FRAGMENTATION OF NATURAL POPULATIONS, GENETICS AND CONSERVATION BIOLOGY.

Robert D. Martin & Franziska von Segesser / Anthropologisches. Institut und Museum Universität Zürich-Irchel.

Abstract

Extensive habitat loss has not only drastically reduced natural populations of many species but has also fragmented them, thus inhibiting gene flow between subpopulations. This is a central problem for conservation of biodiversity, notably with respect to the relationship between genetic variability and minimal viable population size.

General considerations indicate that habitat fragmentation can influence natural populations in various ways, but that genetic effects are of particular significance in the long term. Genetic monitoring offragmented populations under natural conditions and in captive colonies is hence of major importance for conservation biology. Indirect estimation based on species/area relationships indicates that, for primates at least, approximately 20,000 individuals may be needed for long-term survival. Currently, the "50/500" rule for maintenance of genetic diversity and matching new mutation to loss of variation, respectively, is widely applied. But new theoretical considerations indicate that thousands, rather than hundreds, of individuals may be needed for long-term survival. The world population of the Barbary macaque is already less than 20,000 and fragmentation into completely isolated subpopulations makes this a prime test case for examination of genetic diversity (e.g. genetic divergence between the Algerian and Moroccan populations and between the various subpopulations). Preliminary comparison of 5 macaque species with band-sharing values from multilocus DNA-fingerprints indicates lower genetic variability in Barbary macaques than in other macaques, suggesting a past bottleneck. Marked genetic divergence between the Algerian and Moroccan populations is also insdicated.

Ponencia de Clausura

For large-bodied, long-lived species, empirical data combined with theoretical approaches can yield information for management plans for both natural and captive populations. The Barbary macaque -reduced to a wild population of about 15'000 animals in forests of Algeria and Morocco but also present in artificial colonies with differing histories- is an excellent model because: (l) It lives in complex social groups in which group size, male migration, differential male mating success and matrilineal dominance influence genetic structure; (2) The entire world population can be sampled; (3) Samples already exist for most subpopulations (notably the wild Algerian population and the inbred, artificially provisioned colony on Gibraltar); (4) Genetic data now exist for blood proteins, DNA-fingerprinting and microsatellites; (S) Reintroductions from captive colonies can be planned using genetic guidelines from this research; (6) Comparative genetic data are available for other macaque species. Because of its particular history, the isolated colony of Barbary macaques in Gibraltar is of special interest, notably with respect to the potential effects of inbreeding. Preliminary results from a genetic investigation of the Gibraltar macaques are reported.

Conservation Biology and Genetic Monitoring.

In recent years, and not before time, the subject of conservation biology has been increasingly recognised as a distinct discipline in higher education. It is now represented in certain universities in both teaching and research programmes and standard texts are progressively becoming available (e.g. FRANKEL & SOULÉ, 1981; SOULÉ, 1986, 1987; FIEDLER & JAIN, 1992; PRIMACK, 1993, 1995; LOESCHCKE *et al.*, 1994; MEFFE & CARROLL, 1994). Faced with the widespread problems of habitat destruction and consequent threats to the survival of vast numbers of species, biologists have devoted increasing attention to the development of a core of basic principles that can serve as a foundation for effective conservation measures. In the past, responses to conservation problems have often been made on an *ad hoc* basis, with an emphasis on specific and often urgent measures to enhance the survival of individual species. Although such responses have by now yielded an impressive set of highly informative case studies, the main basis for conservation efforts has generally been essentially empirical rather than theoretical. The development of a set of theoretical guidelines for conservation biology is, however, essential if the subject is to be taught at an advanced level to train the experts that will be so sorely needed in the future. Yet the development of such guidelines is still in its infancy and much research is still needed to provide the secure foundation that is required.

The developing subject of conservation biology is necessarily interdisciplinary, incorporating a wide range of topics that may otherwise be treated as relatively independent. In addition to the basic biological disciplines of ecology, ethology and evolution (including genetics), conservation biology must incorporate relevant aspects of disciplines ranging from reproductive biology through natural diseases of plants and animals to education, psychology and law. One key aspect that must also be included is the undertaking of captive breeding of endangered species, which is in turn connected with the practical concerns of reintroduction and translocation. Although captive breeding has often been regarded as a somewhat peripheral concern in conservation biology, it has an essential contribution to make not only to long-term protection of individual species but also to the formulation of guidelines for the discipline of conservation biology. For one thing, captive breeding programmes provide special opportunities for research into many of the topics that concern conservation biologists (e.g. behaviour, reproduction, genetics and evolution, disease, education). More importantly, for many species the remaining wild populations have already been reduced to such a low level that we will be obliged to conduct the combined management of wild and captive subpopulations as an integrated exercise if we are to have any hope of conserving them in the long term. For primates, a classic example of an integrated approach to conservation involving captive and wild populations is provided by the now well-established international programme for the golden lion tamarin, *Leontopithecus* (KLEIMAN *et al.* 1986, 1990; DIETZ *et al.* 1994).

