PRESERVATION OF GENETIC VARIATION IN THE RED PANDA POPULATION

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Genetic management is currently recognized as an important prerequisite to the preservation of (endangered) species in captivity. A number of instances of inbreeding depression in captive populations have made the zoo community realize, that the long term survival of species is guaranteed not only by maintaining a sufficient number of individuals but also requires a well-coordinated breeding program.

Genetic management alone does not constitute a breeding program: behavioural specialisations, husbandry requirements, medical care and even politics can influence management strategies. In fact, measures based on any of these factors alone might even (appear to) conflict with the goal of genetic management (i.e. preservation of genetic variation). This is well illustrated in the breeding program of the red panda, *Ailurus fulgens*. This captive breeding program has, for practical reasons (such as quarantaine regulations), adopted a regional approach (Glatston, 1982). Five regions are recognized: Australia, Continental Europe, Great Britain and Scandinavia, North America and the 'rest' (China, India, Japan). However, genetic variation in the individual regions is too low to ensure future survival of these sub-populations. Therefore,the regions cannot be treated as closed breeding demes (Princée, 1987). However, large-scale migration between regions conflicts with this type of approach. A compromise would seem to be required: a breeding program which is based on limited inter-regional migration.

Such a compromise requires detailed study of the genetic processes which have occurred in the studbook population. Although geneticists can look back on a short, but nevertheless rich, history since Darwin and Mendel, 'zoogenetics' is still very much in its infancy. The complex history of many zoo populations (such as that of the red panda); including the occurrence of inbreeding and overlap of generations, makes the application of theoretical models difficult. An important parameter in the estimation of loss of genetic variation per generation is the effective population size. This parameter cannot be calculated if information on parentage is incomplete. Furthermore, the structure of many zoo populations does not meet the criteria required to calculate effective population size according to theoretical models (e.g. random breeding, no generation overlap, etc). This does not mean that the theoretical models of population genetics are inapplicable to zoo management; they can be used as guidelines.

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One of the recent developments in 'zoo-genetics' is the use of computer simulation models. Such tools allow study of the genetic processes that have occurred in the past and evaluation of measures proposed for the future.

The 1984 studbook population of the red panda was analyzed using the model 'GeneFlow' (Princée, 1988). It was concluded that the loss of genetic variation per generation was too high, given a maximal acceptable loss of 1% (Frankel and Soule, 1981). However, several generations of the red panda population are currently reproductive. Therefore the process of gene flow from one generation to the next occurs simultaneously for several generations. This means that genetic variation in the various generation groups can change with time.

The processes which have caused genetic loss in the past and guidelines to reduce such loss in the future have already been discussed for the 1984 population (Princée, 1988). The current study deals with genetic management of the red panda population in the long term.

Material and methods

The 1985 and 1986 populations of the red panda were analyzed using the computer simulation model 'Gene Flow'. The pedigree data for these analyses were extracted from the 4th edition of the red panda studbook and its update report (Glatston, 1986, 1987). Only data on breeding, or potentially breeding, individuals of the nominate race, *Ailurus fulgens fulgens*, were used in the study; the chinese subspecies *A.f.styani* was not considered. Wild-caught potential breeders are considered potential founders. Dead individuals which have not reproduced, are excluded from the simulation experiments as they do not participate in processes of gene flow.

The generation to which each specimen belongs is determined from the pedigree data. The founder group (P0) consists of real founders (i.e. wild individuals which have reproduced) and potential founders (wild caught individuals with breeding potential).

The generation of an individual is determined by the parent belonging to the latest generation, e.g. the mating of F1 and F2 individuals results in F3 offspring. Individuals which parents are wild-caught are defined as the F1 generation.

The 'Gene Flow' model used in this study expresses genetic variation in terms of gene diversity (or average heterzygosity, as defined by Nei (1975). It is described in detail elsewhere (Princée, 1988). In outline, the model supposes a very large 'wild' population with a number of independent autosomal loci (in this experiment 40). At each locus two alleles with equal frequencies are assumed. In each simulation run, Monte Carlo methods are used to draw random

genotypes for the (potential) founders, and random genotypes, based upon Mendelian segregation of the parental alleles, for the descendants. The gene diversity in the different generation groups and the living animals of the captive (sub-)populations is estimated in each simulation run. The average gene diversity is then estimated over a number of such runs.

