

Stress and adaptation in conservation genetics

R. FRANKHAM

Department of Biological Sciences, Macquarie University, NSW, Australia and Department of Ecology and Genetics, University of Aarhus, Aarhus C, Denmark

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Abstract

Stress, adaptation and evolution are major concerns in conservation biology. Stresses from pollution, climatic changes, disease etc. may affect population persistence. Further, stress typically occurs when species are placed in captivity. Threatened species are usually managed to conserve their ability to adapt to environmental changes, whilst species in captivity undergo adaptations that are deleterious upon reintroduction into the wild. In model studies using *Drosophila melanogaster*, we have found that; (a) inbreeding and loss of genetic variation reduced resistance to the stress of disease, (b) extinction rates under inbreeding are elevated by stress, (c) adaptive evolutionary potential in an increasingly stressful environment is reduced in small population, (d) rates of inbreeding are elevated under stressful conditions, (e) genetic adaptation to captivity reduces fitness when populations are reintroduced into the 'wild', and (f) the deleterious effects of adaptation on reintroduction success can be reduced by population fragmentation.

Introduction

The biological diversity of the planet is rapidly being depleted (Lawton & May, 1995). The current crisis has been referred to as the 'sixth extinction', as the projected losses compare with those from the five major extinctions, revealed in the fossil record (Leakey & Lewin, 1995). However, its cause is different from that of the other mass extinctions, as the current crisis is being driven directly or indirectly by human impacts.

Stress, adaptation and evolution are major concerns in conservation (Bijlsma & Loeschcke, 1997; Hoffmann & Parsons, 1997; Frankham *et al.*, 2002). Populations of threatened species in natural habitats face a continuing array of stresses resulting from climate change, pollution, competitors, introduced predators and novel or changed diseases. Further, threatened species are typically found in nonoptimal habitat (Channell & Lomollno, 2000). Many threatened species have to be bred in captivity to save them from extinction (Frankham *et al.*, 2002). The captive environment is typically stressful, may reduce the

proportion of individuals breeding and may affect their survival. For example, of 246 golden lion tamarins brought into captivity, only 48 have genes represented in the current captive population. Further, animals may attempt to escape noise, humans, etc. and die from injuries sustained from running into fences. Selection for tameness in captive animals was recognized by Darwin and represents a problem when species are reintroduced into the wild. In general, adaptation to captivity results in deleterious effects when species are reintroduced into the wild (Frankham *et al.*, 2002).

Large populations of nonthreatened species are considered to have the ability to adapt to almost any imaginable stress (Lewontin, 1974). However, half or more of plant species evaluated have not evolved tolerance to either heavy metals, or to herbicides (Bradshaw, 1991). Further, a population of a rainforest species of *Drosophila* at the limit of its distribution with extensive molecular genetic variation, exhibited no ability to adapt desiccation stress (Hoffmann *et al.*, 2003).

By contrast, the effects of stress and adaptation in threatened species need to be viewed in the context of inbreeding and loss of adaptive evolutionary potential (Frankham *et al.*, 2002). Threatened species have by definition, small or declining populations where loss of genetic diversity and inbreeding are unavoidable, as

Correspondence: Richard Frankham, Department of Biological Sciences, Macquarie University, NSW 2109, Australia.
Tel.: 612 9850 8186; fax: 612 9850 8245;
e-mail: rfrankha@els.mq.edu.au

indicated by the following equation for neutral genetic variation in a random mating populations:

$$H_t/H_0 = [1 - 1/(2N_e)]^t = 1 - F \quad (1)$$

where H_t is heterozygosity at generation t , H_0 initial heterozygosity, N_e the effective population size, and F the inbreeding coefficient. In random mating populations, the effects of inbreeding and loss of genetic variation are usually inseparable. Loss of molecular genetic diversity is approximately as predicted by this equation in controlled experimental studies (see Montgomery *et al.*, 2000), and the predicted correlations between long-term population size and genetic diversity are found in wild populations (Frankham, 1996). A majority of threatened species have reduced levels of molecular genetic diversity (Spielman *et al.*, 2004b). Threatened species are typically managed to conserve their ability to adapt to environmental changes (Frankham *et al.*, 2002).

