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# Conservation Genetics of the Eurasian Otter in Sweden

JOHANNA ARRENDAL





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#### Abstract

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In this thesis, molecular genetic methods were used to study a threatened species, the Eurasian otter. Estimates of population size and population dynamics parameters were obtained, the genetic effects of a restocking program was evaluated, and a population viability analysis was conducted to assess which demographic parameters are most important for the future viability of an otter population. Many of the studies were based on noninvasive genetic sampling of facees.

In the genetic evaluation of the restocking program, it was found that the released otters had contributed to subsequent generations. However, the effects were to a large degree limited to the near surroundings of the release areas.

Comparison of two census methods, snow-tracking and noninvasive genetic census based on faeces, showed that approximately only half of the otters detected with the genetic census were found with the snow-tracking census. It is recommended to combine these two methods to obtain the most reliable estimates of population size.

A short-term study on population dynamics in otters showed that apparent survival was higher in females than in males and that the rate of addition was also high and likely influenced by migration.

The population viability analysis incorporated both genetics and demography and revealed that survival to first reproduction was the most crucial demographic parameter affecting the viability of the study population. This result suggests that conservation efforts should be focused on protocols that enhance the survival prospects of young females. Environmental stochasticity was also found to have large effects on the probability of extinction of this population.

*Keywords:* Lutra lutra, in situ conservation, individual identification, microsatellites, mtDNA, noninvasive genetic sampling, population dynamics, population size, PVA, restocking

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Till min familj

Cover photos by J Arrendal

## List of papers

This thesis is based on the following papers, which will be referred to by their Roman numerals.

- I Arrendal J, Walker CW, Sundqvist A-K, Hellborg L, Vilà C. 2004. Evaluation of an otter translocation program. *Conservation Genetics* 5:79-88.
- II Arrendal J, Laikre L, Mortensen P, Sjöåsen T. The evolution of a restocked otter population in Sweden. *Manuscript*.
- III Arrendal J, Vilà C, Björklund M. 2007. Reliability of noninvasive genetic census of otters compared to field censuses. *Conservation Genetics* In press.
- IV Arrendal J. Population dynamics parameters obtained by noninvasive genetic methods in a Eurasian otter population. *Manuscript*.
- V Björklund M, Arrendal J. A demo-genetic analysis of a recovering population of otters in central Sweden. *Manuscript*.

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## Abbreviations

DNA mtDNA PCBs PCR deoxyribonucleic acid mitochondrial DNA polychlorinated biphenyls polymerase chain reaction

## Introduction

The survival of a large number of species is currently threatened due to human activity. Many of these are carnivores. As long as carnivores and humans have shared space, carnivores commonly have been hunted for their valuable fur. Additionally, they have been regarded as competitors to humans, as they prey on wild fish and game and, if not prevented, also on domestic animals. Consequently, this has been a second reason for hunting them, which in the past frequently led to organized persecution of the species.

During the 20<sup>th</sup> century other threats were recognized that are induced by the expansion of the human population and society. Habitat destruction has decreased the amount of habitat available, and disturbance from human activity may further reduce the suitability of habitat, even though many populations have adapted to their new human-affected environment (Kranz and Toman 2000). Pollution has caused both direct and indirect mortality, as well as reduced or failed reproduction (Bäcklin and Bergman 1992; Brunström et al. 1994). Competition with other species, introduced on purpose or accidentally or expanding their distribution range due to, for instance, climate change, have in some cases caused population declines (Macdonald and Thom 2001; Tannerfeldt et al. 2002). Finally, traffic mortality, caused by our increasing network of infrastructure, puts extra pressure on small or expanding populations, the extent of which has been hard to assess (Madsen 1996; Philcox et al. 1999).

As a consequence of the threats, carnivore populations have declined and their distributions have been reduced to fragments of their former distribution areas. The increased vulnerability of carnivores to the above mentioned threats mainly arise because they are top predators, often have low reproductive rates, large home-ranges and sometimes complex social systems (Purvis et al. 2000; Cardillo et al. 2005).

Today, a lot of effort and resources are invested in conservation actions to bring endangered species and populations back to their former ranges. Management of threatened species often consists of restoring habitat, limiting factors that cause the population to decline, and monitoring distribution and population size. Sometimes direct actions are taken to bring the species back or increase population growth, such as translocations of conspecifics from other populations (Fisher and Lindenmayer 2000; Breitenmoser et al. 2001).

#### The use of genetics in conservation

The evolution of a population can be visible and measurable in the field, but much can only be revealed at the genetic level. For instance, a population decline reduces the amount of genetic variation in the population (Frankham et al. 2002), which can be measured by population genetic methods even though no negative effects have yet appeared that are measurable on, for instance, survival. Additionally, population genetics can reveal if, for instance, a reduced reproductive rate could be caused by inbreeding depression (Saccheri et al. 1998; Kruuk et al. 2002).

Both basic data on historical processes and current genetic variation and differentiation between populations, as well as more applied studies on, for example, the genetic contribution of translocated individuals to a local population, can generate knowledge important to conservation decisions. Genetics can also be used to identify individuals and their relatedness in a population through obtaining DNA directly or indirectly from the individuals. By doing this, information on population size, survival, reproduction, dispersal, migration and much more can be obtained.

Below I will describe some of the parameters important to conservation that can only or are best obtained, estimated or evaluated with genetic methods. Furthermore, I will describe an important tool for this purpose – the noninvasive genetic technique.

#### Translocations

Translocations of individuals are increasingly used for conservation purposes (Griffith et al. 1989; Breitenmoser et al. 2001). Reintroduction of a species to its former range is often made when it has been assessed that the species has a very low chance of returning on its own within a reasonable time frame (Breitenmoser et al. 2001). Restocking of a population is carried out to support weak populations or to prevent extinction of a species (Parsons 1998; Dobson and Lyles 2000; Snyder and Snyder 2000; Hubbard and Serfass 2004). The release of individuals from another population may also involve captive-bred animals (e.g. Sjöåsen 1996a; Milinkovitch et al. 2004; Brown et al. 2006).

Thorough evaluations of the results of translocations are rarely carried out (IUCN 1987; Fisher and Lindenmayer 2000; Breitenmoser et al. 2001; Laikre et al. 2006). Furthermore, lack of clearly defined aims often obscure the estimates of success. One way to evaluate the success of translocations is to investigate the extent to which the translocated individuals contribute genetically to subsequent generations. This can be done by comparing the genetic composition of the translocated individuals to the local population before and after the restocking event (Ellsworth et al. 1994; Grewe et al.

1994; Leberg et al. 1994; Largiadèr and Scholl 1995; Jones et al. 1996; Nedbal et al. 1997; Rowe et al. 1998; Leberg and Ellsworth 1999).

#### Estimation of population size

Accurate estimates of population size are of paramount importance in conservation and management of threatened species (Gese 2001). These estimates are needed to correctly assess conservation needs, but also to evaluate effects of conservation actions. However, accurate estimates of population size can be very hard to obtain for many species, especially those which are mainly nocturnal and have large home-ranges. For carnivores, population size has often been measured by different field census techniques (e.g. counting tracks, counting active dens, or direct observation; Gese 2001). Capture-mark-recapture techniques have also been used (Thompson et al. 1998) and their estimates are often regarded as highly reliable, but the method has a draw-back in that it depends on directly handling the animals.

During the last few years, noninvasive genetic approaches (see below) have become an alternative to these capture-mark-recapture methods, by giving the opportunity to identify individuals in an area without the need to capture, harm, or even disturb the animals (Taberlet et al. 1996; 1999; Taberlet and Luikart 1999). These methods have during the last years delivered data on population size for species like coyotes, mountain lions, bears, badgers and elephants (Kohn et al. 1999; Ernest et al. 2000; Eggert et al. 2003; Wilson et al. 2003; Frantz et al. 2004; Bellemain et al. 2005). However, noninvasive genetic methods are related to numerous problems, which calls for careful methodologies (Taberlet et al. 1996; Gagneux et al. 1997; Taberlet and Luikart 1999; Lucchini et al. 2002; Miller et al. 2002; Creel et al. 2003; Wandeler et al. 2003; Broquet and Petit 2004). Additionally, it is of great importance to compare the census results of noninvasive genetic sampling to the results of traditional field techniques, to gain further knowledge of actual population size (Eggert et al. 2003; Solberg et al. 2006).

#### Population dynamics

Measurements of population dynamics parameters are important to understand and predict the future of a population and for the development of sensible population viability analyses (Holsinger 2000). Like population size, also these measurements can be hard or impossible to obtain directly, as capture and handling of the animals may harm or disturb them seriously. However, indirect measurements obtained through noninvasive methods can be used (Boulanger et al. 2004; Proctor et al. 2004). Sampling of the population on a regular basis can provide estimates of population rate of change, sex ratio, migration, as well as survival, birth and mortality rates.

