

***Ex situ* conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species**

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Abstract Captive breeding has become an important tool in species conservation programmes. Current management strategies for *ex situ* populations are based on theoretical models, which have mainly been tested in model species or assessed using studbook data. During recent years an increasing number of molecular genetic studies have been published on captive populations of several endangered species. However, a comprehensive analysis of these studies is still outstanding. Here, we present a review of the published literature on *ex situ* conservation genetics with a focus on molecular studies. We analysed 188 publications which either presented empirical studies using molecular markers (105), studbook analyses (26), theoretical work (38), or tested the genetic effects of management strategies using model species (19). The results show that inbreeding can be minimized by a thorough management of captive populations. There seems to be a minimum number of founders (15) and a minimum size of a captive population (100) necessary in order to minimize a loss of genetic diversity. Optimally, the founders should be unrelated and new founders should be integrated into the captive population successively. We recommend that genetic analyses should generally precede and accompany *ex situ* conservation projects in order to avoid inbreeding and outbreeding depression. Furthermore, many of the published studies do not provide all the relevant parameters (founder size, captive population size, H_o , H_e , inbreeding coefficients). We, therefore, propose that a general standard for the presentation of genetic studies should be established, which would allow integration of the data into a global database.

Keywords Genetic diversity · Inbreeding · Outbreeding · Population genetics · Reintroduction · Zoological garden

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Introduction

The conservation of species is a challenging task due to the ongoing threats to biodiversity (Butchart et al. 2010). Although in situ conservation represents by far the most effective way to protect endangered species, it is evident that not all species can be efficiently preserved in their natural habitats. Consequently, the role of zoos and aquariums has changed from historical menageries that collect and exhibit exotic animals to modern institutions that actively contribute to conservation, scientific research and public education. Since the 1980s, many zoological gardens coordinate their breeding programmes in “European Endangered Species Programmes” (EEPs) and “Species Survival Plans” (SSPs). In 1993, the first World Zoo and Aquarium Conservation Strategy (WZACS) was published (WAZA 2005). This document proposes clear goals for zoos and aquariums, including the need to support both in situ and *ex situ* conservation projects. Meanwhile, *ex situ* conservation programmes and reintroductions of captive bred animals have become widespread measures to protect endangered species (Fischer and Lindenmayer 2000; Storfer 1999).

A number of difficulties in captive breeding can affect the survival of a captive population and the success of a reintroduction programme. Among these known difficulties, the requirements for physical health and behavioural anomalies (e.g. stereotypy) have received much attention (Kirkwood 2003). However, even if captive animals are healthy and show no behavioural anomalies, they may adapt genetically to their captive environment (for a review on adaptation in captive breeding programmes see Williams and Hoffman 2009). A major goal of captive breeding programmes is the preservation of maximum genetic variability within a species (Pelletier et al. 2009). For this purpose, founders need to be sampled from several populations and care must be taken that the captive population is not dominated by a small number of genetic lineages. Problems in the genetic representation might be particularly important in species where one population is readily accessible, whereas others are rare. For example, the captive population of the Jamaican yellow Boa (*Epicrates subflavus*) was founded by individuals from only one wild population even though more wild populations were available (Tzika et al. 2009). Indeed, an appropriate representation of genetic lineages is a difficult task, as the right balance between inbreeding and outbreeding must be found. Another important aspect of the genetic management of captive populations is to avoid inbreeding and a loss of genetic diversity (Ralls and Ballou 1986). Captive populations are often started with a low number of founders, either because it is difficult to collect more individuals or because there simply are no more available (Leberg and Firmin 2008). For example, the breeding programme for the Mexican wolf (*Canis lupus baileyi*) was started with the last obtainable seven animals (Hedrick et al. 1997; Hedrick and Fredrickson 2008). This low number of founders increases the risk that deleterious alleles become homozygous and lead to inbreeding depression. The origin and genetic relationships of founders are also often insufficiently known, particularly if confiscated individuals are included in a captive population. Although inbred individuals could be outcrossed rapidly (Hedrick 2005), it is often difficult to obtain new founders from wild populations, especially in the case of endangered species.

Captive breeding programmes should aim at the creation and conservation of healthy, self-sustaining captive populations that resemble their wild counterparts as closely as possible both in behaviour and genetics (Frankham 2008; Robert 2009; Goncalves da Silva et al. 2010; Ralls and Ballou 1986). By taking heed of this, managers can serve two needs: (1) conserving an endangered species in captivity, and (2) creating a captive stock that is suitable as a source for future reintroduction programmes. Inbreeding and the loss of

genetic diversity has been recognized as a major problem in *ex situ* conservation since the 1970s (Ralls et al. 1979; Bouman 1977), which has led to the implementation of captive breeding schemes (Pelletier et al. 2009). Traditionally, the genetic management of endangered species in zoological gardens is based on studbook data only, which is useful for the analysis of inbreeding effects (Ralls and Ballou 1986) and for quantitative genetic research (Pelletier et al. 2009). However, the calculations for inbreeding coefficients in studbooks are based on the assumption that founders are unrelated and non-inbred ($m_k = 0$) and that individuals of unknown origin have a high level of relatedness ($m_k = 0.5$), which may substantially influence the results of such analyses (Ruiz-Lopez et al. 2009; Goncalves da Silva et al. 2010). A recent review analysed the pedigree data of 119 zoo populations to assess the effects of inbreeding on these populations (Boakes et al. 2007). This analysis showed, that inbreeding depression is a common phenomenon in zoo populations. However, without genetic analyses it is not possible to assess the accuracy of studbook data (Boakes et al. 2007). Pedigree data has been shown to be erroneous in several captive populations (e.g. Przewalski's horses (Bowling et al. 2003); Waldrapp ibises (Signer et al. 1994); Arabian oryx (Marshall et al. 1999)). Molecular genetic analyses can provide much more realistic insights into the relationships within captive populations and their population genetic structure. During recent decades, the availability of highly polymorphic genetic marker systems, particularly microsatellites, has led to an increasing number of studies on the genetic structure of captive stocks.