Adaptive evolutionary potential is considered to depend predominantly upon quantitative genetic variation (Franklin, 1980), rather than near neutral molecular variation. For quantitative characters with additive genetic variation and no dominance variation, a similar relationship between proportion of additive genetic variation retained and inbreeding coefficient (or effective population size) to that in eqn (1) is expected. However, there is considerable controversy about this (Cheverud *et al.*, 1999; Whitlock & Fowler, 1999). Further, correlations between molecular and quantitative genetic variation are typically low and not significantly different from zero, for life history (fitness) characters (Reed & Frankham, 2001). We have evaluated the comparative rates of loss in molecular and quantitative genetic variation in 40 or more populations, maintained for many generations at different effective sizes. Quantitative genetic variation for abdominal and sternopleural bristle numbers declined significantly with F , and the regression did not differ significantly from that for allozyme variation (Gilligan *et al.*, 2005). Thus, the low correlations between molecular and quantitative genetic variation are probably because of high sampling variation, especially for measures of quantitative genetic variation. These results do not resolve concerns about loss of quantitative genetic variation for fitness characters that exhibit substantial proportions of nonadditive genetic variation and are subject to natural selection.

In this contribution, I review model experiments using *Drosophila* that we have performed on stress, adaptation and evolution in the context of conservation genetics. Related issues are also covered by Kristensen *et al.* (2005) in this volume.

Inbreeding, loss of genetic diversity, stress and extinction

Inbreeding has deleterious effects on reproductive fitness in all well studied species of naturally outbreeding

animals and plants (Falconer & Mackay, 1996; Lynch & Walsh, 1998; Frankham *et al.*, 2002). Typically, the impacts of inbreeding are more deleterious under stressful, than benign conditions. Disease is one of the most important stresses affecting natural populations. New diseases are arising in nature, other diseases are being spread around the planet because of human activities, and some have crossed species boundaries (Daszak *et al.*, 2000). It is widely presumed that inbreeding and loss of genetic diversity reduce disease resistance, but there is controversy about this point and limited critical data from controlled, replicated experiments (Spielman *et al.*, 2004a). We have evaluated the impact of inbreeding and loss of genetic diversity on resistance to two bacterial diseases (*Bacillus thuringiensis* exotoxin and *Serratia marcescens*) and for both the hypothesized deleterious effects were found (Spielman *et al.*, 2004a). As expected, there was wide replicate variation because of genetic drift.

Wild environments are typically more stressful than captive condition, so inbreeding depression would be expected to be greater in wild than in captivity, and this has been found (Crnokrak & Roff, 1999). Further, inbreeding would be expected to result in higher extinction rates under stressful than benign conditions, and this has been observed by both Bijlsma *et al.* (2000) and by us (Fig. 1).

The second deleterious genetic effect of small population size is expected to be the loss of evolutionary potential, the ability to evolve especially in response to environmental change. I am not aware of any field data that make a scientifically supportable connection between loss of genetic diversity and extinction risk. However, in the laboratory we have tested whether population size restrictions affect extinction risk under condition of increasing levels of a stressful environment,

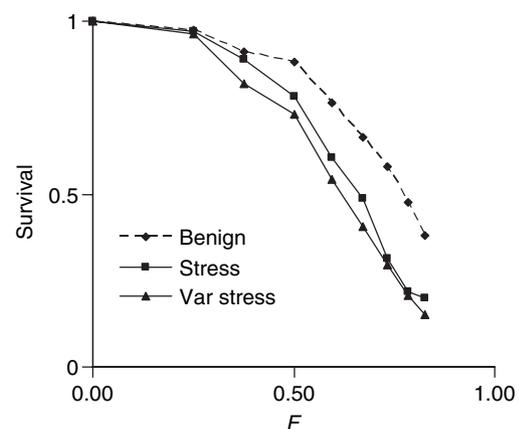


Fig. 1 Proportion of populations surviving at a different inbreeding coefficient (F) for populations inbred using full-sib mating in benign, single stress and variable stress environments (after Reed *et al.*, 2002).

viz. increasing levels of NaCl. Single pair population size bottlenecks for one or three generations resulted in extinctions at lower NaCl concentrations than in non-bottlenecked base population control populations (Frankham *et al.*, 1999). Further, populations maintained for 50 generations at effective sizes of 25, 50, 100, 250 and 500, plus populations maintained with full-sib mating for 35 generations also show elevated extinction risks in treatments with lower genetic variation (Frankham *et al.*, 2002). Thus, extinction risk in stressful environments is elevated by prior inbreeding and loss of genetic variation, as expected. This is expected to occur in both wild habitats and in the laboratory.

An indirect effect of stress is that it may increase the rate of inbreeding compared with the similar sized populations in nonstressful environments, as variances in family sizes may be elevated under stressful conditions. This occurred in an experiment where we were comparing replicated populations designed to have the same effective sizes, but maintained using equal vs. variable family sizes on medium with CuSO₄ added (Frankham *et al.*, 2000). Prior experiments under benign conditions yielded rates of inbreeding in accord with design expectations (Borlase *et al.*, 1993), but in the stressful environment with CuSO₄, the inbreeding coefficient was elevated by approximately 50% in the variable family size treatment. The elevated level of inbreeding on the stressful medium was because of higher family size variation in the stressful environment than under benign conditions (R. Frankham & H. Manning, unpublished data).

