Conserving patterns of genetic diversity in endangered mammals by captive breeding

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Breeding endangered mammals for their conservation requires knowledge about the genetic architecture of the respective species. In taxa with tight genetic cohesion between populations, the definition of management units for captive breeding rarely poses problems, except if there are morphologically well differentiated subspecies grading into one another although they are hardly separate at the molecular level. Species with genetic diversity predominantly between populations can pose serious problems for breeders. Examples are discussed of mammalian species with complex genetic architectures, where decisions have to be drawn whether to select only certain populations for conservation, or to create an artificial taxon. Research into subspecific molecular taxonomy of rare zoo-living wildlife is frequently hampered by small sample sizes available for study, with the risk of spurious molecular taxonomic distances based on marker allele frequencies in populations influenced by genetic drift. "Typological" approaches are suggested for molecular systematics of such study material, with the haplotype organization of polymorphic MHC genes appearing particularly promising. Additional molecular approaches, not easily susceptible to sample size problems, are shortly presented. The implementation of breeding plans to achieve conservation genetic goals may interfere with the social structures of the animals. This group of problems includes the transfer of socially compatible individuals to form new groups, and the provision of suitable sexual partners for mate choice mechanisms to act in their species-specific manner. A survey of scientific research in European zoos is provided to recognize what is being done to fight the current ignorance about basic aspects of the conservation biology of many endangered mammals.

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Introduction

The preservation of genetic polymorphism has been accepted over the last decade as a significant aim of species conservation projects. Breeding programs for endangered species like the 'Europäisches Erhaltungszuchtprogramm' (EEP) which unites some 250 wildlife-breeding institutions throughout Europe (Brouwer *et al.* 1991), implement their captive breeding projects with the objectives to minimize inbreeding, to equalize genetic representation of all founder individuals, and to reach minimum population sizes to retain a defined level of variability over a specified number of years (de Boer 1989a). The proximate aim of these efforts is to avoid inbreeding depression, a problem which has been observed in several zoo-bred mammals but not in others (de Boer 1989b). The ultimate aim is to preserve sufficient genetic plasticity for populations to adapt to changing environments after their reintroduction into the wild. E.g., the interactions between mammals and disease-causing microbes or other parasites require immunogenetic variation of hosts (cf Schreiber 1991, Schreiber and Tichy 1992). Conservation of genetic variability in rare wildlife is usually a prophylactic safeguard rather than the reaction to manifest genetic problems of the species. Conservation biology has been termed a "crisis-oriented science" whose objects, endangered species, are disappearing while the relevant knowledge is slowly being accumulated for their proper conservation. The preservation of genetic polymorphism requires minimum effective population sizes. Depending on the genetic architecture of species, and the species concept underlying the classification, the goal of minimizing the loss of alleles may conflict with the conservation of coadapted genotypes (Greig 1979, Templeton 1986). Although there are elaborated theories concerning the p r o c e s s e s moulding genetic polymorphism for useful application in wildife breeding, p a t t e r n s of genetic variation are much less amenable to generalizing concepts between taxa. The genetic architecture of species which is the result of their evolution in the past is presumably as diverse as there are aggregates of populations which meet the species concept applied in a taxon under concern. Ideally, genetic surveys of natural populations should precede any captive breeding program, in order to define management units and to recognize spatial organization of species into aggregates of differently coadapted genotypes. Since investigations into the genetic architecture of disappearing species may not be feasible apart from exceptional cases, indirect approaches based on the few founder specimens for captive breeding are required. This article introduces into some of the challenges by this field of applied evolutionary biology.

Species concepts and captive breeding

Species are the management units of conservation biologists. However, even within mammals a single species concept probably does not suffice to describe the

multitude of population genetic patterns which lend structure to networks of natural populations. Depending on whether a phenetic, biological (isolation vs recognition), evolutionary or cohesion species concept (Templeton 1989) has been chosen for a taxon, the composition of founder populations for captive breeding will differ when seen from the viewpoint of a geneticist. The growing interest in molecular population genetic patterns may lead to a classification of types of species according to their genetic architecture. Such a typology is lacking at the present time. This article classifies species pragmatically into those whose external phenotype is a sufficient indicator of taxonomic differentiation, and whose cryptic genetic infrastructure is complex, rendering *ex situ* conservation more difficult.

The taxonomic species corresponds to or is less comprehensive than

the biological species

The easiest situation for the conservation biologist are those cases where the described taxonomic species corresponds to the reproductive community in nature, management units can be identified from external morphology, and founder individuals can be included in a captive breeding program if they fit this type (even if their geographic origin remains unknown). Some of these species are confined to distribution ranges the small areas of which have prohibited regional differentiation. Others do range widely but obviously gene flow suffices for close genetic cohesion, i.e. newly arising mutations can spread with sufficient speed throughout the populations to prevent genetic divergence.

