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## Detecting genetic variation

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## INTRODUCTION

As emphasized in the introduction and throughout the different chapters in this thesis, the relative importance of genetic, demographic and environmental factors in the extinction chances of natural populations has not unequivocally been established. Scrupulous investigation of many different populations, taking into account all three components, is essential to gain more insight into the complex, and ever changing interplay of these factors. Assessing the genetic component has been impaired by the fact that classical techniques did not possess enough resolving power to detect very low levels of genetic variation, that sometimes characterize endangered populations. The development of molecular techniques made it possible to reveal these low levels of genetic variation. In this thesis, the applications of molecular techniques for the evaluation of the genetic structure of populations are described.

## GENETIC VARIATION REVEALED BY THE RAPD TECHNIQUE

The Random Amplified Polymorphic DNA technique (RAPD) (Welsh & McClelland, 1990; Williams *et al.*, 1990) has been used in many studies involving mapping strategies and genomic fingerprinting (Welsh *et al.*, 1991; Wilde *et al.*, 1992). The resolving power of this technique to assess genetic variation in *Phoca vitulina* (the harbour seal) was examined in chapter 2. Two populations of the harbour seal (*Phoca vitulina*), known to have low levels of genetic variation, were analysed with the RAPD technique. This technique showed a very low number of polymorphic bands (only 2 out of 50 tested primers generated polymorphic bands). Genetic variation in the harbour seal could not be assessed by this method, the resolving power was not sufficient to measure genetic differentiation. Results found with the RAPD method were in agreement with results found with allozyme electrophoresis, whereby total absence of polymorphism was found for 24 loci (Swart *et al.*, 1996). Thus, although in other population and evolutionary studies genetic variation could be assessed, the amount of variation in the harbour seal was too low to be detected by the RAPD technique (Martin *et al.*, 1991; Welsh *et al.*, 1991; Wilde *et al.*, 1992).

## GENETIC VARIATION REVEALED BY FINGERPRINTING TECHNIQUES

### The harbour seal

The multilocus DNA fingerprinting technique using allozyme electrophoresis and DNA fingerprinting with multilocus DNA fingerprinting probes 33.6 and 33.15 (Jeffreys *et al.*, 1985) revealed genetic variation in the harbour seal populations within the Wadden Sea population and the North Sea population. This indicates a low level of genetic variation. Surveys in geographically isolated populations and species with allozyme fingerprints in other animals (Gilbert *et al.*, 1990; Jeffreys *et al.*, 1991) and the Northern Sea populations and species with allozyme fingerprints (Jeffreys *et al.*, 1985 a,b, 1993).

The fingerprint data on the Dutch and North Sea populations within and between, population genetic variation. Populations are not comparable. Populations cannot be compared based upon these similarities. The data on the population genetic variation leading to higher genetic variation in larger population size of the North Sea population.

The low genetic variation in the North Sea populations of the harbour seal is characteristic for the species *Phoca vitulina* complex (*P. v. vitulina*).

## GENETIC VARIATION REVEALED BY THE MULTILOCUS DNA FINGERPRINTING TECHNIQUE

### The harbour seal

The multilocus DNA fingerprinting technique is a much more sensitive method than allozyme electrophoresis and the RAPD technique. In chapter 2 is described how the multilocus DNA fingerprinting technique was used to determine the amount of genetic variation in the harbour seal. The DNA fingerprint data showed for the two Jeffreys probes 33.6 and 33.15 (Jeffreys *et al.*, 1985a,b) a high degree of band-sharing, approximately 0.80, within and between two populations of the harbour seal: the Wadden Sea population and a Scottish population. This high-degree of bandsharing indicates a low level of overall genetic variation in both populations. Comparative surveys in geographically isolated and small populations showed also less variable DNA fingerprints in other animal species, like the Channel Island fox (Gilbert *et al.*, 1990), the Gir lion (Gilbert *et al.*, 1991), mysticete whales (Amos & Hoelzel, 1990; Van Pijlen *et al.*, 1991) and the Northern Elephant seal (Hoelzel *et al.*, 1993). However, in populations and species with relatively large large population sizes, high levels of genetic variability were generally reported by DNA fingerprinting with the same probes (Jeffreys *et al.*, 1985 a,b; Burke & Bruford, 1987; Georges *et al.*, 1988, Baker *et al.*, 1993).