One core field of conservation biology that has a particularly important contribution to make, in both theoretical and practical terms, is that of genetics. Genetic issues are of major significance for species conservation at various levels. At the highest level, genetic studies can yield invaluable supplementary information on the differentiation of species, permitting more precise definition of individual taxa for conservation efforts. Genetic variation between subpopulations within species is also of major importance, as differentiation within species may be extensive, raising the possibility of outbreeding depression if individuals from widely separated subpopulations are brought together for reproduction (in captive breeding programmes, through reintroductions or as a result of translocations). Last but not least, it is essential to study the genetic structure of small, subpopulations, both in the wild and in captivity, in order to enhance our understanding of genetic processes at the finest level. The problem of inbreeding depression resulting from loss of genetic variability in small, isolated subpopulations, and the identification of natural mechanisms that serve to reduce it, are basic issues of major importance at this level. Reduction of genetic variability is also important with respect to the occurrence of persistent bottlenecks in the history of individual subpopulations or entire species (NEI *et al.*, 1975). There is now convincing evidence for the existence of a past genetic bottleneck in the population history of the African cheetah (MENOTTI-RAYMOND & O'BRIEN, 1993) and an informative case study of a bottleneck affecting an isolated subpopulation of a species has been provided by a study of the lions of the Ngorongoro Crater (PACKER *et al.*, 1991).

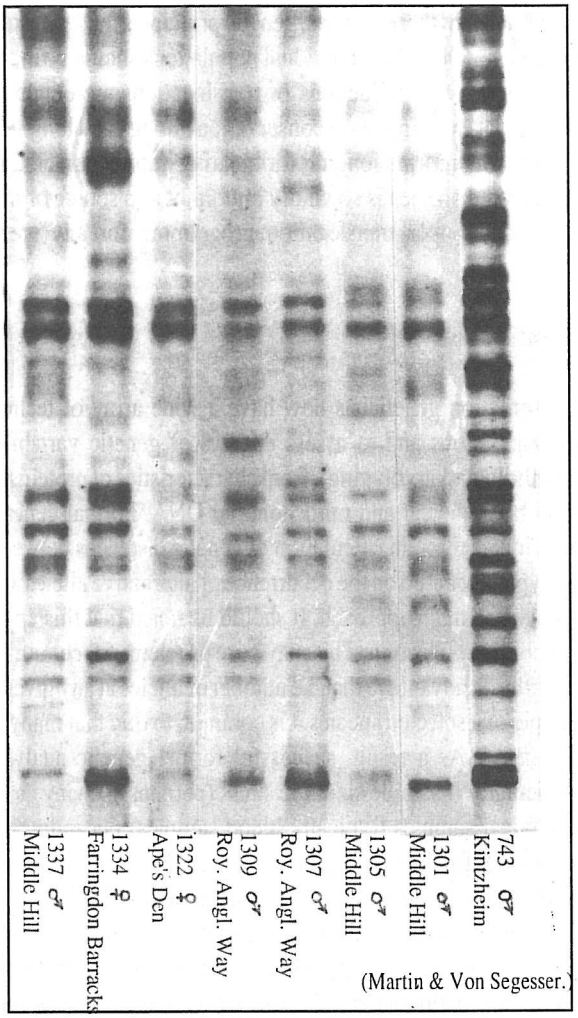

Fig 1. Multilocus DNA fingerprints of 7 Barbary macaques on Gibraltar, compared with an individual from the captive colony in Kintzheim (France). The fingerprints were prepared using the oligonucleotide probe (GTG)5, following digestion with the restriction enzyme Alu 1, and labelled using a chemiluminescent technique. Note that each individual has a unique pattern of bands, despited the expected high level of inbreeding within the Gibraltar colony.

Overall, it is also essential to bear in mind the concept of the metapopulation, according to which any species typically exists as a network of subpopulations, separated to varying degrees and subject to the ever-present risk of local extinction. The development of sound conservation principles requires consideration of demographic processes including the recognition of natural metapopulation structure and the need to preserve (or replicate to an adequate extent) the essential features of that structure in any practical measures that are taken (e.g. see LANDE, 1988). In this context, it is crucially important to note that habitat destruction has not only reduced the total wild populations of many individual species but has also fragmented them, such that the remaining subpopulations have become isolated to an artificially high degree. The topic of habitat fragmentation is hence of central importance in genetic approaches to conservation problems.

For all of these reasons, genetic principles must be a key part of the theoretical core of conservation biology and reliable genetic monitoring of individual populations should be included in practical conservation efforts. This is particularly true in cases where reintroductions or translocations are conducted as conservation measures. Indeed, such actions constitute significant experiments in conservation biology and long-term monitoring of the results can provide one of the most valuable sources of information for formulating future guidelines. Genetic monitoring of artificially modified populations of endangered species is so vitally important as a source of information that it is now little short of irresponsible to conduct a reintroduction or translocation programme for any species without including effective arrangements for long-term monitoring.

Genetic Tools

Conservation geneticists now have a wide array of techniques at their disposal in order to characterize populations or subpopulations and to assess degrees of genetic variability. Valuable information can be obtained from chromosome morphology (karyotyping), from characteristics of proteins (ranging from classical electrophoresis to actual sequencing) and from both nuclear and mitochondrial DNA. Formal genetic analyses (ranging from pedigree analyses for closely related individuals through calculation of genetic distances between subpopulations to estimation of genetic variability of species) are typically based on the occurrence of alternative alleles at individual loci and traditionally protein electrophoresis has been used as the main approach. It should be noted that this approach indirectly reflects coding sequences of DNA, which may be subject to selection, This may pose problems for certain kinds of genetic analyses. Further, protein electrophoresis suffers from the disadvantage that the number of alleles at any given polymorphic locus is typically small (2-3). Indeed, for any given sample of a selected species it is common to find that many loci are monomorphic and hence uninformative for the purposes envisaged. As a result, successful formal genetic analyses commonly require investigation of large numbers of loci, particularly if sample sizes are small (perhaps as many as 50 loci; NEI, 1978) and hence the availability of relatively large biological samples (typically blood samples) per individual. Over the past decade, the progressive introduction of new techniques for direct comparisons at the DNA level, associated with the concepts of DNA typing and of «DNA fingerprints», has revolutionized such genetic comparisons (BRUFORD *et al.*,1992; MARTIN *et al.*,1992). These techniques (which are all based on non-coding DNA sequences) provide a comprehensive basis for reliable identification of individuals, for inference of relationships between individuals (e.g. paternity) and for general studies of genetic variability (e.g. distances between subpopulations; degrees inbreeding within individual subpopulations). Their widespread application has led to emergence of the field of «molecular ecology» (BURKE, 1994).