The present review covers the published results of molecular studies on *ex situ* conservation programmes with a focus on the population genetics of endangered species. Our aim was to synthesize, how breeding programmes influence genetic diversity and inbreeding in captive populations. We were particularly interested to see how population size or founder size influences the genetic structure of captive populations. Also, we propose guidelines for the establishment of captive populations and for the presentation of genetic evaluations of captive breeding programmes. As the problem of management units (MU) has already been reviewed elsewhere (de Guia and Saitoh 2007; Fraser and Bernatchez 2001), the problem of hybridization, outbreeding and defining MUs is not discussed here in depth. Fitness related effects of inbreeding (inbreeding depression) which have been reviewed before (e.g. Keller and Waller 2002; Boakes et al. 2007; Armbruster and Reed 2005) are not analysed here either. Another important problem (adaptation to captivity) is not covered here in detail, as this has rarely been studied in zoo species (but see Williams and Hoffman 2009; Gilligan and Frankham 2003; Frankham 2008).

Methods

Literature search

We conducted an extensive search through the available literature cited by ISI using two databases ("Web of Science" and "Zoological Record"). The term combination used in our search was: "TS = (genetic OR microsatellite* OR allozym* OR SNP*) AND TS = (inbred* OR outbred* OR hybrid* OR heterozyg*) AND TS = (captiv* OR zoo OR *ex situ* OR breed* OR purg*) AND TS = (conserve* OR endang* OR threat*)". Relevant references in the articles found by this search were also included, as well as papers citing these articles. Although it was certainly not possible to cover all published studies in this search (particularly not grey literature such as minor zoo journals or reports),

it is likely that the majority of relevant articles have been incorporated. We included all studies published by December 2010.

Inclusion/exclusion criteria

The criteria for considering a study for this review were the following: (1) The study should deal with a zoo population or captive breeding programme with conservation as its purpose. (2) The study must involve an endangered species and not a domestic breed or game species. (Studies on non-endangered species were considered in the text, if the species served as a model for captive breeding and species conservation or if it was used in experiments on population genetic modelling.) (3) Fish species were only considered if the species was endangered but not used commercially. (4) Studies dealing with reintroduction were considered if captive bred individuals were used. Among the included studies we distinguished between the categories: (a) theoretical work and reviews, (b) studies investigating the genetic information via calculated data from the studbook or reporting inbreeding effects from a captive breeding population, and (c) studies analysing genetic diversity with molecular markers. Based on these criteria we found a total of 188 relevant studies (see supplementary file), 105 of which were presenting molecular genetic data. More than half of the studies used microsatellites ($n = 58$), we focused on these studies to uncover the effects of captive breeding on genetic diversity. The remaining molecular studies used other marker systems (mtDNA, allozymes, AFLPs, RFLPs, RAPDs, ISSRs, karyotype analyses, MHC) and were, therefore, not comparable with microsatellite data.

Variables coded from each study

The information obtained from molecular studies (category c) was entered into a database (Table 1). However, as different molecular methods cannot be compared directly, we decided only to analyse studies using microsatellites ($n = 58$). If studies included multiple subspecies or samples from captive, wild and reintroduced populations (see variable “population status”), all the sampled populations were recorded separately. Where possible the data derived from founders of a captive population was also recorded separately. Studies with mixed samples that were not separately analysed by the authors were excluded.

Data analysis

As most studies did not provide all the parameters needed for our analysis, we extracted different data sets for each parameter (F_{IS} , H_o , H_e). For studies that analysed both captive and wild populations, we performed ANOVAs to test for differences in H_e , F_{IS} and the number of alleles. In order to test for correlations between the number of founders or the size of the captive population with observed (H_o , $n = 48$ studies) and expected heterozygosities (H_e , $n = 54$), we performed linear regression analyses. The numbers of individuals were log-transformed prior to the analysis to improve homogeneity of variances. Since the taxa may differ substantially in their effective population sizes, it would be expected that “taxon” will also affect the data. Therefore, we also performed an ANCOVA with “taxon” and $\log N_{\text{captive}}/\log N_{\text{founders}}$ as explanatory variables. Based upon the regression analysis, we sought to determine N_{founders} and N_{captive} necessary to maintain high genetic variability (H_e). To obtain a desirable value for H_e in captive populations, we

Table 1 Information recorded from each study on the genetic diversity of a captive population using molecular markers

Variable	Meaning
Species	Species or subspecies studied
Population status	This variable explains whether the population was captive, founders of a captive population, wild, reintroduced or reintroduced using a captive source population (“reintroduced from captive”)
n captive pop	Number of individuals in the captive population
n founders	Number of founders for the captive population
n wild	Number of individuals in the wild at the time the study was performed
n sampled	Number of individuals sampled for genetic analysis
Marker	Molecular marker used
n loci	Number of analysed loci
H_e	Expected heterozygosity
H_o	Observed heterozygosity
F_{IS} cal	Inbreeding coefficient calculated from studbook data
F_{IS} gen	Inbreeding coefficient from genetic analysis
n alleles	Average number of alleles per locus

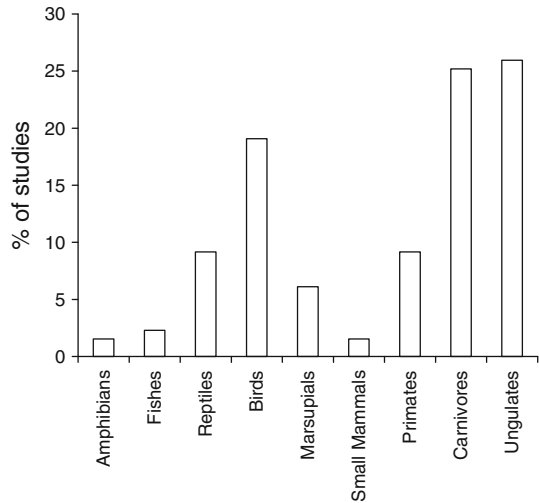
used the mean H_e found in the corresponding wild populations reviewed in this study ($n = 29$). Theory predicts a clear relationship between the number of contributing founders and the loss of genetic diversity in breeding programmes (Frankham et al. 2010). However, it is difficult to compare these theoretical values directly with empirical data, because (1) the number of generations in captivity is usually not given and (2) the number of founders are generally census population sizes (N_c) and not effective population sizes (N_e). The statistical tests were carried out in R 2.10.0 (R Development Core Team 2009).

