Conservation and evolution

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climate which brought about great habitat alteration (the Cretaceous extinction of large reptiles). Throughout the history of life, there has never been as wanton nor as rapid an agent of habitat destruction as twentieth-century man.

3

Population genetics and conservation

3.1 Population size and genetic variability

3.1.1 Introduction

In the previous chapter the process of extinction was discussed in very general terms. In this chapter we turn, rather abruptly, to some very specific, down-to-earth problems. Everywhere, particularly in the tropics, habitats are being lost to a rising sea of humanity. Soon only tiny islands of natural habitat will be left, mostly as arid or cold lands unfit for agriculture, or as isolated nature reserves. Many species will perish completely, and many others will only survive because of the ministrations of man. Hence the need for conservation genetics – the genetics of scarcity.

The scarcity is in numbers. Whether our concern is the wild relatives of cultivated plants or wild animals, the conservationist is faced with the ultimate sampling problem – how to preserve genetic variability and evolutionary flexibility in the face of diminishing space and with very limited economic resources. Inevitably we are concerned with the genetics and evolution of small populations, and with establishing practical guidelines for the practising conservation biologist.

The task has its hazards, not the least of which is the heterogeneity of the biological world. No two species are genetically the same, and no generalization (for example, about minimum population size) can be universally valid. To those who insist on bludgeoning us with this hazard, our response is that Noah must have had similar critics to whom he probably remarked, 'I can either stand here in the rain arguing about precision, or I can start building. Goodbye.'

Do populations suffer a significant genetic deterioration as a consequence of a sudden or gradual decrease in numbers? The fate of thousands of species may hang on the correct resolution of this issue, and on our ability to put principles into practice. To intelligently discuss this question, it is first necessary to describe the relationship between population size and genetic variability, and second, establish the consequences of decreasing genetic

variability. In this chapter we review what is known about the immediate or short-term genetic effects of small numbers on genetic variation and fitness. The relationship between population size and long-term fitness, i.e. evolutionary potential, is the subject of Chapter 4. Readers wishing to pursue these topics in greater depth should consult such standard works as Crow and Kimura (1970), Wright (1977), or less specialized treatments such as Pirchner (1969), Spiess (1977), or Wilson and Bossert (1971).

Fig. 3.1 illustrates the kinds of events with which we are concerned. The 'normal' population size is that found in nature prior to significant inroads by man. The 'crash' or reduction is shown to occur suddenly, portraying, for example, the establishment of a breeding group from a few founder individuals. The decline can also be gradual, such as when habitat destruction diminishes the occupiable territory, as is happening throughout the tropics. The bottleneck is the minimum population size as a result of a crash.

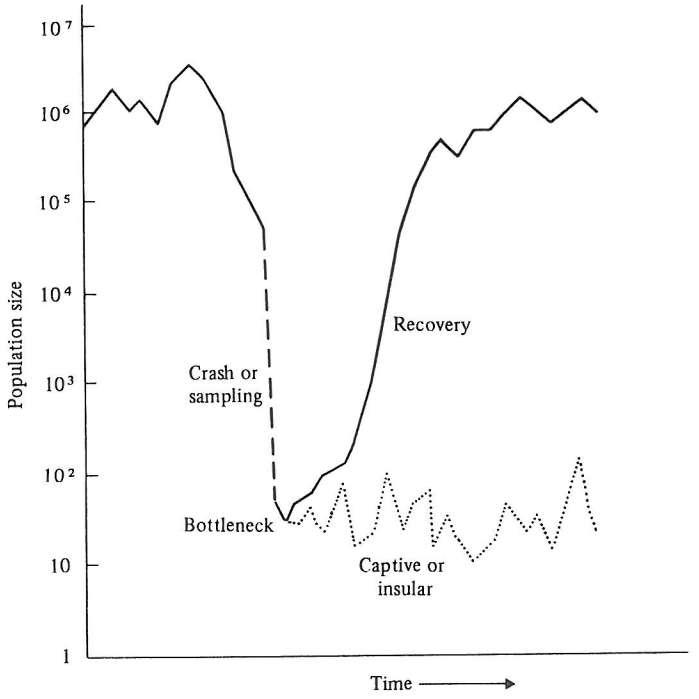

Fig. 3.1 The kinds of changes in population size relevant to conservation genetics.

Whether the population makes a significant numerical recovery or remains in a chronic state of impoverishment depends mostly on the degree of man's hospitality. For economic reasons most captively bred populations will never rebound to their former abundance, whereas some managed

populations, such as whales, have the potential for complete numerical recuperation, requiring only a major revolution in resource utilization by maritime nations.

3.1.2 Effects of bottlenecks on variation and allelic diversity

A bottleneck is an observable and dramatic collapse of numbers. It can be produced by a gradual or sudden environmental change, such as a drought or flood; it can be a natural colonization (founder) event, such as when one or more individuals establish a new population in a previously unoccupied region or on an island; it can be an artificial founder event, such as the establishment of the coastal redwood (*Sequoia sempervirens*) in New Zealand or the Arabian oryx (*Oryx leucoryx*) in Arizona. Whatever the circumstances though, a bottleneck is equivalent to taking a relatively small sample of items, in this case, genes, from a large population. Because small samples rarely are completely representative of the source population from which they are drawn, a bottleneck will usually initiate an interval during which the population lacks some or most of the genetic diversity of the source population. Depending on the degree of genetic 'sampling error' or depauperateness of the bottlenecked population, it may be temporarily or permanently handicapped in ways described in this and the next chapter.

The loss of genetic variability concomitant with a bottleneck event has both qualitative and quantitative aspects. Qualitatively, specific alleles will either be lost or retained, and if lost, it is highly improbable that mutation will replace them as long as the population is small. Quantitatively, the amount of variability for specific characteristics will be reduced. In other words, the *variance* of quantitative traits is reduced by a bottleneck. For example, Fig. 3.2 shows the reduction in the variance of some trait by one-half, the amount of reduction expected when the population size of the bottleneck is a single individual.

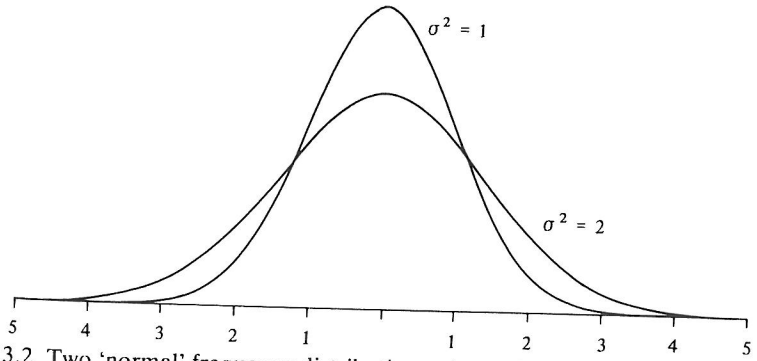

Fig. 3.2 Two 'normal' frequency distributions; the variance of the higher distribution is one-half that of the wider distribution.

Bottlenecks will usually have a greater qualitative than quantitative impact. That is, the loss of alleles, especially rare ones, is much greater than is the loss of genetic variance *per se*. We will examine first, the loss of genetic variance.

An approximation of the amount or proportion of genetic variation or heterozygosity that remains following the sudden reduction of a large population to a small one containing N individuals is

$$1 - 1/2N \tag{3.1}$$

As shown in Table 3.1, most genetic variation is conserved unless the bottleneck is very severe. Even a sample of four or five pairs contains most of the genetic variance of the source population. Experimental data generally support these approximations. Franklin (1980) cites unpublished results of Keith Hammond showing the effects of bottlenecks of sizes 2, 20 and 100 on the loss of genetic variance in abdominal chaetae of *Drosophila*. The results (originally expressed as heritabilities) are shown in the third column of Table 3.1; they are close to the expected values. Unless the number of founders is of the order of two pairs or fewer, the bottleneck, *per se*, is not the villain in a genetic melodrama, at least with respect to genetic variance. Rather, as shown in section 3.1.3, most of the loss that ensues is attributable to events *following* the bottleneck.

TABLE 3.1 *The percentage of genetic variance remaining in founder populations*

No. of individuals in sample	Expected percentage of genetic variance remaining	Empirical results (see text)
1	50	–
2	75	74
6	91.7	–
10	95	–
20	97.5	90
50	99	–
100	99.5	–

The other way of looking at the consequences of bottlenecks is in terms of the loss of alleles. Relatively rare alleles with frequencies of, say, 0.05 or less, contribute little to genetic variance. Yet rare genes, including perhaps genes for disease resistance, may be important in special circumstances, such as during an epidemic. A gene of this kind might be neutral or close to neutral in its effect on fitness for many generations, but during an environmental crisis it could spell the difference between extinction and survival for the population.

How do rare genes fare during bottlenecks? Nei, Maruyama and Chakraborty (1975) and Denniston (1978) have shown that rare genes have a high probability of being lost during bottlenecks. The formula for estimating the number of alleles (n) remaining after a bottleneck of N individuals is

$$E(n) = m - \sum_j (1 - p_j)^{2N} \tag{3.2}$$

where m is the number of alleles prior to the bottleneck, p is the frequency of the jth allele, and N is the effective number of individuals at the bottleneck. For example, consider a diploid species in which each locus has four alleles segregating in the population, one allele of which is common while the other three alleles are rare. Table 3.2 gives these expected values for bottlenecks of various sizes and for two sets of allele frequencies for which $m = 4$.

TABLE 3.2 *The number of alleles retained, beginning with four, in samples of sizes N calculated for two sets of allele frequencies in the source population*

No. of individuals in sample (N)	Average number of alleles retained	
	$p_1=0.70, p_2=p_3=p_4=0.10$	$p_1=0.94, p_2=p_3=p_4=0.02$
1	1.48	1.12
2	2.02	1.23
6	3.15	1.64
10	3.63	2.00
50	3.99	3.60
∞	4.00	4.00

In contrast to the relatively minor effect of bottlenecks on genetic variance, the results in Table 3.2 show that allelic diversity can suffer very seriously during founder events (see Marshall and Brown, 1975). We can only guess about the consequences of such attrition on fitness, however. In the short run, the loss of rare alleles is probably not very important, especially in benign environments. In the long run, though, such alleles might be crucial. The prudent tack would be to hedge our bets: whenever possible, maximize the size of founder groups.

3.1.3 Effects of genetic drift on variation and allelic diversity

A bottleneck is a single event of sampling error, the amount of error and the

loss of variation being proportional to the number sampled. When numbers are low, a population is, in effect, going through a serious bottleneck every generation, and the effects are cumulative because the regeneration of variation by mutation is insignificant in small populations. The random changes in gene frequencies that occur due to sampling error, including the loss of alleles, is called genetic drift.

From equation 3.1 the expected proportion of variation remaining after t generations is

$$(1 - 1/2N)^t \tag{3.3}$$

Some useful results are tabulated in Table 3.3. For example, a population must number at least 100 if it is to retain more than 60% of its genetic variance for 100 generations.

TABLE 3.3 *The retention of genetic variance in small populations of constant size for t generations*

	Percentage genetic variance remaining after 1, 5, 10 and 100 generations			
Population size (N)	1	5	10	100
2	75	24	6	<< 1
6	91.7	65	42	<< 1
10	95	77	60	< 1
20	97.5	88	78	8
50	99	95	90	36
100	99.5	97.5	95	60

Consider the case of a gravid female who establishes a population on an island. In this situation the population will grow until competition or space begin to act as brakes. Assume that the population reproduces annually and that the population size triples every generation. The sequence of sizes over a period of ten years is therefore 2, 6, 18, 54, 162, 486, 1458, 4374, 13 122, and 39 366. In order to estimate the genetic variance remaining in this case, the harmonic mean of the ten values of N (= 13.16) is substituted in equation 3.3 (see section 3.1.5). We see that the amount of variation retained is 67.9%, or ten times the amount retained if the population size had remained at 2. This should partially allay the fears of those concerned that a *single* bottleneck must extract most of the genetic variation in an island population. Again, we wish to emphasize that a bottleneck will not, by itself, erode much of the genetic variance. The crucial issue is whether the

population remains small or grows to a relatively large size. It is perennial low numbers that erode genetic variation.

Another relevant factor in the conservation of variation is the *rate* of population growth. As shown by Nei *et al.* (1975) the proportion of heterozygosity (equivalent to genetic variance for practical purposes) retained subsequent to a bottleneck is negligible if the growth rate, r, is less than 0.1. With $r > 1.0$, however, the post-bottleneck loss of variation is relatively insignificant.

The same conclusions are apparent when considering the number of alleles that survive during an interval of substantial genetic drift. The question becomes one of estimating the number of alleles out of the original m that are retained after t generations. The rather complex mathematics discussed by Denniston (1978) are not reproduced here. Table 3.4 gives the theoretical results for a constant population size of six individuals. Note that after sixteen or twenty generations most populations will have only a single allele remaining at each locus, regardless of how many alleles were present to begin with. Obviously, the leakage of alleles is less in a larger population. However, the prognosis for rare alleles is poor, even at moderate values of N, unless some form of selection elevates the fitness of individuals carrying such genes.