Many initial studies using DNA typing employed multilocus DNA fingerprinting based on minisatellite sequences distributed throughout the nuclear genome. For this purpose, some investigators have used the natural minisatellite probes identifed by Jeffreys (JEFFREYS 1985a, 1985b), while others have employed artificial oligonucleotide probes that were found to yield similar results (EPPLEN *et al.*,1991). In both cases, application of the probes often yielded pattems with numerous bands, and it was commonly found that each individual exhibited a unique pattern (hence the name «DNA fingerprint»). Such many-banded patterns have the advantage that a great deal of information can be obtained from a single gel. However, for the same reason they have two associated major disadvantages. Firstly, the patterns are so complex that it is impossible to identify individual alleles, such that fommal genetic analysis is ruled out. Secondly, because individual bands cannot be reliably recognised (and because the pattem for a single individual may not be consistently replicated in different gels), comparisons can only be conducted between individuals represented on a single gel. However, it is possible to calculate values of a band-sharing index (LYNCH, 1990) for comparisons of the overall degree of genetic similarity

between individuals on a single gel. The general reliability of this approach has been confirmed by the finding for 3 macaque species that there is the expected inverse relationship between average band-sharing values determined from mutilocus DNA fingerprints and average heterozygosity determined from protein electrophoresis (PASTORINI *et al.*,submitted). It has also been shown that band-sharing values may be used as an accessory criterion for patemity exclusion in Barbary macaques, and a progressive reduction of values over generations has been interpreted as an indication of loss of genetic variability in a captive colony (VON SEGESSER *et al.*,1995). However, band-sharing index values provide no more than a diffuse indication of genetic relatedness.

Ideally, practical applications in conservation biology require a technique that combines the abundance of genetic information contained in a multilocus DNA fingerprint with the reliable recognition of individual alleles that is permitted by protein electrophoresis. Such a technique is now available in the form of analysis of single-locus microsatellites amplified using the polymerase chain reaction (PCR). Anaysis of microsatellites is now being used extensively in the field of «molecular ecology» (BRUFORD & WAYNE 1993; BURKE, 1994). Microsatellite regions, also known as simple-sequence repeats (SSRs), are typically short series of repeated dior trinucleotide combinations, within which molecular «slippage» leads to high rates of gain and loss of repeat units (LEVINSON & GUTMAN, 1987). PCR primer pairs can be specially designed to bind to unique DNA sequences on either side of a microsatellite region, such that the length of the amplified product reflects the number of repeat units in the array. Microsatellites occur in all higher organisms tested so far and are both abundant and widely distributed throughout the genome. Initial work on the PCR-amplification of microsatellites just 6 years ago (LITT & LUTY, 1989; TAUTZ, 1989; WEBER & MAY, 1989) unleashed a flood of practical applications that have amply demonstrated the enormous potential of this technique for genetic comparisons at all of the levels identified above. Various studies have used analyses of microsatellites for individual identification (e.g. INOUE & TAKENAKA, 1993; MORIN & WOODRUFF, 1992) and for inter-population studies in mammals (e.g. GOTTELLI *et al.*, 1994; TAYLOR *et al.*, 1994). Microsatellite data have also permitted construction of relationship trees for human subpopulations that reflect their geographic origin with remarkable accuracy (BOWCOCK *et al.*,1994), indicating great scope for applications in conservation biology.

The basic principle of the method is that primer sequences corresponding to the flanking regions of a given microsatellite locus prescribe PCR-amplification of the DNA sequence at that locus, generating vast numbers of copies. The amplification product can then be examined using the standard approch of gel electrophoresis, and allele patterns comparable in replicability to those seen with classical protein electrophoresis are obtained. In many cases, however, the number of alleles found is considerable (often 10 or more), such that far more information is obtained from a single microsatellite locus than from a single coding locus examined by means of protein electrophoresis. On the other hand, the information content of the allele patterns determined from a single microsatellite locus for a number of individuals is commonly markedly less than that determined using a multilocus DNA fingerprint for the same set of individuals. Hence, in order to obtain a comparable density of genetic information, such that individual-specific «fingerprints» are generated, it is necessary to combine the results from a number of different microsatellite loci (e.g. 4-6, depending on the number of alleles found with each microsatellite). This practical disadvantage is compounded by the fact that microsatellites and their flanking sequences can differ markedly between species. Microsatellite primers developed for one species can often be applied to closely related species (e.g. see MOORE *et al.*,1991 for mammals), but application to distantly related species is rarely successful (e.g. in one survey no human primer was found to work with prosimian primates or even with New World monkeys; COOTE & BRUFORD, submitted). The identification of microsatellites and the development of primers for their flanking sequences is a time-

consuming process requiring a considerable grasp of molecular biological techniques, so it is a major undertaking to embark on comparisons using microsatellites for an entirely new group of species.

