CONSERVATION OF GENETIC DIVERSITY FOR ANIMAL IMPROVEMENT

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SUMMARY

Genetic variation is essential for the genetic improvement of domestic animals and plants. With the advent of gene transfer, all species on earth represent a pool of genetic diversity that is of potential value in improvement programs. Plant breeders have already utilized genes from viruses, bacteria, fungi and other plants, and animal breeders have begun to do likewise. Consequently, the focus of conservation efforts in animal breeding should be expanded to encompass all biodiversity.

Genetic diversity is lost through the extinction of populations, and through loss of variation within finite populations. Human induced extinction represents the greatest threat to genetic diversity. To retain maximum genetic variation within a population, the initial variation, the population size and the ratio of effective/census size should be maximized, and the number of generations minimized. Experimental evaluation of the effects of variation in family size, unequal sex-ratios, and fluctuating population size on retention of genetic variation have generally validated theoretical predictions. However, measures of quantitative genetic variation in small populations may not reflect true genetic variation, due to deviations from Hardy-Weinberg and linkage equilibrium. Minimizing kinship is predicted to be the optimum means for managing small pedigreed populations to retain maximum genetic variation.

INTRODUCTION

The first step in any breeding program should be to define the objectives. Genetic variation is essential to permit genetic improvement of domestic animals and plants. This may come from pre-existing variation, or from new mutations (spontaneous, induced, or created by *in vitro* mutagenesis). It is unwise to rely on new mutations as they generally have deleterious effects on reproductive fitness. Conversely, pre-existing variants that have been through the sieve of natural selection are more likely to contribute to selection response without deleterious effects on reproductive fitness. However, it does seem possible to "turn-off" non-essential gene functions with little adverse effect using ribozymes, site specific recombination or anti-sense; the latter has been used to modify ripening in tomatoes (Gray et al. 1992).

Genetic diversity needs to be preserved (i) to allow response to selection for desirable economic characters within current commercial lines, (ii) to cope with changes in consumer preference (eg for leaner meat, or brown eggs), (iii) to cope with changes in production environment (eg dairying in the tropics), and (iv) as a source of novel functions. Plant breeders have already made extensive use of other species to introduce novel functions (see below), and some groups are doing such work in animal breeding. Farm animals are also being developed as expression systems for valuable proteins eg expressing human factor IX and α_1-antitrypsin in milk (Clark 1990).

The agony of choice: what should we conserve?

Resources for conservation of genetic diversity are limited. Animal and plant breeders have focused their conservation efforts on preservation of lines within domestic species, or their close relatives (Frankham 1992). Desirable alleles can be introduced into current commercial lines by crossing or by gene transfer. As most old breeds have performance that is much lower than current commercial lines, the introduction of favourable alleles by gene transfer is the preferred route. Crossing simultaneously introduces many deleterious alleles and is restricted to introducing alleles from the same species. However, gene transfer technology allows the entire biodiversity of the earth to be used as a genetic resource for animal and plant breeding. Consequently, animal and plant breeders should encourage conservation of global biodiversity. Conservation priorities should be to maintain the maximum amount of global genetic diversity (Vane-Wright et al. 1991; Crozier 1992).

Why should livestock breeds be preserved when they contain only a small proportion of total genetic diversity on the planet? They are an irreplaceable resource that must be preserved to cope

with shifts in consumer preference and production environments. Further, they are likely to be a major source of genes for disease resistance (as have lines of domestic plants), and other simply inherited conditions (high fertility in sheep and pigs, feather and eggshell colour in poultry, etc).

Lessons from plant breeding

Plant breeders have been more active than animal breeders in the use of genetic diversity from outside current commercial lines. Genes for disease resistance have been obtained from a wide range of sources. Genes for rust fungus resistance in wheat were initially obtained from *Triticum aestivum*, and introduced by crossing. Subsequently, genes for resistance were introduced from other species of *Triticum* and from related species such as *Aegilops* and *Agropyron* (Sears 1993). As knowledge of the genetics of resistance to animal diseases increases (eg MHC and disease associations; Lie 1990), it is likely that such genetic manipulations will feature increasingly in animal breeding. There has already been manipulations of MHC haplotype frequencies in chickens, but much of the detail is buried in the commercial secrets of breeding companies. Taki et al. (1993) and Zou et al. (1993) demonstrated the feasibility of modifying the mammalian immune system by using site specific recombination to alter an immunoglobulin locus in mice, and to insert human sequences into it.

A major advance in plant breeding has been the use of genetic engineering to introduce novel functions into plant cultivars by introducing genes from other species (Table 1).