There are mammals in which external morphology differs much more extensively between populations than genetic distances would suggest. We determined the electrophoretic standard distance (according to Nei 1988) between Amur cat *Felis* (*bengalensis*) *euptilura* and tropical Bengal cat *F. bengalensis* as 0.018 (Schreiber *et al.* 1993b). This distance probably even overestimates genetic differentiation between both taxa because sample sizes were very small, and the single allozyme locus among 29 which proved different between these cats was found polymorphic in another species, the European wild cat *F. s. silvestris*. A distance of 0.018 would not indicate even subspecies difference in very many mammals but there is no doubt from comparing Amur cat and tropical Bengal cat that both constitute valid taxa, and the taxonomic discussion centers on the question whether both are distinct species or merely very distinct subspecies, differing in body size, craniometry, fur texture, pigmentation, and coat patterns. In a comparable manner, morphologically recognizable tiger subspecies display but small and possibly insignificant electrophoretic protein distances (Goebel and Whitmore 1987, O'Brien *et al.* 1987). As a rule, the genetic parameters usually studied for systematics, allozymes, restriction fragment length polymorphism of DNA or sequences of pragmatically selected macromolecules are probably by-products or results of evolutionary processes, rather than being involved *per se* in organismic evolutionary divergence. This means that the usually screened

marker systems of molecular taxonomy are particularly good markers of organismic phylogeny if they evolve at a rate which is roughly equivalent to overall organismic evolution. Suitable molecular parameters have to be established for various groups of mammals. Research into the molecular genetic basis of adaptive morphological, physiological or behavioural characters of mammals is in a pioneer stage.

In species where genetic differentiation between populations is small, genetic diversity is predominantly present as heterozygosity which can be conserved by planning breeding so as to increase effective population size.

The taxonomic species is more comprehensive than the biological species

Considerable problems for captive breeding may be provided by taxa with complex genetic architecture, whose ignorance may lead to outbreeding depression, or at least to destruction of natural patterns of genetic diversity. Depending on the philosophy of the taxonomist, subspecies in various mammals denote a diversity of evolutionary phenomena which may require different management options. They may represent stages in clines of ecologically selected paramèters, e.g. gradients of size, pigmentation or fur characters. Arbitrary decisions have to be drawn how to delimit such differences, and to judge if they warrant separate management units. The most challenging cases are those taxa where genetic variation is prominent between populations although these cannot unambiguously recognized phenotypically or grade into one another. In particular if various genetic characters, i.e. chromosomes, allozymes or selected DNA systems suggest different patterns of regional differentiation, i.e if the genetic landscape of a taxonomic species is very complex, captive management planners may arrive at the conclusion that a particular genetic architecture is too complex to be preserved by captive breeding. The option remaining is to study genetic differentiation sufficiently to prevent outbreeding depression, and to breed with the aim of conserving a taxonomic type instead of the genetic complexity of the species. Irrespective of the mechanism, whether overall molecular differentiation is largely caused by genetic drift (Nei 1988) or by selectively driven effects (Clarke *et al*. 1988), current opinion about mechanisms driving molecular evolution suggests that even without major environmental changes acting upon a species, time is a very important factor for molecular divergence. Therefore, molecular divergence within taxonomic species lacking efficient genetic cohesion may be expected particularly after long-lasting isolation between populations. Most studies into population genetic infrastructure of large mammals have been performed in northern temperate or boreal zones of America or Europe, in ecosystems which are themselves young. As a rule, these populations have invaded their present ranges after the last glaciation. We know very little about molecular polymorphism, ecotypic variation or clines of genetic markers in tropical large mammals although some of their habitats are ancient. In addition, expansions and reductions of tropical habitats during phases of differing climatic humidity in the Pleistocene may have

led to secondary contact between differentiated populations. Most endangered mammals are of tropical origin.