On the other hand, comparisons using microsatellites have the major advantage that PCR-amplification can yield results from very small biological samples, in the extreme case from a single cell nucleus. Although multilocus DNA fingerprinting requires considerably smaller samples (e.g. of blood) than classical protein electrophoresis, only PCR-amplification of microsatellites can be applied to samples as small as a few cells. It is possible, for example, to conduct analyses using residual traces of blood samples that are too small to permit multilocus DNA fingerprinting and there is great potential for application to trace material that may possibly be collected non-invasively and possibly without the need to trap individuals (a major practical limitation in many field studies). For example, it was shown some time ago that PCR-amplification of microsatellites can be used with single human hairs (HIGUCJI *et al.*,1988) or sperm (LI *et al.*, 1988), and this technique has now been used for hair samples from various other mammals such as chimpanzees (MORIN *et al.*,1992; MORIN & WOODRUFF, 1992; MORIN *et al.*,1993; MORIN *et al.*,1994; TAKASAKI & TAKENAKA, 1991), Barbary macaques (our study) and bears (TABERLET *et al.*, 1993). More recently, PCR-amplification of microsatellites has been successfully applied to faecal samples from bears (HOSS *et al.*,1992), baboons (CONSTABLE, 1995) and bonobos (GERLOFF, 1995).

In the light of these introductory comments on conservation biology and the genetic tools that are now available, the rest of this paper will deal first with the general implications of habitat fragmentation and then with certain aspects of conservation genetics. The specific case of the Barbary macaque (*Macaca sylvanus*) will be taken as an exemplary model, illustrated with some of the results of our own research. The Barbary macaque is a particularly informative test case in that it is possible to sample the entire remaining world population of this species, which is divided into subpopulations of various sizes and histories both in the wild and in captivity.

Habitat Fragmentation and Conservation

The Problem of Population Fragmentation

Natural populations of many animal species have suffered drastic reduction in overall size because of extensive habitat loss. Reduction in total population sizes is recognized as a primary problem in conservation biology. At the same time, however, such residual populations have typically become fragmented, with resulting disruption or complete suppression of gene flow between subpopulations. An early discussion of the botanical effects of fragmentation was provided by CURTIS (1956) and this issue has since received increasing attention as the major implications for conservation biology have been appreciated. Natural, undisturbed populations are commonly subdivided into subunits with varying patterns and levels of gene flow between them (hence the concept of the metapopulation), but widespread habitat destruction leads to a breakdown of the natural genetic structure. As a result of such abnormal fragmentation, small isolated populations are subjected to increased levels of inbreeding. Long-term separation of subpopulations followed by natural or artificial transfer between them may also lead to outbreeding depression. Study of the genetic effects of population fragmentation is therefore of central importance for conservation biology and increasing research interest is now being devoted to this topic (HARRIS, 1984; HARRIS & SILVA-LÓPEZ, 1992; LOESCHCKE *et al.*, 1994; LOVEJOY *et al.*, 1983; PRIMACK, 1993; ROLSTAD, 1991; WILCOX & MURPHY, 1985).

Habitat fragmentation can affect natural populations in many different ways. At a minimum, the following effects can be recognised (list expanded from PRIMACK, 1993): (1) decreased total area of available habitat; (2) increased exposure to environmental fluctuation; (3) increased vulnerability to risks such as fire, exotics, pest species and pollution; (4) increased proportional effect of edge effects; (S) induced changes in microenvironment at habitat edges; (6) reduced potential for dispersal/colonization; (7) increased exposure to demographic fluctuation; (8) increased genetic effects of small population size (e.g. loss of heterozygosity; genetic drift). Genetic effects are only part of the picture and they interact with other effects of fragmentation in complex ways. For example, reduced dispersal/ colonization is of particular importance with respect to gene flow and the associated concept of metapopulation. It has been shown for Amazonia that many forest-living species (insects, birds and mammals) fail to migrate across even very short stretches of open country (see BIERREGAARD *et al.*, 1992). In the long term, the cumulative genetic effects of habitat fragmentation are undoubtedly crucial to the survival of a species.

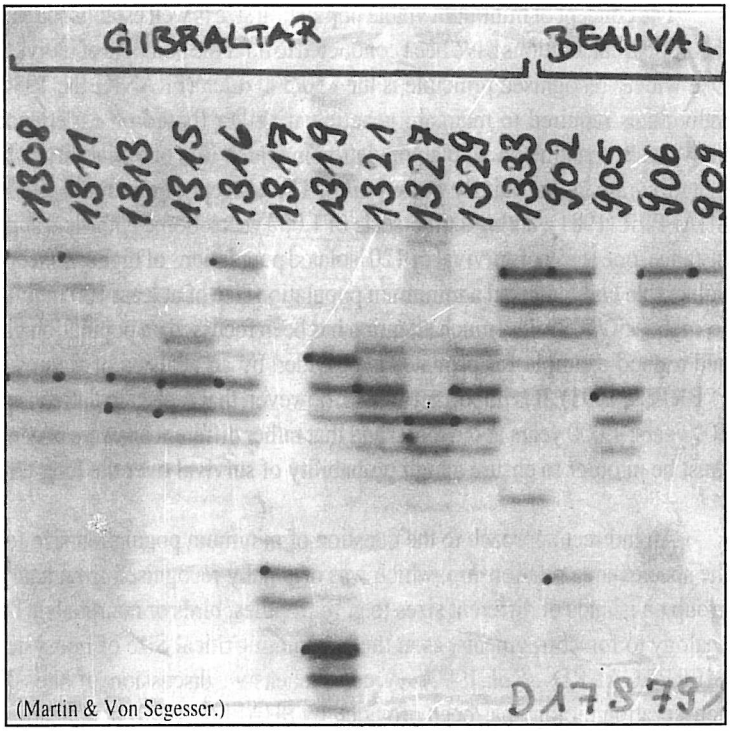

Fig 2. Microsatellite patterns for 11 Barbary macaques on Gibraltar and for 4 in the colony at ZooParc Beauval (France), obtained using the human primer D17S791. Main bands for alleles are indicated with an ink-dot; other (lighter) bands are artifacts generated by «slippage» during the replication process. Heterozygous individuals have 2 main bands (e.g. 902, 1319); homozygous individuals have only one (e.g. 905, 1317). In all cases, DNA extracted from a small number of hairs was used. Labelling was conducted using a chemiluminescent technique.

