 pair of siblings. Different sizes of these four bottlenecks reflected limitations in the numbers of individuals in some of the smallest surviving lines. Only 2 of the 12 original lineages were available for use in our experiment; the others had gone extinct, either failing to reproduce or producing too few offspring to establish populations in subsequent years. After each bottleneck, populations were allowed to expand for four generations, reaching sizes of up to 300 individuals.

In our experiment, the six populations in both the control and single bottleneck treatments were each represented by two mesocosms, one of which was founded by siblings. Each of the two lineages in the serial bottleneck treatment was represented in 12 mesocosms (half founded by siblings). We treated each lineage experiencing serial bottlenecks as a separate treatment, but because the mean responses of the two lineages were never significantly different ($P > 0.10$ in all cases), we discuss them together here. We assessed differences in population growth by measuring population size after the populations had grown for approximately two generations. Size is a reasonable surrogate for growth because all replicates were established at the same time and with the same number of founders.

We tested the null hypothesis that there would be no differences in population growth among bottleneck history treatments based on relatedness of founding individuals. If purging had an effect on inbreeding depression, we would expect a significant interaction between bottleneck history and founder relatedness on population growth, with the differences in growth of populations founded with siblings and nonsiblings being the smallest in the serial bottleneck

treatment. Because some founders failed to successfully establish populations and we were unsure that the cause of failure was related to the experimental treatments, we compared estimates of population size for all populations and for only populations with successful reproduction. Failure to reproduce during the 2 months of our experiment was not the result of the experiment being too short to observe reproduction. Pools that contained only a pair of fish at the end of the experiment held no additional fish when surveyed 6 months later.

Results and discussion

Successful reproduction occurred in only 33 of the 48 mesocosm populations. Regardless of whether we considered all populations or only those with successful reproduction, the populations experiencing four previous bottlenecks were much smaller than the populations that experienced fewer bottlenecks ($P < 0.001$, Fig. 2). There was no significant difference in population size between populations experiencing zero or one previous bottlenecks ($P > 0.700$ in all comparisons). This observation of reduced population recovery after multiple bottlenecks would be predicted if severe bottlenecks led to fixation of deleterious alleles. There is no evidence that populations that had been through bottlenecks had been purged of deleterious recessives to the point that their growth approached those of the controls.

When considering all populations (Fig. 2, top), there was no significant effect of founder relatedness on population size. However, in the analysis of populations with reproduction, populations founded with siblings were smaller than those founded with nonsiblings ($P = 0.036$; Fig. 2, bottom). Thus, population growth was influenced by relatedness of population founders, as was observed in *Gambusia holbrooki* (Leberg 1990b). The purging hypothesis would predict that the difference between the growth of populations founded by siblings and nonsiblings should decrease with the number of past bottlenecks. There was, however, no evidence of an interaction between history of bottlenecks and relatedness of founding individuals ($P = 0.658$).

History of bottlenecks had a large effect on the failure of founders to establish populations ($P = 0.027$; Fig. 3). Success for founders from lineages with a history of multiple bottlenecks was lower than for founders from populations with no past history of bottlenecks or that had experienced one past bottleneck. Apparently, fixation of deleterious alleles increased susceptibility to extinction in populations that experienced multiple bottlenecks. However, relatedness of founders had no effect on the likelihood that founders established populations ($P = 0.723$; Fig. 3), and there was no interaction between founder relatedness and history of bottlenecks on reproductive success

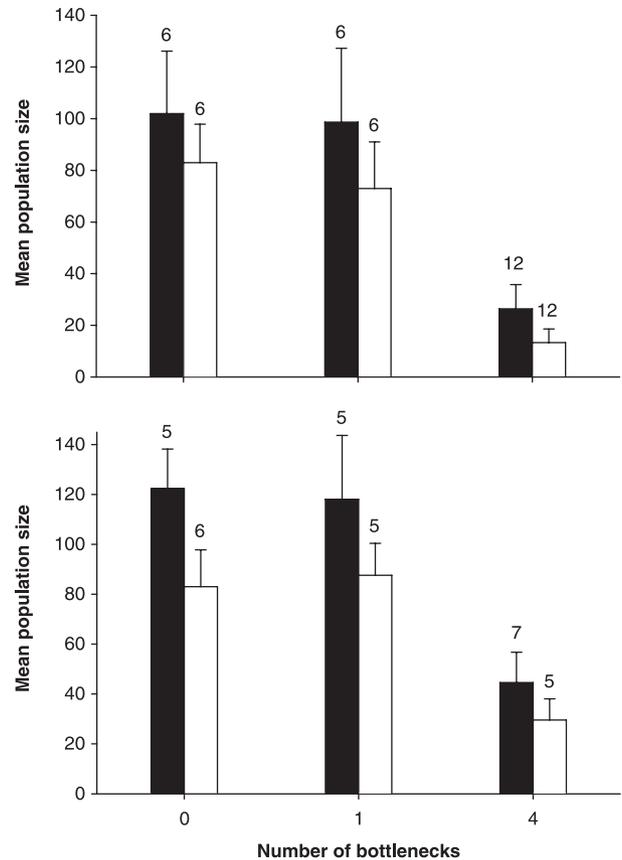


Fig. 2 Mean population sizes of treatments with different levels of past bottlenecks. Black and white bars represent populations founded with siblings and nonsiblings, respectively. The top graph is for all populations ($n = 48$), the bottom graph is for populations that had > 2 individuals ($n = 33$). The number of replicates in each treatment is shown above the error bars, which represent one standard error.