#### Population viability analyses

There is a strong interest in predicting the future of small and threatened populations, as an aid to make decisions concerning conservation efforts. The concepts and methodologies of population viability analyses (PVA) are intensively discussed and developed (Boyce 1992; Beissinger and Westphal 1998; Brook et al. 2000; Coulson et al. 2001; Brook et al. 2002; Ellner et al. 2002; Lindenmayer et al. 2003). Ideally, to make a proper PVA detailed information concerning basic demographic parameters such as survival and birth rates across age classes and the variance of these estimates are needed (Saether and Engen 2002). By using this knowledge, predictions concerning future population sizes and risk of extinction can be derived by simulations.

However, classic PVAs only concern the changes in numbers over time, while the negative effects of loss of genetic variability is not considered (Allendorf and Ryman 2002; Reed and Frankham 2003). Few attempts to incorporate both demographic and genetic factors of population long-term survival have been done (e.g. Lehmann and Perrin 2006). By doing this the demographic parameters that are most important for the growth rate of the population can be identified and the effects of demographic and environmental stochasticity can be evaluated.

#### Noninvasive genetic techniques

Noninvasive genetic sampling refers to sampling of DNA sources without the need of capturing or handling the animals, which otherwise may cause harm or disturbance. Instead, DNA can be obtained from what the animals leave behind. For example, DNA can be found in faeces, urine, hairs, sloughed skin, or feathers (Taberlet et al. 1996; 1999; Taberlet and Luikart 1999). Since the 1990's, noninvasive genetic methods have been applied to a number of populations and species (e.g. Gagneux et al. 1997; Reed et al. 1997; Kohn et al. 1999; Ernest et al. 2000; Lucchini et al. 2002; Eggert et al. 2003; Wilson et al. 2003; Frantz et al. 2004; Hedmark et al. 2004; Hung et al. 2004; Bellemain et al. 2005).

In conservation genetics, noninvasive methods can be used for several reasons. For instance, when a study is based on genotyping DNA, noninvasive sampling can be used instead of sampling the population directly through capture of individuals for obtaining e.g. blood or tissue samples, and thus avoid the harm and disturbance that handling of wild animals may cause (e.g. Flagstad et al. 2004). Additionally, when a study is based on physical capture-mark-recapture of individuals (e.g. to obtain population size estimates), or on detailed field observations (e.g. to estimate movements or litter size), instead these studies can be based on genetics and noninvasive sampling without the need to catch or even observe the animals (e.g. Taberlet et al. 1997; Lucchini et al. 2002; Flagstad et al. 2004).

However, the amount of DNA from the target species in noninvasivly obtained samples is often much smaller than in e.g. muscle tissue (Taberlet et al. 1999; Taberlet and Luikart 1999). Additionally, the DNA is often degraded to some extent and, in the case of faecal samples, these may contain substances that inhibit chemical processes during laboratory work (Taberlet et al. 1999; Taberlet and Luikart 1999). These factors may all lead to overall worse performance of the samples in the laboratory, which implies that more sophisticated methods are needed and repeated genotyping of the same sample must be carried out to obtain a reliable genotyping result (Taberlet et al. 1996; 1999; Taberlet and Luikart 1999). The costs of laboratory analyses of noninvasive sampling is therefore often much higher than for genotyping of samples from sources rich in DNA, like muscle tissue samples.

The most frequent problem in genotyping noninvasive samples is allelic dropout, that is when one of the two alleles is not amplified during PCR (Taberlet et al. 1996; Gagneux et al. 1997; Taberlet and Luikart 1999; Lucchini et al. 2002; Miller et al. 2002; Creel et al. 2003; Wandeler et al. 2003; Broquet and Petit 2004). This may happen when the number of templates is low and one of the templates is amplified more efficiently during the early stage of the PCR. The occurrence of misprinting, or false alleles, is another problem of PCR-based origin (although not as frequent as allelic dropout; Taberlet et al. 1999). During the early stage of amplifications, a fragment may be generated that is one or more repeat units shorter or longer than the true allele. If this fragment is amplified sufficiently efficient, a false allele will result.

To control for genotyping errors and obtain reliable genotypes from noninvasive samples, a multiple-tubes approach should be used (Taberlet et al. 1996; 1999; Taberlet and Luikart 1999). With these methods each DNA extract is replicated independently several times for each locus. The success rate in obtaining reliable genotypes have shown to be dependent on factors like sampling conditions (Lucchini et al. 2002; Hedmark et al. 2004; Nsubuga 2004; Hajkova et al. 2006), storage and extraction methods (Frantzen et al 1998; Nsubuga 2004), and the diet of the sampled animal (Reed et al. 1997; Farrell et al. 2000; Murphy et al. 2003).

#### Study species

The Eurasian otter (*Lutra lutra*) is a threatened semi-aquatic carnivore. On the Swedish red list it is regarded as "vulnerable" according to criterion D1 (the number of sexually mature individuals is less than 1000; Gärdenfors 2005). The Eurasian otter has been subjected to most of the threats mentioned above and thereby gone through a severe population decline (Mason and Macdonald 1986; Olsson et al. 2006).

#### Taxonomy and distribution

The Eurasian otter is member of the family Mustelidae, which also incorporates for example stoats, weasels, minks, martens, badgers and the wolverine. This otter species has a large distribution area that covers approximately the whole of Europe and Asia and also the northern-most parts of Africa (Mason and Macdonald 1986). However, today there are large gaps in the distribution, as otter populations have decline with onset in the early 20<sup>th</sup> century (Mason and Macdonald 1986). Today, some populations are thriving, some have started to grow, and some remain small, whereas others still decline.

In Sweden the otter population started to decline during the 1950's (Olsson et al. 2006). The species was protected by law in the whole country in 1968, but continued to decline (Erlinge 1971). During the 1980's the population reached its minimum size, which was estimated to less than 1000 otters (Ahlén and Tjernberg 1996; Fig. 1). The otter was by then locally extinct from large parts of Sweden. In the northern half of the country it was sparsely distributed, whereas in the southern half it only existed in small remnant populations in the provinces of Småland, Östergötland, Södermanland and Uppland. During the 1990's populations started to grow in most parts of the country (Länsstyrelsen Gävleborg 1998; Bisther 2000; Bisther 2005; Bergström et al. 2006; Hammar 2006; Fig. 2).



Figure 1. Otter distribution in Sweden during 1980-1989. Grey-shaded areas show otter distribution within surveyed areas during 1980-1989, where light-grey corresponds to surveyed areas with no otter signs, and medium-grey is surveyed areas with otter signs (map provided by the Swedish Museum of Natural History).



Figure 2. Otter distribution in Sweden during 1986-1995 (left) and 1996-2005 (right). Grey-shaded areas show otter distribution within surveyed areas, where light-grey corresponds to surveyed areas with no otter signs, medium-grey and dark-grey is surveyed areas with otter signs, with higher otter densities within the dark-grey areas. The distribution data are split on two maps, as surveys differ in time and space. Together they provide a more complete picture of the otter distribution (maps provided by the Swedish Museum of Natural History).

#### Threats

The reasons for the population decline could be numerous. All threats mentioned above have more or less partially contributed, but the main factor seems to be pollutants (PCBs; Roos et al. 2001). PCBs are no longer used in Sweden, but still exist in nature although overall levels have declined.

#### Species biology

The Eurasian otter lives in all kinds of waters (in seas, lakes and rivers). It has an elongated body with several adaptations to a life in water. However, the otter also often moves across land, as it takes short-cuts between water courses, or sometimes have their holts several hundreds of meters from the shore line (Kruuk 1995).

The otter mostly prey on fish and other water-living animals, like crayfish, but occasionally also to a varying extent on frogs, sea birds and rodents (e.g. Erlinge 1971; Kruuk and Moorehouse 1990; Jedrzejewska et al. 2001). It is nocturnal in most of its distribution area, but diurnal in some areas (Erlinge 1971; Kruuk 1995).

The otter has a litter size of between one to four cubs, with a mean varying around two in many European countries (Jenkins 1980; Mason and Macdonald 1986; Kruuk et al. 1991). In Sweden it averages 1.9; Erlinge 1967). Cubs can be born all year round, but in Sweden births have been shown to peak during spring time (Erlinge 1971). The cubs follow the female during the first year and together they form a family group. The otter male does not rear the cubs.