Minimum Viable Population Size

Understanding of the genetic effects of isolation and fragmentation of populations can be furthered through theoretical analyses, through experimental studies and through empirical investigation of particularly revealing case studies. For large-bodied, long-lived animals -which are often the first to be threatened by habitat destruction- empirical case studies offer the best opportunities for obtaining short-term answers to pressing conservation questions. For certain aspects, for example the influence exerted by complex social structure on metapopulation dynamics, such studies are essential. Good empirical data for such species combined with appropriate theoretical approaches can yield information of immediate value for conservation measures, particularly with respect to the central notion of minimum viable population size, which can be defined as «the smallest number of individuals necessary to give a population a high probability of surviving over a specified period of time» (PRIMACK, 1993). This applies not only to management plans for natural populations but also to captive breeding programmes.

Ponencia de Clausura

The concept of minimum viable population size is well established in the literature on conservation biology and various theoretical calculations have been conducted to infer the numbers of individuals required for effective conservation measures. One widely recognised principle is the «50/500 rule» (FRANKLIN, 1980). This states that 50 is the minimum number of individuals required to maintain genetic variability (based on experience from domestic animal breeding) and that 500 individuals constitute a viable population in which loss of variability is balanced by new mutation (based on data for the mutation rate in the fruit fly *Drosophila*). The concept of minimum viable population size has been discussed in detail by SHAFFER (1981), taking a time-scale of 1,000 years. Some empirical support has been provided for the «50/500 rule». For instance, one study of survival of 120 isolated populations of bighorn sheep confirmed that small populations are particularly vulnerable and indicated a minimum population size of at least 100 individuals (BERGER, 1990). As a practical extension from the «50/500 rule», much attention has been focussed on population viability analyses (see BOYCE, 1992, for a review) and a good example for primates is provided by an analysis of figures for the Tana River mangabey (KINNAIRD and O'BRIEN, 1991). It is important to note, however, that such calculations are often made from a short-term perspective (often 100 years; 1,000 years at the most) and that rather different answers may emerge if we wish to know how large a population must be in order to ensure a high probability of survival over the long term (many thousands of years).

An indirect approach to the question of minimum population size for long-term survival can be made on the basis of the species-area relationship, which was originally recognised from analyses of numbers of species of a given taxonomic group on islands of different sizes (e.g. for reptiles, birds or mammals). The model developed for islands can be applied by analogy to forest fragments, as in the Minimum Critical Size of Ecosystems Project in Amazonia (LOVEJOY *et al.*1983; BIERREGAARD *et al.*,1992). A comprehensive discussion of the significance of the species-area relationship for conservation biology has been provided by SHAFER (1990). Application of the species-area relationship to conservation planning has attracted considerable controversy in one particular direction because of the «SLOSS debate» (discussion of whether it is best to aim for a single large reserve or several small ones), but it has undoubted potential in other directions. For example, using an empirical relationship (species:area curve) derived for numbers of primate species on islands of different sizes in South-East Asia, it was inferred that an island area of at least 300 km^2 is required for long-term survival of a single species (VASARHELYI & MARTIN, 1994). Taking the long-tailed macaque (*Macacafascicularis*) as a widely distributed representative primate species of middling body size, it was then calculated that such an island area would correspond to a total population in the region of 20,000 individuals. In this case, it is possible to work out a likely time-scale for survival of a population of this size. 18,000 years ago, during the last ice age, the islands concerned were all part of the continental landmass of Sundaland. As the sea level rose following the retreat of the ice, the islands became progressively isolated, such that they probably became effectively separated from one another at least 10,000 years ago. It can be presumed that the fauna of each island originally contained a relatively large number of primate species corresponding to the mainland distribution pattern, but that various species progressively disappeared following isolation of the islands, such that each of them eventually retained a number of species corresponding to its size. Under these circumstances, it would seem that survival of a single species over 10,000 years corresponds to a population of about 20,000 individuals rather than to the much lower figure suggested by the «50/500» rule.

As part of the same study, it was possible to investigate the relationship between genetic polymorphism and island area (presumably providing a rough indication of population size). Using data on protein polymorphism obtained from populations of long-tailed macaques living on different islands (KAWAMOTO *et al.*,1984), it was found that genetic variability (as indicated by heterozygosity or proportion of polymorphic loci) increased progressively with increasing island area. This increase continued for populations living on islands well above the minimal area of inferred for long-term survival,

indicating that even a subpopulation of 20,000 individuals may contain only a fraction of the genetic variability present in the species as a whole. A similar finding has been reported for genetic variation in subpopulations of the dioecious coniferous shrub *Halocarpus bidwillii* in New Zealand (BILLINGTON, 1991). In that case, genetic diversity continued to increase with increasing population sizes up to at least 100,000 individuals. Thus, it would seem that considerable loss of genetic variability is involved even if a few thousand individuals of a given species remain following reduction of the original habitat area.