Table 1. Examples of genetically engineered plant cultivars

Trait	Source of introduced gene	Reference
Virus resistance	Virus coat protein genes	Weising et al. (1988)
	Virus polymerase genes	Anderson et al. (1992)
	Satellite RNA	Weising et al. (1988)
Herbicide tolerance	*Streptomyces*	Weising et al. (1988)
	Salmonella	Weising et al. (1988)
	Plant gene forced to evolve resistance	D'Halluin et al. (1992)
	Resistance gene from another plant	D'Halluin et al. (1992)
Insect resistance	*Bacillus thuringiensis*	Weising et al. (1988)
	Cowpea proteinase inhibitor gene	Weising et al. (1988)
Increased seed phosphate	*Aspergillus*	Pen et al. (1993).

The introduction of novel functions from other species has been done in chickens, and is being attempted in sheep. In a manner reminiscent to engineering virus resistant plants, transgenic chickens carrying avian leukosis virus (ALV) sequences were resistant to the ALV virus (Crittenden and Salter 1990). Bacterial genes encoding steps in the pathway to sulfur amino acids are being inserted to improve wool yields, bacterial glyoxalate pathway genes to improve feed utilization (Ward and Nancarrow 1991), and a plant chitinase inhibitor to produce resistance to the Australian sheep blowfly (Kevin Ward *pers. comm.*).

Forms of preservation of genetic diversity

Preservation takes two forms: (a) within current commercial lines, or (b) in gene banks (live animals, frozen embryos, frozen semen, cell cultures, preserved DNA, seed banks, and freeze dried microbes). Preserved DNA is the simplest and most inexpensive of these to store and is sufficient as a genetic resource. However, it is unlikely to be adequate in practice as it does not allow screening for function as can be done in living organisms.

Loss of genetic diversity

Genetic diversity is lost through the extinction of populations, and through loss of variation within finite populations. Extinction of species through human activities represents the greatest threat to genetic diversity. Loss of habitat, over exploitation, pollution and introduced species are creating

an extinction crisis as severe as the major extinctions found in the geological record. (see WCMC 1988).

Inbreeding and extinction

A fundamental assumption underlying the application of genetics within conservation biology is that inbreeding increases the risk of extinction. Since inbreeding depresses components of reproductive fitness in naturally outbreeding species (Wright 1977; Ralls et al. 1988; Falconer 1989), it is assumed to increase the risk of extinction, though this is hotly contested by non-geneticists (see Caro and Laurenson 1994). This presumption is supported by correlations between cumulative extinctions and inbreeding in laboratory and domestic animals (Soulé 1980). However, genetic and non-genetic causes of extinction were not delineated. Methods were devised to separate genetic and non-genetic causes of extinction in inbred populations and applied to published data sets from *Drosophila melanogaster, D. funebris* and mice (Frankham 1994a). Inbreeding markedly increased rates of extinction in all animal species with appropriate data. All showed a threshold relationship between incremental extinction and inbreeding with low initial extinction, but sharply increased extinction beginning at intermediate levels of inbreeding. Small populations, especially those that are not being closely monitored, may give little warning of impending inbreeding crises. The risk of extinction is greatly increased in small populations, due to inbreeding depression, demographic stochasticity and environmental stochasticity (Gilpin and Soulé 1986).

Immigration into partially inbred lines improves reproductive fitness

The obvious solution to inbreeding depression in small populations is to introduce immigrants from elsewhere. However, it is important that rare breeds retain as much of their unique genetic characteristics as possible. Consequently, it is desirable to minimize the number of immigrants. Introduction of a single immigrant into small partially inbred populations of *Drosophila* caused an approximate doubling of reproductive fitness (assessed after three generations) and returned fitness approximately half-way back to that of the wild base populations (Spielman and Frankham 1992). All 10 replicates showed improvements in fitness. Low levels of immigration can be recommended to alleviate inbreeding depression in small populations of rare breeds.

Methods for preserving genetic variation within populations

Most authors assume that heterozygosity represents evolutionary potential. However, Allendorf (1986) and Fuerst and Maruyama (1986) have stressed the need to retain allelic diversity. Methods for retaining heterozygosity do not necessarily maximize the retention of allelic diversity, especially when there are population bottlenecks. This issue needs to be resolved empirically.

The expected proportion of genetic variation (heterozygosity H_t) retained within a population after t generations is given by equation 1 (after Crow and Kimura 1970).

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

Where H_0 = initial heterozygosity, N = population size, N_e = effective population size and t = number of generations.