of founders ($P = 0.126$; Fig. 3). Sibling founders did not have significantly reduced success establishing populations relative to nonsiblings in any treatment, so the purging hypothesis could not be evaluated with regard to reproductive failure.

Both population recovery and the ability of founders to establish populations were affected by a history of serial bottlenecks. While both our study and Leberg (1990b) found that *Gambusia* populations founded with siblings grew more slowly than populations founded with nonsibs, we found that the effects of founder relatedness on population growth and extinction were much smaller than the effect of past bottlenecks. Single past bottlenecks tended to have little effect on population size or reproductive success of population founders. Only multiple severe bottlenecks had a large effect on population viability, suggesting that the accumulation of relatedness resulting from bottlenecks might have a threshold effect on population viability (Frankham 1995b).

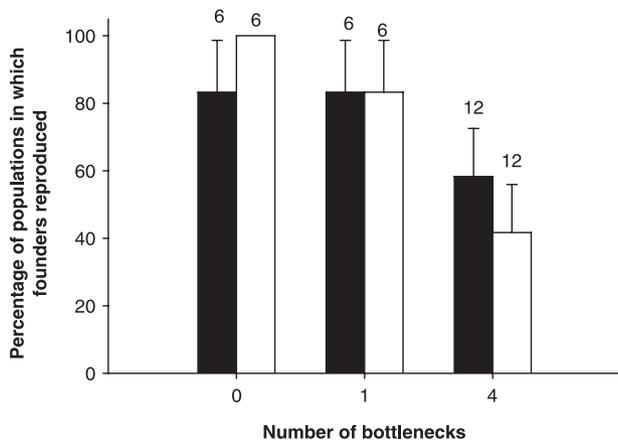


Fig. 3 Percentage of founder pairs that successfully established populations with different histories of past bottlenecks. Black and white bars represent populations founded with nonsiblings and siblings, respectively. The number of replicates in each treatment is shown above the error bars, which represent one standard error.

Effects of inbreeding were not reduced in populations that had experienced multiple bottlenecks, indicating that purging had not reduced inbreeding depression. Purging is expected to take place within the first few generations of intense inbreeding (Fu *et al.* 1998), so we should have detected purging if it had occurred. Our results do not support the prediction that populations that have experienced bottlenecks will be less susceptible to future inbreeding depression than more outbred populations.

Regardless of whether purging effectively alters inbreeding depression of individual viability in captive breeding programmes or in the laboratory, the use of inbreeding as a management tool may increase extinction risk. This problem was evident in 45% of the populations that had experienced multiple bottlenecks and never grew beyond their initial number of founders. Furthermore, the extinction of 84% of the populations that we attempted to use in our four bottleneck treatment suggests that bottlenecks, and associated inbreeding and fixation of alleles, are major threats to the viability of these fish populations.

Conclusions

Several factors affecting the effectiveness of purging may have been responsible for the lack of purging in *Gambusia* populations, including a high component of mildly deleterious alleles or overdominance contributing to inbreeding depression, an insufficiently stressful selective environment, and drift overwhelming selection. More importantly for the management of small populations, our populations that experienced multiple bottlenecks appear to have become fixed through time for alleles that may not be lethal but still affect individual reproduction or survival.

Once fixation occurs during a bottleneck, further inbreeding cannot remove these alleles through selection.

Purging is an important evolutionary force in small populations (Crnokrak & Barrett 2002). There are, however, too many unknowns concerning the genetic basis of inbreeding depression in most species, the stochastic effects associated with bottlenecks, and variation in the efficiency of purging in different environments to suggest using purging in the management of small populations (Hedrick & Kalinowski 2000; Wang 2000). Because purging is unpredictable, the negative effects of inbreeding depression are best avoided by the traditional approaches of minimizing the severity and duration of population bottlenecks and the use of captive breeding designs that avoid matings between close relatives.

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This work is an extension of Paul Leberg's studies of the effects of demographic bottlenecks on the components of genetic diversity and on population viability. Currently, he and his students are studying the effects of fragmentation on genetic structure of avian populations. The experimental results represent a portion of the thesis research of Brigitte Firmin. She is currently working on conservation and regulatory issues with the US Fish and Wildlife Service.