Otters have home-ranges that they scent-mark with spraints (faeces and anal secretions; Fig. 3). Males predominantly have larger home-ranges than females, and are more territorial (Erlinge 1968). Male home-ranges may overlap with other males, but they tend to avoid conflicts as far as possible (Erlinge 1968). Female home-ranges are referred to as feeding areas with quite sharp boundaries against other females, especially when having cubs, however, overlapping with male home-ranges (Erlinge 1968).



Figure 3. Otter spraints on grass and tracks in the snow. Photo: J Arrendal.

#### Research and conservation efforts in Sweden

Much effort has been devoted to the otter in Sweden. Pioneering research on otter ecology was conducted by Erlinge in southern Sweden during the 1960's-1970's (e.g. Erlinge 1967; 1968; 1969). Many organizations have funded and carried out conservation efforts. Habitat has been restored to better suite otters. Research on pollutants and their effects on otters have been conducted for several decades (e.g. Roos et al. 2001). Data on otters

found dead are continually being collected (stored at the Swedish Museum of Natural History).

During the severe population decline, restocking of otter was carried out in areas in southern Sweden, with the aim to support the fragmented and partially isolated populations in those areas, induce population growth and provide connectivity among otters in the southern and northern parts of the country (Sjöåsen 1995). Fifty-four wild-caught and captive-bred otters originating from northern Norway and Finland were released in an area in the province of Södermanland (N=47) and another area in the province of Uppland (N=7) (Sjöåsen 1997; Larsson and Ebenhard 1994).

The restocking program gave an opportunity to conduct further research. The translocations were evaluated both through radio-tracking of two-thirds of the released animals (Sjöåsen 1996a; 1997) and surveys on otter distribution (Sjöåsen 1996b; Sjöåsen 1997). Radio-tracking showed that the survival during the first year was 54% (Sjöåsen 1996a), and that at least 44% of the released otters established home-ranges (Sjöåsen 1997). The distribution surveys, conducted in 1983, 1988 and 1996, indicated an expanding population (Sjöåsen 1996b; 1997).

Monitoring of otter distribution has been organized since the 1980's. Surveys have been conducted in most parts of Sweden and repeatedly in some areas (e.g. Länsstyrelsen Gävleborg 1998; Bisther 2000; Bisther 2005; Bergström et al. 2006; Hammar 2006).

Fauna passages at bridges have been built since the 1990's to decrease the number of otters being road-killed (e.g. Hammar 1999; Lindström and Martinsson 2002). Currently, more resources are being devoted and the monitoring and measures of suboptimal bridges are increasing.

An action plan has recently been adopted, which summarizes what was known until 2006 and presents proposals on how a sustainable and viable otter population can be guaranteed in Sweden (Bisther and Aronson 2006). The time frame of the action plan is 2006-2010.

#### Genetic diversity in the Eurasian otter

The Eurasian otter has quite low genetic diversity overall in the mitochondrial DNA (Effenberger and Suchentrunk 1999; Mucci et al. 1999; Cassens et al. 2000; Ferrando et al. 2004; Ketmaier and Bernardini 2005). This has probably been caused by recent population declines, but also due to founder events during postglacial recolonisation (Effenberger and Suchentrunk 1999; Mucci et al. 1999; Cassens et al. 2000; Pertoldi et al. 2001) or to anthropogenic pressure during the last 2000 years (Pertoldi et al. 2001). Of the mtDNA control region one haplotype has been found to be dominant and found in all over Europe and also in Russia (Ferrando et al. 2004). The largest number of mtDNA haplotypes have been found in the eastern parts of Europe and in Russia (up to four haplotypes; Cassens et al. 2000; Ferrando et al. 2004), but often only one or two haplotypes exist within a country (Mucci et al. 1999; Ketmaier and Bernardini 2005). In Sweden two haplotypes have been found (Giulianelli 1995).

In nuclear microsatellite markers, which have higher mutation rates than the mitochondrial genome, otters have low to moderately high variation depending on the population history (Dallas et al. 1999; Pertoldi et al. 2001; Dallas et al. 2002; Randi et al. 2003). Population declines have fragmented otter populations and caused differentiation between them (Dallas et al. 1999; Dallas et al. 2002; Randi et al. 2003).

## The need of knowledge for enhanced conservation of the otter in Sweden

The otter population in Sweden has been subjected to a severe population decline and has also been supported through the release of otters from other areas in Fennoscandia (Olsson et al. 2006; Sjöåsen 1995). Although otters currently are increasing in numbers in most parts of the country, large areas remain empty and recolonization seems to be slow (e.g. Bisther 2000).

To enhance the conservation of the population, knowledge is needed on several issues. Estimates of genetic variation among different parts of the population are important to understand future performance of the population (Frankham et al. 2002). This also relates to the restocking of otters that has been conducted. It is of great interest to know if and to what extent the released otters have contributed to subsequent generations (IUCN 1987; IUCN/SSC 1998; Fisher and Lindenmayer 2000; Breitenmoser et al. 2001).

Estimates of population size are obtained from snow-tracking in Sweden and in other northern countries (e.g. Reid et al. 1987; Sidorovich and Lauzel 1992; Aronson 1995; Sulkava 1995; Sidorovich et al. 1996). However, this method has a weakness as it is dependent on snow and ice during a sufficiently long time period. In some parts of Sweden and in large parts of the species distribution area, these criteria cannot be found. Hence, it is important to develop a methodology that can be used under more diverse conditions. Noninvasive genetic sampling has been used with variable results on otters (Jansman et al. 2001; Dallas et al. 2003; Hung et al. 2004; Hajkova et al. 2006) and if population size can be estimated through this method it could aid in estimating otter numbers when snow-tracking cannot be used. It is also important to compare the two methods to understand the limits of the methods and assess which method delivers the most accurate estimate (Eggert el al. 2003; Solberg et al. 2006).

Measurements of population dynamics parameters are hard to obtain on wild otters. Nevertheless, they are needed to understand and predict the future of the population. Parameters that underlie the population dynamics of Eurasian otters have been estimated through radio-tracking and observations of live animals (e.g. Erlinge 1967; Kruuk et al. 1991; Kranz 1995; Sjöåsen 1997) and from examination of otter carcasses (Kruuk and Conroy 1991; Ruiz-Olmo et al. 1998; Hauer et al. 2002a; 2002b). However, estimates on live animals are often based on following only a few individuals for a limited time and estimates from otters found dead can be highly biased as only a very small proportion of the dead otters can be expected to be found (Kruuk and Conroy 1991; Ruiz-Olmo et al. 1998; Hauer et al. 2002a; 2002b). Estimates of population rate of change, sex ratio, migration, as well as survival, birth and mortality rates can be obtained indirectly through noninvasive genetic methods and would fill a gap of knowledge about otters in Sweden.

Population viability analyses can aid in assessing the risk of future extinction and determine which parameters are the most important for survival and reproduction. A PVA on otters in Sweden was performed during the 1990's (Larsson and Ebenhard 1994). It was largely based on data from the period when otter numbers were at minimum and showed that small and isolated otter populations had a severely risk of extinction within only 50 years. The incorporation of genetics into PVA and the performance of sensibility analyses could contribute with additional and updated information.

## Aims of the thesis

My main aim of this thesis is to perform investigations that contribute to the knowledge of genetics and ecology of wild populations of Eurasian otter in Sweden. This knowledge may in turn contribute to the conservation of the species in Sweden and other parts of its distribution area. More specifically, I focus on the following issues:

- 1. Genetic evaluation of the otter restocking program in Sweden.
- Comparison of two census method for otters: a genetic census based on noninvasive sampling of faeces and a field census method based on snow-tracking.
- 3. Estimation of population dynamics parameters through noninvasive genetic sampling on an otter population in central Sweden.
- 4. Performance of a population viability analysis of otters in a population in central Sweden, which incorporates both genetics and demographics.

## Present investigations

#### Evaluation of an otter translocation program (I)

In southern Sweden, 54 otters of Norwegian origin were released during 1987-1992 to support the severely declining population (Sjöåsen 1997). The survival of the released otters was 54% after one year and a subsequent population growth was found in the area (Sjöåsen 1996a; Sjöåsen 1996b). In this study, we used genetics to evaluate the effects of the restocking program on the genetic structure of the Swedish otter population. If translocated individuals had successfully contributed to the gene pool of the resident otters, we expected the genetic composition of populations in areas neighbouring the release sites to show similarities to that of the group of released otters.

#### Methods

Samples from a total of 114 otters were used for the analysis: 20 from released otters originating in northern Norway (N) and 94 from Sweden. Northern Sweden (NS) was represented by 28 samples and from central Sweden (CS) 23 samples were used. The samples were grouped into populations based on their geographic origin (Fig. 4). For the samples in southern Sweden we differentiated the samples dating from before the release of Norwegian otters (SB, N=15) from those collected afterwards (SA). Additionally, we separated the otters from southern Sweden post-dating the releases into two populations (SA1, N=8 and SA2, N=20). Only one of these populations (SA2) was directly subjected to the translocations (included release site 2, Fig. 4), but both of them could be indirectly influenced by dispersing individuals from the other release site. Due to lack of samples we were unable to study the area surrounding release site 1.