Results

Study characteristics

Most of the surveyed studies (70%) dealt with specific breeding programmes for endangered species. Some articles contained theoretical considerations on inbreeding and the effect of captive breeding on genetic diversity or disease risks (20%). Others focused on developing optimal management strategies or the influence of captive breeding on the survival of reintroduced populations using model species (10%). The studies dealing with specific breeding programmes showed a marked bias in research efforts (and possibly also a publication bias) towards certain taxa (Fig. 1). Most of them studied carnivores, ungulates (including elephants) or birds (25, 26 and 19%, respectively), but there were also a few on primates (9%) and reptiles (9%). Studies on small mammals, marsupials and fish species were scarce (2, 6 and 2%, respectively). There were only two studies on endangered amphibian species (Kraaijeveld-Smit et al. 2006; Beauclerc et al. 2010) and none at all on endangered invertebrates. These results mirror the well-known bias towards charismatic vertebrates in conservation, research and publication policy (Artacho 2006; Maslin 2006; Gippoliti and Amori 2007). Most studies considered only one species or subspecies (90%). About 70% analysed only captive individuals, whereas 30% compared a captive

Fig. 1 Percentage of specific analyses on certain taxa (both genetic and non-genetic studies), $n = 131$. Out of practical reasons the two elephant studies were included in the ungulate category



population with either a natural wild population or reintroduced populations (or both). Only few species or populations (like the Mexican wolf or the black-footed ferret) were reassessed some years later.

The year of publication ranged from 1979 to 2010, but most of the research ($\sim 60\%$) was carried out during the last decade (Fig. 2). Studbook analyses were performed throughout the whole period (sometimes in combination with genetic data), but many of them were labelled as “genetic analysis”. Genetic studies using molecular markers already started in the late 1980s, but most were carried out after 2000. There is a clear temporal trend in the use of different molecular marker systems (Fig. 2). DNA fingerprinting and allozymes were mainly used during the 1990s. AFLPs were mainly used for fishes (but altogether not very often), mtDNA was applied rarely (due to its limited usefulness for population genetics) but throughout the whole period. Microsatellites were first applied in the mid 1990s and have now become the most frequently used marker system (52% of all the studies used microsatellites). Only 16 out of 105 studies combined different marker systems, mostly mtDNA and microsatellites.

Many studies presented some relevant basic data on the species or population studied, but unfortunately the quality of this information differed substantially. The data often included the size of captive and wild populations or the number of founders for captive or reintroduced populations, but no study provided all of this information and the given data was usually only for a captive subpopulation. The expected and observed heterozygosities (H_e and H_o) were given in 90 and 79% of the studies, respectively, but only 67% gave both measures. Despite the fact that most statistical programmes used for population genetic analysis almost automatically calculate a genetic inbreeding coefficient (F_{IS}), and although it presents important information for an *ex situ*-conservation project, only 38% of the studies mentioned this measure. For 15 studies, we calculated F_{IS} values from the information on H_e and H_o according to Wright (1931): $F_{IS} = (H_e - H_o)/H_e$. An inbreeding coefficient calculated from studbook data was documented only in 18% of the studies. There were only three studies reporting a significant correlation between relatedness derived from studbook data (f) and the inbreeding coefficient (F_{IS}) (Nielsen et al. 2007; Ruiz-Lopez et al. 2009) or observed heterozygosity (H_o) (Ellegren 1999). Only four studies calculated the effective population size (N_e) of the captive population.

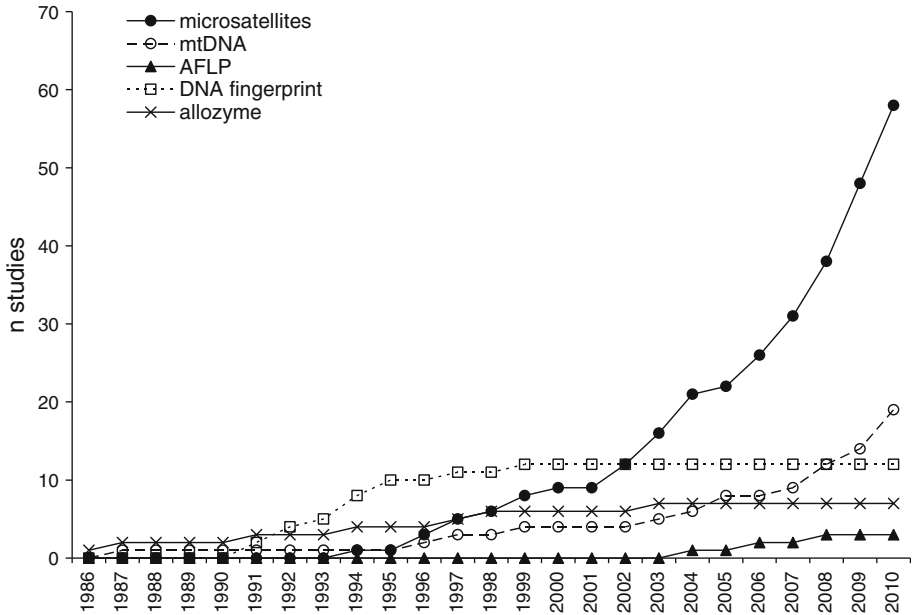


Fig. 2 Cumulative number of studies using certain molecular markers since 1986. Only the five most frequently used methods have been considered