3.1.4 Effective population size: unequal sex ratio in dioecious species

So far in this chapter we have made the assumption that the number of males

TABLE 3.4 *The expected number of alleles after t generations in a population of six individuals given three different starting frequencies*

	Number of alleles when:		
No. of generations	$m=2, p_1=p_2=1/2$	$m=4, p_j=1/4$	$m=12, p_j=1/12$
0	2.00	4.00	12.00
1	1.99	3.87	7.78
2	1.99	3.55	5.88
4	1.91	2.94	4.08
8	1.67	2.18	2.64
16	1.34	1.52	1.68
20	1.24	1.36	1.44
56	1.01	1.02	1.02
∞	1.00	1.00	1.00

From Denniston (1978).

and females contributing to each subsequent generation is the same. This permitted us to sidestep a major complexity in genetic calculations, namely, the problem of the genetically effective population size, N_e. Kimura and Crow (1963) should be consulted for a comprehensive treatment of this subject. N_e is not necessarily the same as the actual number of breeding individuals. Unless the sexes are equal, N_e is less than N.

The reason for this can be seen intuitively. Consider a herd of zebra comprised of a male and nine mares. All the offspring in such a group will be half-sibs or full sibs. Now, in a population comprised of five males and five females, the progeny will, on the average, be much less closely related. Clearly the chance of an allele becoming lost is greater in the former population. That is, the amount of genetic drift in the herd with the skewed sex ratio is higher than the amount in the herd in which the sexes are equal. To be precise, the formula for N_e when considering the sex ratio is

$$N_e = \frac{4N_m N_f}{N_m + N_f} \tag{3.4}$$

where N_m and N_f are the number of breeding males and females, respectively. In the zebra example, N_e for the skewed herd is 3.6. In other words, the sampling error in a population of 3.6 individuals with an equal number of males and females is equal to the sampling error in a population of 10 individuals with a 9:1 sex ratio. Thus, N_e is the size of an ideal population that is subject to the same degree of genetic drift as a particular real population. In this definition 'ideal' means a randomly breeding population with a 1:1 sex ratio, and in which the number of progeny per family are randomly (Poisson) distributed.

The zebra example is really not far-fetched. In zebras, like other equids, the reproductive group is a harem, and a single male may sexually monopolize as many as 6 mares (Klingel, 1969). If the average size of a harem is 5 mares, a herd of 100 individuals will consist of, say, 60 females (12 harems of 5 each), 12 stallions, each with a harem, and 28 bachelor males. From the above formula, the effective size of the herd is $4(12 \times 60)/(12 + 60) = 40$. In other words, the amount of random genetic drift in these 100 adult zebra is equal to that in an 'ideal' population made up of 20 males and 20 females mating randomly.

3.1.5 Effective population size: population fluctuation

Real populations fluctuate in size. Much of the discipline of ecology deals with the causes and consequences of this phenomenon. In insects, fluctuations of several orders of magnitude are common, especially in temperate-zone species. Vertebrates tend to fluctuate less violently, but changes in food

abundance, weather and pathogens often account for large swings in numbers. Even in the tropics, long thought to be synonymous with stability, there are dramatic changes in the numbers of animals (Gilbert, 1980; Foster, 1980).

When populations decline or 'crash', the survivors are the progenitors of all future generations, and any deviation in the genetic make-up of these progenitors from the gene pool of the original population will be reflected in future generations. More particularly, if the progenitors contain but a sample of the kinds of genes that existed in the original population, future generations will have a corresponding deficit in genetic diversity. In more quantitative terms, the effective size of a population when the number per generation varies over time is the harmonic mean of the effective number of each generation, or

$$\frac{1}{N_e} = \frac{1}{t}\left(\frac{1}{N_1} + \frac{1}{N_2} + \ \ldots \ + \frac{1}{N_t}\right) \tag{3.5}$$

In section 3.1.3 this formula was used to calculate the effective size of an exponentially growing population. A more typical situation for the practising wildlife manager would be a herd of large animals confined to an area of finite size. For example, say that we wish to maintain a stock with an effective size of at least 100, but that we can expect the population size to decline to 25 on the average of once in 10 generations. In order to maintain an effective size greater than 100 the population must be allowed to grow to a larger size during the 'good' years. A simple calculation will show that this larger size, or carrying capacity, K, is 150. If the minimum size over the 10-generation interval is 15 individuals, K must be 270. K reaches infinity if the minimum size is 10. It follows that the maintenance of a reasonably large effective size will require space or facilities for more individuals than might have been anticipated.

3.1.6 Effective population size: progeny distribution

One of the characteristics of a genetically ideal population is that the number of progeny is randomly distributed among the families; i.e. a Poisson distribution should describe the frequencies of offspring number. Such a distribution probably holds for organisms with non-overlapping generations, but large organisms as a rule have overlapping generations. Hill (1977) points out that 'with overlapping generations, even if there are no fertility differences among survivors and there is random death of breeding individuals, the inbreeding rate will be higher than [expected] since the distribution of lifetime family size is not Poisson. With an exponential distribution of deaths the rate can be nearly *three times as high* as in the simple formula (Felsenstein, 1971)' (emphasis added).

The effect of a non-random distribution of offspring among families on N_e is easily estimated. Let $k_1, k_2, \ldots$ represent the number of gametes contributed by different individuals to the next generation, and let $\bar{k}$ and V_k be the mean and variance, respectively, of the k's. Then

$$N_e = \frac{N\bar{k}}{\left(\frac{N}{N-1}\right)\frac{V_k}{\bar{k}}(1+F) + (1-F)} \tag{3.6}$$

where N is the actual number of parents and F is the inbreeding coefficient (Kimura and Crow, 1963), the latter defined in section 3.2.2. In an infinitely large (ideal) population both $\bar{k}$ and V_k equal 2.0, and F is a very small number. A few 'thought experiments' with formula 3.6 should convince you that N_e will be less than N when some families have no offspring and others have many. At the other extreme, where all families have exactly the same number of offspring $N_e = 2\ (N-1)$. Thus in a closely managed population, such as in a zoo or possibly a remnant herd of a large mammal in a nature reserve, it is possible for the effective size to be twice the number of breeding individuals. This principle is probably the most powerful weapon in the hands of captive breeders. It should be noted, however, that the exploitation of this method forestalls the operation of natural selection and would rarely if ever be appropriate in populations numbering several hundred or more individuals.

One way of reducing V_k and thus increasing N_e is by culling offspring from larger families, thereby levelling out the genetic contributions of parents. Culling of excess offspring is often necessary in managed populations, so it might as well be practised with this genetic purpose in mind. The following example from Denniston (1978) demonstrates the efficacy of culling to increase N_e. Removal of offspring is done as follows: if one individual is culled, it is taken from the largest family; if two or more individuals are culled, individuals are taken from the largest family until it is reduced to the size of the second largest family, then individuals are removed alternatively from both families until they are reduced to the size of the third largest family, and so on. Denniston's population is monogamous and consists of fifteen families contributing 0, 0, 1, 1, 1, 1, 1, 6, 6, 7, 7, 7, 7, 8 and 9 progeny, respectively. Here, the value of $\bar{k}$ is 4.13, and V_k is 11.05. The impact of culling in this manner is shown in Fig. 3.3. Note that half of the offspring can be removed without decreasing N_e. In fact, if there is significant inbreeding in the population, culling in this manner actually increases N_e. The mathematically inclined reader should refer to James (1962) and Latter (1959).

3.1.7 Effective population size: close management of breeding

In situations where individuals can be identified and where breeding can be manipulated (such as in zoos), it is possible to minimize the effects of genetic drift and inbreeding. Animal breeders have developed several such breeding schemes; they include *maximum avoidance of inbreeding, circular half-sib mating* and *double first cousin mating*. These schemes require precise pedigree information which in turn permits the calculation of inbreeding coefficients (Wright, 1977).

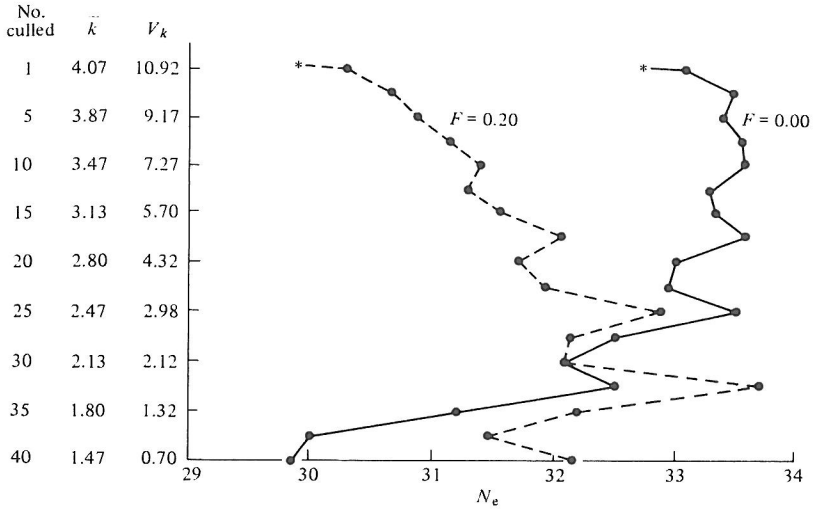

Fig. 3.3 The effect on effective population size of culling progeny from the largest of fifteen families contributing 0,0,1,1,1,1,1,6,6,7,7,7,7,8 and 9 offspring, respectively. Asterisks indicate the N_e values for the unculled population.

The impact of such schemes on the effective size of a breeding group varies according to the scheme. The effect of maximum avoidance of inbreeding is essentially the same as that produced by equalizing progeny number among families – there is a doubling of the effective population size compared to random mating. Circular half-sib mating is less effective in the early generations but surpasses maximum avoidance later on (Kimura and Ohta, 1971). In practice, however, we feel that these schemes rarely will be implemented. The reason is that specific matings would be dictated by the pedigree rather than by social position, but most zoo breeders are reluctant to separate established and productive breeding pairs and to disrupt social hierarchies by shifting animals from one group to another. In fact, such manipulations can often result in infanticide and other forms of mayhem (Kleiman, 1980).

In any case, there is virtually no advantage in using these schemes over the equalization of progeny number among families. The doubling of N_e in the maximum avoidance system, for example, is almost entirely attributable to each parent contributing the same number of progeny to the next generation of parents. The breeder is well advised, therefore, to use common sense in

the management of breeding, and to emphasize the practice of culling offspring (as described in the preceding section) for the minimization of genetic attrition.

3.2 Genetic variation in natural populations: data, models and hypotheses

In section 3.1 we reviewed the principles and statistical tools which permit us to predict the consequences for genetic variability in those situations where population size is low. In this section we attempt to go one step further – to determine the effect on *fitness* of reduction in genetic variation, the fundamental question of conservation genetics. Before fully engaging this topic, however, one must be somewhat familiar with three related subjects: (1) the methods currently in use for estimating levels of genetic variation in natural populations; (2) the generalizations about genetic variation in different taxa and how these might be affected by population structure; (3) some of the models used to account for the existence, persistence and heterogeneity of genetic variation in populations. Readers already familiar with these topics should skip to section 3.2.3.

3.2.1 Estimation of genetic variation in natural populations

Several authors have recently reviewed the literature on biochemical variation in natural populations (Powell, 1975; Selander, 1976; Soulé, 1976; Nevo, 1978; Wright, 1978). Each author tends to champion a particular hobbyhorse, but on some points the data speak for themselves, and there is general agreement. But before discussing these generalizations, it is necessary to take a short excursion on methodology.

There are many ways to express genetic variability at the gene product level (Wright, 1978). With respect to a local population, one can consider the proportion of the observed loci which have more than one variant (allelozyme or allozyme), the percentage polymorphism, P. A common convention is to consider as polymorphic only those loci at which the commonest variant (allele, loosely speaking) has a frequency less than 0.95. Also at the level of the local population, one can estimate the observed heterozygosity, H, the percentage of observed genotypes at which the average individual is heterozygous. The two measures are highly correlated as shown in Table 3.5.

When considering many populations or the species as a whole, however, the correlation may break down completely. For example, local populations of an inbreeding plant may each be fixed for a unique constellation of alleles, thus giving, for the species as a whole, a high estimate for P, but zero H. The same phenomenon, though less extreme, is seen in highly subdivided populations of outbreeding species of plants and animals. Thus in inbred, subdivided or poly-typic species, the greatest fraction of the observed variation may manifest itself among rather than within individuals.

TABLE 3.5 *Estimates of genetic variation in natural populations based on electrophoretic data*

Taxonomic group	No. of species	P Mean	P SD	H Mean	H SD	$r(P, H)$
Mammalia	46	0.147	0.098	0.036	0.025	0.838[b]
Aves	7	0.150	0.111	0.043	0.036	0.900[a]
Reptilia	17	0.219	0.129	0.047	0.003	0.605[a]
Bony fishes	51	0.151	0.098	0.051	0.034	0.883[b]
Plants	15	0.259	0.166	0.071	0.071	0.206
Insecta (exc. *Drosophila*)	23	0.329	0.203	0.074	0.081	0.680[b]
Amphibia	13	0.269	0.134	0.079	0.042	0.785[a]
Invertebrata (exc. Insecta)	27	0.399	0.275	0.100	0.074	0.788[b]
Drosophila	43	0.431	0.130	0.140	0.053	0.637[b]

From Nevo (1978).
P = proportion of loci polymorphic per population.
H = proportion of loci heterozygous per individual.
$r(P, H)$ = correlation between P and H over all species.
[a] $p < 0.01$.
[b] $p < 0.001$.