In the population of approximately 30 anoas (*Bubalus* sp.) available for captive breeding in European zoos, complements of 44, 45, 47 or 48 chromosomes have been found, comprising translocation polymorphism within two of the import lines, and karyotypic differences between import lines which probably indicate taxonomic separation (Schreiber *et al.* 1993a). Anoas are endemic wild buffaloes from Sulawesi (Indonesia), an island which is well-known for its complex zoogeography, with allopatric animal species commonly found confined to single ones of its mountaineous peninsulas (Whitten *et al.* 1988). Anecdotal evidence from naturalists indicates that anoas are territorial inhabitants of closed tropical forests. Anoas combine morphological characters of buffaloes (i.e. bovines) and antelope-like bovids, and probably represent a phylogenetic lineage persisting since a time when others among today's bovid genera had not yet diverged. There is a possibility that anoas from various regions may live in isolation from each other for fairly long periods of time. Molecular investigations into the variation of allozymes or serum proteins, and restriction fragment length polymorphism of MHC genes revealed considerable polymorphism, when compared to other bovids (Schreiber 1991, Schreiber *et al.* 1993a). The proportion of polymorphic loci amounted to approximately 30% even in the zoo stock obtained from very few founder specimens. Genetic variation is reflected by a bewildering multitude of body sizes, horn shapes, pelage characters and pigmentation which formed the basis for several taxonomic revisions which remain controversial. On the basis of limited museum material collected from certain regions from within Sualwesi, some taxonomists recognized three taxa which have been allocated to one or two species (Weise 1979). However, several individuals of the captive population did not fit any described morphological type, the supposed taxonomic characters being present in variable combination (A. Schreiber, unpubl.). Hardly anything reliable is known about the biology of these buffaloes in the wild, including the geographic ranges of various kinds of anoas. Our study revealed that the genetic architecture of anoas is complex although we are unable to suggest a breeding plan to conserve genetic patterns of this taxon without studies of free-living anoas or specimens with known origin from within Sulawesi. The limited evidence available suggests that the African buffaloes *Synceros* sp. which are not too distantly related to anoas exhibit a chromosomal hybrid zone in western Uganda, where red buffaloes from rain--forests and Cape buffaloes meet (Buckland and Evans 1978).

Ryder *et al.* (1987) found two cytotypes separating two import lines of Kirk's dikdik *Madoqua kirki* from Kenya to North American Zoos, with 2n = 46 in one population, and 2n = 47 in males and 2n = 46 in females, in the other, the cytotypes differing by multiple chromosomal rearrangements, including inversions, X-autosome translocation, and tandem fusions. While female offspring from crossings of both cytotypes proved to be fertile, male F_1 hybrids never bred themselves. Evidently, taxonomic species and biological species are not congruent in Kirk's dikdik.

As the closest relative of the lab mouse which perhaps is the genetically best-
-studied mammal, the wild house mouse *Mus musculus* of Europe has been the
subject of very many genetic studies into its wild populations (reviews: Sage 1981,
Bonhomme and Gunet 1989). Although not a candidate for captive conservation,
it can demonstrate how complex taxonomy can become in a well-investigated
mammal. In Europe, two widespread taxonomic subspecies used to be recognized
north of the Alps (in addition to local forms), *Mus musculus domesticus* in western
Europe, and the nominate form *M. m. musculus* in the east, separated by a hybrid
belt from Jutland to Bulgaria. The nominate form has a longer tail, a darker-
-coloured belly and is better able to hibernate outside human buildings. At least
in the northern part of the contact region, extensive flow of mitochondrial DNA
has been occured across a narrow subspecies hybrid zone of 20 – 30 km width.
There are Swedish mice with predominantly nuclear genomes of *musculus* al-
though their cytoplasmic DNA is of the *domesticus* type. In Greece, the reverse
has been found. As to the nuclear genes, a lowered fitness of hybrids keeps the
transition zone stable. However, within these two descibed subspecies, there are
complex genetic landscapes if the distribution of chromosomal variants of the
Robertsonian translocation type is considered. When some 400 wild house mice
from an area of 500 km^2 in South Germany were karyotyped (Adolph and Klein
1983), 16 different patterns of Robertsonian translocation were encountered.
Chromosome numbers ranged from 38 or 40 in the Tübingen area to 30 near the
Bodensee. On the basis of this single character, mice of this region were classified
into five cytogenetic populations, each of them polymorphic for some trans-
locations. These populations turned out to have evolved *in situ*. However, there
were "hybrid zones" connecting these populations. Even greater chromosomal dif-
ferences are known from house mice in valleys from the Alps or the Abruzzi
mountains. *Mus musculus* comprises individuals with 40 acrocentric chromosomes
down to multimetacentric patterns of 22 chromsomes. Mice from adjacent villages,
isolated farm houses or neighbouring mountain valleys may exhibit different
karyotypes in certain areas. At least some populations belonging to different
cytotypes cannot be well separated by allozyme electrophoresis which suggests
that cytogenetic differentiation is recent. In contrast, *musculus* and *domesticus*
are easily recognized from electrophoretic patterns. It is thought that within one
Italian population, 9 chromosome mutations arose sequentially and spread over
7500 km^2 within less than 8000 years. Crosses between multimetacentric popu-
lations differ in the degree of reduced fertility, from apparently unimpaired
fertility to sterility. If house mice were an endangered species to be bred in zoos
and the genetic data assembled by dozens of scientists not available, conservation
biologists would have to debate whether the small subspecies characters between
musculus and *domesticus* are sufficient to start two breeding projects. Genetic
analysis reveals that sampling of founder specimens from very many geographic
regions would be required to conserve a part of the genetic diversity of the
aggregate genetic complexes concealed under the taxon name *Mus musculus*