The samples were genotyped for fragments of mtDNA control region I and six microsatellite loci. Genetic variation in, and differentiation between, the populations were analyzed. An assignment test was used to calculate the likelihood of each individual multi-locus genotype having originated from each of the defined populations.



Figure 4. Populations of Eurasian otters included in the study. Populations marked in grey were sampled after the release of otters from Norway (from population N). The release sites are indicated.

#### Results and discussion

Two mtDNA haplotypes were found among the samples. Haplotype A occurred in all sampled populations (93% of the samples), but haplotype B was only found in the group of translocated otters (11%) and in SA2 (35%)(Table 1). The genetic variation varied between the populations from low to moderately high and was comparable to what has been found in other areas in Europe (Dallas et al. 1999; Effenberger and Suchentrunk 1999; Mucci et al. 1999; Cassens et al. 2000; Pertoldi et al. 2001).

In SA2, seven otters were released (release site 2, Fig. 4) and our results showed that this population might have been affected by the restocking. One microsatellite allele was present in a high frequency only among the released otters and in SA2 (Table 1) and additionally, mtDNA haplotype B was present only in these two populations (Table 1). Neither the allele nor the haplo-type had been observed in the otter population in southern Sweden before the releases (SB).

| Population | Haplotype A (no. of ind.) | Haplotype <i>B</i> (no. of ind.) | Allele 4<br>(frequency) |
|------------|---------------------------|----------------------------------|-------------------------|
| N          | 17                        | 2                                | 0.225                   |
| NS         | 28                        | 0                                | 0.036                   |
| CS         | 23                        | 0                                | 0.022                   |
| SB         | 15                        | 0                                | 0.000                   |
| SA1        | 7                         | 0                                | 0.000                   |
| SA2        | 11                        | 6                                | 0.275                   |

Table 1. Distribution of mitochondrial DNA control region haplotypes and microsatellite allele 4 at locus Lut832 in the populations.

Otters from SA1 were characterized by a low genetic diversity (Table 2), which is expected in a population that has gone through a sharp decline. The release of 47 otters from Norway at release site 1 (Fig. 4), to the north of SA1, did not seem to have resulted in the arrival of new alleles or an increase of diversity. In fact, the otters in SA1 were most similar to otters in SB, the area before the releases.

Perhaps as a result of the arrival of new alleles with the released otters, the allelic diversity in SA2 had not been as depleted as in SA1 (Table 2). Probably random genetic drift induced by the small population size, had made this population very divergent from all the others (Hartl and Clark 1997). However, even though the genetic composition of SA2 had been affected by the releases it was still most similar to SB (Table 4), suggesting that the effect of the releases were limited.

Table 2. Sample size (*N*), Nei's (1978) unbiased estimation of the expected heterozygosity ( $H_E$ ), observed heterozygosity ( $H_O$ ), average number of alleles per locus (standard deviations in parentheses) and inbreeding coefficient  $F_{IS}$ .

| Population                                                                          | N  | $H_{\rm E}({ m SD})$ | $H_0$ (SD)    | N. alleles (SD) | $F_{\rm IS}$ |  |
|-------------------------------------------------------------------------------------|----|----------------------|---------------|-----------------|--------------|--|
| Ν                                                                                   | 20 | 0.683 (0.051)        | 0.715 (0.041) | 5.33 (0.82)     | -0.048 (ns)  |  |
| NS                                                                                  | 28 | 0.745 (0.028)        | 0.656 (0.037) | 6.17 (1.72)     | 0.122 **     |  |
| CS                                                                                  | 23 | 0.695 (0.028)        | 0.696 (0.039) | 5.67 (0.52)     | -0.001 (ns)  |  |
| SB                                                                                  | 15 | 0.694 (0.030)        | 0.656 (0.050) | 4.83 (0.75)     | 0.057 (ns)   |  |
| SA2                                                                                 | 20 | 0.613 (0.048)        | 0.642 (0.044) | 3.67 (1.51)     | -0.048 (ns)  |  |
| SA1                                                                                 | 8  | 0.451 (0.091)        | 0.500 (0.072) | 2.50 (0.55)     | -0.116 (ns)  |  |
| ** Significantly different from 0 at D=0.01: no. not significantly different from 0 |    |                      |               |                 |              |  |

\*\*Significantly different from 0 at P<0.01; ns, not significantly different from 0.

Since we were unable to obtain samples from the area where the majority of the releases were done, we do not imply that these released otters did not contribute to the gene pool of the resident otters. However, the absence of an apparent effect of the translocation to otters from an area not very distant (SA1) indicated that the effects may not have been far-reaching. Consequently, the results of this study implied that the growth of the otter population observed in southern Sweden during the 1990's (Hammar 1996; Sjöåsen 1996b; Bisther 2000), may not have been as closely related to the restocking program as initially suspected. Together, these results seem to indicate that the release of otters from northern Norway had a limited impact on the local population and only at a reduced spatial scale. Similar results have been experienced for translocations of white-tailed deer in southeastern United States (Leberg and Ellsworth 1999). It seems that long distance dispersal of otters had been quite low, perhaps due to the overall low population density in the south of Sweden and subsequent large availability of vacant home ranges.

## The evolution of a restocked otter population in Sweden (II)

This paper is a follow-up on the previous genetic study of the first 5-10 years after restocking (see paper I). The major objective of the present study was to provide a temporally and spatially extended evaluation of the genetic effects of the otter restocking. We reviewed an almost twenty year long period from the first release and covered a wider geographic area than in the first genetic study. We obtained more detailed information and managed to sample the released otters thoroughly and analyzed their relative contribution to subsequent generations.

We wanted to know to what extent the otters in the surroundings of the release area in Södermanland (Fig. 5), the area that could not be evaluated in the previous study, were affected by the restocking. Additionally, we wanted to ascertain if the initial contribution of the released animals remained stable over time. As most populations in Sweden have shown growth in distribution and numbers during recent years (Länsstyrelsen Gävleborg 1998; Bisther 2000; Bisther 2005; Hammar 2006; Bergström et al. 2006) it is likely that dispersal patterns change with increasing otter densities, with increased gene flow as a result.

#### Methods

Eight microsatellite markers and the variable fragment of the mtDNA control region I were used in the study. We also used noninvasive sampling (faecal samples, method see paper III and IV) together with recent tissue samples from otters found dead in Södermanland to evaluate the effect of the release of otters in this area. The gender of the faecal samples was obtained with a marker on the male-specific SRY gene.

A total of 188 samples (including 14 individuals identified from the noninvasive sampling) collected from between 1966 and 2006 were analyzed. Twenty-five of the 54 released animals were sampled in the study and additionally, we could infer and include the genotypes of 17 more captive-bred individuals, as their four parents (two females and two males) were geno-



Figure 5. Distribution of sampled populations in Sweden. Grey-shaded areas show otter distribution within surveyed areas during 1980-1989, where light-grey corresponds to surveyed areas with no otter signs, and dark-grey is surveyed areas with otter signs (distribution data provided by the Swedish Museum of Natural History). The two release areas are indicated by stars.

typed. Consequently, we used a total of 42 released otters. As in the previous study, southern Sweden before the releases was represented by pooled samples from the provinces of Småland, Östergötland, Södermanland and Uppland (N=22; Fig. 5). For the time period after the restocking Småland (N=8+22), Östergötland (N=12), Södermanland (N=31) and Uppland (N=24+27) were treated as separate populations when studying the effects of the released animals (Fig. 5). For two of the areas (Småland and Uppland) sample sizes were large enough for dividing them into two time periods, older post-release samples (1991-1999) and recent post-release samples (2000-2006), to evaluate the effect of the releases over time.

| Population                           | H <sub>NB</sub> | (SE)    | $H_0$ | A    | F <sub>IS</sub> |     |
|--------------------------------------|-----------------|---------|-------|------|-----------------|-----|
| Released (42)                        | 0.609           | (0.149) | 0.643 | 5.38 | -0.058          | ns  |
| Southern Sweden before releases (22) | 0.686           | (0.176) | 0.555 | 5.25 | 0.196           | *** |
| Småland, older post-release (8)      | 0.496           | (0.304) | 0.594 | 2.63 | -0.215          | *   |
| Småland, recent (22)                 | 0.525           | (0.221) | 0.551 | 3.38 | -0.051          | ns  |
| Östergötland (12)                    | 0.529           | (0.246) | 0.538 | 3.63 | -0.018          | ns  |
| Södermanland (31)                    | 0.661           | (0.114) | 0.640 | 4.75 | 0.033           | ns  |
| Uppland, older post-release (24)     | 0.610           | (0.106) | 0.630 | 3.75 | -0.033          | ns  |
| Uppland, recent (27)                 | 0.645           | (0.091) | 0.611 | 4.25 | 0.054           | ns  |
| ***, P < 0.001,                      |                 |         |       |      |                 |     |

Table 3. Expected ( $H_{\text{NB}}$ ) and observed ( $H_{\text{O}}$ ) heterozygosities, mean number of alleles per locus (A), and inbreeding coefficient  $F_{\text{IS}}$ . Sample sizes are shown within parentheses.