Reed *et al.* (2003) investigated several aspects of the relationships between stress, inbreeding, fitness and adaptation. Outbred and inbred populations of *D. melanogaster* were maintained under benign, constant stressful (CuSO₄ or methanol added to medium), or variable stressful condition (CuSO₄ or methanol added to medium in alternating generations) for four generations and then forced to adapt to a novel stressful environment (no sugar in the medium) for seven generations. Our findings were; (1) populations inbred in a variable stressful environment had almost 50% higher rates of adaptive change when placed on a novel stressful environment than those inbred in a constant stressful or benign environment, (2) populations adapted to a prior stressful environment had more fitness when reared in a novel stressful environment than those less adapted to stress, (3) inbred populations had lower fitness and adapted at about half the rate of the outbred populations they were derived from and (4) strong lineage effects were detected across environments in the inbred populations.

Genetic adaptation to captivity

Populations containing genetic diversity adapt through natural selection to changed environmental conditions (Endler, 1986; Mousseau *et al.*, 2000). Consequently,

threatened species brought into captivity are expected to adapt genetically to the captive environment. Adaptations to many conditions have been reported. We have observed genetic adaptation to captive conditions involving high larval and adult density (Frankham & Loebel, 1992; Gilligan *et al.*, 2003), NaCl (Frankham *et al.*, 1999), CuSO₄ (Frankham *et al.*, 2000), low sugar in the medium (Reed *et al.*, 2003) and to benign condition with single adult pairs in vials (Woodworth *et al.*, 2002).

How rapidly does genetic adaptation occur and what is the pattern of adaptation? The extent of adaptive genetic change has often been very large (Frankham & Loebel, 1992). In an experiment, over 88 generations with modest crowding bottles (25 pairs per bottle) and an effective size of approximately 300, the final fitness was approximately three-times the initial value (Gilligan *et al.*, 2003). Adaptation was complete after approximately 60 generations. The pattern of adaptation was one of diminishing rate with time until a plateau was reached. Similar total magnitude of adaptive change was observed when wild rats were brought to the laboratory and allowed to adapt for 25 generations (King, 1939).

As adaptation to one environment typically reduces fitness in other environments, genetic adaptation to captivity would be expected to reduce fitness when populations are reintroduced into the wild. This has been observed in a wide array of experiments in fish, *Drosophila*, biocontrol insects and plants (see Frankham *et al.*, 2000, 2002).

As the objective of many captive breeding programmes for endangered species is to preserve the option of reintroduction into the wild, it is important to consider means for minimizing genetic adaptation to captivity. The amount of genetic adaptation to captivity is expected to be (Frankham & Kingslover, 2004):

$$R_t = Sh_0^2 \sum_{i=1}^t [1 - 1/(2N_e)]^{t-i} \quad (2)$$

where, R_t is response to selection after t generations (genetic adaptation), S is the selection differential and h_0^2 is the initial heritability. From this, we can predict that genetic adaptation is minimized by:

- minimizing generation in captivity (t),
- minimizing selection in captivity (S),
- minimizing genetic diversity (h^2),
- minimizing effective population size (N_e).

Migration from wild to captive populations will also reduce genetic adaptation, but is usually not an option for endangered species, as there are few or none left in the wild. Minimizing generations in captivity can be done using cryopreservation of gametes, zygotes or individuals or by breeding from individuals at later ages (Frankham *et al.*, 2002). Unfortunately, cryopreservation is available only for few threatened species, usually those related to domestic animals. In plants, seed storage can be

used for many species and cryopreservation is successful for many species. Breeding from animals at later ages is not favoured, as individuals that do not breed early in life may not breed at all (Frankham *et al.*, 2002).

Minimizing selection in captivity is dependent upon minimizing mortality and upon minimizing the differences between the captive and wild environments and there are serious constraints on this in zoos (Frankham *et al.*, 2002). The former is performed, but the latter is difficult as threatened animals are highly valuable and ethical and social considerations limit the ability to maintain species in captivity with the normal range of disease, parasites and predators, as do concerns about the spread of disease and parasites to other species.