domesticus. Without very intensive genetic sampling within small areas, fertility problems arising from crossing wild-caught mice from two adjacent villages would probably be explained by inbreeding depression since certainly the concept of "mouse populations" would be applied with a too wide mesh.

Numerical taxonomy and the effects of limited sample sizes

Ideally, studies of natural patterns of genetic variation should precede any breeding program, and they should include as many genetically determined characters as possible. In practice, such studies are hardly feasible in the majority of highly endangered species where such work would be most urgent. Even wild--caught founder specimens are not available in several species whose captive preservation depends on assembling specimens from different (and sometimes obscure) origins. Any genetic research with such limited study material is faced with problems of sample size effects. In a species as polymorphic as anoas, one cannot prevent that the founding of each new subpopulation influences allele frequencies, because the numbers of individuals involved are necessarily tiny. This problem is exacerbated by the aggressive social behaviour of anoas which precludes to keep more than a handful in one zoo. Numerical taxonomy is based on the quantitation of genetic differences. If sample sizes are as small as is typical in conservation genetics, and influenced by stochastic distortion of marker allele frequencies, spurious distances are a real risk. Unfortunately, pedigrees are insufficiently known in several captive relict populations to estimate the bearing of genetic drift upon the study population. Among the options remaining for molecular genetics, those approaches deserve attention which permit a partly typological approach in molecular taxonomy, i.e. which introduce qualitative character states typical of certain populations instead of measuring frequencies of freely segregating alleles. Three approaches have been suggested in this context: private alleles confined to single populations, population-specific linkage groups involving highly polymorphic loci, and the use of concerted evolution to produce population-specific restriction fragment patterns in repetitive DNA. Private alleles are those which are confined to certain populations within a species. They can yield valuable information on gene flow (Slatkin and Barton 1989), and thereby on the genetic cohesion of a species, and they enable allocation of individuals carrying such a marker to one population. In ungulates at least, protein electrophoresis yields rarely alleles which are confined to local populations within described taxa which rather differ in allele frequencies, and the few exceptions tend to be rare alleles whose utility would suffer even from relatively mild bottle-necks (Schreiber 1991). The other extreme is provided by rapidly evolving markers provided by multilocus-fingerprinting of tandemly repeated DNA which are so "private" indeed that they may indicate family genealogy instead of demes or regional populations. In our lab, we found the Major Histocompatibility Complex

(MHC) of genes to be so hyperpolymorphic in many ungulate species of conservation concern, and its alleles to be phylogenetically so stable, that hybridization with several MHC gene probes will detect informative restriction fragments in many cases (Schreiber 1991, Schreiber and Tichy 1992). The MHC systems of mammals are organized into two linkage groups which consist of several loci each. Although polymorphism differs between MHC loci, the majority of MHC systems studied so far in mammals displayed considerable to extreme polymorphism. In addition to allelic variation at certain MHC loci, characteristic linkage groups of alleles from different loci within the MHC are not rarely encountered. In our lab, MHC haplotype patterns which were specific to particular breeding-lines of large mammals have been found in cervid and bovid species (A. Schreiber, unpubl.). Insufficient data concerning MHC polymorphism in wildlife precludes judging whether these hybridization patterns are characteristic of a population in the sense that they are exclusively confined to it, or if only frequencies are so different to make patterns appear specific when in practice only limited sample sizes are covered. However, the linkage disequilibrium encountered in MHC genes, which has been well characterized in the intensively studied primate and murine MHCs (Klein 1986) appears to be very useful for studying intraspecific systematics since the technique of trans-specific hybridization with gene probes from the well-known mammalian MHCs has become possible. Klein *et al.* (1991) found haplotypes of polymorphic primate MHC genes to be phylogenetically stable enough for persisting relatively unchanged for millions of years. The term "frozen haplotypes" has been coined to characterize this phenomenon. Schreiber and Tichy (1992) speculated about a possible adaptive function of conserved haplotype organization in the MHC. In any case, these linkage patterns should be resistant to inbreeding or drift, which is a difference to allele frequencies. Thus they appear to represent a promising tool to type populations of which certain linkage groups are typical if only their geographic distribution is understood in respective taxa. A third approach for molecular typology utilizes the peculiarities of evolution in families of dispersed satellite DNA. Mutations in these sequence families can spread within the genome by a mechanism called "molecular drive", the molecular processes of which are probably unequal cross-over or biased gene conversion (Dover 1982). The stage of spread of a point mutation in a repeated element of non-coding DNA which occurs at various localities within the genome of each of two individuals to be compared could yield an indication of the time since the ancestors of those individuals have become genetically seperated (Ohta 1983). Like in the case of MHC haplotypes, possibly a single individual per deme would suffice for allocation to a taxon/population. If molecular drive acts sufficiently fast to resolve short periods of isolation, 'fingerprinting' or sequencing of a suitable sequence family could yield important information about population phylogeny. Turner *et al.* (1991) used this approach to study the divergence of populations in the fish *Cyprinodon variegatus*. All these approaches require considerable additional methodic development, particularly tests in populations systems whose relationship and temporal