The fingerprint data can be used to calculate similarity between the harbour seal populations in the Dutch Wadden Sea and Scotland. No significant differences, either within and between, populations were found. These results indicate that the two populations are not completely isolated, and that some geneflow between these populations cannot be excluded. Nevertheless, when heterozygosities were estimated based upon these similarity values, a slight difference between populations was detected. The data on the population from Scotland showed a more frequent occurrence of rare alleles leading to higher values for estimated heterozygosities, which may reflect the larger population size of the Scottish population.

The low genetic variation found by multilocus DNA fingerprinting, in the two North Sea populations of the harbour seal could indicate that such low levels are characteristic for the species *Phoca vitulina*. In chapter 3 three subspecies of the *Phoca vitulina* complex (*P. v. vitulina* (East Atlantic), *P. v. concolor* (West Atlantic) and *P. v.*

*richardsi* (East Pacific)) were analysed with respect to the level of genetic diversity by multilocus DNA fingerprinting. Bandsharing similarities between subspecies were significantly lower than within subspecies. This indicates that gene flow between subspecies is limited, which could be expected from the geographical position of the diverse subspecies. Especially, the subspecies *P. v. richardsi* is clearly separated from the other subspecies. These data indicate that low levels of genetic variation are not characteristic for all *Phoca vitulina* subspecies. Moreover, the grey seal (*Halichoerus grypus*), a marine carnivore living in the same habitat, has significantly higher levels of genetic variation, revealed by DNA fingerprinting. Data on cytochrome *b* sequences have indicated that the grey seal should be included in the *Phoca* genus (Arnason *et al.*, 1995), which implies furthermore that the low level of overall genetic variation is not characteristic for the genus.

The pauperization of genetic variation found in the subspecies in the North Sea area shown by the heterozygosities estimated from DNA fingerprinting data, could also be due to historical events, and more specifically by the last Northern Hemisphere glaciation. The latter event caused a dramatic reduction of size and fragmentation of populations in many large mammal species (Marshall *et al.*, 1982; Menotti-Raymond & O'Brien, 1993). The subspecies *P. v. richardsi* and *P. v. concolor* probably did experience significantly less severe bottlenecks during the last glaciation than the subspecies *P. v. vitulina*, because both the West Atlantic and East Pacific regions were less covered with ice (Peltier, 1994). The date of the bottleneck, causing loss of almost all minisatellite variation in *P. v. vitulina*, was estimated in chapter 3. The present level of genetic variation in the subspecies *P. v. vitulina* does agree with the assumption of a bottleneck some 10.000 years ago.

### The Seychelles warbler

In chapter 4 the use of multilocus DNA fingerprinting to reveal the amount of genetic variation in the Seychelles warbler is described. The world population of the Seychelles warbler population consisted of 26-29 birds, between 1959 and 1968, and the population was confined to Cousin Island. The warbler population started to recover from 1968 with numbers rising to 320 in 1982, since then the population fluctuated around that number. The genetic variation was estimated to determine the effects of a ten year bottleneck in the Cousin Island population and the possible effects of the

translocation to Aride island to establish a new population high (about 0.50), when compared to other species (Burke & Bruford, 1987). Bandsharing similarity between subspecies by DNA fingerprinting no effect of

In addition, estimates of population size by DNA fingerprinting and demographic data suggest that the bottleneck reduced the level of heterozygosity. In the Seychelles warblers have a feature, the population density of founding a new, rapid increase in other avian species. A population of 0.76, this population started with a population size of around

Low levels of genetic depression, which can be found in the Seychelles warbler reported, though, of course of genetic load by earlier events occurred. However, as a result be reduced and therefore And although no current changes of such a population

### GENETIC VARIATION

The microsatellite method of variation. Advantage of to score codominant markers follow genetic processes

translocation to Aride island. In 1988 29 birds were translocated to Aride Island, in order to establish a new population. Mean bandsharing similarity within both populations is high (about 0.50), when compared to values, around 0.25, obtained from outbred avian species (Burke & Bruford, 1987; Wetton *et al.*, 1987; Westneat, 1990). No effects on bandsharing similarity between populations on both islands are observed, thus with DNA fingerprinting no effect of the translocation could be assigned.