Which of these simple measures is best for conservation genetics? Limiting ourselves to diploid, outbreeding species (and most large animals and tropical plants belong to this category), the important variable is the amount of genetic variation within the group of individuals that is actually or potentially the target of a conservation programme.* Such a group will most often be (1) a natural geographic remnant of a once widespread species, or (2) a synthetic mixture of individuals from two or more such remnants. For the present purpose, the difference between these two kinds of groups (natural and synthetic) can be ignored because it will disappear after a single generation of breeding (although certain genetic problems, such as inbreeding depression, might appear with greater frequency in the natural group, whereas other problems, such as genetic incompatibility, may be more common in synthetic groups). The simplest measure of actual genetic variability in such circumstances is observable heterozygosity. In the absence of dominance and epistasis, heterozygosity is the same as additive genetic variance, the selectable component of total genetic variance.

Table 3.5 from Nevo (1978) summarizes the general levels of elec-

* Note discussion in Chapter 1, p. 8, on the genetics of scarcity.

trophoretic variation in natural populations. The taxonomic groups are ranked in order of increasing H. The data for plants and birds could be misleading because of the small sample size and because most of the plant species so far studied have been inbreeders (Brown, 1978). In addition to the plants, hermaphroditic snails (Selander and Kaufman, 1973a) and non-flying Orthoptera (Nevo, 1978) also have very low levels of heterozygosity, and the inclusion of these groups in the table has reduced the H values for the invertebrates and the insects, respectively. There is no way to avoid this problem when averaging together species which differ among themselves in breeding system, population size, vagility and history.

3.2.2 Models for the maintenance of genetic variation in natural populations

In chapters 3 and 4 we have generally and implicitly assumed that deleterious recessive genes are the cause of the decrease in fitness that accompanies inbreeding. The term applied to this category of genetic disability is *mutational load.* Actually inbreeding depression can also be the result of over-dominance, the superiority of the heterozygote over both of the homozygotes. Genetic load resulting from over-dominance is referred to as *segregational load.*

The consensus today is that little or no overdominance exists at individual gene loci, or even for individual morphological or reproductive traits (Eberhart, 1977). Comstock (1977) concurs, and in reviewing the state of the art of quantitative genetics states that 'Studies of genetic variance components indicate that overdominance is not a major feature in the genetics of single quantitative traits, not excepting such highly heterotic ones as grain yield of maize.' Falconer (1977) comes to the same conclusion based on his selection experiments in mice. The only well-documented exception in all the scores of traits that have been genetically defined, whether simple or complex, is sickle-cell anaemia, but even this textbook example turns out to be a case of 'conditional' heterosis, since the heterozygote advantage disappears with the removal of the selective factor, falciparum malaria (cf. Berger, 1976).

One way of rescuing single gene overdominance was originally suggested by Levene (1953). Levene showed how a polymorphism could be maintained in a heterogeneous environment even though the heterozygote was nowhere superior to both homozygotes. The necessary condition is that the relative superiority of the homozygotes changes from one site to another. An analogous model could be applied to tissues within individuals or to the different life history stages. That is, if AA is superior to aa in the adult stage (or the gut epithelium), while aa is better in the larva (or brain), then the heterozygote, Aa, would be the most efficient overall (Fig. 3.4). This model requires no environmental heterogeneity.

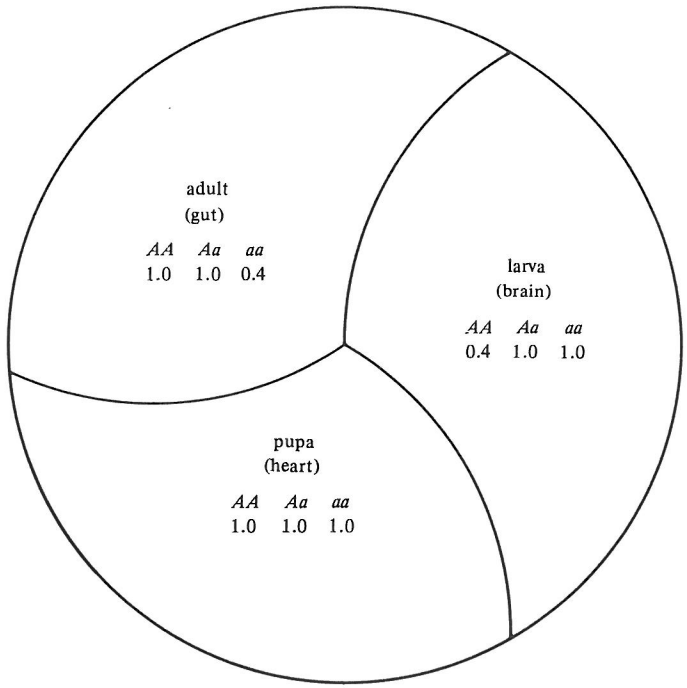

Fig. 3.4 A model of the maintenance of a structural gene (protein) polymorphism due to life cycle or tissue heterogeneity. The numbers below the genotypes are hypothetical biochemical efficiencies. Averaged over all stages or tissues, the heterozygote is superior to either homozygote, yet there is no heterosis *per se* in any single biochemical environment.

Such models, stemming from Levene and others, bear the name *marginal overdominance*, and they have attracted considerable attention and extension in recent years (Hartl and Cook, 1973; Karlin and Lieberman, 1975; Gillespie and Langley, 1974; Hedrick, 1974; Gillespie, 1977). Here, however, we have an example of theory outdistancing facts; there are simply no sophisticated tests of marginal overdominance. Nevertheless, the genetically knowledgeable conservationist should be aware that the downfall of pure (or unconditional) overdominance need not imply that the masking of deleterious recessives is the only advantage of heterozygosity. The genetic polymorphisms existing in natural populations might enhance fitness, even in the absence of deleterious recessives, assuming the reality of marginal overdominance. In summary, environmental variation in space or time (including somatic space and ontogenetic time) could account for the persistence of some fraction of allelic polymorphisms.

On the surface, this conclusion might appear to contradict our belief that

the amount of genetic variation in natural populations is strongly dependent on population structure, particularly population size (see section 4.2.2). Actually it does not. Even if most polymorphisms are selected, the selection coefficients must be small, on the average, and genetic drift will still be a major force in determining the overall number of such polymorphisms.

Quite a different conceptual approach, one favoured by ecologically minded population geneticists, invokes the metaphysical concept of niche width as a principal cause of differences in genetic variation between populations. This theory posits that heterozygosity can enhance the ecological amplitude of a population, either by (1) allowing the production of more kinds of individuals, or by (2) enhancing the flexibility of each individual. These ideas, known collectively as niche-variation hypotheses, (see review by Hedrick, Ginevan and Ewing, 1976) predict that a population inhabiting a wide niche can more successfully exploit its resources by generating phenotypic variation of one or both of the above two kinds. For example, when the variance of prey size is large, a predator species that is variable in size might be able to maintain a greater population size compared to a species that is less variable; this is selection of the first (1) type. Another version of the niche-variation hypothesis assumes that heterozygous individuals can cope with environmental extremes and variation more efficiently than can relatively homozygous individuals; this is selection of type (2), above.

Marginal overdominance of the niche-variation hypotheses are mutually compatible; more precisely, the former provides a mechanism that could account for the latter. That is, a 'wide niche' is a mosaic of selection regimes permitting marginal overdominance to be expressed, whereas a 'narrow niche' could be thought of as a single regime in which selection coefficients of genotypes are fixed.

These ideas have spurred considerable debate. One of us (M.E.S.) has argued that the niche-variation hypotheses, while logical and intuitively appealing, are based on a simplistic view of genetic organization, and that all the available data can actually be explained more parsimoniously by an alternative hypothesis, namely that the differences in average heterozygosity among populations are explicable in terms of population structure and history as well as differences in the strength of directional selection (Soulé, 1976). Others base their models on predictability and reliability of food categories in the environment (Valentine, 1976). Both of these latter schools are in agreement that natural selection has a role in determining the differences in genetic variability among populations and species; they also agree that the simpler theories of the late 1960s and early 1970s (e.g. Selander and Kaufman, 1973b; Nevo, Kim, Shaw and Thaeler, 1974) do not account for the patterns of variation that have emerged in recent years.

Still another model that can account for the selective maintenance of heterozygosity is frequency-dependent selection; Lewontin (1974) gives a comprehensive discussion. While there is some laboratory evidence for a negative correlation between an allele's frequency and its contribution to fitness (an allele improves relative fitness when rare, decreases it when common), the relevance of this in nature is unknown.

Yet another class of models, a class that is in some ways quite realistic, makes an important distinction regarding the kinds of genetic load (Wallace, 1970). Wallace points out that some genotypes are unfit in all environments and at all population densities (density-independent fitness); i.e. 'hard' selection will tend to eliminate such genotypes at all places and times. Other kinds of genotypes will prosper when competition or population density is low, but will suffer when conditions are less favourable (density-dependent fitness); he refers to such conditional selection as 'soft selection'. It is hard to see how such a distinction will be of much interest to practical conservationists, however; as is discussed in chapter 5, the survival of *many* species of large vertebrate or plant is going to require intensive management, including density management, and such phenomena as hard versus soft selection, or unconditional versus marginal overdominance, will probably not be applicable (or detectable) in practice.

Finally, there are the 'neutralists' (Nei, 1975, reviews the thinking of this school) who espouse a modern version of the 'classical' position in population genetics (see Lewontin (1974) for a thorough discussion). The neutralists believe that the heterozygosity we observe is mostly irrelevant to evolution and fitness because the alleles are physiologically and biochemically indistinguishable. Ohta and Kimura (1975) have proposed a model which is classical in spirit, but can explain the persistence of much allelic diversity in terms of an equilibrium between mutations producing slightly deleterious alleles and their elimination by stabilizing selection. Wright (1978) offers another option, taking into account variation in space; he also assumes complex dominance and epistasis interactions.

3.2.3 Heterozygosity and fitness in natural populations

Immediate fitness is all those phenomena that contribute to survival (viability) and reproduction. There are two categories of genetic phenomena which can have a direct effect on fitness, independent of their effects on particular characteristics; these are *heterosis* and *inbreeding depression*. Both of these are related to N_e and to the level of genetic variability or heterozygosity, and in many situations they might even be considered opposite sides of the same coin. Nevertheless, we shall treat them separately (for reasons of convenience and didacticism), while acknowledging the somewhat arbitrary nature of this dichotomy. We begin with heterosis.

Among geneticists and breeders there is a *heterozygosity consensus*; this is the belief based on extensive laboratory and farm experience that fitness (viability, vigour, fecundity, fertility, etc.) is enhanced by heterozygosity, and that any decrease in genetic variation will be paralleled by a diminution of fitness. Enhancement of fitness due to increased heterozygosity is called heterosis, and it is virtually universal in outbreeding domesticated plants and animals.

The question we address here is whether a decrease of heterozygosity in a *natural* population will lead to a corresponding decrease in fitness. If it does, some species could become moribund or extinct for no other reason than loss of heterozygosity, *per se*. Another way of phrasing this question is this: how much of fitness depends on being heterozygous at a significant proportion of an individual's loci? There are many possible functional relationships between fitness and heterozygosity in natural populations. Some are illustrated in Fig. 3.5. Model 1 portrays the 'null hypothesis', that of no effect of heterozygosity *per se* on fitness. (The intercept for this curve is arbitrary.) Model 2 portrays a linear relationship – the more loci are heterozygous, the more fit the individual, and each additional heterozygous locus, on the average, adds a constant increment of fitness. Model 3 portrays an asymptotic hypothesis model: each additional heterozygous locus confers less benefit.

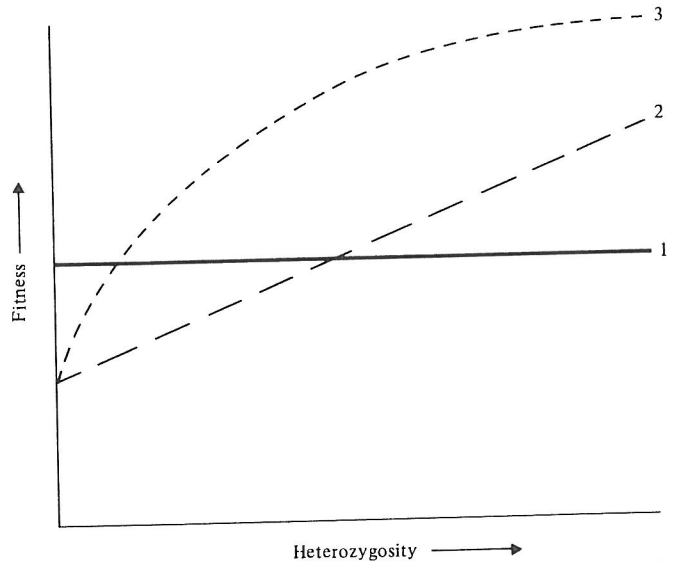

Fig. 3.5 Three possible relationships between genetic variation and fitness. The intercept is shown to be greater than zero in order to indicate that viable populations can exist without any heterozygosity. The intercept of curve '1' is arbitrary.

The central theoretical problem of conservation genetics is to decide which among these models provides the closest fit to reality. One approach is to compare the heterozygosities and fitnesses of individuals within populations. Until recently it was impossible to assay the relative differences in heterozygosity among individuals, unless, that is, their pedigrees were known. Since about 1965, however, electrophoretic techniques have permitted us to rank individuals according to their electrophoretic variation (Lewontin, 1974). The most promising strategy for demonstrating the superiority of relatively heterozygous individuals is the 'longitudinal' sampling of a cohort, i.e. repeated sampling of a year class in order to test the hypothesis that individuals which are relatively heterozygous have a higher than average probability of surviving a long time. The next best thing is a 'vertical' (one-time) sampling of the age classes, although this method introduces the possibility of error because of heterogeneity due to birth or development during different seasons or years. Data from both kinds of studies are slowly accumulating.