Temporal trends in genetic diversity and comparisons with wild populations

Six studies tested the temporal course of the genetic structure of captive populations. This was done by comparing recent samples with the founders or historical samples from museum specimens ($n = 4$) or by reassessing the population after some years ($n = 1$). One study (Kraaijeveld-Smit et al. 2006) compared the genetic diversity of the founders with the genetic diversity found in short-term and long-term captive populations. The results from these comparisons were mixed, but the data indicated that long-term captive breeding leads to a loss in expected heterozygosity and the number of alleles. When captive populations were compared with wild populations (18 studies including 33 captive and 29 wild populations), the mean difference in H_e , F_{IS} and number of alleles was not significant (ANOVAS, $P > 0.1$, $H_{e\text{ wild}} = 0.6 \pm 0.02$; $H_{e\text{ captive}} = 0.57 \pm 0.02$; $F_{IS\text{ wild}} = 0.05 \pm 0.02$; $F_{IS\text{ captive}} = 0.04 \pm 0.02$; $n\text{ alleles}_{\text{wild}} = 4.71 \pm 0.25$ $n\text{ alleles}_{\text{captive}} = 4.14 \pm 0.23$). However, there was some variation in the responses of these three genetic parameters to captivity (Table 2). Twenty-four studies tested for differences between H_o and H_e in captive populations, nine of which (37.5%) were significant. Except for one, in all of these cases H_o was lower than expected (arithmetic mean: 0.036).

Correlations of population size or number of founders with H_e , H_o and F_{IS}

Our analysis revealed a strong correlation with H_e of both captive population size and the number of founders. The expected heterozygosity was positively correlated with the log number of founders ($r^2 = 0.28$, $P < 0.001$; Fig. 3a) and also with the log size of the captive population ($r^2 = 0.20$, $P = 0.013$; Fig. 3b). A similar pattern was observed for H_o (correlation with $\log N_{\text{founders}}$: $r^2 = 0.19$, $P = 0.011$). The correlation between H_o and

Table 2 Effects of captive breeding on genetic diversity compared to wild populations

Species group	Species	Effect of captive breeding on			
		H _e	n alleles	F _{IS}	References
Amphibian	<i>Alytes muletensis</i>	Negative	Negative	–	Kraaijeveld-Smit et al. (2006)
Amphibian	<i>Peltophryne lemur</i>	Neutral	Neutral	Neutral	Beauclerc et al. (2010)
Bird	<i>Falco naumanni</i>	Neutral	Neutral	Neutral	Alcaide et al. (2010)
Bird	<i>Falco peregrinus</i>	Neutral	Neutral	Neutral	Jacobsen et al. (2008)
Bird	<i>Gypaetus barbatus</i>	Positive	Positive	Positive	Gautschi et al. (2003)
Bird	<i>Gyps fulvus</i>	Neutral	Neutral	Negative	Le Gouar et al. (2008)
Carnivore	<i>Canis lupus</i>	Neutral	Neutral	–	Ellegren et al. (1996); Ellegren (1999)
Carnivore	<i>Ailuropoda melanoleuca</i>	Negative	Neutral	Positive	Shen et al. (2009)
Carnivore	<i>Canis lupus signatus</i>	Neutral	Neutral	Negative	Ramirez et al. (2006)
Carnivore	<i>Mustela nigripes</i>	Positive	Neutral	Negative	Wisely et al. (2003)
Chiroptera	<i>Pteropus rodricensis</i>	Negative	Negative	Positive	O'Brien et al. (2007)
Marsupial	<i>Onychogalea fraenata</i>	Negative	Negative	Neutral	Sigg (2006)
Marsupial	<i>Perameles bougainville</i>	Positive	Positive	–	Smith and Hughes (2008)
Marsupial	<i>Macrotis lagotis</i>	Negative	Negative	–	Smith et al. (2009)
Reptile	<i>Alligator mississippiensis</i>	Positive	Positive	Negative	Glenn et al. (1998)
Reptile	<i>Epicrates subflavus</i>	Negative	Negative	–	Tzika et al. (2009)
Ungulate	<i>Capra ibex (ibex)</i>	Neutral	Negative	Neutral	Maudet et al. (2002)
Ungulate	<i>Tapirus bairdii</i>	Positive	Positive	Neutral	Norton and Ashley (2004)

log N_{captive} was not significant ($r^2 = 0.08$, $P = 0.12$). If the highest values of N_{captive} and N_{founders} are deleted, the correlation even improves. No significant effect of the variable “taxon” was detected (ANCOVA, $F_{8,30} = 0.55$, $P = 0.81$).

One aim of captive breeding programmes is to maintain 90% of the natural genetic diversity for 100 years (Frankham et al. 2010). It is evident that microsatellite loci differ in their variability and taxa also may differ in their natural genetic diversity. Hence, it is rather difficult to obtain a standardized threshold value for H_e , which should be reached in captive breeding programmes. Based upon the mean H_e of 0.60 (± 0.02 SE) for wild populations calculated above, a desirable 90% threshold would be $H_e = 0.54$. Our analysis shows that captive populations with $N_{\text{founders}} > 15$ consistently exceed this threshold. For N_{captive} this value was 100 individuals. There were only isolated cases in which a higher number of founders (*Perameles bougainville*: Smith and Hughes 2008) or captive individuals (*Alligator sinensis*: Xu et al. 2005; *Oryx leucoryx*: Marshall and Spalton 2000) failed to reach these values. $F_{IS} > 0.2$ also only occurred in populations with less than 15 founders or 100 captive individuals. Due to the logarithmic relation between H_e and N the addition of single individuals has a strong impact when the number of founders (or captive individuals) is still low. With increasing N the impact of each individual substantially decreases. For example, an addition of one individual to $N_{\text{founders}} = 2$ increases H_e by 2.7%, whereas it is only 0.33% for $N_{\text{founders}} = 20$.