Consequently, retention of heterozygosity is maximized by

(a) Maximizing initial heterozygosity

(b) Minimizing number of generations

(c) Maximizing population size, and

(d) Maximizing N_e/N ratio

I will consider each of these in turn:

Maximizing initial heterozygosity

Maximizing initial heterozygosity is achieved by initiating populations with a reasonable number of founders (typically a minimum of 20-30). Where possible populations with high levels of genetic variation should be chosen (little can be done about this in most cases). A population size bottleneck of n individuals is predicted to reduce heterozygosity and selection response by the proportion 1/2n (James 1971). Bottlenecks have been shown to reduce allozyme variation (see Leberg 1992). Experimental evaluations of this theory, using bristle characters in *Drosophila* that exhibit predominantly additive characters, have validated James' predictions (see Robertson 1966; James 1971; Franklin 1980; Frankham 1980). However, Bryant et al. (1986) reported results from

houseflies that conflicted with predictions; the bottlenecked lines showed elevated genetic variation for characters that exhibited non-additive genetic variation, as indicated by inbreeding depression. Further, Lopez-Fanjul and Villaverde (1989) showed elevated selection response for a fitness character in bottlenecked lines of *Drosophila.* Models containing non-additive genetic variation can account for the observations (see Willis and Orr 1993). However, the relevance of the Bryant et al. (1986) and Lopez-Fanjul and Villaverde (1989) results to conservation remains obscure, as the long-term evolutionary potential of bottlenecked populations is unlikely to be increased.

Many captive populations of wildlife have been founded when only small numbers are left (see Hedrick 1992). These founders often contribute unequally, such that the rate of inbreeding and the loss of genetic variation is increased. Consequently, it is recommended that such populations be managed to equalize founder representation. Our experimental evaluation of this theory showed benefits in terms of reduced rate of inbreeding and loss of genetic variation, but no benefits in terms of reproductive fitness (Loebel et al. 1992). To our surprise, changes in founder representation were almost completed in the first generation. Computer simulations subsequently confirmed this effect for populations with non-overlapping generations. The mating of individuals connects them, such that the fates of under and over represented founders can become inextricably bound.

Minimizing generations

Generations can be minimized by either extending the generation interval by breeding from older animals, or by using cryogenics (Moore et al. 1992). Embryo freezing and semen freezing offer great prospects for minimizing the loss of genetic variation. While embryo freezing technology is available for most domestic animals and is used in routine stock maintenance in mice, it is not available for most wildlife or for domesticated birds. Semen freezing is in essence half as good as it applies the technology to one sex. It is available for essentially all domestic animals including chickens and turkeys, but is not available for most wildlife. For the majority of non-domesticated species, live individual conservation is the only available method.

Maximizing population size

Robertson (1960) predicted that long-term response to selection would depend on the population size of lines. This prediction has been validated in *Drosophila* (Jones et al. 1968; Weber 1990; Weber and Diggins 1990) and mice (Eisen 1975). Maximizing population size is the means for minimizing loss of genetic variation in commercial lines.

Maximizing N_e/N ratios

The genetic consequences of finite population size are predicted to depend on the effective population size, rather than the census size (Wright 1931). N_e is predicted to depend not only on the census size, but on variation in family size, inequalities in sex ratio (especially harem structures) and on fluctuation in numbers over generations (Wright 1969; Crow and Kimura 1970; Falconer 1989). The simple single locus theory assumes that there is no mutation, natural selection or linkage. There are significant deviations from predictions of related theory on inbreeding and heterozygosity (Mina et al. 1991; Rumball et al. 1994). While this theory has been widely used, most of its predictions had not been subjected to experimental evaluation until we set out to do so.

N_e/N ratios

Until recently, effective population sizes were assumed to be 50-80% of census sizes (see Falconer 1989). However, it is now evident that N_e/N ratios are highly variable among species, and that many species have much lower ratios than previously suspected (Table 2). Low ratios are prevalent in highly fecund species. These species would be expected to have high variances in family sizes, and perhaps large variation in N across generations. For species with lower fecundity (lizards and below in the Table), there is a trend for lower ratios for those with unequal sex-ratios of reproductives (polygyny). This effect is almost certainly underestimated as paternity is usually unknown. It can no longer be safely assumed that N_e/N ratios fall within the range of 0.5-0.8, particularly in fish, oysters and polygynous species. Explicit tests of the effects of these three variables on N_e/N ratios, genetic variation and reproductive fitness are described below.

Table 2. N_e/N ratios in a range of species

Species	N_e/N ratio	Comment	Reference
Insects			
Drosophila melanogaster	0.004-0.28	High fecundity	Briscoe et al. (1992)
D. pseudoobscura	0.01-0.04	High fecundity	Briscoe et al. (1992)
Olive fruit fly	0.10	High fecundity	Nei and Tajima (1981)
Fish			
Chinook salmon	0.013,0.043	High fecundity	Bartley et al. (1992)
Sea bass	0.40	High fecundity	Bartley et al. (1992)
Rainbow trout	0.90	Pair mated	Bartley et al. (1992)
Pacific oysters	10^{-6}	High fecundity	Hedgecock et al. (1992)
Amphibian			
Great toad	0.01-0.1	High fecundity	Easteal and Floyd (1986)
Lizard	0.61-0.71	Monogamous	Tinkle (1965)
Birds		Low fecundity	
Red-crowned crane	0.45	Monogamous	Mace (1986)
Galapagos finches	0.30, 0.40	Monogamous	Grant and Grant (1992)
Mammals		Low fecundity	
Grevy's zebra	0.28	Polygyny	Mace (1986)
Scimitar horned oryx	0.20	Polygyny	Mace (1986)
Grizzly bear	0.28	No harem	Allendorf et al. (1991)
Tiger	0.41	No harem	Smith and McDougal (1991)
Bison	0.084-0.296	Polygyny	Shill and Tipton (1987)
Moose	0.20-0.36	No harem	Ryman et al. (1981)
White-tailed deer	0.35-0.42	No harem	Ryman et al. (1981)
Japanese macaques	0.2-0.76	Polygyny	Nozawa (1972)
Eastern barred bandicoot	0.11-0.41	Polygyny	Robinson et al. (1990)