In addition, estimates of effective population size were made based on DNA fingerprint and demographic data. The estimates indicate a reduction of effective population size by 67-78%, compared to the situation in outbred avian species (Burke & Bruford, 1987; Wetton *et al.*, 1987; Westneat, 1990). The multilocus DNA fingerprint data suggest that the bottleneck of 29 animals during at least ten years has considerably reduced the level of heterozygosity of the Seychelles warbler population. Concluding the Seychelles warblers have probably undergone considerable genetic erosion. Despite this feature, the population did recover after protective measures on Cousin and was capable of founding a new, rapidly growing population on Aride. Similar results were found for other avian species. A population of the Canada geese showed band similarity values of 0.76, this population started with 5 individuals and was rapidly increasing to a current population size of around 30 - 50,000 individuals (Tegelström & Sjöberg, 1995).

Low levels of genetic variation are associated with the occurrence of inbreeding depression, which can cause impaired fitness. Despite the low levels of genetic variation found in the Seychelles warbler populations, no flagrant deleterious effects have been reported, though, of course, no outbred population is available for comparison. Purging of genetic load by earlier inbreeding periods, including the 10-year bottleneck may have occurred. However, as stated by Hedrick (1995), the mean fitness of the population may be reduced and therefore the risk of extinction in the future may have been increased. And although no current effects are visible, the adaptive potential to environmental changes of such a population may have been severely affected.

#### GENETIC VARIATION REVEALED BY MICROSATELLITE ANALYSIS

The microsatellite method has a high resolving power to reveal low levels of genetic variation. Advantage of this method, compared to DNA fingerprint analysis, is the ability to score codominant genotypes with exact allele sizes. This provides the opportunity to follow genetic processes, like gene flow. Microsatellite analysis have been applied to the

analysis of genetic variation in the harbour seal (Chapter 6). Five specific microsatellite markers were developed for the harbour seal and were used to study the genetic structure of harbour seal populations in the North Sea, the West Atlantic and the East Pacific Ocean.

The degree of heterozygosity of the five microsatellite loci proved to be very low in both the North Sea and the West Atlantic populations of the harbour seal, and the highest heterozygosity was found in the East Pacific population, which is in agreement with the multilocus fingerprinting data (Chapter 3). No specific alleles were found for the North Sea populations, the populations from the West Atlantic and the East Pacific showed 2 and 7 population specific alleles, respectively. A distance tree was constructed based on Nei's genetic distance, and provisionally it can be concluded that all populations are genetically separated, with exception of the Scottish populations.

The DNA fingerprinting method provides many loci, which are assumed to be independent, but specific alleles cannot be assigned and therefore gene flow and migration cannot be determined. With microsatellite analysis specific alleles can be assigned and with the data an estimate of population divergence and migration can be made based on  $R_{ST}$  measures (Slatkin, 1995). This measure is based on the estimated variances in allele sizes (see chapter 6). The  $R_{ST}$  values found for the North Sea and the West Atlantic populations are relatively high, indicating a distinct population substructure. For the North Sea populations a substructure could be detected within the North Sea. Based on  $R_{ST}$  values the Scottish populations seemed to be one panmictic population and the migration between the Dutch Wadden Sea population and the Scottish populations is estimated to be low. Therefore it is not to be expected that the low levels of genetic variation can be replenished by exchange with neighbouring gene pools. Although the Dutch Wadden Sea population is recovering and increasing in population size, the genetic impoverished situation has not improved. Protective measures, installed for the Dutch Wadden Sea area, however, may have improved the environment, and therefore this area may become more attractive for seals to breed. Thus, a part of the increase in numbers of the harbour seal population in the Dutch Wadden Sea could be due to immigration from animals from e.g. Denmark, Germany or Britain, therefore the isolation of subpopulations in the North Sea can be relieved.

## CONCLUDING REMARKS

With molecular techniques the harbour seal and the Seychelles were examined. Several techniques were used to study the genetic structure. Our results indicate several opportunities to detect the genetic structure of populations. To measure the level of genetic variation and population structure a method of multilocus fingerprinting, based on microsatellite polymorphism and the amount of genetic variation, was used. The genetic structure of the harbour seal populations (Chapter 3 and 5). To investigate the genetic variation, like the harbour seal, because no specific loci can be assigned, and therefore, genetic structure cannot be determined. The data assessed by the various techniques is compared. The comparison of the data is subject to interpretation of the data collected from different organisms with various methods.

Although, the molecular techniques used and on the genetic structure of the harbour seal, the obtained data. The significance of the data with respect to the risk of population extinction, the impact of loss of variation, and the interaction between genetic structure and environment in the future.

Currently, in consequence of the importance of genetic structure in population extinction. Low genetic variation, which means decreased genetic potential. The latter trait is a result of genetic drift (Chapter 3 and 4). Thus, the genetic structure of populations or species presently seem