\*, P < 0.05,

ns, Not significant.

The geographical distribution of mtDNA haplotypes among the samples were analyzed using the Geographic Information System (GIS). The relative contribution of the released otters was assessed from the results from telemetric studies (Sjöåsen 1996a) and known dates of death (data from Swedish Museum of Natural History) and was compared to the distribution of the haplotypes among the populations. Genetic variation in, and differentiation between, the populations were analyzed and recent movements between the populations were estimated by an assignment test.

#### Results and discussion

The genetic variation in Småland, Östergötland and Uppland was low (Table 3), probably as a result of the severe population decrease. However, the negative trend seemed to have stopped and the genetic variation had started to increase again, as visible in the recent populations of Småland and Uppland (Table 3).

The same two mitochondrial haplotypes (A and B) as in the previous study (paper I) were found and haplotype B only existed in the group of released otters and in two of the populations after the releases: Uppland and Södermanland. The geographic distribution of haplotype B was concentrated around the release sites (Fig. 6).

It was clear that Södermanland was most affected by the releases, as inferred by distribution of the alleles from the released individuals, estimates of genetic differentiation and the assignment test (see factorial correspondence analysis, Fig. 7). However, probably also the few native otters that remained in the area contributed, as the current population was also similar to the population in southern Sweden before the releases (Fig. 7). The other area where otters were released, Uppland, seemed to be affected by the re-



Figure 6. Schematic spatial distribution of mitochondrial DNA haplotypes A and B among the samples in Södermanland and Uppland. The two release areas (in Södermanland and Uppland) are indicated by rectangles.

leases to a lesser extent, but additionally, the population showed clear evidence of isolation and genetic drift and had differentiated from all populations (Fig. 7).

The southern-most populations, Småland and Östergötland, were largely unaffected by the released otters. MtDNA haplotype B could not be found there and the microsatellite genotypes were similar to southern Sweden before the releases (Fig. 7). However, there was evidence of sporadic effects. Only the assignment test, which shows recent movements, gave support for more frequent movements between Småland/Östergötland and Södermanland. It is possible that growing populations induces dispersal as otter densities increases and that the populations will mix gradually with time. However, the distribution of mtDNA haplotype B clearly showed that the effects of the releases still congregate around the release areas (Fig. 6).



Figure 7. Factorial correspondence analysis (FCA) for the genotyped samples in the different populations.

Increased similarities to the released otters in estimates of genetic differentiation (as could be studied in Småland and Uppland), may imply that translocated otters might have contributed to a larger extent than the native otters to subsequent generations. Another indication of this might be that mtDNA haplotype B was spread among the samples in Uppland and Södermanland in the same proportions as they were represented among the released individuals, when relative contribution to subsequent generations of the released individuals was accounted for (Table 4). If the released individuals would have contributed equally or less than the native otters, haplotype B would have been found to a lesser extent in the areas, following dilution among native otters.

Success of the released otters could result from superiority of these individuals compared to the residents. The arrival of new otters may have acted, to some degree, as a genetic rescue, where the arrival of immigrants increased the fitness or growth rate of the resident population (Vilà et al. 2003). If the translocated otters were more successful than native otters, this could affect the genetic variation of the otters in Sweden in less favorable ways (e.g. local adaptations could be lost; Frankham et al. 2002; Allendorf and Luikart 2007), as the released otters originated from a smaller number of individuals with a limited amount of genetic variation. On the other hand, these otters have also contributed with new genetic material and the resulting effect of the restocking program on the Swedish otter population can be hard to predict. Therefore, continued evaluation in the future to reveal possible changes would be of great interest to the conservation of the species.

#### Reliability of noninvasive genetic census of otters compared to field censuses (III)

Accurate estimates of population sizes are hard to obtain, but fundamental in wildlife conservation and management. Tracking (snow or mud) is considered a cheap and potentially effective census method for otters (Ruiz-Olmo et al 2001). The preferred census method in areas that are covered with ice and snow in the winter is snow-tracking (e.g. Reid et al. 1987; Sidorovich and Lauzel 1992; Aronson 1995; Sulkava 1995; Sidorovich et al. 1996). The method is based on finding, separating and counting tracks from different otter individuals on snow, which can be difficult for semi-aquatic animals. The method is highly dependent on weather conditions.

During the last few years, noninvasive genetic approaches have become an alternative census method. However, estimates based on noninvasive genetic censuses can suffer from different biases such as the ability to sample all or most of the individuals, older samples may belong to dead individuals, or genotyping errors.

In this study, we compared the result of a noninvasive genetic census based on faeces, to population size estimates obtained from snow tracking. We also investigated the factors affecting the genetic census in order to design a sampling strategy that would facilitate gathering reliable results.

#### Methods

Snow tracking and collection of faeces for genetic analyses were conducted in an area of about  $2500 \text{ km}^2$  in central Sweden (in the province of Uppland) during winter conditions. The study population was not isolated or closed. We visited 110 places, where tracks and faeces from otters were likely to be found.

Snow tracking was conducted following Aronson (1995) and Sulkava (1995). Two different estimates of the number of individuals were produced: a minimum number ( $N_{min}$ ) of otters that without doubt could be separated, and a maximum number ( $N_{max}$ ) of otters that might be an overestimation of the actual number of individuals.

All sampling and laboratory work was performed so as to avoid crosscontamination of samples. Probability of identity for siblings was measured from tissue samples (N = 20) collected from dead otters in the study area and its surroundings. DNA extraction for the faecal samples was performed using QiaAmp DNA Stool Mini Kit (Qiagen). The samples were genotyped for eight microsatellite loci, plus one additional marker in case a higher resolution was needed. For sex determination we used a marker on the malespecific SRY gene. To avoid incorrect genotyping due to allelic dropout and misprinting (false alleles) heterozygote genotypes were replicated twice and homozygotes three times, following a variant of the multiple tubes approach (Taberlet et al. 1996). The genotyped samples were checked with Microsatellite Toolkit (Park 2001) for matching pairs. An estimation of population size was generated by a capture-mark-recapture program designed for noninvasive samples (CAPWIRE; Miller et al. 2005).

#### Results and discussion

A total of 150 otter faeces were collected from 42 locations. DNA could be obtained from 31-63% of the samples (complete genotypes and DNA amplified in a first sorting event, respectively), which fell within the ranges of other studies of carnivores, including otters (e.g. Taberlet et al. 1997; Kohn et al. 1999; Jansman et al. 2001; Dallas et al. 2003; Hedmark et al. 2004; Hung et al. 2004). To reduce genotyping costs, it is important to focus only on higher quality samples. In this study, fresh or old frozen faeces provided amplifiable DNA more often, which is in concordance with other studies (Lucchini et al. 2002; Hedmark et al. 2004; Hajkova et al. 2006). It has been suggested that the diet (especially fish based) of the species could affect the chances of isolating DNA due to the presence of PCR inhibitors. Our results did not support that, however 82% of the samples contained fish remains.

The markers used seemed to have enough power to differentiate between individuals in the area, as the probability of identity for siblings was  $5.47 \times 10^{-3}$ . Dropout rate tended to increase with marker length, which calls for avoidance of long loci, although there were notable differences between the markers.

Ideally, the two census methods that we compared in this study should give comparable results, but noninvasive genetic analysis of faeces do not always agree with estimates based on other techniques (Solberg et al. 2006, but see Eggert el al. 2003). In our case the two methods resulted in different estimates. Through noninvasive genetic analysis of faeces we found approximately twice as many otters in the study area as with snow tracking (23 and 10-15, respectively; Fig. 8). CAPWIRE generated an estimated population size of 31 otters (range: 23-40). However, it is possible that the software tends to overestimate the number of otters because faeces were not collected at random: samples were intended to represent the entire study area, avoiding oversampling in a limited area. The snow tracking method might tend to underestimate the number of individuals even when the weather conditions are reasonably good. It is difficult to track otters, especially when there are



Figure 8. Distribution of the otter individuals found: a) with noninvasive genotyping (each number corresponds to a different individual); b) with snow tracking (each letter corresponds to a different individual).

large areas of open water. However, even the maximum number of otters estimated from the snow tracking was well below the estimate obtained from the noninvasive genetic analysis. Ruiz-Olmo et al. (2001) concluded that for mud tracking it is possible to underestimate the true number of otters where otter densities are high (>0.6 individuals/km). Our study only gave rough measures of otter densities, but higher densities were found in the southern parts of the study area. Accordingly, the largest differences between the two methods were also found there (see Fig. 8) and we agree that tracking methods can be less suitable in areas with high otter densities.