The inference that thousands, rather than hundreds, of individuals are required for retention of sufficient genetic variability for long-term survival has recently received support from a theoretical analysis of the genetic implications of population size (LANDE, 1995). The original calculations underlying the «50/500 rule» were based on consideration of alleles subject to relatively strong selection. If alleles subject to much weaker selection («quasi-neutral alleles») are also taken into account, it emerges that much larger populations (at least 5,000 individuals) are probably needed to permit a reasonably high probability of long-term survival. This suggests that conservation strategies will require efforts to maintain populations at higher levels than has commonly been thought, combined with management measures that replicate natural pattems and levels of gene flow between subpopulations.

It is important to note, incidentally, that theoretical calculations of the minimum population size required for survival refer not to total population size but to effective population size (N_e) which takes into account the differential participation of individuals in reproduction and may be a relatively small fraction of the total population. Effective population size is influenced by a number of variables, such as variation in number of offspring, and it declines as the sex ratio between individuals involved in reproduction deviates from 1:1. Thus, social structure will influence effective population size and must be taken into account in considering genetic processes. For a total population size of 20,000 macaques, for example, the effective population size might be of the order of 5,000-7,000.

Barbary Macaques: A Case Study

The Barbary Macaque as a Model for Conservation Biology

Although the Barbary macaque originally occurred in several parts of Europe during the Pleistocene, the modem natural distribution of the species is confined to certain forested areas of North Africa, in Algeria and Morocco (FA, 1984). Because of progressive habitat destruction, the natural populations of both countries have been drastically reduced, such that the species is classified as «vulnerable» by the IUCN and listed in Category II by CITES. The total surviving population in Algeria is now estimated as approximately 5,000 individuals, while that in Morocco is estimated at some 10,000 individuals. The total estimated wild population of approximately 15,000 is quite close to the minimum indicated above for long-term survival of a single population of a macaque species. Within both Algeria and Morocco, however, deforestation has subdivided the Barbary macaques into isolated subpopulations of different sizes, thus presenting a typical example of anthropogenic population fragmentation. It seems likely that, because of such fragmentation into a number of completely isolated subpopulations, the Barbary macaque species will eventually disappear in the absence of appropriate management intervention. In addition to the basic question of the genetic effects of such relatively recent isolation of subpopulations within Algeria and within Morocco, there is also the crucial question of the degree of genetic separation between the Algerian and Moroccan populations. For the planning of future conservation measures, it is vital to assess the degree of genetic divergence between these two main geographical populations. One practical issue that has already arisen, for instance, is whether it would be justifiable in genetic terms to release captive-bred Barbary macaques of Moroccan origin in Algerian forests.

Ponencia de Clausura

The Barbary macaque is also a particularly suitable subject for study because of the existence of substantial colonies outside North Africa. Firstly, there is the provisioned free-ranging colony that was initially established on Gibraltar over 200 years ago and was last re-stocked with new individuals (reportedly from Morocco) over 50 years ago (FA, 1986, 1987). This artificially maintained colony, which has recently been allowed to expand to 170 individuals, was constrained to about 35 individuals (often by deliberate intervention) for many years. As a potentially highly inbred subpopulation, for which reasonably good documentation exists, it constitutes an extremely valuable test-case. Secondly, there is a system of 3 semi-free-ranging captive colonies (at Kinzheim and Rocamadour in France and at Salem in Germany) maintained by a single organisation and containing about 300 individuals at each site. These 3 captive colonies are derived from about 260 founders, all imported from the Moroccan Moyen Atlas in 1969, 1971 and 1974, and they are beginning to experience initial inbreeding (VON SEGESSER *et al.*,1995). Finally, there are various small zoo colonies of 10-30 animals derived from various sources and exhibiting various levels of inbreeding. Taken together, these captive subpopulations of the Barbary macaque present a unique, graded array of opportunities for assessing the genetic consequences of population fragmentation and varying degrees of isolation in a long-lived species with complex social organization.

Our study of the genetics of the Barbary macaque has been designed with the following aims in mind:

(l) Collection of evidence concerning the possible existence and timing of a past population bottleneck leading to reduced genetic variability in the species *Macaca sylvanus* as a whole.

(2) Investigation of levels of genetic variation within and between isolated wild subpopulations in Algeria and Morocco.

(3) Assessment of current levels of genetic variability in various captive populations (e.g. the large breeding colonies in Kintzheim, Rocamadour and Salem and individual zoo colonies such as that at Zooparc Beauval, France). Determination of reduction in genetic variability over time. Specific assessment of the effects of long-term isolation on genetic variability in the colony on Gibraltar and use of genetic information to test and expand historical information on the origin and development of this colony.

(4) Examination of the degree of genetic differentiation between the 2 major geographical populations of *Macaca sylvanus* in Algeria and Morocco. Estimation of the time of separation between the 2 populations.

(5) General modelling of the relationships between genetic variability, social organization and demography in the Barbary macaque, with special emphasis on the concepts of metapopulation structure and effective population size.

(6) Contribution to the formulation of an overall management plan for the Barbary macaque, taking into account the genetic effects of population fragmentation and the need to replicate natural metapopulation processes. Establishment of practical guidelines for long-term combined management of the wild and captive populations. The existence of various captive breeding programmes can provide a basis for subsequent reintroductions, which could be planned according to genetic guidelines established in the course of this research project.

The Barbary macaque presents a number of advantages, which together make it a particularly promising model for conservation biology generally, notably with respect to examining the genetic effects of population fragmentation. Firstly, it is a large-bodied, long-lived species living in complex social groups in which group size, male migration, differential mating success of males and matrilineal female dominance will all influence genetic structure. Secondly, it is possible to obtain an effective sample of the entire extant world population of this species, including both wild and captive subpopulations. Finally, comparative data are available for a number of other macaque species, providing a broader perspective for the interpretation of genetic data from the Barbary macaque.