Expected genetic drift (H) in generation groups is calculated according to the equation:

(a)

$$H = 1/(2N)'$$ (Wright, 1931)

where N is the size of the previous generation.

The effective size of a population with an equal sex ratio, in which each pair produces an equal number of offspring (k) is calculated according the equation:

(b)

$$Ne = \frac{N \times k}{(1 - H)}$$ (modified from Frankel and Soule,1981)

where N is the population size and H is genetic drift.

Generation time and reproductive life span were estimated from studbook data.

Results

Genetic variation in regional populations

Genetic variation at the end of 1985 and 1986 in both the world and regional populations is presented in figure 1. It can be seen that the amount of variation in the Australian population increased by 5% over this period, while that in the total world population and in the other regional populations showed no significant change. In addition, genetic variation in the world population is larger than the arithimetic mean of these regional populations.

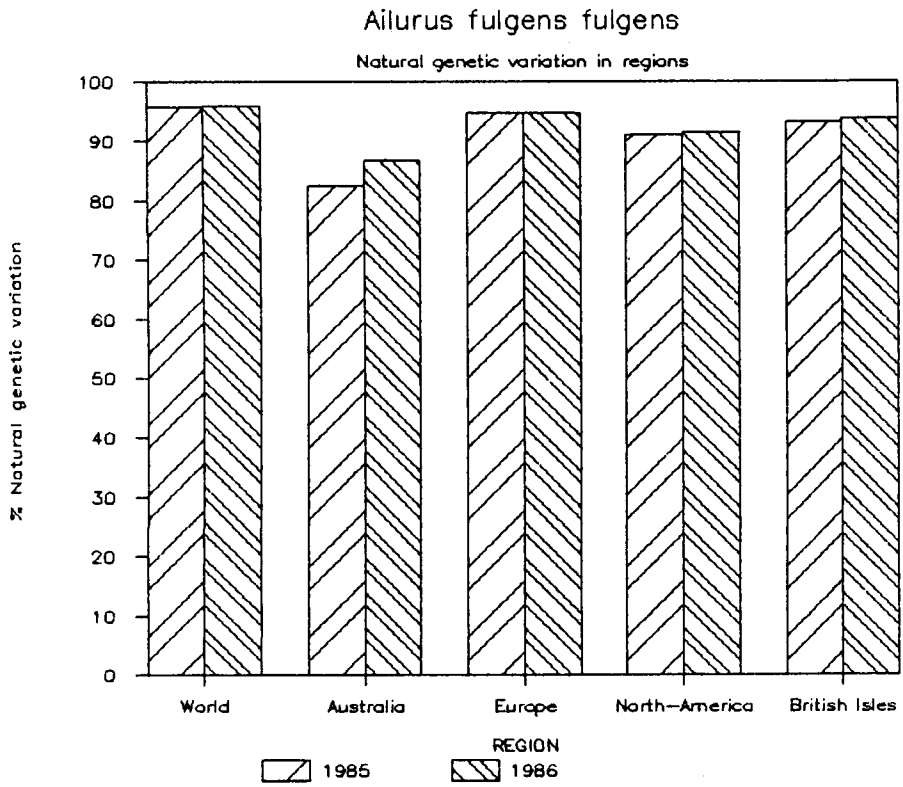

Fig. 1. Natural genetic variation in the regional subpopulations.

Genetic variation and genetic loss per generation

Figure 2 shows genetic variation in the generation groups at the end of 1985 and 1986. The generation groups only consist of (potential) breeders. The genetic loss per generation is presented in figure 3. In these figures it can be seen that levels of variation in the later generation groups (F3, F4, F5) increased during 1986 and that genetic loss in the third generation was approximately only 0.05%. This means that most genes present in the F2 generation are also found in the F3 group.