If the costs of noninvasive genetic censuses do not allow their use over extensive areas, an alternative could be to combine the two methods. The two census methods could then be used to complement one another, with the field census as the cheaper background method for extensive censuses over large areas and noninvasive genetic census would provide more detailed information in smaller areas.

### Population dynamics parameters obtained by noninvasive genetic methods in a Eurasian otter population (IV)

Estimates of population dynamics parameters are important in order to understand and predict the future of a population. For threatened animal populations these measurements can be hard or impossible to obtain directly.

The Eurasian otter has exhibited unstable fluctuations in numbers and occurrence throughout its former distribution area. Parameters that underlie the population dynamics of Eurasian otters have been estimated through radiotracking and observations of live animals (e.g. Erlinge 1967; Kruuk et al. 1991; Kranz 1995; Sjöåsen 1997) and from examination of otter carcasses (Kruuk and Conroy 1991; Ruiz-Olmo et al. 1998; Hauer et al. 2002a; 2002b). However, estimates on live animals are often based on following only a few individuals for a limited time and estimates from otters found dead can be highly biased as only a very small proportion of the dead otters can be expected to be found (Kruuk and Conroy 1991; Ruiz-Olmo et al. 1998; Hauer et al. 2002a; 2002b).

In this study, I used seasonal noninvasive genetic sampling of faeces in three subsequent years to estimate population dynamics parameters in an otter population in the province of Uppland, central Sweden. By identifying individual genotypes I was able to estimate population size, sex ratio, population rate of change, apparent survival, rate of addition, dispersal and births.

#### Methods

The study area covered about  $3000 \text{ km}^2$  (in the first year a smaller area of  $2500 \text{ km}^2$ ) and most of the water systems had permanent otter occurrence, but some parts were unoccupied at the start of the study. The study population was not isolated or closed. Sampling of faeces was conducted under winter conditions. The search sites were spaced so that they would likely allow the location of all individuals existing in the area.

All sampling and laboratory work was performed so as to avoid crosscontamination of samples. Probability of identity for siblings was measured from tissue samples (N = 27) collected from dead otters in the study area and its surroundings. DNA extraction protocols have been described in paper III. The samples were genotyped for eight microsatellite loci and sex determined with the male-specific SRY gene. Genotyping was performed as in paper III.

An estimation of population size per year was generated by CAPWIRE (Miller et al. 2005). Apparent survival ( $\Phi$ ) was estimated directly from the data and using the software MARK (White and Burnham 1999). Rate of addition (*f*) was estimated as the number of new individuals in the area at time t + 1 per individual at time *t*. The population rate of change  $\lambda$  was calculated from apparent survival and rate of addition. Dispersal within the study area was estimated through individual large-scale movements between the years. The birth rate was based on the rate of addition.

#### Results and discussion

A total of 99 otters (58 males, 41 females) were genotyped in the study area over the three years (Figs. 9-11). Sex ratios varied between the years, with a male to female mean of 1.32 (range 0.857 – 1.875, ns different from one in all years). The number of otters found per year was 23 in 2002, 55 in 2003 and 52 in 2004. CAPWIRE resulted in population sizes estimates of 24 (range 23-25) in 2002, 57 (55-59) in 2003 and 58 (52-65) in 2004. In 2003 and 2004 a larger area was studied than in 2002 and these years gave similar population estimates (55 and 52, respectively), much larger than that obtained for 2002 (23). The population was still expanding and re-establishing into new areas. Therefore, it is likely that the population size was actually smaller in 2002. However, data on proportions of genotyped samples and rate of addition indicated that fewer faecal samples yielded genotypes that year.

Apparent survival was overall moderate to low for males (0.27), but higher for females (0.74) and averaged 0.45 per year (Table 4). The results from MARK were similar with an apparent survival for females of 0.79 (95% CI: 0.46-1, combined over years), and 0.51 for males (95% CI: 0.15-0.98). Many males seemed only to be able to hold their home-range for one or two years, but it is likely that many males moved out of the study area, as



Figure 9. Distribution of the individuals found with noninvasive sampling in 2002.



Figure 10. Distribution of the individuals found with noninvasive sampling in 2003. Red numbers correspond to individuals also found in 2002. Blue numbers correspond to individuals new in 2003.



Figure 11. Distribution of the individuals found with noninvasive sampling in 2004. Red numbers correspond to individuals that were found already in 2002. Blue numbers correspond to individuals that were found for the first time in 2003. Green numbers correspond to individuals new in 2004.

|                   |         | 2002-2003 | 2003-2004 | Mean |
|-------------------|---------|-----------|-----------|------|
| Rate of change    | total   | 2.13      | 0.95      | 1.54 |
| Apparent survival | total   | 0.57      | 0.33      | 0.45 |
|                   | females | 1.00      | 0.48      | 0.74 |
|                   | males   | 0.33      | 0.20      | 0.27 |
| Rate of addition  | total   | 1.57      | 0.62      | 1.09 |
|                   | females | 1.75      | 0.64      | 1.20 |
|                   | males   | 1.47      | 0.60      | 1.03 |

Table 4. Population rate of change, apparent survival and rate of addition over the years in the population. Estimates for the total population, as well as for females and males, respectively, and means are given. For 2002-2003 data from the smaller area was used and for 2003-2004 data from the larger area was used.

long-distance dispersal seemed to be larger for males than females, maybe due to male-male competition or possibly higher mortality rates in males (natural, competition induced, or from accidents). As I did not measure migration, it is unknown how large the surviving part of the population was – dispersal and long-distance movements within the study area implied that emigrants might make up a certain portion of the otters that disappeared from the study area and, thus, generate a lower apparent survival.

The rate of addition was quite high and averaged 1.09 (Table 4). However, if data from 2002 was excluded, then the estimate was lower (0.62; Table 4). Estimates of population rate of change indicated a population growth between the first two years, even though this growth might have been exaggerated due to a presumed underestimated population size in 2002. The estimates of population rate of change varied between the two periods and averaged 1.54 (Table 4). Between 2003 and 2004 the population did not seem to increase in size ( $\lambda = 0.95$ ).

Birth rate was estimated to 1.36 offspring per female per year, assuming no immigration. However, litter sizes observed in the field have been shown to be around two for most populations in Europe and females do not have cubs every year (Erlinge 1967; Jenkins 1980; Mason and Macdonald 1986; Kruuk et al. 1991). Additionally, dispersal within the study area indicated that large-scale movements occurred and, hence, that immigration might be quite large. Thus, this was an upper estimate.

Dispersal within the study area was found to be male-biased, given that a larger proportion of males than females made large-scale movements. Malebiased dispersal is common among carnivores, e.g. brown bears (*Ursus arctos*; Proctor et al. 2004) and North-American river otter (*Lontra canadensis*; Blundell et al. 2002). Males sometimes also changed the locality of their home-ranges between years. Several of the males in the study area were found in a smaller area the first year and a larger (or much larger) area the following year or years. It has been shown that male otters increase their home-ranges at sexual maturity (Sjöåsen 1997) and, therefore, the results might represent age-related changes in home-range. Females tended to be more sedentary, which might imply that young female otters settle as close to their natal birthplace as possible, provided there is vacant habitat to do so.

In this short-term study, the quality of the data was shown to vary. However, with precautions, all of these parameter estimates contribute useful information that will facilitate the development of optimal management of this population and species. It would be of high relevance to estimate the amount of migration between otter populations to be able to obtain accurate estimates on survival and mortality. Low migration rates might imply a slow population expansion.

## A demo-genetic analysis of a recovering population of otters in central Sweden (V)

Classic PVAs only concern the changes in numbers over time, while the negative effects of loss of genetic variability is not considered (Allendorf and Ryman 2002; Reed and Frankham 2003). One of the few attempts to incorporate both demographic and genetic factors of population long-term survival was given by Lehmann and Perrin (2006). They defined a global index of resistance to genetic drift and extinction,  $\lambda_T$ , which is a product of the classic intrinsic rate of growth measured as the leading eigenvalue of the demographic transition matrix,  $\lambda_P$ , and the leading eigenvalue for the corresponding genetic matrix,  $\lambda_G$ .