A caveat before proceeding with a discussion of the results: there have now been hundreds of electrophoretic surveys of natural populations and only a small fraction of these report any significant excess of heterozygotes. By emphasizing the latter studies and ignoring the rest there is obviously a danger of bias. On the other hand, there are two reasons why this possible bias is not so serious. First, most studies use relatively small sample sizes per population, so statistical significance for departure from Hardy–Weinberg frequencies would not be expected unless the heterozygote excess was quite large. Second, few studies were designed to sample age classes, either horizontally or vertically, and the chance of finding a heterozygote advantage is very much decreased when age classes are pooled.

One final technical point: on *a priori* grounds we might expect never to observe an excess of heterozygotes when many loci are sampled in a series of age classes. This is because, as Sved, Reed and Bodmer (1967) have shown, the variance of the number of heterozygous loci per individual in a randomly mating population is very low. For example, in a population in which there are 10 000 polymorphic loci, each segregating for two equally frequent alleles, the average individual will be heterozygous for 5000 with a standard deviation of fifty; this means that 95% of the individuals will have between 4900 and 5100 heterozygous loci. With such a tiny differential in heterozygosity, natural selection might be hard pressed to distinguish between the extremes. Later (p. 53) we suggest that structured populations, which are probably the rule for many terrestrial species of plants and animals, have much higher heterozygosity variances than would be the case for the ideal, randomly mating population, in turn allowing much more latitude for natural selection to distinguish individuals based on their total heterozygosity.

3.2.4 Intrapopulation studies

In some investigations only one or two polymorphic loci have been examined. One of the first and most widely cited of such studies was a survey of transferrin groups in species of tunas by Fujino and Kang (1968). They divided a sample of 790 skipjack tuna from Hawaii into five age (size) classes in order to test for differential fitness of the transferrin classes with length of survival. They claimed to show that the smallest fish (31–40 cm) had a deficiency of homozygotes and an excess of heterozygotes. There are several ways to look at these data, however, and it is by no means established that overdominance (heterosis) exists. In any case, the putative heterozygote advantage decreases with the age of the fish, so there is no evidence for the superiority of heterozygotes throughout the life of the adult fish.

Chaisson, Serunian and Schopf (1976) reported that ribbed mussels (*Modiolus demissus*) living in the Wild Harbor salt marsh, Cape Cod, Massachusetts, apparently have a higher probability of surviving to adulthood if they are heterozygous at the tetrazolium oxidase locus. At two localities in the marsh, the large mussels showed considerable excesses of heterozygotes, but the young mussels at the same sites did not. Koehn, Turano and Mitton (1973) earlier came to the same conclusion using the same material; they found that newly settled mussels have a deficiency of heterozygotes, whereas large individuals have no deficiency or have a slight excess. Analogous results were obtained by Tracey, Bellet and Graven (1975) and by Koehn, Milkman and Mitton (1976) in studies on another genus of mussel. An unresolved issue in these studies is why the populations are deficient of heterozygotes just after settling.

Similar trends to those just described for mussels have been reported by other investigators employing only one or two polymorphic loci. These include Tinkle and Selander (1973) using the lizard *Sceloporus graciosus*, Watt (1977) using *Colias* butterflies, and Converse and Williams (1978) who found that heterozygotes in man for the *HLA-B* locus live longer than do homozygotes.

A clever departure from the usual protocol of such studies was carried out by Singh and Zouros (1978) on the American oyster *Crassostrea virginica*. They noted a great heterogeneity in shell size of oysters of the same age, and asked whether the faster growing individuals were more heterozygous than slower growing ones in the same cohort. Electrophoresis was performed on 372 one-year-old oysters, half of which were the smallest (slow growing), weighing less than 1 g, and half of which were the largest (fast growing) in their sample weighing more than 4 g. Five loci were found to have substantial levels of polymorphism; they were *Lap–2, Pgi, Pgm, Est–3* and *Got–1*. All except *Got–1* were deficient in heterozygotes for the small class. Singh and Zouros divided the large class of oysters into three subgroups: 4–6 g, 6–8 g, and 8 g and over. The general result was a correlation between growth rate and heterozygosity (Fig. 3.6); in the largest class, none of the four loci showed a heterozygote deficiency. For *Got–1*, the pattern was the opposite with an excess of heterozygotes in the small class and a deficiency in the largest. The authors attribute the *Got–1* result to locus specific effects on fitness. The overall slower growth in relatively homozygous individuals, they think, is probably related to genetic load i.e. homozygosity for deleterious genes: on the average the slower growing individuals are more inbred and thus suffer from a mild degree of inbreeding depression (see section 3.3.3). Regardless, this study provides evidence for the superior viability or vigour of relatively heterozygous individuals.

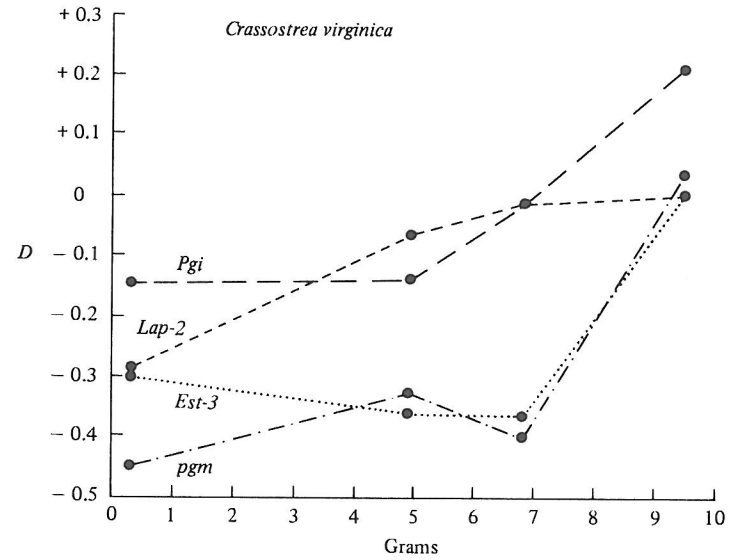

Fig. 3.6 Deviations from expected heterozygosity at four loci in the American oyster. The ordinate is the deviation: observed heterozygosity (H_o) minus expected heterozygosity (H_e) divided by H_e. The abscissa is weight in grams. After Singh and Zouros (1978).

One of the most convincing studies of this kind was performed on the perennial herb *Liatris cylindracea* by Schaal and Levin (1976). *Liatris* is a self-incompatible member of the Compositae occurring locally throughout the dry prairies of the American midwest. The perennating organ is a corm which can be aged by counting the rings of pigmented cells deposited annually in this population. The average age of individuals at the study site was nineteen years, and the range was one to forty-four years. The sample was divided into six age classes and heterozygosity estimates were obtained

by electrophoretic surveys of corm tissue, using fourteen polymorphic loci. Survival is clearly related to heterozygosity as shown in Fig. 3.7. As in Fig. 3.6, the ordinate is deviation from expected heterozygosity. The youngest plants are excessively homozygous, probably because of inbreeding, but this excess decays with age, apparently due to the disproportionate mortality of homozygous individuals.

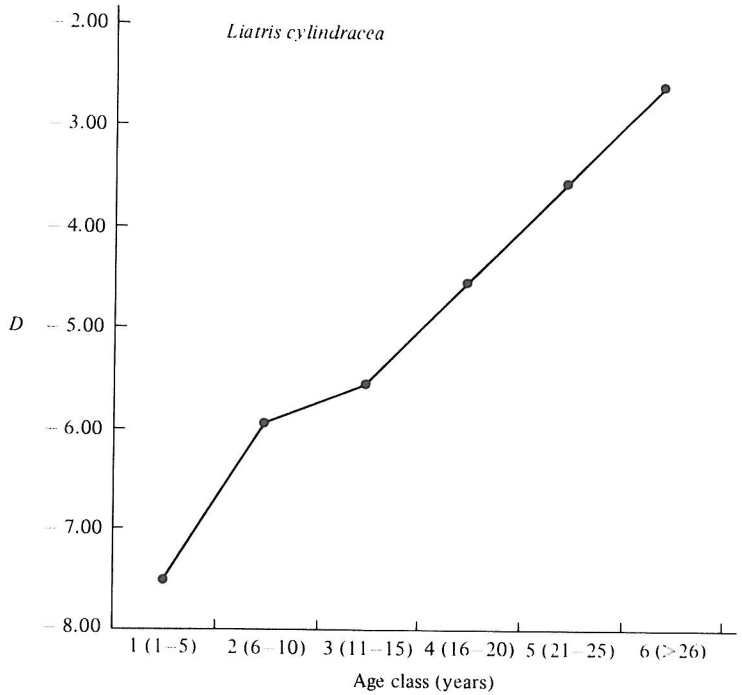

Fig. 3.7 The decrease in heterozygote deficiency with age in a natural population of *Liatris cylindracea*. Ordinate is deviation from expectation assuming random mating. Data from Schaal and Levin (1976).

Schaal and Levin exploited the relative ease of manipulation in plants, performing greenhouse tests for relationships between heterozygosity and (1) age at sexual maturity, (2) reproductive output and (3) vegetative output. All these experiments confirmed the association between heterozygosity and fitness. For example, *Liatris* seedlings were grown in the greenhouse for two years and were then divided into those that had flowered (in the field, flowering usually commences at between five and ten years) and those that had not reached maturity. The average heterozygosity of those that had flowered was 8.1%, while that of the non-flowering plants was 5.2% ($P<0.001$). Overall, this study quite clearly demonstrates natural selection favouring excessively heterozygous individuals.

Further evidence for the superiority of relatively heterozygous individuals in natural populations comes from studies (Mitton, 1978; W. F. Eanes, 1978) in which both external morphological as well as electrophoretic measurements are made on the same individuals. Both Mitton, whose organism was the killifish, *Fundulus heteroclitus*, and Eanes, whose organism was the monarch butterfly, *Danaus plexippus*, reach the conclusion that individuals which are heterozygous at randomly chosen polymorphic loci are less variable for the morphological traits than are homozygous individuals. Eanes and Mitton view their results in light of laboratory studies showing that homozygotes are less well developmentally buffered than heterozygotes. Even if this interpretation is incorrect, the fact remains that phenotypically extreme individuals are less likely to survive and reproduce than typical individuals (Cavalli-Sforza and Bodmer, 1971; Fox, 1975; Franklin, 1980 refers to several examples), and that fitness is therefore inversely related to an individual's deviation from the average character state for quantitative traits.

At this point we step aside from our catalogue of relevant studies and briefly discuss the issue raised on p. 49 regarding the effect of population structure on the theoretically narrow range of heterozygosities occurring in an ideal population. Recall that in a large, panmictic population, all individuals should have about the same level of heterozygosity. Actually such an ideal population is one of the extremes on a continuum of possible population types; near another extreme are highly structured populations in which small and semi-isolated subpopulations exchange individuals at a very low rate. In the latter case there will be an excess (compared to random breeding) of homozygotes, and the proportion of the genome that is homozygous will vary considerably among individuals. In addition, rather large arrays of linked alleles can be maintained in non-random or non-equilibrium combinations for several generations (Wills, 1978). In other words, structured populations will produce a much greater range of individual heterozygosities. Consequently, the chances are good of finding evidence of heterozygote superiority when assaying a random sample of loci in such populations because even neutral alleles will often be linked to genes that confer an advantage to the heterozygote. As a result, we can expect to observe heterozygote advantage much more easily in a structured population, such as *Liatris*, than in a more vagile species.

It is important, however, to appreciate that heterozygotes may be superior even in species where such fitness differences are undetectable. That is, we may not always be able to resolve the slight differences in heterozygosity existing among individuals. In such species another approach is required. One such approach is to compare the fitnesses of individuals from populations differing in average heterozygosity.

3.2.5 Interpopulation studies

Just such a study was performed by Garten (1976) on the relationship between aggressive behaviour and heterozygosity in the oldfield mouse, *Peromyscus polionotus*. Garten obtained mice from populations differing in mean electrophoretic heterozygosity and tested their aggressiveness by staging paired encounters in small arenas. He found very significant correlations between various components of aggression and heterozygosity (Fig. 3.8). As in all such studies, the danger of spurious correlation is ever present. For example, the correlation between mean body size and mean heterozygosity among populations was also very high, so that the aggressive superiority could be the result of the actual differences in strength among individuals, or, perhaps, it could have resulted from the intimidation of the smaller individuals by the larger. Other, more subtle spurious correlations cannot be ruled out.