divergence is known or amenable to reconstruction by other techniques to provide a framework of interpretation.

Captive breeding of taxa with doubtful systematics

In practice, decisions have frequently to be drawn, upon insufficient data bases, whether founder populations of one taxonomic species should be split into subunits to be managed separately to meet the desire of preserving genetic diversity between populations, or to increase the management unit by grouping all inter-fertile specimens into one management unit to maximize effective population size and thereby the preservation of heterozygosity and rare alleles. Although this conflict continues to stir debates among conservation biologists there may be a solution to satisfy both purposes of species conservation. There is a strategy which avoids the crossing of animals which possibly belong to different intraspecific taxa until taxonomy has been better analyzed, while being entirely compatible with the aim of retaining polymorphism in the global population. The rational of such a cautious approach is as follows: Genetic drift cannot be avoided in any population of animals, and will lead to the loss of molecular polymorphism. If a captive population is subdivided into isolated breeding groups, the population size of management units will diminish, and genetic drift be accelerated. Increasing effective population size will delay this fate. However, it being largely a matter of chance which allele from a polymorphic locus will reach fixation in a small population, different sub-groups will probably be fixed at different alleles. There-fore, global variation will be less affected if the stud had been subdivided before drift set in than if one single world herd reaches fixation: a specific allele has a greater chance of being retained in at least one subline. Templeton (1991) dis-cussed this approach in greater detail. There are some prerequisites for the imple-mentation of this strategy. First, isolation between subgroups must be efficient since even a low rate of exchange will counterbalance differential drift. In practice, this may be difficult to fulfil during the initial trial-and-error phase of captive husbandry of wildlife, when the import of animals to replace the loss of key individuals may be frequently required. Second, the breeding in small subgroups of already very limited population numbers should not result in inbreeding de-pression severe enough to endanger the project. It should be empirically tested if inbreeding depression is a problem in the taxon under concern. Third, management in separate lines must not result in genetic divergence to a point where subsequent crossing between lines to recombine variable genotypes for reintroduction is hampered. Given the usual temporal framework of captive breeding projects, the latter aspect may be of less significance considering generation lengths of large mammals. Since this strategy reshuffles genetic variation from heterozygosity to between-population variance, the response of the less polymorphic breeding lines to undesirable selection, and adaptation to the artificial zoo environment will be slowed down.