Equalization of family size (EFS)

EFS is predicted to double N_e and so reduce loss of genetic variation, inbreeding and inbreeding depression (Wright 1969). Consequently this is recommended in the captive breeding of rare breeds and endangered species. We performed a controlled, replicated study of this procedure in *Drosophila* (Borlase et al. 1993). Ten replicate populations of both EFS and variable family size (VFS controls) were tested in a paired comparison. As predicted, EFS led to greater N_e, retention of allozyme variation and reproductive fitness, and slower inbreeding. Less extensive, but related results have been obtained in *Tribolium* (Bray 1966), mice (Eisen and Hanrahan 1974; Farid et al. 1986; Falconer 1989), and Japanese quail (Havenstein et al. 1988). Surprisingly, there was no significant difference between our treatments in quantitative genetic variation for abdominal bristle number, and there was not even a trend in the predicted direction (Frankham 1994b). The expectation was that additive genetic variance would decline in proportion to the inbreeding coefficient. On the basis of multi-locus theory and computer simulations, Bulmer (1980) predicted that random deviations from Hardy-Weinberg equilibrium and linkage equilibrium would cause substantial random deviations from true genetic variances in finite populations. Consequently, a difference in the predicted direction should be evident when our lines had attained Hardy-Weinberg equilibrium and linkage equilibrium after maintenance for many generations as large populations. This prediction was verified. Single locus models are inadequate to predict the detailed behavior of quantitative genetic variation in small populations.

Harems

Harems are predicted to reduce the effective population size and so increase the rate of inbreeding and loss of genetic variation. Our studies (Briton et al. 1994) confirmed these predictions.

Consequently, harem breeding structures should be avoided as far as possible in programs for conservation of rare breeds. Where this is not possible, their effects should be minimized by use of smaller harems, rotation of males, etc.

Fluctuating population size

Fluctuating population size is common in natural populations of animals. This is predicted to reduce the effective population size, so increase the rate of inbreeding and loss of genetic variation. These predictions have been experimentally validated (Woodworth et al. 1994). Such fluctuation should be minimized in breed conservation programs.

Optimum genetic management of small pedigreed populations

A major advance in the management of small pedigreed captive populations has been provided by Ballou and Lacy (1994). Using computer simulations, they showed that minimizing kinship is equal to or superior to maximum avoidance of inbreeding, genome uniqueness and random choice of parents for retention of heterozygosity and allelic diversity. The advantage arises when there is unequal representation of founders. This advantage is mostly gained in the first generation of management. Their predictions need to be evaluated in a living organism.

Minimum viable population sizes

How big do populations have to be to (i) avoid inbreeding depression, and (ii) retain their quantitative genetic variation? Franklin (1980) and Soulé (1980) suggested that an N_e of 50 was sufficient to avoid inbreeding depression in the short-term. Recently, Latter and Mulley (1994) have shown inbreeding depression in long-term populations with N_es of about 50. We have studies underway to investigate this question.

Franklin (1980) argued that an N_e of about 500 should be sufficient for indefinite retention of quantitative genetic variation due to a balance between drift and mutation. Lande and Barrowclough (1987) reached similar conclusions based on a model incorporating stabilising selection. For reproductive fitness characters, mutations are predominantly deleterious, so the average mutation rate is not appropriate; the required N_e may be larger. We have work underway to obtain empirical evidence on this issue.

CONCLUSIONS

1. The total biodiversity on earth represents a resource that should be conserved to supply genetic diversity for animal improvement.
2. Breed conservation is required to cope with changes in consumer preference or productive environment, to supply genes for disease resistance and other simply inherited characteristics, and for aesthetic reasons.
3. Where available, cryopreservation is the most cost effective means of conservation.
4. Maximum retention of genetic variation within populations occurs when initial variability, population size, and N_e/N ratio are maximized, and number of generations are minimized. The N_e/N ratio is maximized by equalizing family sizes, and the sex-ratio of reproductives, and by minimizing fluctuations in population size over generations.

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