The number of living animals in each generation group and the number of (potential) breeders in these groups on 31 December, 1986 are presented in table 1 together with the number of offspring produced by each group in 1985 and 1986. The breeder group includes dead animals which have reproduced as well as all living animals of that generation. The (potential) founder population

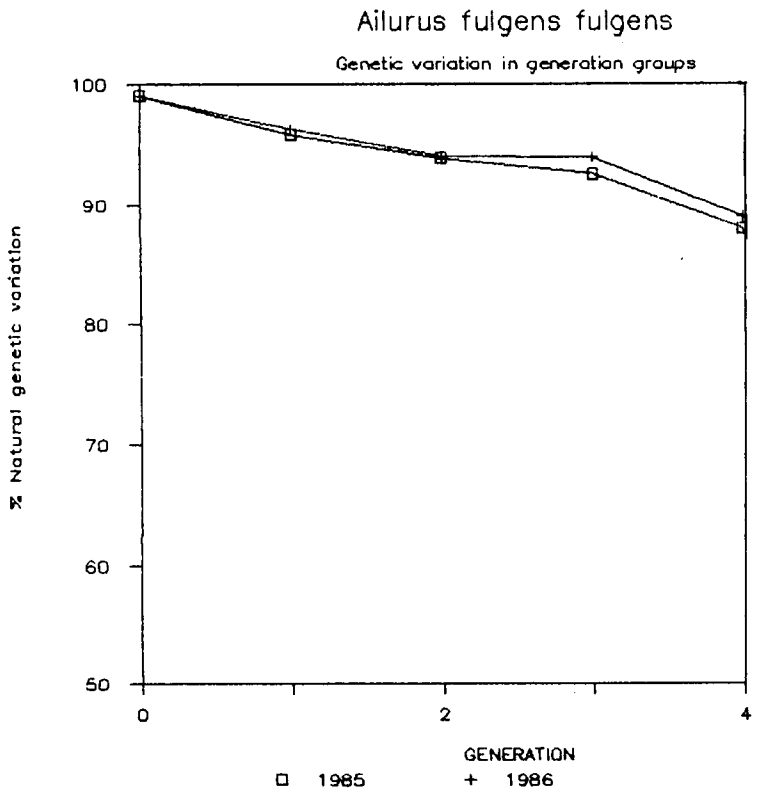

Fig. 2. Genetic variation present in the different generations.

consists of 51 wild-caught animals: 14 of these were still alive in 1986 but of these not all had reproduced. For the purposes of this analysis these animals are considered as potential founders.

The various generations of the red panda zoo population overlap. On 31 December 1986 the population consisted mainly of F2, F3 and F4 individuals (table 1). The P2 and P3 groups, which produce F3 and F4 offspring respectively, produced most young in both 1985 and 1986 and no wild-caught animals were imported in either year.

Future projections of genetic variation

Future projections of genetic variation in the studbook population are presented in figure 4. The initial natural genetic variation in these projections is taken

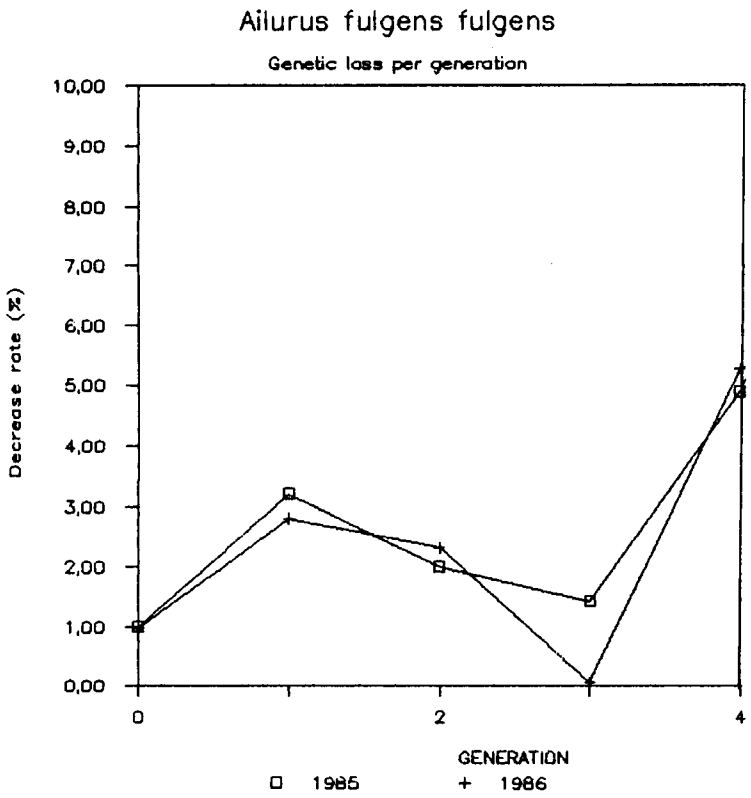

Fig. 3. Rate of genetic loss per generation.