The degree of genetic polymorphism differs widely between even closely related species. Population genetic theory and knowledge has identified several factors that exert an influence upon molecular variation in populations (Hartl 1985). However, there is hardly any species for whose variability can the relative impact of those factors be determined since the various hypotheses are difficult to test rigorously. A definite influence is exerted by (repeated) bottle-necks or (prolonged) phases of reduced population size. These may have been due to human influence although natural bottle-neck events can be expected during colonization of new habitat patches, or catastrophes reducing stocks to small remnants. Several students of speciation claim that peripatric species would originate preferentially during phases of prolonged reduction in population number (cf Schreiber 1991). Genetic transilience in small populations is explained by genetic drift which may help a population to pass the phase of lowered fitness which separates the coadapted genotypes of the ancestral and the new species. Another argument to emphasize the role of bottle-necks for speciation is the modification of epistatic effects upon major genes because by decreasing heterozygosity, genetic variance will be more of the additive type, and thus expose various genotypes more directly to natural selection than if smoothened by epistacy of a variable genetic back--ground. One attractive consequence of assuming that genetic variance within a species may be correlated with historic events like speciation would be that the sometimes astonishing differences in genetic polymorphism between closely related species could be explained. These are difficult to understand if they were due to influences by different ecology or lifestyles of those very similar species. Schreiber *et al*. (in press) used the immunological technique of Comparative Determinant Analysis to derive determinant formulas for up to 22 serum proteins in selected species of Eutherian mammals. Antigenic determinants are portions of proteins composed of 5 – 10 amino acids. If a mutation substitutes an amino acid, the loss of cross-reactivity between the mutated determinant and the antiserum used for its recognition may result. It is much likely that any subsequent mutations will affect another amino acid within the epitope instead of restoring the former determinant structure by exactly reversing the previous mutation. Thus, there is polarity in character transformation, with losses of recognized determinant being much more frequent than convergent evolution of epitopes. This permits to infer ancestral character states which are symplesiomorphic for two diverging phylogenetic lineages. If there has been a radiation phase giving rise to several lineages in 'rapid' succession, the ancestral character state will be similar to the plesiomorphic situation of these lineages, and their retaining the ancestral determinants may serve as indicators of substitution rates in the particular clades. We found higher rates of determinant substitution in Micro- *versus* Megachiroptera, and in bovids *versus* pigs. Although it is difficult to disentangle the many possible influences upon substitution rates, our findings are compatible with the quoted model correlating species-richness of a lineage and substitution rate. The substitution rate is a function of the fixation probability of molecular polymorphisms.

In addition to historic influences of bottle-neck phases, several ecological and demographic factors have been suggested to influence genetic polymorphism in mammals, e.g. living in coarse- or fine-grained niches, reproducing as k- or r-strategists (Hartl and Reimoser 1988), modulation of effective population sizes by social structures and mate choice mechanisms, etc. At present, there is no species whose degree of genetic variation can be satisfactorily deduced from the many possible relevant influences, some of which are difficult to define rigorously. Much additional work is required to understand why populations of free-living mammals differ so widely as to their genetic variability.

Conflicts between genetic management and the conservation

of social structures

Evolutionary biology is a science which implies a considerable impact from theories guiding the interpretation of data. Discussions about the ontology of species concepts are as old as the science of systematics, and the dichotomy between supporters of a more static ("morphological") species concept and those focusing upon processes ("reproductive communities") as more important denominators of species has persisted in the biological literature since at least the early nine-teenth century. Empiric work is required to fill terms like population, species, and other pragmatic contructs with the contents needed to understand the specific object to be managed in *ex situ* conservation. All populations are composed of individuals, but some aspects which are targets of conservation geneticists, such as taxa, coadapted genotypes, etc. affect rather aggregates of individuals instead of these individuals themselves. Clearly, there is an ethic responsibility to balance the interests of specimens of endangered species with the plan to conserve phenomena of classes of individuals. E.g., for the preservation of a defined level of genetic polymorphism within a breeding line, selected individuals have to be exchanged between institutions, and bred a specified number of times with defined mates. The transports required to meet this aim frequently impose stress on the animals, sometimes with fatal consequences. Social incompatibility between individuals chosen for producing offspring poses prominent difficulties for the species manager. Pragmatically, two types of social incompatibility may be recognized. First, social conflict may be caused by behavioural traits of the particular specimen's individuality. Only regrouping of individuals which have difficulties of being accepted in a new social setting, i.e. trial and error, can overcome this problem, with all risks for the animal's welfare implied. Alternatively, groups established with animals belonging to particular sex, age or rank classes may lead to conflicts in a more predictable manner. Maiwald and Schreiber (1991) summarized studies about mechanisms of mate choice, and their modification by learned or inborn behaviour patterns. The current state of knowledge in this field is of limited value for captive conservation breeding, both as to the depth of understanding, and as

to the obvious diversity of involved mechanisms between species. However, there is a certain upper level of social conflict, and a certain minimum stability in interindividual interactions, shall a breeding group yield the desired number of socially competent offspring. The behavioural competence of animals bred in zoos contributes to the ultimate success of captive breeding projects, and in no small part determines the success of reintroduction projects into the wild. Zoo-born animals require sufficient social stability, and interactions with partners of their own and of the opposite sex for their behaviour to mature in their species-specific manner.