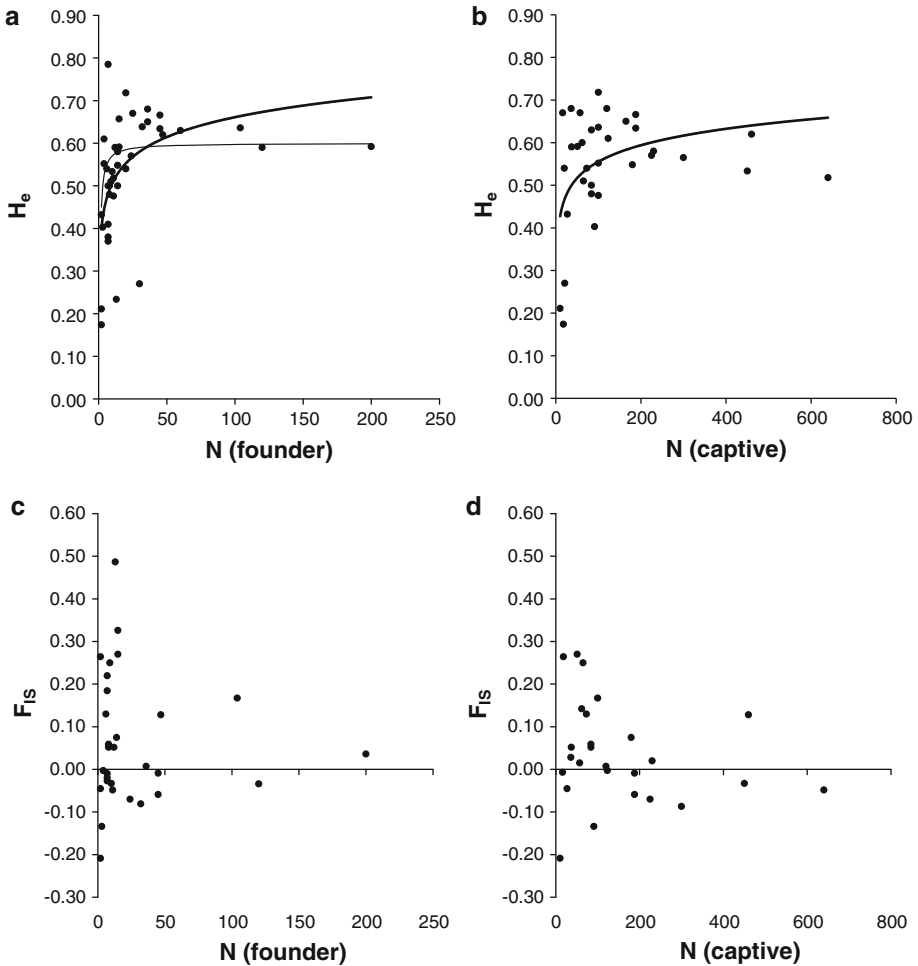


Fig. 3 Correlation between expected heterozygosity (H_e) and the founder size (a) and the size of the captive population (b). The *thin line* in (a) indicates the amount of retained heterozygosity after one generation predicted by theory (Frankham et al. 2010) based upon a mean H_e of 0.6 as found in the wild populations (see text). The *lower graphs* show how the number of founders (c) and the size of the captive population (d) correlate with the inbreeding coefficient (F_{IS})

For F_{IS} the variation of values decreased with an increasing number of founders and an increasing captive population size (Fig. 3c, d). The mean F_{IS} across all studies was 0.060 ± 0.02 SE ($n = 42$). There were only four captive populations with an $F_{IS} > 0.25$. Among the 26 studies that analysed studbook data, 23 dealt with the effects of inbreeding on captive populations (studies that analysed the same species were counted as one study: $n = 18$ covering 20 species or subspecies). In nine studies inbreeding had a negative effect on the captive population. Negative effects included higher infant mortality, lower semen quality, lower litter size and hereditary defects (for an overview on hereditary effects caused by inbreeding in several carnivore species see Laikre 1999a, b).

Discussion

Our analysis shows that inbreeding and loss of genetic diversity can be minimized in captive breeding programmes by thorough population management. The genetic consequences of captive breeding are highly variable depending on a number of factors, such as the number and relatedness of founders or the period for which breeding has been coordinated by a studbook (Ruiz-Lopez et al. 2009). Franklin (1980) and Soulé (1980) suggested that an effective population size of 50 would be sufficient to avoid inbreeding. Lacy (1987) proposed that a higher number (100 individuals) was needed and experimental data with fruit flies also suggests that a number of 50 would be far too low (reviewed in Frankham et al. 2010). The results of our analysis support the recommendation that a captive population should number at least 100 individuals (based on N_c , see below) to effectively counteract inbreeding and a loss of genetic diversity (Fig. 3). However, if the goal is to retain evolutionary potential, the effective population size needs to be much larger (e.g. Franklin 1980: 500; Lande 1995: 5000). A recent analysis showed that 67% of *ex situ* populations in AZA (Association of Zoos and Aquariums) institutions have a population size of less than 100 individuals (Baker 2007) and the mean N_e of SSP (species survival plan) populations is 41 (Frankham et al. 2010). This indicates that zoo stocks still require a lot of restructuring in order to make captive breeding a more valuable contribution to species conservation (Baker 2007; Leader-Williams et al. 2007).