Genetic Studies of the Barbary Macaque

Using a combination of blood protein electrophoresis and DNA-fingerprinting with both «natural» multilocus probes (JEFFREYS *et al.*,1985a; JEFFREYS *et al.*, 1985b) and oligonucleotide probes (EPPLEN *et al.*,1991), various genetic studies of two endangered primate species -Goeldi's monkey (*Callimico goeldii*) and the Barbary macaque (*Macaca sylvanus*)- have been conducted at the Anthropological Institute in Zurich over the past 6 years. Multilocus DNA-fingerprinting has been established as a routine procedure and special emphasis has been placed on introduction of techniques that do not require isotopic labelling. The studies completed to date have permitted reliable identification of individuals, investigation of familial relationships and assessment of levels of genetic variability at different levels of population structure. Preliminary results from DNA-fingerprinting conducted with our captive Goeldi's monkeys have been reported within the framework of a general discussion of potential contributions to primate conservation genetics (VASARHELYI & MARTIN, 1994).

One subproject specifically devoted to genetic aspects of Barbary macaques has already been completed using samples from the colony of this species maintained at the «Montagne des Singes» in Kintzheim (France). This involved an investigation of genetic relationships (especially paternity) within a single social group containing approximately 50 animals. Using a combination of protein electrophoresis and DNA-0fingerprinting, this study (VON SEGESSER *et al.*,1995) permitted an overall level of 75% exclusion of potential males from paternity and reliable inference of paternity for 73% of

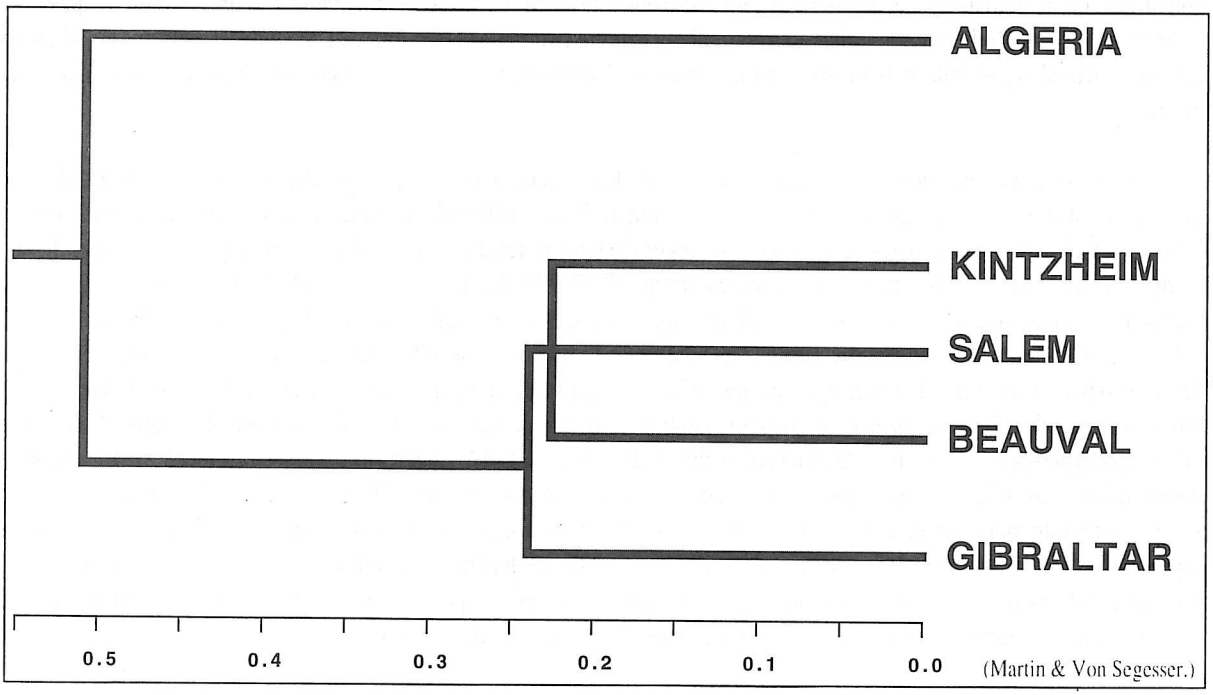

Fig 3. Preliminary dendrogram showing genetic distances between different samples of Barbary macaques based on combined analysis of data from 5 microsatellites amplified using 2 Japanese macaque primers (MFGT2, MFGT17) and 3 human primers (D75503, D17S791 and D15707). The Gibraltar sample groups with the samples from Salem and Kintzheim, which are exclusively of Moroccan origin and with the sample from Beauval, which is predominantly of Moroccan origin. All of these samples are well separated from the Algerian sample.

the infants born over a period of 3 years. It was shown that multiple paternity of offspring occurred within this group and that there was no relationship between male age (indicative of social rank) and breeding success. Despite the relatively low levels of variability found in blood proteins of this species, all individuals studied to date have been found to exhibit unique DNA-fingerprints. Overall, however, a relatively high degree of band sharing in DNA-fingerprints was found even in the absence of inbreeding, and this suggested that the natural population of the Barbary macaque in Morocco may itself be characterized by an alarmingly low level of genetic variability (possibly indicating a persistent bottleneck effect at some time during the history of the population).