Ideally, the social organization of a species, including patterns of dispersal and group formation should guide breeding exchange: specimens from those sex and age classes which would disperse in nature should be those to be exchanged between zoos. In small populations, insufficient sample sizes available for chosing require the transfer of specimens which would never leave their group in the wild, or which cannot be expected to be easily integrated in a new social setting, if this is the only option to preserve the alleles from a certain ancestral line. Those factors which stabilize local social groups and genetic demes, and those which disrupt long-term aggregations of individuals (with their stabilized social relations and the possibility to transmit behavioural traditions), and thereby prevent locally--distributed genealogical clusters should lead to an overall degree of genealogical relationship in a population. However, there is no possibility to rationally infer what average level of genealogical relationship should be planned for any one of the presently endangered captive-bred mammals. We are completely ignorant whether outbreeding beyond that level would imply decreased social competence in offspring (cf Maiwald and Schreiber 1991). Pragmatic considerations have to decide on details of exchange between breeding groups, with recommendations provided by theoretical population genetics serving as frame to guide decisions but not to enforce them.

In the Przewalski's horse *Equus przewalskii* which is managed in zoos according to a well-defined breeding plan for maintainaing allelic polymorphism, transfers sometimes led to problems. As far as can be inferred from the sociology of feral domestic horses, Przewalski's horses live in stable harem groups, consisting of a stallion and several adult mares. The stallions are challenged by rivals and tend to be replaced after some years. The juvenile offspring leaves the group usually at an age from 1.5 to 3.5 years, the exact age depending, among other factors, on the advent of the first estrous in females or the availability of playmates (Klingel 1972, Penzhorn 1984, Hoffmann 1985, Berger 1986). Young mares may change group membership several times until final integration in an existing herd, or founding a new herd in company with a mature male. It is not known whether there are additional factors which determine the end of the straying period of a young female, other than maturation of estrous-related behaviour (Klingel 1972). Young males join a bachelor group of non-harem stallions of different ages where they gain the skills for leading a harem, which is unlikely to occur before the age

of six years (Hoffmann 1985, Berger 1986). In captive breeding management, the majority of transfers between groups affect adult stallions. Provided that these males are fertile, this practice does not lead to major problems of social integration or breeding success. Young males are kept in bachelor groups before their use as breeders (Kolter and Zimmermann 1988a). Those few cases when young mares of 2 – 3 years of age had been transferred to new groups did not pose problems of social integration. In contrast, male aggression was elicited when adult females were to be integrated into existing herds. This aggressive behaviour required temporary separation of the imported mares, and rendered breeding almost impossible. It is not possible to conclude from the few cases whether the import of adult female Przewalski's horses was incompatible with the social flexibility of the species, or should be explained by individual characteristics or the limited space of zoo enclosures. In two studied populations of feral horses, up to 30% of the adult females changed their group attachment occasionally (Rutberg 1990, Stevens 1990): ecological factors like food availability and the attentiveness of stallions facilitated the change between groups. In zoo-living wild horse, severe and persistent male aggression specifically towards a specific mare is sometimes observed in well established herds, too, with the same stallion showing normal behaviour to other females of his group (Mackler and Dolan 1980, Kolter and Zimmermann 1988b). A recent survey of the behaviour of wild horses which lived in European zoos until 1988 revealed that 8 out of 27 stallions (30%), and 10 out of 121 mares (8%) were affected (L. Kolter, unpubl.). In the wild, this behaviour presumably serves to remove conspecifics not belonging to the harem (Feist and McCullough 1976). Most of the concerned mares displayed abnormal socio-sexual behaviour as described by Mackler and Dolan (1980) and Kolter and Zimmermann (1988b), or failed to display estrous behaviour. At least four of the concerned mares never produced offspring.

With the captive population of Przewalski's horse numbering several hundreds, the frequency of mate rejection is too low to interfere with genetic goals of the global breeding plan. Selectivity among partners affects captive breeding more significantly in predominantly solitary animals with contacts between sexes only during the reproductive season, or in those living in permanent monogamy. Chosing inappropriate mates easily precludes reproduction for a whole breeding season or even lifetime. E.g. merely 20% of tree shrews *Tupaia* sp. which had been 'paired' arbitrarily in captivity formed succesful pairs (D. von Holst, pers. comm.). Another 60% of partners lived together without overt aggression, but also without attachment. Immediate fighting was observed in another 20% of the pairs chosen by man, and had to be separated to prevent losses. A tree shrew not accepted by one partner may harmonize with another individual. The factors determining this social attraction are not understood. In cheetah *Acinonyx jubatus*, low male libido and reduced sperm quality, presumably correlated with phylogenetically fixed inbreeding depression are thought to constitute the major reason for the poor reproductive performance of the species in zoos (e.g. de Wildt *et al.* 1983, 1987).