as 94%. This value is based on the level of genetic variation in the F2 generation group (see discussion). The amount of variation after 45 generations is 30 or 90% for decrease rates of 2.5% and 0.1%, respectively.

Discussion

Future prospects for the red panda in captivity

As indicated in the introduction, the survival of a species in captivity is not guaranteed by a large population size. The population needs to be armed against changing or fluctuating environmental conditions (e.g. new viral strains). In genetic terms: the genes needed to survive under different condi-

Table 1. Number of births in 1985 and 1986 in different generation groups of the red panda population (*Ailurus f. fulgens*), living animals in these groups as on 31/12/1986 and number of breeders.

Generation	Wild	F1	F2	F3	F4	F5
Births 1985	–	5	5	34	23	12
Births 1986	–	2	2	19	13	6
Living 1986	14	22	39	46	34	6
Breeders*	51	43	45	53	36	6

* The breeder group consists of dead animals which reproduced and living animals.

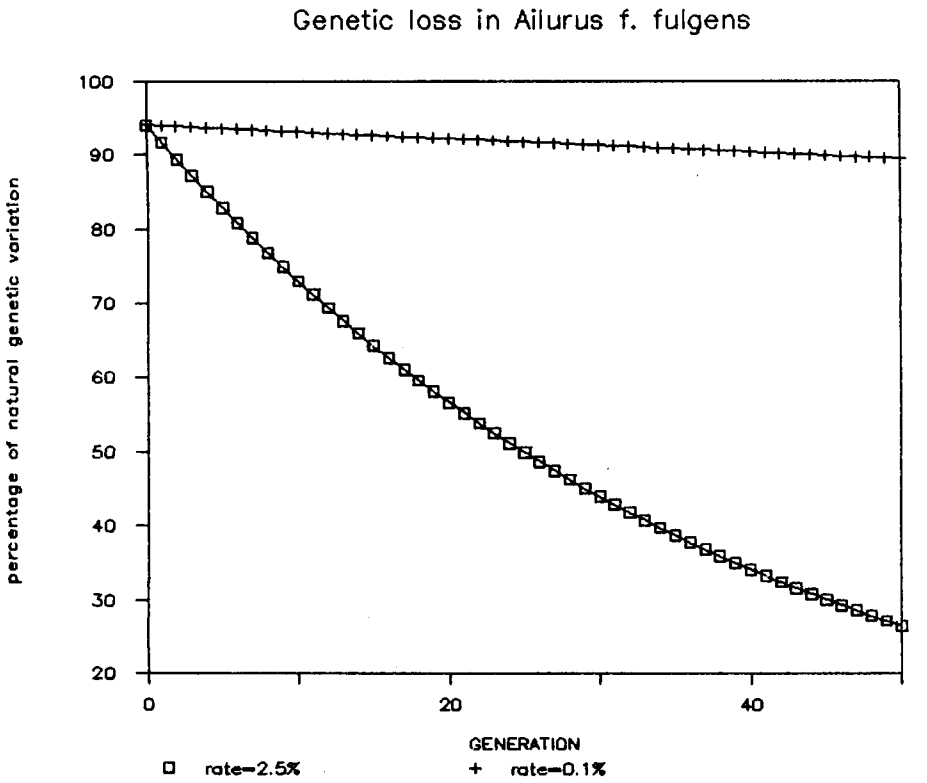

Fig. 4. Predicted rates of genetic loss per generation.

tions must be present in the population. Since the rate of mutation in small populations is almost negligible, these genes must be present in the population at the time it is founded. As a result of genetic drift and inbreeding, small populations loose genetic material with each new generation. Thus, the probability

that a small population possesses the genes required to survive under future conditions also decreases with each generation.