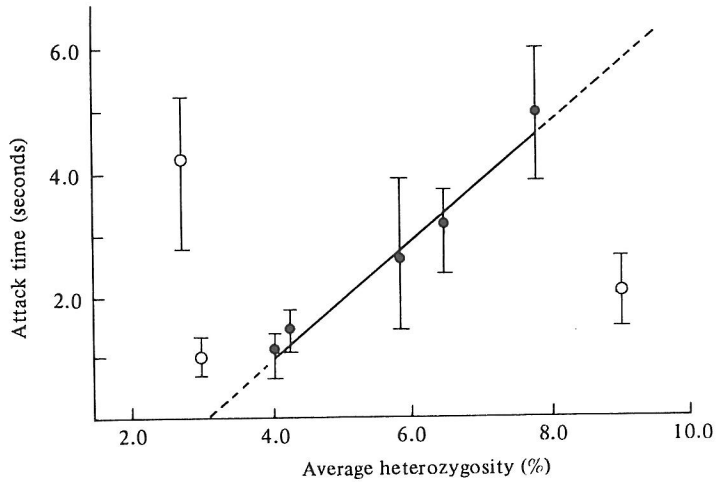

Fig. 3.8 Relationship between mean accumulated attack time and average percentage heterozygosity of male oldfield mice from five mainland (dots) and three island (circles) localities. The regression line is based on mainland samples only. After Garten (1976).

Another interpopulation study was recently reported by Soulé (1979) who reanalysed data from earlier studies on lizard populations in order to test the relationship between mean heterozygosity and fitness. Fitness was defined in terms of developmental stability or homeostasis as measured by asymmetry of bilateral morphological traits. The assumption is that asymmetry reflects accidents or 'noise' during development, and that genetically superior individuals will be less asymmetrical than genetically inferior ones.

Fig. 3.9 suggests that there may be a negative correlation between heterozygosity (estimated by electrophoresis of proteins) and asymmetry. If future studies of this kind verify a correlation between developmental stability and heterozygosity, we will be led to conclude that individuals from populations rich in genetic variation are more fit in this regard than individuals from genetically depauperate populations.

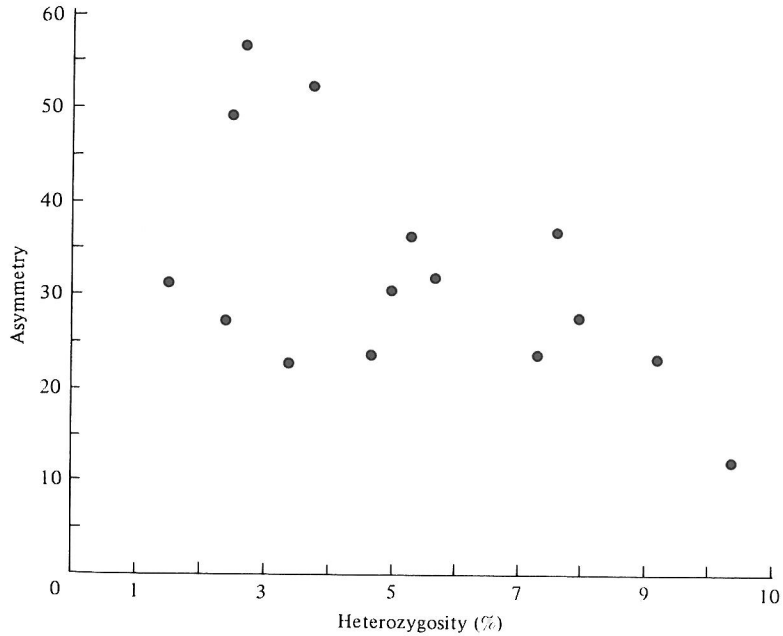

Fig. 3.9 The relationship between fluctuating asymmetry (sum of ranks) and percentage heterozygosity in one mainland and fourteen island populations of the sideblotched lizard. From Soulé (1979).

The studies discussed so far point to the ubiquity of heterozygote superiority. On the other hand, it is possible to carry this conclusion too far. Just as cake is edible without icing, so might populations be viable without any heterozygosity, at least in the short run. Nor is the mere existence of allelic variation in a population evidence for its contribution to immediate fitness. Icing can be made with salt instead of sugar, and allelic variation can be neutral or even deleterious. In any case, populations are known in which allelic variation is nil.

Some predominantly inbreeding plants, for example, have relatively high levels of heterozygosity, while others seem to have none. Four such species were included by Brown (1978) in a recent survey of genetic structure in plants. The heterozygosity data are given in Table 3.6. The important thing

TABLE 3.6 *Genetic variability in a sample of predominantly inbreeding plants*

Species and region	Number of populations	No. of loci scored	No. of loci polymorphic	H_e		
				Mean	Min	Max
Oenothera biennis						
Cook County	16	20	1	8	0	50
Oenothera biennis						
S. Illinois	28	20	4	22	0	50
Avena barbata						
California	16	5	5	7	0	48
Hordeum spontaneum						
Israel	28	28	25	11	0	20
Lycopersicon pimpinellifolium						
Ecuador & Peru	43	11	11	14	0	27

H_e is the mean expected panmictic heterozygosity.
From Table 1 of Brown (1978).

to note is the range of the values. Some populations in each species had no heterozygosity; some had 50%. Except for the obvious conclusion that heterozygosity is not a necessary condition for survival in selfing plants, it may be imprudent to generalize further.

Yet, the very presence of segregating polymorphisms in some populations of predominantly inbreeding species is something to wonder at, and it has been interpreted as prima-facie evidence for heterozygote superiority. For example, Clegg, Allard and Kahler (1972) studied experimental populations of barley (*Hordeum vulgare* L.); these lines are over 99% self-fertilizing. Later they (Clegg and Allard, 1972) found that some populations maintain substantial heterozygosity for tightly linked, co-adapted gene complexes. As Clegg and his coauthors point out, the fitness of the heterozygotes in these populations must be twice as high as that of the homozygotes to maintain these polymorphisms in the face of such intense inbreeding.

Rick, Fobes and Holle (1977) also observed twice the expected level of heterozygosity in polymorphic populations of *Lycopersicon pimpinellifolium*, a close relative of the tomato. Inbreeding in these populations ranged from about 45% to 100% per generation. Add to this the evidence for heterozygote advantage in other predominantly self-pollinated grasses (Jain and Marshall, 1967), and one cannot dismiss the possibility that heterosis is common in inbreeding species.

Thus, in inbreeding plants we see (Brown, 1978) an entire spectrum of

phenomena relating to heterozygosity. On the one hand there are completely homozygous populations; on the other there are populations with large and significant excesses of heterozygosity. In the middle are populations with some heterozygosity, but with no evidence, as yet, for heterozygote superiority. If generalizations are applicable to such species, they will not be widely accepted until many more studies of the Schaal and Levin model are performed.

3.2.6 The significance of monomorphic populations

Selfing plants are not alone in providing examples of negligible variation in proteins. Animals, too, occasionally lack such polymorphisms. For example, an elephant seal population with no detectable electrophoretic heterozygosity (Bonnell and Selander, 1974) is growing very rapidly, following near extinction from hunting. Other animals which are virtually lacking in detectable electrophoretic variation are an isolated gopher species (Selander, Kaufman, Baker and Williams, 1975 and references therein), lizards on tiny islands (Soulé, 1980), and a facultatively, self-fertilizing land snail (Selander and Kaufman, 1973a). Indeed, it is very likely that most of these populations are evolutionary dead-ends, but at least it is clear that survival in nature, albeit short-term, is possible despite virtual homozygosity.

Is the appearance of virtual total allelic impoverishment in the above plants and animals to be taken at face value? Admittedly, there could be considerable cryptic genetic variation in these populations, but even granting this, it still appears certain that some species (and some populations) are able to sustain themselves even though they contain much less than the average level of heterozygosity.

3.2.7 Conclusions

In this section we have asked whether there is empirical support for the hypothesis of a positive effect of heterozygosity on fitness in natural populations. Many of the foregoing studies would not, by themselves, be particularly convincing. Collectively, however, and in the absence of contradictory evidence, the judicious conclusion is that fitness in natural populations is a positive function of heterozygosity. Tentatively, then, we can rule out Model 1 in Fig. 3.5. Essentially this means that any loss of genetic variation, at least in outbreeding populations, is tantamount to erosion of immediate fitness. Further, there is no logical basis for assuming that there is a 'safe' level of fitness detriment. Given, that is, the general applicability of either Model 2 or Model 3 in Fig. 3.5, it is evident that each increment of genetic simplification costs the population an increment (not necessarily constant) of welfare.

The study by Rick *et al.* (1977) on genetic variation in *Lycopersicon* provides a tantalizing clue favouring Model 3 versus Model 2. As shown in Fig. 3.10, the excess of observed heterozygotes over the expected appears to be related to percentage cross-pollination. Heterozygosity of these populations and cross-pollination are highly correlated ($r = 0.81$; $P<0.01$), so the negative association of heterozygote excess with outbreeding suggests that the locus-specific enhancement of fitness increases as the overall heterozygosity decreases among populations. This may be the first evidence from natural populations for an asymptotic relationship between genetic variation and fitness. Earlier experimental work by Crow and his colleagues (Temin, Meyer, Dawson and Crow, 1969; Crow, 1970) provides additional support for the asymptotic model. They found that as *Drosophila* were inbred, their viability drops off slowly at first, and more rapidly as inbreeding reached higher levels.

It would be premature to base a conservation strategy on such a subtlety, but if Model 3 turns out to be a general rule in population genetics, it would mean that the absence of obvious inbreeding effects on the viability or fecundity of a managed population during the first few generations of captive breeding or intensive management is no guarantee that such immunity would persist at higher levels of inbreeding.

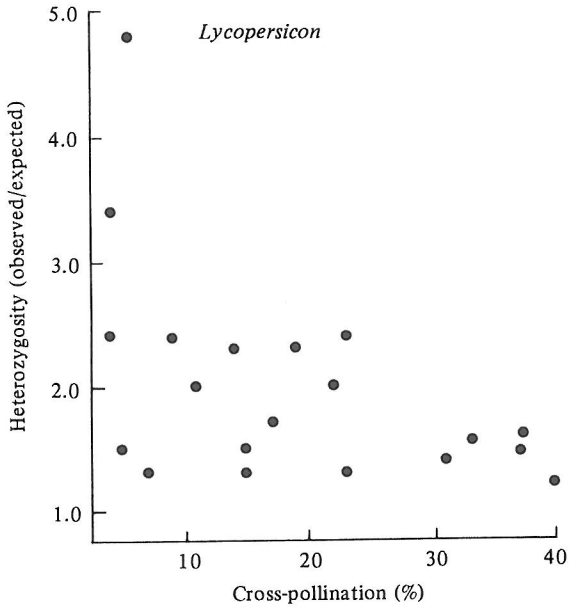

Fig. 3.10 The inverse relationship between cross-pollination and heterozygote excess in *Lycopersicon*. After Rick, Fobes and Holle (1977).

Finally, the correlation of fitness with heterozygosity in natural populations need not signal the existence of single gene overdominance. An alternative is dominance i.e. recessive deleterious genes; this is mentioned (p. 51) in the discussion of the work of Singh and Zouros (1978). One of the major tasks of empirical population genetics is to discover the mechanisms underlying the purported correlation.

3.3 Inbreeding depression

3.3.1 Inbreeding defined

We begin our discussion of inbreeding with an experiment performed on Poland China swine almost forty years ago (McPhee, Russel and Zeller, 1931). The experiment was designed to establish the effects of sib (brother–sister) mating. As it turned out, the experiment was rather short-lived, lasting only two generations. The reason for its discontinuation at this point was a precipitous drop in the fitness of the inbred line. As shown in Table 3.7 the mean number of pigs per litter dropped from 7.15 in the general herd to 4.26 in the second generation of inbreeding. This decline in fecundity was accompanied by a drop in survivorship of pigs by more than half. When the values for fecundity and survivorship are multiplied, one obtains the number of surviving offspring per litter. In the general herd this number is four; it is only one in the F_2 inbreds. Concomitant with this 75% loss in productivity was an equally serious change in the ratio of males to females; it changed from 1.1:1.0 in the general herd to 1.6:1.0 in the F_2 inbreds, thus further aggravating the decline in fecundity and survivorship.

The implications of results like these for the conservation of large organisms are profound, not only for the breeders of endangered species in zoos, but also for the managers of wildlife reserves. But before pursuing this topic we must first define inbreeding in a more quantitative fashion, and explain

TABLE 3.7 *Vital statistics of a herd of Poland China swine and the progeny of two generations of sib mating*

	No.	Inbreeding coefficient		Size of litter	Percentage born alive	Percentage raised to 70 days	Sex ratio
		Dam	Litter				
General herd	694	0+	0+	7.15	97.0	58.1	109.7
F_1 inbred	189	0.09	0.33	6.75	93.7	41.2	126.1
F_2 inbred	64	0.33	0.42	4.26	90.6	26.6	156.0

From McPhee, Russel and Zeller (1931) after Wright (1977).

some of the theories that attempt to account for its effects. Those familiar with inbreeding genetics might wish to skip to section 3.3.4.

One of the most important points to grasp about inbreeding is that it is a *relative* concept. For example, John may be homozygous for deleterious genes at many more loci than Mary, but Mary, strictly speaking, may be more inbred. This is because the formulation of inbreeding is in terms of the proportion of homozygous loci in an individual relative to that proportion in the general population. That is, John may be a member of a tribe having a strong taboo against incest and inbreeding, and yet everyone in the tribe can trace his ancestry back to a single matriarch and her husbands. Mary, on the other hand, might be a typical American of mixed ancestry, as well as being the daughter of first cousins. In genetic terms, John is more homozygous, but Mary is more inbred.

Another way of saying this is that an individual who is a product of inbreeding within a very heterozygous base population can be more heterozygous than a non-inbred individual from a genetically homogeneous population. We will return to this point in the discussion of the relationship between inbreeding and genetic load.