The number and identity of founders is a major factor determining the gene pool of the captive population. Therefore, it obviously should have a greater impact than the captive population size. Frankham et al. (2010) proposed that an N_e of 20–30 founders is needed in order to maintain genetic diversity. According to our analysis a minimum founder size of 15 individuals (N_c) seems to be sufficient to preserve an H_e of 0.54 (i.e. 90% of the mean H_e in wild populations). We only found one exception (Western barred bandicoot: *Perameles bougainville*), in which H_e was low (0.27) despite the number of 30 founders (Smith and Hughes 2008). However, in this case the sampling of the captive population was rather low ($N = 8$) and H_e was also low in the wild populations. Furthermore, a second captive population had an even lower founder size ($N = 20$) and substantially higher genetic diversity ($H_e = 0.54$).

It has to be noted, that all population sizes presented in the analysed publications refer to the census size (N_c) and not to the effective population size (N_e). These two values might differ considerably within a population depending on the contribution of each individual to reproduction, which is influenced by factors like the mating system, sex ratio, genetic bottlenecks and ploidy (Hedrick 2005). The relatedness between the founders also plays an important role for N_e of captive populations. Closely related individuals (family groups, herds, regional subsamples) might be sampled for practical reasons, while recruiting breeding stocks. Some species are much more prone to such non-random sampling due to their social structure and breeding system. Also, the susceptibility of the species to inbreeding needs to be considered. Species with small ranges (for example on islands) are thought to be less affected by inbreeding depression, because the genetic load may have been reduced by natural purging (Leberg and Firmin 2008; Princée 2001). For inbred *in situ* populations the number of founders as well as the captive population size needs to be much greater and it should be aimed at obtaining specimens from additional populations.

It has to be mentioned, that our analysis did not consider the time that a population has been kept in captivity. The genetic effect of the time spent in captivity has only been studied in a single species (Kraaijeveld-Smit et al. 2006). The results of this study confirmed that the time spent in captivity is negatively correlated with genetic diversity, which

is also predicted by theory (Frankham et al. 2010). In many captive breeding programmes, additional individuals are successively introduced into the *ex situ* population, which can help to avoid genetic erosion.

In general, it is advisable to use as many founders as possible to start a captive breeding programme, which should be non-inbred and unrelated (Soulé et al. 1986; Ralls and Ballou 1986). Later introductions of additional individuals might be necessary, but it would be ideal if the specimens were genotyped before being incorporated into the population, in order to clarify their origin and relatedness with other captive individuals. Evidently, the minimum numbers given above are still rough estimates and will not ultimately prevent a loss in genetic diversity. Significant adaptation to captive conditions can occur within some tens of generations (Gilligan and Frankham 2003). Therefore, it has been proposed that captive breeding programmes for reintroductions should not start sooner than is necessary and “prophylactic” captive breeding should be avoided (Snyder et al. 1996). In order to prevent adaptation to captivity, *ex situ* conservation needs to be optimized in a way that animals will be returned to the wild as soon as possible (Snyder et al. 1996). However, there is a trade-off between capturing a maximum of genetic diversity found in the wild and delaying *ex situ* conservation as long as possible. Furthermore, such a strategy requires sound knowledge of the population trends in wild populations. A possible solution to prophylactic captive breeding would be to regularly augment the captive populations with individuals from the wild, thereby reducing adaptation to captivity to a minimum.

Although these general recommendations on *ex situ* conservation strategies have already been proposed decades ago (e.g. Snyder et al. 1996; Ralls and Ballou 1986), there is still a discrepancy between theoretical knowledge and practical implementation. More than 10,000 species are currently kept in zoological gardens (ISIS 2010), but according to WAZA only 850 taxa are currently managed by studbooks (international and regional) and/or breeding programmes (WAZA 2010). However, only 118 of the international studbooks were labelled as “active”. Indeed, a high number of critically endangered species definitely still need *ex situ* conservation programmes. Appropriate breeding programmes need to be established for these species or the existing programmes need to be assessed and optimized. In fact, it has been stated, that fewer than 200 threatened mammals are sustainably propagated in zoos (Conway 2007). Current zoo stocks still comprise a high number of species that are either common, severely inbred or outbred or just kept for commercial purposes. We have to admit that keeping charismatic megavertebrates is crucial for the economic survival of zoos and that these species have an important function as flagship species for conservation and to attract visitors. On the other hand, legal obligations to keep some large mammal species, such as the African elephant, are increasing and some zoos have to allocate large areas of their premises in order to keep this species. This is in stark contrast to the fact that the African elephant certainly can be protected efficiently in the wild and the population trend is increasing (Blanc 2008). Zoological gardens need to restructure their stocks and should aim at achieving a balanced portfolio of charismatic flagship species and highly endangered species that are kept for *ex situ* conservation (Leader-Williams et al. 2007; Conde et al. 2011).

Management units

Another important problem in *ex situ* conservation is that the phylogenetic relationships of the available individuals, populations or species are often not sufficiently known. A number of examples exist where zoos accidentally mixed individuals that belonged to different species, subspecies or other management units for lack of knowledge about the

systematic relationships. A well-known example is the frequent hybridization of the Bornean Orang-utan (*Pongo pygmaeus*) and the Sumatran Orang-utan (*Pongo abelii*). These two species had formerly been managed as a single species, but subsequently it became evident that they are strongly differentiated (Zhi et al. 1996). Therefore they are now listed as two distinct species in the IUCN red list (Singleton et al. 2008; Ancrenaz et al. 2008). The multitude of phylogenetic studies carried out during the last decades reveal that many taxa consist of several cryptic species or subspecies, which might be morphologically similar but show significant genetic differentiations (Hochkirch and Görzig 2009). In a captive population of dik-diks, infertile individuals turned out to be crosses between cryptic chromosomal races (Ryder et al. 1989). As speciation is often a long lasting process with many intermediate stages, genetic differentiation may also affect populations within a species. A genetic survey of the Asian elephant revealed two matrilinear clades and male hybrids between these clades show reduced fertility (Fickel et al. 2007).