Soule *et al.* (1986) suggest that at least 90% of the original (i.e natural) genetic variation must be retained in captive populations for them to be able to adapt to changes in environmental conditions. Given this criterion, the red panda population as of 31 December 1986, contained sufficient genetic variation (96%, see figure 1). Whether this will remain sufficient in the future depends on how long the red panda population is maintained in captivity without the introduction of fresh blood from the wild population. Given the 200 year management strategy suggested by Soule *et al.* (1986), 90% of the alleles which are present in the current wild population must be retained in the captive red panda population until the year 2186.

The prognosis for genetic variation is made for an 'ideal' population, where the genetic loss per generation is estimated from effective size. If the generation length is known, the total genetic loss over 200 years can be estimated for this population (see Soule *et al.*, 1986). The red panda population differs from the ideal population in that it consists of a mixture of generation groups (table 1), where the F5 generation contains 83% of natural variation (see figure 2) and the entire population 96% (figure 1). Given this, it does not seem justified to base the prognosis for the red panda on the latest generation.

Although the generations overlap, most of the (potential) founder and F1 individuals belong to the older age classes and can be expected to die soon. These groups, despite their small size, make a major contribution to the total genetic variation in the living population (Princée, 1987). When the (potential) founder and F1 groups die out, genetic variation in the population will decline to the level represented in the F2 group. Since reproduction in the founder and F1 groups is limited (table 1), gene flow from the founders to F1, and F1 to F2 is low. This means that genetic variation in the F2 group is unlikely to increase markedly in the future. During 1986, the variation in this group did not change at all (figure 3). Therefore, the genetic variation in the world population will decline to approximately 94% (figure 2) in the near future. However, the genetic variation of the entire population will not equal exactly that estimated for the F2 (breeder) group (figure 2), since this group also includes dead individuals which have reproduced. Therefore, 94% must be considered as a rough estimate.

The rate of genetic loss in the F1 and F2 generation groups is 2.8% and 2.3% respectively. Projections of future trends can be based on the average genetic loss for these generations (2.5%) and the genetic variation in the F2 generation. The generation length of the red panda is approximately 4.5 years. Thus, 45 generations can be expected over 200 years. Using these parameters, 30% of the genetic variation will remain at the end of this period. In fact, within 10 years (i.e. 2 generations), the amount of variation in the population will be less than 90% of that required (figure 4).

It is clear that measures should be taken to prevent this enormous loss of genetic material. In doing so, a maximum loss of 0.1% per generation could be used as a guideline for the red panda population. In that case, after 200 years, the population would still have 90% of natural genetic variation (figure 4).

Strategies

Different management plans can be developed to reduce genetic loss to 0.1% per generation. Theoretically, an effective size of 500 individuals per generation group is required to reduce genetic loss to 0.1% (formula a). This effective size is achieved when each generation consists of 125 pairs, each of which produce two offspring (formula b).

The number of (potential) breeders per generation group in the current population is small (table 1). The F2 group consists of 45 individuals, of which 39 are still alive. Assuming that 20 pairs of the F2 group will all contribute to the F3 generation, each pair must produce 12-13 offspring to achieve an effective size of 500 (formula b). This will result in a F3 group of 240-260 individuals from which 125 pairs can be formed. A stable population of 125 breeding pairs per generation, each of which produce two offspring would be sufficient to ensure the future survival of the red panda population. The carrying capacity required for this strategy must be sufficient for 500 individuals (parents and offspring).

The strategy presented above is theoretical and it is doubtful whether it would be feasible in practice. It is almost impossible for 20 pairs of the F2 group to produce sufficient offspring. The reproductive life-span of captive born, female red pandas averages eight years (from 2 to 10 years of age). This means that each female must produce 12-13 viable offspring in that time.