Inbreeding means mating of close relatives, individuals, that is, who are likely to share some of their genes because they have one or more ancestors in common. The most widely used measure of inbreeding, or consanguinity, is the inbreeding coefficient of Wright (1921), denoted by F. This coefficient is most easily understood as the probability that the two alleles of a particular locus in an individual are identical by descent. For example, consider individual E in Fig. 3.11; she is the product of a brother–sister (sib) mating, so she has only one set of grandparents. When considering only one gene (we use gene and locus interchangeably), the total number of copies present in her grandparents was four. We don't mean that the two grandparents had four biochemically distinct alleles; these four copies may have been molecularly identical, but for our purposes they are still considered distinct. Now we ask what is the probability (this probability is called F) that E is homozygous for any one of these four copies present in the grandparents. The answer is 1/4. This can be seen in the following way. The probability that A transmitted a copy of one of his two alleles, say a', to C is 1/2. The probability that another copy of a' was transmitted to D is the same, 1/2. Therefore the probability that C and D both received one copy of the identical gene a' is 1/4. Given that both C and D received one copy of a', the probability that E is homozygous for a' is 1/4; therefore, the probability that E is homozygous $a'a'$, *and* that both of the a' alleles came from A is $1/4 \times 1/4 = 1/16$. Because A and B together had four alleles of the gene in question, and because the probability is 1/16 that E will be homozygous for a *particular* one, the probability that she is homozygous by descent for *any* of the four copies is $1/16 + 1/16 + 1/16 + 1/16 = 1/4$. Precise methods for calculating inbreeding coefficients can be found in Cavalli-Sforza and Bodmer (1971), Crow and Kimura (1970), Pirchner (1969) and Falconer (1960).

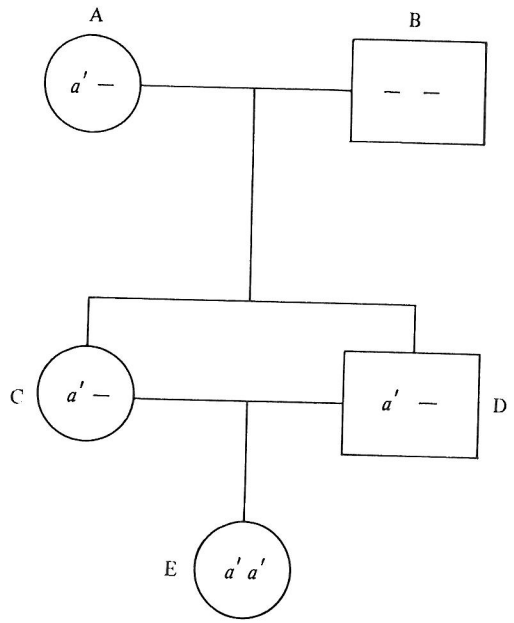

Fig. 3.11 A pedigree showing identity of a gene by descent. See text.

Another way to interpret F is in terms of the relative amount of heterozygosity. In the previous example we showed that an individual who is the offspring of a sib mating has 25% chance of being homozygous by descent at a given polymorphic locus. This is equivalent to saying that she will have alleles identical by descent at 25% of all of the loci which are not already fixed (that is, were polymorphic) in the base population. Putting it slightly differently, she will be, on the average, 75% as heterozygous as the average individual in the base or source population. F, then, is a direct estimate of the genetic variability *relative* to the variability in the non-inbred population.

3.3.2 The rate of inbreeding

Next, we examine a question of critical importance in conservation genetics – the *rate of inbreeding*. The rate at which genes become fixed (alternatively, the rate at which heterozygosity is lost) depends on the breeding system. The closer the relationship between parents, the higher the rate of fixation. The most intense form of inbreeding is selfing or self-fertilization. In theory, half of the remaining heterozygous loci become fixed every generation in a

selfing population. Hence, the per generation inbreeding is $F = 0.5$. Sib mating or offspring–parent mating gives a per generation loss of heterozygosity of 0.25, as we saw above. The inbreeding coefficients for sib mating as well as for other systems are given for five generations in Fig. 3.12. Note that the per generation change in the inbreeding coefficient, ΔF, is a constant for any breeding system.

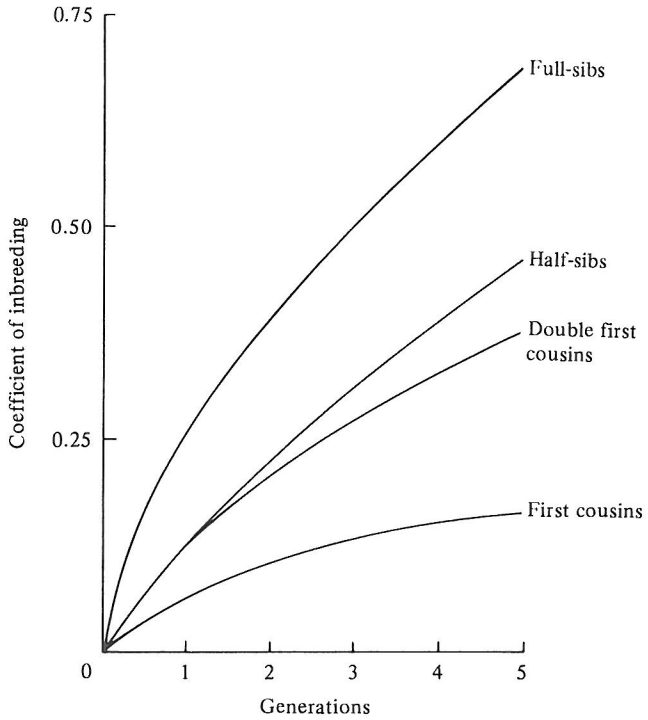

Fig. 3.12 Increase in homozygosity during inbreeding. After Underwood (1979).

3.3.3 Inbreeding depression: theory

Aside from its effect on heterozygosity, phenotypic changes are often observed in inbred lines. For simple Mendelian traits, the changes are random. Sewell Wright (1977) recounts his ability to distinguish his inbred lines of guinea pigs due to unique colour patterns and other traits that had become fixed in each line. More is said on this topic in section 3.5.5.

Inbreeding also causes a change or shift in the means of some genetically determined quantitative characters. But this shift, unlike genetic drift, is directional; *it is always towards the direction of the phenotype expressed by (homozygous) recessive alleles.* This is where the term *inbreeding depression*

comes from. One might ask why this is necessarily so – why do recessive alleles when homozygous, produce inferior or 'depressed' phenotypes? The answer has to do with exposure. Dominant alleles are always exposed, or subject to natural selection; hence, a deleterious dominant is readily eliminated in a population. On the other hand, a deleterious recessive can persist at low frequency indefinitely, since it is rarely 'seen' in the homozygous state. The effect of inbreeding on a trait, in the presence of recessives, can be stated more precisely: if the variation for a particular phenotypic character has any degree of dominance or overdominance (heterozygote superiority), then there will be a shift in the average expression of the character towards the homozygous recessive phenotype, as the following example shows.

In an infinitely large, randomly breeding population, the equilibrium (Hardy–Weinberg) genotype frequencies, considering a single locus, are

$$p^2 + 2pq + q^2 = 1$$

where p is the frequency of allele a_1, the dominant allele, and q is the frequency of a_2, the recessive allele. Upon inbreeding, these Hardy–Weinberg frequencies change to

$$(p^2 + pqF) + (2pq - 2pqF) + (q^2 + pqF) = 1$$

Note that the proportion of homozygotes increases at the expense of the heterozygotes. Now, the reason the mean shifts towards the recessive (a_2) side is illustrated in Fig. 3.13. Assume a population is subdivided into a number of lines, each of which is inbred at a level F. The frequencies of the two alleles a_1 and a_2 within a line are p and q, respectively, and the average frequencies for the whole population (all lines together) are $\bar{p}$ and $\bar{q}$. For the sake of simplicity we have set $p = q = 0.5$ and we have set d, the genotypic value (average phenotype) of the heterozygote, equal to the genotypic value of the dominant, or 1.0; the respective value of the recessive is -1.0. This is merely a mathematical way of expressing complete dominance. Upon inbreeding, the change in the mean is $-2d\bar{p}\bar{q}F$, and the mean genotypic value of the average completely inbred ($F = 1.0$) population is (Falconer, 1960)

$$\begin{aligned} M_F &= M_0 - 2dpqF \\ &= 0.5 - 2(0.25) \\ &= 0 \end{aligned}$$

where M_0 is the mean genotypic value of the randomly breeding population and M_F is the value for the average inbred population. Of course, a completely inbred population will be composed of individuals that either are all a_1a_1 or all a_2a_2; 50% of lines will be monomorphic for one genotype and 50% will be monomorphic for the alternative genotype. It is by averaging together all the inbred lines that we obtain $M_F = 0$. As long as there is any

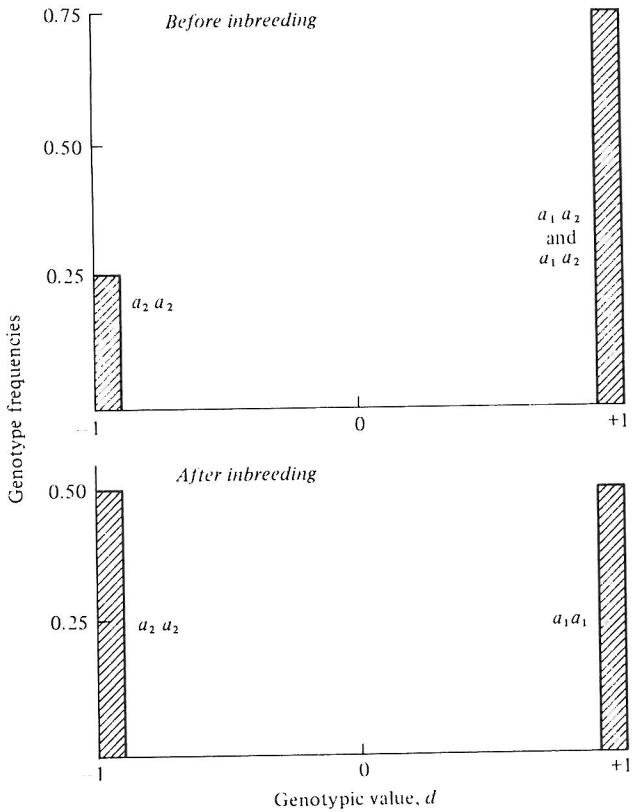

Fig. 3.13 Inbreeding depression as the result of dominance. The genotypic value of the dominant allele is 1; that of the recessive is −1. Before inbreeding, the mean genotypic value for the population is 0.5, as indicated by the symbol M_0. After inbreeding (below), all lines are homozygous, half for allele a_1 and half for allele a_2. Therefore, half of the lines have a genotypic value of −1 and half have a genotypic value of 1; the mean (M_F) is zero. After Falconer (1960, p. 113).

dominance, inbreeding always produces a phenotypic shift towards the recessive.

To counteract this tendency it is common practice for breeders to introduce 'outside' genes from relatively unrelated colonies. This procedure reduces the frequency of unfit homozygous recessives in the mixed population. Bruce (1910) explained this effect mathematically for a single locus. Assume for example, that we have two lines each having a different recessive allele with frequencies q and q', respectively. If we pick at random the same number of individuals from both lines, the overall proportion of unfit homozygous recessives is $(q^2 + q'^2) / 2$. But if we produce a *new* line by

crossing individuals from one line with individuals from the other, the frequency of homozygous recessives becomes $(q/2)^2+(q'/2)^2+2(q/2)\ (q'/2)$ which is less than or equal to $(q^2 + q'^2)/2$. This assumes (conservatively) that qq' is as unfit as qq or $q'q'$.

Crossing has an even more salubrious effect if the two lines tend to have recessives at different loci. For example, line A might have an unfit recessive, m, at locus M, and line B has unfit recessive n at locus N. Assume the frequency of m and n has the value 0.2 in each population respectively. The proportion of homozygous recessives in a new line formed by crossing equal numbers from A and B will be $2(0.1)^2 = 0.02$, in contrast to 0.04 in line A or B.

3.3.4 Inbreeding depression and fitness

In this section we delve more deeply into the actual forms taken by the deleterious effects of inbreeding. The expression of inbreeding depression is biased towards certain kinds of characteristics. That is, inbreeding depression neither affects all characters uniformly, nor are these effects random or unpredictable with respect to the traits in which they appear. In fact, we can predict quite accurately which characteristics will be depressed the most upon inbreeding.

The careful reader will have anticipated this predictability of inbreeding effects. Recall that the average phenotype of traits upon inbreeding moves away from the dominant or overdominant phenotype and towards the recessive. Traits, therefore, which have a significant amount of dominance or overdominance (non-additive genetic variance) will change the most. Furthermore, a shift towards the phenotypic expression of recessive genes is tantamount to a decline in fitness because, as already mentioned, a disproportionate number of deleterious alleles are recessive.

Now, in what kind of characters is dominance typically observed? By and large, they are traits related to reproduction; in the parlance of quantitative genetics, they are 'fitness characters' (Robertson, 1955). This means that we can expect to observe the most inbreeding depression in characters such as fecundity (total reproductive output), fertility (ability to produce viable gametes or zygotes), developmental rate or age at sexual maturity, litter size and analogous traits. In contrast, characters, the states of which are not critical to reproduction, are less affected by inbreeding. Finally, the traits which are least affected by inbreeding are those that can vary greatly without affecting the viability or reproductive contribution of individuals.