It is important that management units are defined and breeding programmes focus on threatened lineages. For example, the North American raccoon (*Procyon lotor*) is frequently held in European zoos, even though it is common in its native range and even invasive in parts of Europe and Asia (Schmidt 1999). In contrast, the critically endangered Pygmy raccoon (*Procyon pygmaeus*) is rarely held (but work is in progress to establish a captive breeding programme; Cuarón et al. 2008). Conservation priorities are not always as clear as in this case, however. The definition of Evolutionary Significant Units (ESUs) and Management Units (MUs) has been the subject of much debate and the underlying problems are similar to those in the discussions on species concepts. Up to now several concepts and definitions have been proposed (see de Guia and Saitoh 2007 for a review). In order to assign populations to optimal management units, many kinds of information should be considered, such as life history traits, ecological and demographic data, as well as molecular genetic data (Allendorf and Luikart 2007). The approach proposed by Crandall et al. (2000) uses genetic and ecological inexchangeabilities over recent and historical times to identify separate MUs. It thus considers adaptive distinctiveness, combines genetic and ecological principles and is testable in the form of null hypotheses (Fraser and Bernatchez 2001). However, conducting such an expensive and time consuming study is not always feasible. Particularly in highly endangered taxa quick assessments are often necessary. In these cases provisional MUs can be defined based on significant differences in allele frequencies at neutral marker loci and/or mitochondrial DNA haplotypes (Avice 2004). Nevertheless, it should always be kept in mind, that these MUs might not capture traits like environmental adaptations or learned behaviour. Hence, they can lead to insufficient protection of possibly unique populations. On the other hand, the use of highly polymorphic molecular markers can also lead to an exaggerated splitting of populations into separate MUs (Allendorf and Luikart 2007). This can happen, if significant differentiation is caused by anthropogenic population fragmentation and subsequent gene drift which does not reflect biologically meaningful adaptation. Thus, if possible, the history of the populations in question should also be considered. In any case, the definition of management units should not rely on a single marker system, particularly not just on mitochondrial DNA, which may easily be introgressed into the gene pool of related species.

A guide to implementing *ex situ* programmes

From the considerations discussed above, we conclude that detailed information on the status of a species is needed in order to plan and manage a successful captive breeding

programme. We therefore recommend following the steps below in establishing a new captive breeding programme:

- (1) Obtain data on the natural genetic structure of the wild populations of endangered species; possibly also include populations that are extinct in the wild by using museum material.
- (2) Define management units based on the genetic data and prioritize these management units within the species (considering local adaptations, avoiding outbreeding).
- (3) Collect basic information on the existing captive population and holders.
- (4) Analyse the genetic structure of the captive population and assign captive individuals to the management units defined in step 2.
- (5) Select suitable individuals for a captive breeding programme for a defined management unit and create a studbook.
- (6) If possible, collect wild individuals in order to maximize the genetic variability and represent missing genetic lineages for a management unit.
- (7) Reassess the genetic structure of the captive population after some generations.

This list shows that genetic analyses are valuable at several stages of an *ex situ* conservation project. They can help to determine founder origin, founder relationships and uncover erroneous pedigree information, as has been shown for the Przewalski's horse and the Asiatic lion (for a short overview see Ivy et al. 2009).

Management strategies

Inbreeding has been widely recognised as a major problem in captive breeding (e.g. Boakes and Wang 2005; Frankham et al. 2010; Leberg and Firmin 2008). Purging (deliberate inbreeding in order to eliminate recessive, deleterious alleles) has sometimes been proposed as a management tool in *ex situ* conservation, but studies have shown that the results of purging are unpredictable. Therefore, it should not be applied in the management of captive populations (see Leberg and Firmin 2008; Boakes et al. 2007), and particularly not to endangered species. The "traditional" approach to avoid inbreeding depression is to minimize potential bottlenecks and to avoid matings between closely related individuals based on studbook data. However, although the concept of studbooks is persuasive, some underlying assumptions are often not met in real world *ex situ* populations. First of all, there are usually only a few individuals available to establish a captive population (Leberg and Firmin 2008). More importantly, the genetic relationships of the founders (and also of individuals later integrated into the breeding stock) are usually not known, but inbreeding calculations for studbooks are based on the assumption that founders are unrelated and non-inbred (Ruiz-Lopez et al. 2009). This assumption may be logistically necessary, but it does not accurately describe the true relationships between the founders (Russello and Amato 2004) and might therefore lead to severe underestimates of inbreeding coefficients (Ruiz-Lopez et al. 2009).

The most widely accepted management approach is Minimizing Kinship (MK) (Falconer and Mackay 1996), where the overall level of relationships in the population is minimized to maximize the retention of gene diversity (Ballou and Lacy 1995; Saura et al. 2008). This approach is generally recommended in conservation breeding programmes (Saura et al. 2008), but detailed pedigree data for the captive population is needed to find optimal breeding pairs (Ballou and Lacy 1995; Caballero and Toro 2000). A related, but more simple approach is the Maximum Avoidance of Inbreeding scheme (MAI) (Kimura and Crow 1963). MAI involves equalizing family sizes and a circular mating pattern,

where females are mated to males of different subpopulations each year (Windig and Kaal 2008; Frankham et al. 2010). This scheme is recommended when a large proportion of the pedigree is unknown (Ballou and Lacy 1995). Simulations show that high levels of genetic variation can be maintained by using MAI (Ballou and Lacy 1995). But due to the complex breeding scheme which has to be followed precisely, this strategy is sometimes very difficult to apply and it is only fully effective, if it is applied from the first generation (i.e. the founders) on (Frankham et al. 2010). Another strategy is to use a breeding circle. Breeding circles are rotational breeding schemes, where each subpopulation provides males for its neighbouring subpopulation. This approach has many practical advantages; no pedigree data is needed, new subpopulations can easily be incorporated into the scheme, geographical distances for translocations between subpopulations can be minimized by careful design of the breeding circle and the scheme can not suffer from incompatibilities between individual owners (Windig and Kaal 2008). This last approach is especially useful if species are maintained as groups without individual pedigree information, as is often the case for invertebrates, fishes and some bird species. Generally, the management strategy for any *ex situ* population should be adapted to the special social structure, mating system, generation time, size, trophic level and other bionomic variables of each species.