The most practical means for minimizing genetic adaptation to captivity in most threatened animal species are to (i) equalize family sizes, so that selection is only within families, resulting in the halving of selection, and/or (ii) fragmenting populations so that genetic variation within each population is reduced. We have tested each of these options. Equalizing family sizes resulted in the expected halving of rate of adaptation to captivity (single pairs in vials on medium containing CuSO_4), but produced little improvement in 'reintroduction' success (Table 1). It also did not prevent substantial adverse genetic effects upon reintroduction into the 'wild' from benign captive conditions in *Drosophila* populations studies for 50 generations (Woodworth *et al.*, 2002). Populations with effective sizes of 500 declined by 1.7% per generation in fitness when moved from benign conditions (single pairs of parents per vial) into crowded, competitive conditions. Minimizing kinship, the recommended genetic management regime for captive populations of threatened species, is equivalent to equalizing family sizes when there is equal founder representation in the first generation, so this is already part of captive management for many endangered species (Frankham *et al.*, 2002). It is not clear why equalization of family sizes fails to reduce adverse impacts on reintroduction success. One possibility is that the conditions of full-sib competition under equalization of family sizes favours reduced competitiveness and less male-female conflicts

Table 1 Genetic adaptation to captivity over 25 generations in populations maintained with equalization of family sizes (EFS) or variable family sizes (VFS), compared with the outbred base population. Number of offspring per female in captivity is shown, along with relative fitnesses, compared with the base population when the populations were transferred to the 'wild' (from Frankham *et al.*, 2000).

	# Offspring	'Wild'
EFS	+8.8%**	-38% ^{ns}
VFS	+17.5%	-43%
Base populations	113	100%

** $P < 0.01$ for the comparison of EFS and VFS.
ns, not significant.

(Holland & Rice, 1999), and this is deleterious upon reintroduction to more stressful and competitive environments.

Fragmentation in the absence of population extinctions, is predicted to reduce genetic adaptation to captivity and lead to greater retention of genetic diversity, for the same total population sizes. The magnitude of selection response increases with population size (Robertson, 1960; Jones *et al.*, 1968; Eisen, 1975; Weber & Diggins, 1990). Consequently, fragmenting a population of effective size N into r isolated sub-populations reduces the effective population size in each sub-population to N/r and so is expected to reduce the rate of genetic adaptation in each sub-population, compared with a large population of size N . This effect has been verified in experimental populations of *Drosophila* (Fig. 2). In all comparisons, 'reintroduction success' was higher in several small (pooled) populations than in single large populations and the advantage generally increased with the degree of fragmentation. Theoretical analyses predict that, there will be greater retention of genetic diversity when a population of size N is fragmented into isolated sub-populations, than in a single large unfragmented population of the same total size (Kimura & Crow, 1963; Lande, 1995). We verified this prediction in our study (Margan *et al.*, 1998). Nevertheless, fragmentation is not a deliberate part of current captive management of threatened species (Frankham *et al.*, 2002). While endangered species are spread over several institutions to avoid catastrophes, movement of animals is used to manage the total population as a single population. Fragmentation has other potential benefits of

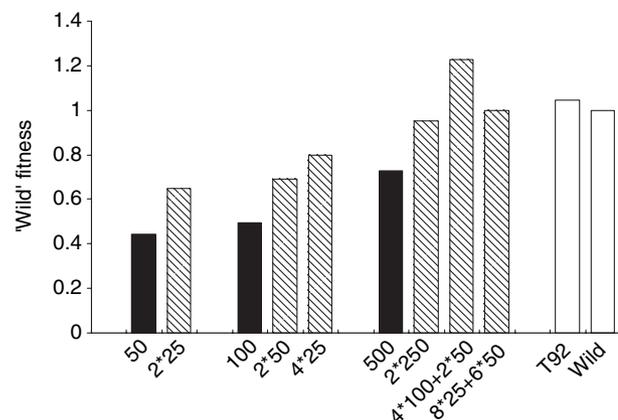


Fig. 2 Reproductive fitness under 'wild' conditions of populations previously maintained under benign captive conditions for 50 generations as either single large populations (solid columns), or as several small populations of the same total size and pooled after generation 50 (hatched columns), along with the T92 wild outbred base population and a new sample from the wild (T95) (open columns). The numbers at the bottom refer to the effective population sizes of the populations.

reducing the cost of moving animals and reducing the risk of spreading diseases in zoos. Management using fragmentation could be implemented by keeping populations in different institutions isolated, until inbreeding reaches an unacceptable level (say around $F = 0.1-0.2$), followed by a round of migration, a further period of isolation, etc.

Conclusions

Stress, adaptation and evolution are major concerns in conservation genetics and conservation biology. Threatened species face an array of stressful conditions. Stress typically amplifies the deleterious effects of inbreeding and loss of genetic diversity and elevates extinction risks. Consequently, inbreeding and loss of genetic variation need to be minimized in threatened species. Genetic adaptation to captivity, typically reduces fitness when species are returned to the wild. Equalizing family sizes reduces genetic adaptation to captivity, but shows little benefits upon reintroduction success. Fragmentation of populations in the absence of extinction reduces genetic adaptation, has beneficial effects on reintroduction success and improves retention of genetic diversity.

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