Another way of expressing the above breakdown of traits is in terms of *heritability*. A long exegesis on heritability would be inappropriate here (see Bodmer and Cavalli-Sforza, 1976 for an excellent summary). For our purposes it is sufficient to point out that heritability is a measure of the genetic determinism of a trait or of its correlation among close relatives. Such

correlations are high if the genes that control a trait are acting in an additive fashion, that is, if there is relatively little dominance or overdominance. The experience of breeders has led them to generalize that reproductive traits generally have low heritability (little additive variation) while traits which are apparently less relevant to reproduction and survival have high heritabilities.

Table 3.8 summarizes the heritabilities of some traits in animals. Note that the heritabilities for coat pattern, tail length and body conformation all tend to be high (50% to 90%), whereas those for reproductive characters are usually 20% or less. The rule of thumb is that the characteristics with the lowest heritabilities are those which will be the most depressed by inbreeding, assuming a relatively large amount of dominance variation.

At the beginning of this chapter we gave an account of inbreeding depression in Poland China swine. This example is just one of several hundred in the animal and plant breeding literature. A complete review of the literature would serve no purpose here, but some additional examples are helpful in reaching some general conclusions.

Sewell Wright (1977) recently summarized his extensive and long-term studies on inbred guinea pigs. Three points are especially significant. First, out of thirty-five original lines undergoing inbreeding, only half survived for nine years, and only five were vigorous enough to warrant intensive study. Second, in these five relatively vigorous lines, the effective fecundity, compared to non-inbred controls (young raised per mating year) was only 30%.

TABLE 3.8 *Heritabilities of various traits*

Trait	Heritability	Source
Finger print ridges in humans	0.95	Holt (1961)
Amount of spotting in Friesian cattle	0.95	Falconer (1960)
Stature in human males	0.79	Osborne and De George (1959)
Femur length in mice at 3 months (male-son)	0.79	Leamy (1974)
Tail length in mice	0.6	Falconer (1960)
Length of wool in sheep	0.55	Falconer (1960)
Abdominal bristle number in *Drosophila*	0.5	Falconer (1960)
Skull length in mice at 3 months (male-son)	0.50	Leamy (1974)
Combined molar length in mice	0.30	Bader (1965)
Milk yield in cattle	0.3	Falconer (1960)
Egg production in poultry and *Drosophila*	0.2	Falconer (1960)
Litter size in pigs and mice	0.15	Falconer (1960)

Third, the overall fitness of the inbred lines compared to the controls is worst in unfavourable (nutritionally poor) environments. That is, the inbreds are less resistant to stressful conditions (Lerner, 1954; Parsons, 1971).

Bowman and Falconer (1960) studied the effects of inbreeding on litter size in mice. The decline was from 7.77 to 4.58 in five generations; expressed in terms of F, this is 0.6 of a mouse (7.7%) per 10% increase in F. (Recall that ΔF, equal to 10% is slightly less than the ΔF in a half-sib mating scheme.) Only one out of twenty inbred lines remained at the control level for litter size after generation twelve.

The difference between inbred and outbred strains of Holstein-Friesian cows was studied by Tyler, Chapman and Dickerson (1949). They found little effect of inbreeding on body dimensions (note that body dimensions are not fitness characters) but recorded a depression in milk and butterfat production of 6.2% and 5.8%, respectively, for 10%ΔF.

In gallinaceous birds, the effect of inbreeding appears to be related to the history of the species in captivity; the longer they have been domesticated, the less the inbreeding depression. Table 3.9 summarizes the results of several studies of Abplanalp and coworkers (Abplanalp, 1974). Note that the decline in fitness is least in chickens and turkeys, and most in Japanese quail and chukar partridge; the latter two species are much less domesticated than the former two. Domesticated species have a history of selection and inbreeding; i.e. they have been partially purged of their deleterious genes, so inbreeding depression concomitant with further inbreeding is relatively less severe.

Wright (1977), in reviewing the history of inbreeding in maize, reiterates that the fitness characters show the most drastic decline. Most lines

TABLE 3.9 *Effect of 25% inbreeding on the relative performance of four gallinaceous species; performance of non-inbred lines equals 100*

	Performance as percentage of non-inbred birds			
Trait	Chicken	Turkey	Japanese Quail	Chukar
Hatchability: embryo inbred	90.0	83.4	72.2	71.3
hen inbred	97.0	92.1	89.3	89.1
Fertility	99.1	98.8	79.2	71.1
Viability of females	94.3	90.7	81.5	92.1
Egg production	90.4	89.5	83.9	84.1
Total reproduction	74.4	61.6	35.9	34.1
'B' = inbreeding depression	1.183	1.938	4.098	4.303

From Abplanalp (1974).

deteriorated so rapidly upon inbreeding that they could not be saved. Those that survived to nine or ten generations of selfing deteriorated in height (27%), length of ear (28%) and, most dramatically, in yield (61%).

In sum, these studies point to the universality of inbreeding depression. More particularly, there is remarkable agreement between the inbreeding coefficient and loss of fitness, namely, a ΔF of 10% generally corresponds to a 5–10% decline for a particular reproductive trait. When considering *total reproductive performance* (rather than isolated characteristics) the decline in fitness jumps to a stunning 25% or so for species or lines that have not been extensively inbred in the past (Tables 3.7 and 3.9).

The decrease in fitness is less for most domesticated species and for previously inbred or selected lines of experimental organisms. As suggested above, this apparently less severe inbreeding depression is probably the result of the 'purging' effect of selection and inbreeding. Any inbreeding is likely to remove some of the deleterious genes from a line. For example, Roberts (results cited in Falconer, 1960) performed inbreeding experiments on mice stocks obtained by crossing inbred laboratory strains. Only two out of thirty lines were lost after three generations of sib mating. Such a high survival rate of lines is probably a consequence of the rarity of deleterious recessives in the original inbred strains.

The experience of plant breeders also leads to the conclusion that a history of slow or episodic inbreeding tends to decrease the severity of later inbreeding episodes. Many more plants than animals are prevailingly self-fertilizing, and it is possible to ask if species which are predominantly selfing have less inbreeding depression than would be expected. Young and Murray (1966) noted that the degree of inbreeding depression in domesticated plants correlated with the typical amount of self-pollination; in order of increasing cross-pollination and inbreeding depression, these plants are barley, cotton, tomato and corn. In general, self-pollinating species show less heterosis and less inbreeding depression than do out-crossing forms.

There are 'costs', however, to the purging of a stock by inbreeding. First, most attempts fail, so one must start with many lines if one hopes to pull some through the 'inbreeding crisis'. Second, a successfully purged inbred line, once obtained, will be genetically different from the outbred population in many, random, directions. Notwithstanding such costs, it is apparent that once a line has safely passed through one bout of inbreeding, it is more likely to make it through others because it will retain progressively fewer deleterious recessives.

It is clear from the previous paragraphs that inbreeding can be seen to produce two opposing effects: on the one hand it can purge some deleterious genes; on the other hand, it can fix some deleterious genes. Whether a line survives a period of inbreeding, therefore, is a matter of chance. If by chance no lethals or subvital genes are fixed, the line will survive. If, however, some

such genes become fixed or reach a high frequency, then the line will probably be lost. *The critical question then is what is the ratio between lines that are purged and survive versus lines that go extinct?* The value of this ratio determines whether inbreeding is a potentially useful tool for conservation.

On this question the data speak rather loudly. Above we noted that only half of Rommel and Wright's guinea pig lines survived for nine years (approximately 11.5 generations of sib-mating). Bowman and Falconer (1960) inbred twenty lines of house mice; only one line survived after generation 12, and only ten lines survived to generation 5. Using wild mice, Lynch (1977) found that only two out of fourteen lines survived for six generations of sib-mating. The results for *Drosophila* are essentially the same; only about 10% of lines survive more than ten or twenty generations of sib-mating (Clayton, Knight, Morris and Robertson, 1957; Wallace and Madden, 1965).

Abplanalp reported that only eight out of 279 lines survived a large inbreeding experiment with white leghorn chickens. The material for the above studies was domesticated stocks, so it is probable that they were all partially purged of their deleterious recessives by population bottlenecks and selection. Thus, one could anticipate that the survivorship of inbred lines would be even less with a foundation stock fresh from nature. In summary, between 5% and 20% of lines survive after F reaches values above 0.80.

One of the myths about inbreeding is that there exists an inbreeding depression minimum, and that once a line succeeds in traversing safely this genetic purgatory, it is cleansed of deleterious genes. The basis for this misconception is the existence of apparently fit inbred domesticated lines such as white rats. Indeed, there exist apparently* homozygous lines, but it is not usually appreciated that these lines typically are severely handicapped with respect to many traits, and would certainly not survive in nature. For example, Lindstrom (1941) found that 677 *useful* lines of inbred maize had been produced by experimental stations in the US, but these were only 2.5% of all those lines that had been started. Further, the average yield of these was only 30% that of F_1 hybrids. It is probably extraordinarily rare for an inbred line to be as fit overall as an outbred population.

3.3.5 Inbreeding depression and behaviour

Up to this point we have stressed the effects of inbreeding depression on the reproductive functions. Certainly reproductive characters are usually profoundly depressed by inbreeding, but other kinds of traits can be seriously

* It is now accepted that inbred lines are never as homozygous as predicted from simple theory (e.g. Enfield, 1977; Eriksson, Halkka, Lokki and Saura, 1976); natural selection apparently acts through heterozygote superiority to impede fixation.

affected as well. The reason that we have emphasized reproductive depression is that breeders and experimenters find it practical and relevant to study these. But an overemphasis on such traits as fecundity could lead one to believe that if an inbred stock is relatively fecund, for example, then it should be relatively fit overall, a very misleading assumption. Fecundity and viability in the laboratory, farm or zoo is probably useless in predicting how a stock will perform in nature. This because viability and reproduction in nature depend on a vast and subtle integration of physiological and behavioural phenomena. Whereas an inbred stock might perform well in a sheltered environment, it is likely to be severely handicapped when completely on its own. White leghorn poultry, for example, are champion egg layers, but no farmer would bet on their success in a hedgerow.

To take a more concrete example, laboratory mice (*Mus musculus*) are rarely exposed to the environmental extremes encountered by their wild cousins. Hence, a deterioration of certain abilities necessary for survival in the wild might be expected in laboratory mice, and many such evolutionary changes would go unnoticed. For example, the loss of the capacity to make a warm, protective nest would, in a laboratory strain, have little or no effect on fitness, but such a change in a wild population would lead to death of young from exposure, and thus to extinction. Are behavioural traits of this type likely to be depressed by inbreeding?

They are. Lynch (1977) determined that the amount of nesting material used by mice each day (nesting score) behaves genetically in the same way as fitness characters such as litter size. That is, inbreeding decreases nesting scores within lines, and crosses between inbred lines give substantial heterosis. Fig. 3.14 shows how nesting score varies among some inbred laboratory strains in comparison with a cross between them and in comparison to a wild strain. One of the six inbred strains, *BALB*, builds much bigger nests than the wild strain (this could be as non-adaptive as building too small a nest), and two of the inbred strains build very small nests. Such aberrant behaviour, while perhaps of little significance in the laboratory, could prove disastrous in nature.

There is a rich supply of anecdotes regarding inbreeding effects. A recent example concerns the brown-eared pheasant *Crossoptilon mantchuricum*. In the West all brown-eared pheasants (descended from a male and two females captured in northern China in 1864) have been producing mostly non-fertile eggs. Eggs obtained from artificially inseminated hens are fertile, and the infertility was traced to the low libido of the cocks, according to researchers at Cambridge University (Anon., 1977). Inbreeding problems are notorious in popular breeds of dogs; hip dysplasia and ocular diseases are particularly common.

Behavioural deterioration in traits related to competition will be detrimental in nature, if not on the farm or in the field. Several studies suggest

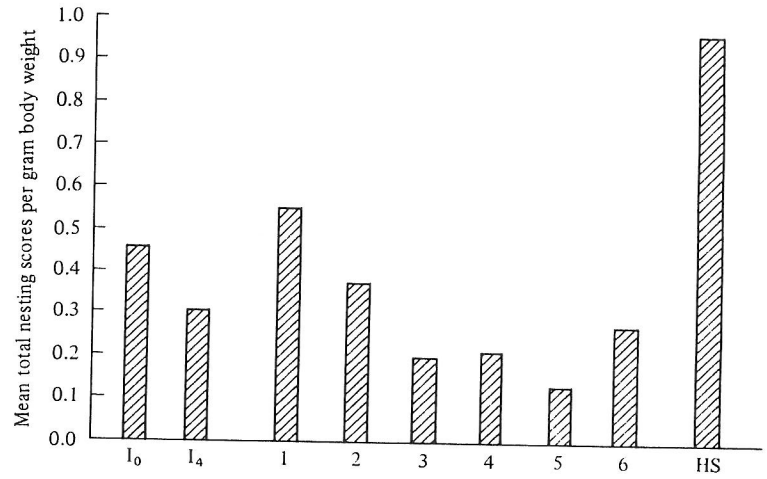

Fig. 3.14 Mean nesting scores for matings between wild caught mice (I_0), the fourth generation of full-sib matings derived from this natural population (I_4), six inbred strains (1 is *BALB/cJ*, 2 is *CBA/J*, 3 is *C3H/HeJ*, 4 is *C57BL/6J*, 5 is *DBA/1J* and 6 is *A/J*) and an eight-way cross among inbred strains (HS). After Lynch (1977).

that inbreeding severely depresses competitive ability. Latter and Robertson (1962) showed a strong effect of inbreeding on competitive ability in *Drosophila*. Mertz, Cawthorn and Park, (1976) ran competition experiments between two species of flour beetle, *Tribolium castaneum* and *T. confusum*. One of the variables in these experiments was the level of inbreeding. In a reanalysis of their results, J. W. Senner (in preparation) demonstrated a very strong depression of competitive ability, apparently attributable to inbreeding. In these examples the depression in competitive ability could be just another expression of a loss of fecundity, since any decline in reproductive fitness should reduce competitive ability.