Even though we need detailed information to conduct a robust PVA, such information is very hard, or even impossible, to obtain for many species. Noninvasive methods, such as genetic analysis of faeces, can be used to get estimates concerning population size and apparent survival (see paper IV). Direct estimates of birth rates and age distribution cannot be obtained by these methods, but we have to rely on indirect derivations. We believe that the problems facing this study are shared by many other studies with conservation implications, especially those that focus on mammals. Yet, the need for an understanding of the future population size is nonetheless urgent.

In this study, we used the demographic and genetic data obtained in paper IV to conduct a demo-genetic analysis of the long-term survival of an otter population in the province of Uppland, central Sweden. We aim to identify the demographic parameters that are most important for the growth rate of the population, as well as the effect of demographic and environmental stochasticity.

#### Methods

Survival was estimated in MARK (White and Burnham 1999) using a markrecapture model. Based on the estimated adult survival rate we estimated life expectancy (*E*) after reaching maturity. Females in the population were found to become at least six years old (paper IV). Therefore, we used that as a maximum age. This lead to E = 2.59, and thus we assumed that females reproduce on average three times after reaching maturity. From paper IV we had two measures of annual birth rate (b = number of daughters born per female per year); if there is no net immigration then b = 0.68, assuming a net input of four females reduces this to 0.6. This leads to a generation time of 3.5 years.

From these data we constructed a Leslie matrix, which was analyzed using different parameter combinations of survival, birth rates and fecundity. From this matrix we calculated the intrinsic rate of growth measured as the largest positive eigenvalue ( $\lambda$ ). We calculated elasticities (Caswell 2001), which are the proportional change in  $\lambda$  of perturbations in the matrix. To assess the effect of demographic stochasticity, we calculated the risk for extinction. We also used the model by Lande (2002) and Saether and Engen (2002) where  $\lambda$  is allowed to vary due to stochastic reasons. In total we ran this 1000 times for each  $\lambda$ -value and recorded the proportion of runs in which the population went extinct for different values of environmental and demographic stochasticity. Different kinds of effective population size were estimated from genetic data from noninvasive sampling of faeces during the years 2002-2004 (paper IV).

#### Results and discussion

Using simple models and making simulations over a set of realistic parameter values, we were able to obtain qualitative information on the factors that are likely to affect population growth in this population. This can help to clarify the importance of the various factors that may underlie population viability, and thus increase the knowledge-base upon which conservationbased management decisions can be applied. Although we were forced to make many assumptions in this study, the results demonstrated that the PVAs were fairly robust to many of these assumptions. Furthermore, since some background data for this species was known, the assumptions were made within reasonable limits.

The population seemed to have a possibility of increase in the future ( $\lambda_P >$  1; Fig. 12), but it was clear that if survival dropped to under 0.7, then fecundity had to be considerably larger than estimated in this population in order to keep  $\lambda > 1$ . However, data was based on a number of assumptions, and the analysis of the robustness of the data shows several very important features of the population dynamics of this population. The most important factor



Figure 12. Values of  $\lambda$  in relation to birth rate and survival assuming all age classes have the same survival and birth rates. Isoclines refer to parameter combinations resulting in similar values of  $\lambda$  (values along the lines).

was survival to first reproduction (Fig. 13). If we compared changes in survival to first reproduction and number of daughters at first reproduction, it was clear that only very slight changes in survival corresponded to large changes in fecundity (Fig. 14). Viewed together with the genetic data, however, there were no indications of immediate problems to the population, through either extinction or the loss of genetic diversity.



Figure 13. Elasticity of  $\lambda$  for survival and fecundity for different age classes.



Figure 14. Values of  $\lambda$  for different combinations of survival to and birth rate at first reproduction. Lines indicate  $\lambda = 1$ . a) assuming adult birth rate = 0.68, b) assuming adult birth rate = 0.6.

Inbreeding and variance effective population sizes were lower than the actual number of individuals, consistent with most studies, which could indicate that genetic drift can operate rapidly and reduce genetic variability. This was contradicted by the eigenvalue effective population size, which is a measure of the rate of loss of alleles. Here, the undefined eigenvalue effective size was the most informative as this must mean that gene diversity is increasing.

a)

The effect of survival to first reproduction was surprisingly strong. Since the survival of this group of females is so essential for the long-term survival of the population, the potential risks to survival at this age stage need to be identified. A general recommendation for management is to focus on increasing the survival prospects of females reaching their first reproductive event, since this group of individuals makes a pivotal contribution to the long-term population dynamics.

Environmental stochasticity was very important, and even small increments in the variance led to a drastic increase in probability of extinction. Even though genetic diversity currently is increasing, it is essential that this population is not isolated but have connections with other populations. An isolated population will lose genetic variation due to inbreeding over time (Björklund 2003).

## Concluding remarks

In this thesis, genetic methods were utilized to generate data on the threatened Eurasian otter, that may be directly applied to the ongoing conservation and management of the species. In particular, I focused on obtaining estimates of population size and population dynamics parameters, evaluated the genetic effects of a restocking program on the Swedish otter population, and conducted a population viability analysis to assess which demographic parameters are important for the future viability of this population. Most of the studies were based on noninvasive genetic sampling of faeces and this sampling method facilitated in increasing the sample size in one of the studies.

In the genetic evaluation of the restocking program, we found that the released otters contributed to subsequent generations. They may also have been relatively more successful than the native otters. Otters in areas surrounding the release sites were genetically affected, however, the effects were not far-reaching. Otters in areas more distant from the release sites remained largely unaffected, although there were indications of sporadic gene flow. The long-term effects of the releases may be difficult to predict.

Using a comparison of two census methods – snow-tracking and noninvasive genetic census based on faeces – we found that approximately only half of the otters found with the genetic census were found with snow-tracking. Based on the results of this study, it is recommended to combine the two methods to obtain the most reliable estimates of population size.

A short-term study on the population dynamics in an otter population yielded data that varied in quality between years. It seemed clear that apparent survival was higher in females than in males and that the rate of addition was also high and likely influenced by migration. Both these parameters indicated a quite high turnover of individuals in otter populations in Sweden. If used cautiously, these parameter estimates will facilitate the development of optimal management strategies for this population and the species in general.

By utilizing a population viability analysis that incorporated both genetics and demographics, we found that survival to first reproduction was the most important demographic parameter influencing the viability of this population. This calls for conservation efforts that focus on increasing the survival prospects of females reaching their first reproductive event. It was also shown that environmental stochasticity can have large effects on the probability of extinction. The studies presented herein have demonstrated that genetic methods and noninvasive genetic sampling can provide reliable data that are of high relevance to future conservation efforts and management strategies regarding otters and other endangered populations and species. Continued monitoring of otter populations is important to ensure their future survival.

Genetic analyses and the use of noninvasive samples are being used in the management of several carnivores in Sweden, such as wolves, wolverines and bears. These methods are used to obtain long-term data on these threatened populations to improve the design of the action plans for their conservation. Likewise, studies that investigate the genetic and demographic parameters influencing otter population dynamics, provide data that will be essential for continued development of a robust and effective conservation management policy for the Swedish otter population. By monitoring genetic changes and population trends in various parts of its distribution, the negative factors that risk population viability can be revealed. The identification of individuals through noninvasive methods could also be useful both in the estimation of population size in areas of particular conservation interest, as well as in more applied situations, for instance in cases in which problematic individuals, which negatively affect human economic activities, would need to be removed.

## Sammanfattning på svenska

#### Genetiska studier för bevarandet av uttern i Sverige

Många arter på vår planet hotas av utrotning till följd av mänsklig aktivitet. Den euroasiatiska uttern (*Lutra lutra*) är en av dem. Uttern har minskat i världen sedan början av 1900-talet och i Sverige sedan 1950-talet. På 1980-talet var populationen som minst i vårt land och uppskattades till färre än 1000 individer. Orsakerna till minskningen är exempelvis förstörda livsmiljöer (habitat), störningar från mänsklig aktivitet, trafikdöd, död orsakad av fiskeredskap, samt jakt. Det största problemet för uttern har dock varit miljögifter (speciellt PCB).

Många insatser har gjorts för att bevara uttern och sedan 1990-talet ökar arten åter till antal och utbredning i vårt land. Bland annat har användandet av vissa miljögifter förbjudits, habitat har restaurerats och faunapassager för utter vid broar byggs för att minska risken för att uttern springer över vägbanan. Utterns förekomst inventeras för att följa populationens utveckling och för att upptäcka eventuella minskningar i tid. Ett åtgärdsprogram har skapats för att ge riktlinjer för utterns bevarande i vårt land.