How to perform a genetic assessment of a zoo population

Microsatellites are currently the best neutral marker system to study the population genetics of zoo populations (although other promising methods are currently arising, see below). Currently, the disadvantage of microsatellites is that the development of specific primers can be time consuming and expensive. Although the costs for primer development are falling, they may still be too high for the budgets of small projects. Fortunately, primers are already available for many bird and mammal species, and the number is steadily increasing. Furthermore, cross-species amplification is possible in many cases (Barbará et al. 2007). As mentioned above, a genetic study of field populations should precede or accompany *ex situ* studies in order to obtain information about the natural genetic variation and the identity of management units. A sufficient number of polymorphic microsatellite loci should be used (at least 10 loci) and it is advisable to combine this marker system with sequencing mtDNA to facilitate the identification of introgression events. It is also necessary to sample a sufficiently high number of specimens from the captive population. Ideally, all individuals should be genotyped and only genotyped individuals should be included in a studbook. Another frequent problem in microsatellite analyses is the occurrence of scoring errors. Possible pitfalls of microsatellite analyses have been treated elsewhere (e.g. Birnbaum and Rosenbaum 2002; Selkoe and Toonen 2006).

The statistical analysis depends on the research objectives, but some standard measures should be presented in any *ex situ* genetics study. This is particularly true for observed and expected heterozygosities as well as the inbreeding coefficients obtained from pedigree (f) and molecular data (F_{IS}). The pedigree inbreeding coefficient f measures the theoretical cumulative inbreeding over generations based upon observed (or assumed) relationships. It is based on the—often erroneous—assumption that the founders are unrelated and non-inbred (Ruiz-Lopez et al. 2009). Genetic studies revealed that studbooks often contain erroneous or incomplete pedigree information (e.g. Signer et al. 1994). The molecular inbreeding coefficient F_{IS} describes the deviation between observed and expected heterozygosities (assuming Hardy–Weinberg–Equilibrium). It thus measures non-random mating and is influenced by unknown population structure (Wahlund effect), scoring errors (null alleles), homoplasy (particularly in rapidly mutating microsatellites) and inbreeding.

It is, therefore, unlikely that values for f and F_{IS} will be identical. However, cumulative inbreeding will affect both measures. A correlation between both values is, therefore, intuitive and has been reported (Nielsen et al. 2007). A comparison of both measures in a meta-analysis may provide valuable insights, but most of the hitherto published studies only mention f , even though genetic data was available. Other measures that should also be given include the size of the captive population and the number of founders. It is also advisable to calculate the effective population size (N_e) from the genetic data. If the presentation of data is standardized, it could be fed into a database, which could be maintained by international organisations (e.g. WAZA, IUCN) and be made accessible to breeders and scientists. This would provide valuable information on the efficiency of *ex situ* conservation programmes.

Knowledge gaps and future research priorities

The majority of papers on *ex situ* conservation genetics analysed only the captive population. Comparisons with wild populations are very scarce, although it is exactly this comparison that is needed in order to evaluate whether the goals of breeding programmes for endangered species are really being met. This data would simplify the work of studbook coordinators by providing more detailed knowledge on the genetic variability of the breeding stock. Additionally, it is sensible to compare the genetic structure of reintroduced populations with the captive stock. Such studies are important as a major goal of *ex situ* conservation is to establish a suitable source for reintroductions. Long-term assessments of the genetic trends in *ex situ* populations are also very sparse. This is probably caused by the short-term funding of research projects. A general problem in *ex situ* conservation genetics is the incompleteness of population sampling. Often, not all holders are willing or able to contribute samples. Even if all holders participate, a number of administrative requirements can impede sample acquisition. The convention on the international trade in endangered species of wild fauna and flora (CITES) is an important and successful instrument of nature conservation. However, its regulations complicate the exchange of material, which can discourage potential participants. Also, the administrative effort required is often not compatible with the time scale of regular research projects. It would, therefore, be advisable to establish simplified procedures for scientific purposes that would facilitate sample acquisition.

Another important issue, which needs to be addressed in empirical studies using genetic markers is adaptation to captive environments (Williams and Hoffman 2009). Experimental studies with fruit flies and fishes have shown that adaptation to captive conditions can occur rapidly and might thus negatively influence even short-term breeding programmes (e.g. Ford 2002; Gilligan and Frankham 2003; Araki et al. 2007; Heath et al. 2003). For a review on adaptation to captivity see Frankham (2008) or Williams and Hoffman (2009). In order to avoid complications during reintroduction programmes, it is crucial to increase our knowledge on adaptation processes in captive breeding programmes for endangered species. For this purpose, non-neutral markers need to be studied, such as the major histocompatibility complex (MHC), which has been studied extensively in the wild (reviewed in Bernatchez and Landry 2003). Although MHC studies on captive populations of endangered species also exist, most of them focus on loss of genetic diversity rather than on adaptation to captivity (e.g. Hedrick et al. 2000; Sachdev et al. 2005; Marsden et al. 2009). On the other hand, other genes have received less attention and Radwan et al. (2010) caution not to focus on MHC diversity alone. The recent development of new techniques (SNPs, next generation sequencing) will certainly improve the options

for empirical research on genetic adaptations to captivity. In the future, these techniques will also become less expensive (Mardis 2006) and thus become affordable for applied conservation research. The rapid progress made in the field of genomics will probably help to understand processes of inbreeding and adaptation in greater detail. Captive populations represent ideal study objects for this kind of research.

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