The results of Garten (1976), discussed on p. 54 are also relevant here. Garten found that intraspecific competitiveness, as measured by aggression in oldfield mice, was positively correlated with mean heterozygosity in the population from which the mice were collected. Such studies reinforce our suspicion that *any* loss of genetic variability, whether due to natural causes (small population size, bottlenecks, directional selection) or to artificial inbreeding, is going to reduce the chances of survival in the wild.

3.3.6 Advantages of inbreeding

For certain purposes, inbred lines have clearcut advantages over non-inbred lines. Their most attractive characteristic is phenotypic uniformity under controlled conditions. In biological experimentation, the most repeatable

results are obtained when the experimental material is genetically homogeneous. Much pure and applied research in physiology, developmental biology, immunology, endocrinology and pharmacology depends on inbred strains. The problem with inbred organisms, however, is that they are highly sensitive to variation in the environment, particularly nutrition and temperature. The best of both worlds, that is homeostasis in a fluctuating environment and phenotypic uniformity, is achieved by crossing two or more inbred lines with good 'combining' characteristics. The hybrid offspring are genetically identical and heterozygous, the latter tending to minimize their sensitivity to environmental heterogeneity.

Another apparent advantage of hybrids between some inbred lines is their superior productivity. The best known example is hybrid corn. This brings us to a very important question: Why isn't it possible to assemble all the best genes together in a single homozygous line, assuming heterosis results from the masking of deleterious recessives? Indeed, this is a possibility for specific agricultural or laboratory purposes, but because of the thousands upon thousands of genes involved, a project of this magnitude would be extraordinarily tedious and difficult, especially for slowly reproducing species. Even for maize, which has been bred for many decades, such an ideal strain is unlikely to replace hybrids between inbred lines for the forseeable future (Eberhart, 1977).

Whereas it is our opinion that inbreeding under most circumstances is anathema for conservation programmes, special circumstances may permit special means. In an organism, for example, that for some reason is already very homozygous, further inbreeding might do little harm and could make it easier to maintain in a domesticated or semi-domesticated state. Just such a protocol was recommended by Slatis (1960) who found that inbreeding depression in the European bison, *Bison bonasus* was nearly absent. Perhaps this is because this species has never been abundant in historical times and recently passed through a bottleneck of seventeen *related* individuals. It should be recognized, however, that the consequence of intense inbreeding, assuming the unlikely result of survival without serious depression in viability and fecundity, is the relinquishing of future adaptive options, as well as the appearance of random changes in the phenotype.

3.4 The basic rule of conservation genetics

It would appear that there is no safe amount of inbreeding for normally outbred organisms. And even changes in level of heterozygosity, such as occur normally in natural populations, might cost populations a certain amount of fitness. Of course, all species cannot be maintained in a completely outbred state, but it behoves us to minimize inbreeding and the loss of genetic variation. Notwithstanding this, inbreeding at low intensities is

quite common in nature, as well as in domestic stock, so, *a priori*, it would appear that deleterious genes can be eliminated by natural selection before being fixed, given that the rate of inbreeding is low enough. Theory and experience tell us that the smaller the population, the more difficult is selection's task in the weeding out of such genes; the reason is that selection is no match for genetic drift at very small population sizes.

Is there, then, a threshold rate of inbreeding, above which fitness relentlessly declines, and below which fitness can be maintained? The answer is a qualified 'yes'. It is a qualified response because no two populations are alike: those with a large load of deleterious genes will tolerate less inbreeding than those with a lower genetic load. Incidentally, species with high levels of heterozygosity may have relatively high genetic loads (Soulé and J. W. Senner, in preparation) so there is some hope of making rough predictions of the inbreeding tolerance of different species or stocks.

The basis for our 'yes' answer regarding a maximal rate of inbreeding is an empirical rule of thumb used by animal breeders. The rule is that natural selection for performance and fertility can balance inbreeding depression if ΔF per generation is no more than about 1% (Franklin, 1980) or 2–3% (Dickerson *et al.*, 1954; Stephenson, Wyatt and Nordskog, 1953). If ΔF values are higher than this, natural selection is unable to offset the tendency for the fixation of deleterious recessives. Apparent exceptions are very rare. We prefer the lower (1%), more conservative value, for two reasons. First, domestic animals from which the rule is derived are already inbred to some degree and therefore have less genetic load than their wild progenitors. As any genetics student knows, it is virtually impossible thoroughly to eliminate a complete recessive from a population, even if it is lethal as a homozygote; nevertheless, such genes can be driven to relatively low frequencies, and in small populations, genes at low frequencies tend to be lost.

Second, domestic animals can tolerate more random phenotypic change than can stocks destined for reintroduction into unmanaged habitats. Breeders of domestic animals may safely ignore genetic and phenotypic changes in their organisms that would completely debilitate a species that had to cope with predators, inclement weather and other conditions occurring in nature. Egg ranchers for example, do not mind if their animals have genes that cause a poor body configuration, poor feather distribution, myopia, or poor predator escape behaviour. These sorts of genes often accumulate in a stock during the course of inbreeding and artificial selection.

We refer to the 1% rule as the *basic rule of conservation genetics* because it serves as the basis for calculating the irreducible minimum population size consistent with short-term preservation of fitness. An even more stringent criterion for conservation genetics is given in Chapter 4 in the context of long-term protection of adaptive potential.

How does the basic rule translate into population size? Fortunately, the

relationship between ΔF and N_e is a simple one. The per generation rate of loss of heterozygosity is related to population size as given by Wright's (1931) expression

$$\Delta F = \frac{1}{2N_e} = \frac{1}{8N_m} + \frac{1}{8N_f} \qquad (3.7)$$

so that the effective size must be fifty or more if the loss of heterozygosity (or increase in homozygosity) is not to surpass 1%.

As discussed in section 3.1, a census size = 50 individuals does not always mean an effective size of fifty. To take only one example, the sexes of the reproducing adults, if not equal, will require a larger census if our goal is $N_e \geqslant 50$. This is illustrated in Fig. 3.15. Note that the *minimum* number for the least numerous sex is fifteen. In other words, if a herd has less than fifteen breeding males (and between seventy and eighty females), ΔF will be above 1%, which is in the danger zone according to the rule. If a slightly more

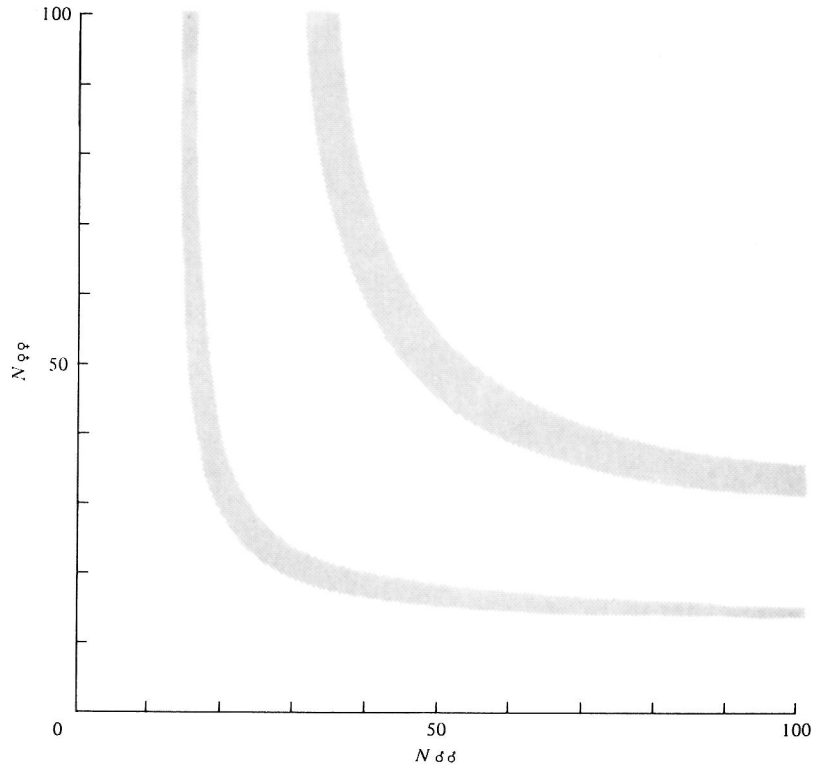

Fig. 3.15 The numbers of breeding males and breeding females needed to satisfy the 1% or $N_e = 50$ criterion (the lower left shaded region) and the 0.5% or $N_e = 100$ criterion (the upper right shaded region).

conservative criterion is employed, say ΔF of 0.5%, the equivalent minimum numbers are thirty males and ninety females. Similar effects result from population fluctuations and progeny distributions when $V_k > \bar{k}$. In the following chapters we shall apply the basic rule to various hypothetical and real conservation problems.

3.5 Mutation–selection equilibria

One final point. We have chosen to discuss the mutational origin and replacement of variation in the following chapter, so only a brief comment is made here. One might hope that the alleles that are lost in small populations could be regenerated by mutation. Such a sanguine expectation is untenable. Wright (1937) discussed this problem while examining the effects of joint pressures on the distribution of gene frequencies at single loci. The essential conclusion is that selection pressure is relatively powerless to prevent loss or fixation of alternative alleles (including new mutations) in a small population. Not only will small populations rapidly lose genetic variation, but *the beneficial alleles have roughly the same probability of being fixed as do the deleterious ones.*

3.6 Summary

1. A population bottleneck, here defined as a single generation event during which a population is severely reduced in size, has the following effects on genetic variability:
   (a) The loss of genetic variation or heterozygosity is not severe; even two individuals retain 75% of a population's genetic variance;
   (b) In contrast, the loss of alleles, especially those at low frequencies, is serious. If these alleles are ever important for survival, such as during an epidemic, then their attrition would increase the expectation of extinction;
   (c) The total amount of genetic variation that is lost following a bottleneck depends on how fast the population grows to a moderate (several hundred or more) size.
2. Chronically small population size produces random gene frequency changes and fixation or loss of alleles; such random fluctuations are referred to as genetic drift. Small populations continually 'leak' alleles and genetic variance, and a few generations of genetic drift is much more erosive of genetic variation than a bottleneck followed by a rapid recovery of numbers.
3. The impact of genetic drift is directly related to the effective population size, not the census number of individuals. The effective size, N_e, is extremely sensitive to unbalanced sex ratio among breeding adults. In

a polygynous system in which the females are monogamous (e.g. Pere David's deer, hamadryas baboon, elephant seal, zebra) N_e can be an order of magnitude smaller than the census number.

4. Large fluctuations in population size can severely depress N_e. The reason is that N_e is most strongly influenced by sampling error (drift) occurring during the 'crash' part of the sequence of generations. Mathematically, N_e is the harmonic mean, not the arithmetic mean, of the numbers in each generation.
5. If for any reason the reproductive output of some families is especially great, and/or that of other families is especially poor, N_e will be less than it would be if the number of progeny were randomly distributed among families. The converse is also true: as the variance of progeny per family approaches zero, N_e approaches twice the actual number of breeding adults.
6. It is incumbent on managers to be aware of these effects and to maximize N_e to the extent consistent with other management programme criteria.
7. The central problem of conservation genetics is the relationship between change in genetic variation and fitness. In natural populations, genetic variation can vary in space (between populations) as well as time (within populations). A review of the literature leads us to conclude that fitness within and between *natural* populations is often correlated with measures of heterozygosity. The superior viability of relatively heterozygous individuals is most apparent when sampling different age classes, especially in species with 'structured' populations. There is also some evidence that homozygous individuals are phenotypically more extreme compared to heterozygotes. Among populations of the same species, at least for non-flying vertebrates, there is evidence for a correlation between mean heterozygosity and fitness.
8. Predominantly inbreeding plants span virtually the entire range of heterozygosity levels observed in outcrossing species, though a relatively high proportion of them have no detectable allelic variation. In those populations in which heterozygosity is retained there is some prima-facie evidence for heterozygote superiority.
9. The existence of some populations of plants and animals lacking detectable genetic variation means that survival is possible without heterozygosity, at least under some circumstances. Since such populations cannot evolve, however, their extinction probability is very high.
10. There is some evidence for the asymptotic model of genetic variation and fitness, suggesting that the more genetic variation is lost, the more deleterious the losses become.
11. Inbreeding always reduces fitness in animals; the decline for reproductive traits (inbreeding depression) resulting from a 10% increase in the inbreeding coefficient is usually between 5% and 10%. For total reproductive performance, the decline may be two to five times this high. Behaviour traits and competitive ability are also depressed by inbreeding.
12. Intense inbreeding results in the loss of 90% or more of lines unless the stock has a long history of domestication or slow inbreeding.
13. The basic rule of conservation genetics, based on the experience of animal breeders, is that the maximum tolerable rate of inbreeding is 1%. This translates into an effective population size of fifty.