Does this mean that the red panda has no future with respect to the maintenance of genetic variation? The strategy presented above is based on genetic variation in the current F2 group. The degree of generation overlap found in the red panda is ignored in this strategy. However, an alternative approach would be to consider the current reproductive group as a generation. From a biological standpoint this is more meaningful; the reproductive group will produce the next 'biological' generation. This group comprises 119 individuals (F2, F3 and F4 specimens). Suppose that this group consists of 60 pairs. If each pair produced four viable offspring, the effective size would be approximately 500 (formula b.

How much genetic variation is present in the reproductive group?

The living F2 and F3 groups both contain 94% of natural genetic variation, while 88% is found in the F4 group (figure 2). The measure used to express genetic variation in this study is gene diversity (Nei, 1975). The total gene diversity of groups with different levels of diversity is not necessarily equal to the arithmetic mean of those levels (Princée, 1988). A simulation model which estimates genetic variation in the reproductive group is required. Since such a model has not yet been developed, it is assumed that genetic variation in the current reproductive group is about 94%.

Inbreeding depression

High rates of juvenile mortality are often an indication of inbreeding depression (e.g. Ralls and Ballou, 1983; O'Brien *et al.*, 1985). Inbreeding depression can occur when a population looses genetic variation due to genetic drift or consanguineous matings. Two main types of inbreeding depression which affect the viability of the population can be recognized:

1) The population has a reduced adaptive potential, i.e. alleles which are required for survival under changing or fluctuating conditions are lost.
2) Expression of deleterious alleles due to increased homozygosity.

The adaptive potential of a population is more or less determined by the percentage of natural genetic variation which it has retained. The amount of genetic variation in the current reproductive group is assumed to be sufficient to allow for adaptive potential. However, the population consists of different generation groups. The chance that deleterious alleles have been fixed is higher in the later generations. Inbreeding depression resulting from the expression of deleterious alleles can occur in crosses between individuals from these groups. Back-crossing with previous generations is recommended to prevent this type of inbreeding depression.

Management of regional populations

Princée (1988) discusses the small sizes of the regional populations and the resultant large loss of genetic variation due to inbreeding and genetic drift. It can be seen that genetic variation in these sub-populations will soon be below the 90% level. Although genetic variation in the world population might seem

sufficient for future survival, inbreeding depression could occur within these regional populations. Therefore, they must not be considered closed breeding demes.

In order to avoid inbreeding depression within regional populations, unrelated animals should be introduced. These animals do not have to be imported from the wild. As mentioned previously, the total gene diversity of groups with different levels of gene diversity is not necessarily equal to their arithmetic mean. Since genetic drift is a random process, the qualitative effect will differ in each region. This effect can be illustrated with the following (hypothetical) example. Suppose there are two sub-populations of mice. All animals in the first sub-population are homozygous for the fur color 'black', while in the second sub-population they are all homozygous for 'white'. Thus there is no genetic variation at the 'fur color' locus in either group. However, on the population level (i.e. both sub-populations) there is genetic variation: 'black' and 'white'. In genetic terms two alleles are found at the 'fur colour'locus: 'black' and 'white'.

The effect of migration on genetic variation in the captive red panda population is illustrated in figure 1. During 1986 four individuals were introduced in the Australian population and this increased genetic variation by 5%; from 82.6 to 86.7% (figure 1).

For practical reasons, limited migration between regions is preferable. This means that each region must be as independent as possible. Therefore it is recommended that regional populations be considered more or less closed demes. A minimum of 90% genetic variation should be maintained per region. Migration is required when genetic variation falls below this level. Furthermore, introduction of unrelated individuals from other regions is essential if inbreeding cannot be avoided. Otherwise inbreeding depression can occur within the regional population.

Conclusion

The amount of genetic variation in the current studbook population of the red pandas is sufficient to develop a long-term preservation program. Genetic loss over the next 200 years must not exceed 0.1% per generation (i.e. per 4.5 years). This can be achieved if the current reproductive group (which is considered as a generation group) produces a new generation of at least 250 surviving offspring within 4.5 years (i.e. generation time). In future generations a population comprising 125 pairs which each produce two viable offspring will reduce genetic loss to 0.1%. The minimum carrying capacity for the red panda zoo population should be sufficient for 500 specimens (parents and offspring).

It is recommended that a minimum of 90% genetic variation is maintained

in the regional populations. These sub-populations can then act as more or less closed demes.

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