Studies at the San Diego Wild Animal Park did not reveal a correlation between poor semen quality (also observed in these cheetah) and breeding success (Lindburg *et al.* 1993). Husbandry conditions, such as the separation of sexes, estrous detection in females which did not display typical felid estrous behaviour, and mate preferences were found to play important roles for breeding cheetahs in captivity (Schumann 1991, Stearns 1991). Strong individual mate preferences by both sexes have been reported from two institutions (Schumann 1991, Stearns 1991). A large number of potential mates to permit choice is thought important to breed cheetahs, a view supported by stud-book data (Marker-Kraus 1991): only 21 of the 149 institutions keeping cheetahs bred the species, but all of those successful zoos but two institutions kept more than a single pair. There are no data about mate choice behaviour in cheetah, neither from zoos nor from wild animals.

Several of the smaller felid species show poor breeding performance in zoos. Reminiscent of the example from tree shrews, there are pairs which avoid both aggression and contacts, as well as highly aggressive pairs. Appropriate sexual behaviour and reproduction are confined to harmonizing partners. Experience during early ontogeny and the relationship to keepers appear relevant for the development of competent sexual behaviour, with hand-reared specimens being less likely to behave normally. Clouded leopards *Neofelis nebulosa* are extremely difficult to breed, with only 20% of the captive population producing offspring. They develop strong and long-lasting pair bonds if acquainted to each other before they are half a year old (Mellen 1992).

Primate societies being typically highly individualized, their stability depends on long-term intimacy between partners. Foreign intruders frequently experience agonistic behaviour. The exchange of individuals between breeding groups is usually possible only if members of certain age classes, and one particular sex are imported. Nevertheless, integration of foreigners into breeding groups may prove very time-consuming and can lead to decreased reproduction of the group. Along with the relatively low reproductive rates in primates, caused by their long gestation periods, the age required until females can reproduce for the first time, the small number of offspring per female, and a sometimes considerable infant mortality, any regrouping of zoo populations, desirable as this may be for the preservation of rare alleles from underrepresented founder lines, can lead to negative development of demographic structure. E.g., if breeding plans meant to preserve rare alleles suggest new partner constellations in callitrichids, the formation of completely new pairings is usually required, which leads to practical difficulties of housing these new families. Species or breeding lines of apes which contain a significant proportion of individuals which had been rejected by their mothers and reared by humans are very difficult candidates for the implementation of recommendations by statistical genetics, because a lowered social competence of these animals diminishes the likelihood of regrouping to prove successful.

Cutting the knot

It is evident that each captive breeding project has to be addressed individually to cover pertinently the various, and sometimes conflicting, demands from disciplines which share a general aim, species conservation, although they focus on selected aspects of an animals' biology. Perhaps the most serious challenge to conservation biology is posed by the necessity to integrate data from various, and traditionally unconnected research disciplines. In fact, there is hardly any field of zoology where a false balance between various disciplines leads so mercilessly to irreversible desaster. For the first time in history, man aims to manage the genetics of organisms not with the purpose of increasing a narrowly-defined performance, but instead attempts on a large scale to replace the natural ecological niches in their function to shape species until possibly natural selection will again be able to take over this job after reintroduction into what will remain of 'the wild'. Evolutionary biology has entered into an applied phase of large-scale experiments which determine the survival of an appreciable portion of large mammals: a very demanding and stimulating goal indeed which puts the scientific rigour of several zoological disciplines to a merciless test. At present, conservation breeding is best based on experience gained separately for each species, with generalizations being of value to formulate hypotheses for subsequent empirical test.

Against the background of the aspects discussed in the preceding pages (and of additional problems of veterinary medicine, nutrition, physiological rhythms, behaviour, etc.), it is evident that the ultimate success of captive breeding programs will depend on the quality and the quantity of research projects. A survey of various bibliographic sources revealed that some 1713 scientific reports have been published since 1981 by scientists working in European zoos, either as zoo employees or guests. Of the 1262 papers selected for further analysis, 32% were by students of animal behaviour, 32% treated questions of husbandry, 17% veterinary medicine, 13% genetics, reproductive biology or nutrition, and merely 7% taxonomy, morphology, and aspects of reintroduction. Mammals were the subject of 62% of these publications (birds of 21%, reptiles of 5%, amphibians of less than 1%, and fish of 2%), with primates and ungulates predominating. Only single attractive mammals, like gorilla or red panda, attracted very much attention from research workers. Approximately 20% of those papers covered species which had been selected for coordinated captive conservation breeding within the 'Europäisches Erhaltungszuchtprogramm'. Less than 30% of European zoos, and less than 30% of the scientists employed by them published results of research work of any kind. Several research papers are the result of casual observations, and not very many report findings of longer-term research projects. The possible problems of applying results by specialized research disciplines in the last few dozen survivors of a species without considering other needs of these animal's biology call for multidisciplinary approaches of biologists with complementary expertise. Such projects remain exceptional.

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