Since 1990, close collaboration with a research group from the Station Biologique de Paimpont (Université de Rennes, France) has permitted combined analysis of behavioural and genetic data from the wild population of Barbary macaques in Algeria. Over a lO-year period, the research team from Paimpont has collected behavioural data on known individuals from a number of social groups (MÉNARD & VALLET, 1993a, 1993b). By means of joint trapping campaigns in 1991 and 1993, blood samples were collected from many individuals from those groups and (for comparative purposes) from other wild Algerian subpopulations. An initial analysis using a combination of protein electrophoresis and multilocus DNA-fingerprinting permitted a relatively high level of inference of paternity in natural social groups and also yielded information on genetic variability within and between groups (MÉNARD *et al.*, 1992; SCHEFFRAHN *et al.*,1993). Demonstration of the occurrence of multiple paternity in natural groups of the species confirmed reports from the captive colony maintained at the «Affenberg» in Salem, Germany (KUSTER *et al.*, 1992; PAUL *et al.*,1992a). It was also shown for the wild Algerian macaques that, as with the captive colony at Salem (PAUL *et al.*,1992b), there is no particular relationship between males and infants involved in the special pattern of «agonistic buffering». In this behaviour, a male picks up an infant to «present» to another male, with the outcome that aggression between the two males is inhibited. The study of wild-living Algerian macaques was also particularly interesting in that it was possible to examine genetic aspects of the natural fission of a social group.

Among primates, macaques count among the best-studied species with respect to genetic analyses. The genus *Macaca* is also one of the most species-rich primate genera, so there is an additional advantage in that informative comparisons between species can be conducted (e.g. for assessment of the relative level of genetic diversity in any given species). In one study that was completed recently in our research group (PASTORINI *et al.*, submitted), DNA-fingerprints were used to conduct a comparative study of genetic variability in 5 macaque species (*M. arctoides, M. fascicularis, M. mulatta, M. sylvanus, M. tonkeana*). One specific aim of this study was to determine whether genetic variability is, indeed, lower in *Macaca sylvanus* than in other macaque species and hence indicative of a persistent bottleneck in the past. Values for the band-sharing index for a representative range of samples from *M. sylvanus* were found to be markedly higher than for the other 4 macaque species examined. Another aim of this study was to use DNA-fingerprints to assess the level of differentiation between Algerian and Moroccan populations of *Macaca sylvanus.* As samples of wild-living individuals were available only for the Algerian population, early-generation individuals of Moroccan origin from captive colonies were taken as representative of the wild population in Morocco.) The results indicate that there is a quite marked genetic distinction between the 2 populations (relatively low band-sharing index values; presence of population-specific bands), suggesting that the Algerian and Moroccan populations have been separated for some considerable time.

Over the last 3 years, in addition to DNA-fingerprinting, PCR-amplification of microsatellites has also been progressively established as a routine procedure within the framework of a «Special Programme Environment» project, supported by the Swiss National Foundation («Genetics of Endangered Primate Species»). The first results from our

microsatellite analyses are now beginning to emerge. So far, we have used 2 microsatellite primer pairs specifically developed for Japanese macaques (*Macaca fuscata*; see INOUE & TAKENAKA, 1993) and 3 human microsatellite primers for routine analysis of samples from Barbary macaques. These 4 microsatellites exhibit between 4 and 10 different alleles in *Macaca sylvanus*, and hence yield a relatively large amount of information on genetic variability. DNA has been successfully extracted and amplified from hairs of Barbary macaques, producing very good results. In most cases, hair root cells from a very small number of hairs (approximately 4) have generated enough DNA to conduct microsatellite analyses. Currently, pilot work is being conducted on extraction and analysis of DNA from faecal samples.

In order to expand our work on conservation genetics, a 2-week programme of sample collection was conducted with the provisioned colony of Barbary macaques on Gibraltar in October 1994. In addition to blood and hair samples, we also collected faecal samples to examine possibilities for extraction of DNA from samples that can be collected without trapping. The colony on Gibraltar is of particular interest because of its long-term history as a small, isolated subpopulation. Given this prior history, it is to be expected that there should be a high degree of inbreeding. Indeed, during our sample collection we noted that a few individuals exhibited a drooping eyelid affecting one eye. This condition is known from human medicine under the name of «ptosis» and in some cases it is genetically determined, reflecting a recessive homozygous condition. This may therefore be one indication of inbreeding in the Gibraltar colony. During a survey conducted in 1995, a drooping eyelid was recorded for 5 individuals (all males) in 2 different troops (W. MING, pers. comm..)

Somewhat surprisingly, our preliminary results indicate that there is a relatively high level of variability in DNA in the Gibraltar macaques. Multilocus fingerprints show individual-specific patterns (Fig. l) and there is considerable heterozygosity in single-locus microsatellites in comparison with the larger, more recently established captive colonies in Kintzheim and Salem (Fig. 2). As a further finding, a preliminary dendrogram based on allele frequencies for 5 microsatellite systems (Fig. 3) links the Gibraltar macaques to the captive macaques of Moroccan origin rather than to the Algerian macaques, confirming the likely Moroccan origin of the Gibraltar population.

A field project has just been conducted by a student from our instutute (W. MING) on the feeding behaviour and social organization of 2 of the 6 free-ranging groups of Barbary macaques on Gibraltar. This study included a general census of the entire population (total in September 1995 = approximately 170 animals). Further collection of samples of hair and faeces from known individuals was also conducted, such that a detailed examination of genetic characteristics in relation to social structure should be possible in the near future.

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This paper is dedicated to the memory of the late Gerald Durrell (1925-1995), who as founder of the Jersey Wildlife Preservation Trust inspired many people throughout the world to take the subject of conservation biology seriously.

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