Även mera direkta åtgärder har gjorts för att få uttern att öka i Sverige. När situationen för uttern såg som sämst ut på 1980-talet startades ett utplanteringsprojekt för att stödja de spillror av utterstammen som fanns kvar i södra Sverige. Mellan 1987 och 1992 sattes 54 uttrar med nordnorskt och finskt ursprung ut i Södermanland (47 st) och Uppland (7 st).

Trots att uttern som art är tämligen välbekant finns det kunskapsluckor. Om denna kunskap kunde inhämtas skulle bevarandearbetet kunna göras mer effektivt. Mitt syfte med denna avhandling har varit att ta fram kunskap inom några av dessa områden.

I och med att uttern är nattaktiv och rör sig över ganska stora områden är den svår att observera i fält och därmed svår att studera. Idag kan mycket information om en art inhämtas med hjälp av studier av dess gener. Material till denna forskning kan erhållas utan att man observerar eller fångar uttrar i naturen. Uttern är statens vilt och döda uttrar som lämnats till Naturhistoriska riksmuseet kan ge material till genetiska studier. För studier där man vill ha noggrann kunskap om individerna i en population, är antalet prover från döda uttrar otillräckligt. Genetiskt material (DNA) från en individ finns i allt som individen släpper ifrån sig, såsom spillning, urin och hårstrån. Genom att samla in exempelvis spillning från uttrar i ett område kan man få prover från alla, eller nästan alla, utterindivider som lever i området.

Grundläggande kunskap om den genetiska variationen hos uttern kan ge en inblick i vilken överlevnadspotential populationen har både i ett kortare och i ett längre tidsperspektiv. Stor genetisk variation ger en större möjlighet för populationen att kunna anpassa sig till framtida förändringar i miljön och på så sätt överleva. Liten genetisk variation innebär det motsatta – det finns mindre möjligheter för populationen att anpassa sig till framtida förändringar, men i ett kortare perspektiv kan även överlevnad och reproduktion påverkas negativt. I minskande populationer minskar den genetiska variationen till följd av bortfallet av individer. När populationen väl är liten kan variationen fortsätta att minska till följd av slumpfaktorer – vissa individer bidrar mer än andra till kommande generationer och det blir mer påtagligt när populationen är liten.

Studier i Europa har visat att uttern generellt har liten genetisk variation. Detta är till följd av att små grupper av uttrar vandrade in och etablerade sig i Europa efter istiden, men även det jakttryck som uttern har utsatts för under de senaste 2000 åren och det senaste århundradets hotfaktorer har påverkat. Mina studier visade att mängden genetisk variation varierade mellan olika områden i Sverige. Den var, jämfört med uttrar i andra länder, relativt hög i norra och södra Norrland, men betydligt lägre i södra Sverige. Detta var förväntat i och med att populationsminskningen var som svårast i södra Sverige. Det fanns även signifikanta skillnader mellan uttrarna i de olika delarna av landet, vilket visar att delarna har blivit mer eller mindre isolerade från varandra, även detta till följd av minskningen av antalet uttrar.

Det är av stor vikt att bevarandeåtgärder följs upp för att utvärdera om resultaten är de förväntade. Vad gäller de utplanteringar av utter som har gjorts i Sverige är det av stort intresse att få reda på hur mycket de utplanterade djuren har bidragit till kommande generationer, dvs hur väl utplanteringsprojektet föll ut och till vilken grad det har bidragit till populationsökningen. Detta kan göras genom att studera den genetiska sammansättningen på populationen före och efter utplanteringarna och jämföra dem med den genetiska sammansättningen hos de djur som sattes ut. Vi kunde se att de utplanterade individerna hade bidragit till kommande generationer, men att effekten var begränsad till den lokala utterpopulationen runt utsättningsområdena i Uppland och Södermanland. Till Småland och Östergötland hade den genetiska effekten av utplanteringarna i stort sett inte nått, även om det fanns sporadiska tecken. Det är möjligt att den pågående populationstillväxten gör att effekten sprids mera framöver.

Det är svårt att förutspå resultatet av utplanteringarna i ett längre tidsperspektiv. Det fanns tecken på att de utplanterade individerna var relativt sett mer framgångsrika än de som redan fanns i området. De utplanterade uttrarna har bidragit med nytt genetiskt material, vilket kan vara viktigt för att öka populationens tillväxttakt. De nya uttrarnas gener kan däremot riskera att förstöra genetiska anpassningar till den lokala miljön, vilka har utvecklats hos den lokala populationen under århundraden och som kan vara viktiga för populationens överlevnad.

Utterns utbredning är relativt lätt att konstatera med inventeringar av spillningsförekomst under barmarksförhållanden. Antalet uttrar har uppskattats med hjälp av snöspårning av de olika individerna i en population. Denna metod är inte tillämpbar när snö- och isförhållanden är dåliga och därför behövs andra sätt att mäta populationsstorleken. I och med att alla individer är genetiskt olika (förutom enäggstvillingar) kan man med hjälp av så kallade genetiska markörer särskilja individer från varandra med hjälp av DNA. DNA-analys av utterspillning har provats med olika framgång. Om metoden visar sig fungera väl, skulle man på detta sätt kunna uppskatta antalet uttrar i ett område oberoende av snötillgång.

Genom att utveckla metodiken gjorde vi en uppskattning av antalet uttrar i ett område i Uppland och jämförde den med snöspårningsmetoden för att få en uppfattning om vilken metod som gav den mest korrekta antalsuppskattningen. Med snöspårningsmetoden hittades bara hälften av de uttrar som kunde hittas med hjälp av DNA-analysmetoden. I och med att DNA-analys av spillning är en relativt dyr inventeringsmetod bör de båda metoderna kombineras för att på så vis ge en tillförlitlig uppskattning av antalet uttrar i ett område.

Överlevnad, rekrytering och spridning är parametrar som inverkar på utvecklingen i numerär hos en population. För många arter, såsom uttern, kan dessa parametrar vara svåra att mäta. De är däremot angelägna att mäta i och med att de har betydelse för populationens framtida utveckling. Även här kan man använda DNA-analys av spillning och därigenom följa de olika individerna genom åren. Då kan man mäta antalet individer som finns kvar i området år efter år (överlevnaden), antalet individer som kommer in som nya till populationen (föds eller immigrerar), antalet som försvinner (dör eller emigrerar) och hur uttrarna sprider sig inom området.

Genom att studera ett ca 30 kvadratmil stort område med uttrar i Uppland vintertid under tre års tid kunde jag uppskatta populationsstorleken och dess förändringar mellan åren. Även om studien gjordes över ett fåtal år, genererade den intressanta resultat. Överlevnaden var låg till medel hos hanar, men högre hos honor. Antalet nya uttrar per år var relativt stort och kompenserade för bortfallet av individer. Däremot kunde inte mängden migration mätas, även om spridningen av individer (speciellt hos hanar) inom området talade för att migrationen stod för en viss del av de försvunna och nya individerna. Om migrationen skulle visa sig vara låg, skulle det kunna innebära en långsammare expansion av populationen. Långsam populationstillväxt har observerats i olika delar av utterns utbredningsområde, bland annat i Sverige.

En sårbarhetsanalys är en bedömning av hur stor risken är att en population ska dö ut inom en tidsangiven framtid. En sårbarhetsanalys kan också analysera vilka parametrar som är allra viktigast för populationens överlevnad. Demografiska parametrar (exempelvis antalet individer som överlever, föds, dör och migrerar per år) har alltid utgjort grunden i sårbarhetsanalyser, men eftersom även de genetiska förutsättningarna kan vara begränsande i en population är det viktigt att inkorporera även mått på genetisk variation. På detta vis kan vägledning ges för att ta fram lämpliga och riktade bevarandeåtgärder.

I avhandlingen genomfördes en sårbarhetsanalys med demografiska data från en utterpopulation i Uppland. Analysen innefattade även genetiska data. Målet var att ta reda på vilken demografisk parameter som mest påverkade populationens tillväxt och överlevnad. Det visade sig att överlevnaden för unga honor till deras första reproduktion var av mycket stor betydelse för populationens tillväxt i det långa perspektivet. Därför är det viktigt att i bevarandearbetet satsa på åtgärder som syftar till att öka möjligheterna för denna grupp av uttrar att överleva. Slumpmässiga förändringar i miljön hade också stor påverkan på risken för populationens utdöende. Även om den genetiska variationen i dagsläget ökar i den här populationen är det viktigt att populationen inte isoleras, utan får vara sammanknuten med andra delar av den svenska utterpopulationen, för att inte mer genetisk variation ska gå förlorad.

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