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Research article

Long term vs short term impact of founder relatedness on gene diversity and inbreeding within the European Endangered Species Programme (EEP) for the Nepalese red panda (*Ailurus f. fulgens*)

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Abstract

Traditionally, studbooks have been used as the major tool for the scientific management of ex-situ populations organised in breeding programmes. Well documented pedigree information enables managers to sufficiently monitor population size, demographic stability and the level of genetic diversity. However, breeding programes can only maintain the genetic information which is brought into captivity by wild caught founder animals. In most studbooks little is known about those individuals. This is also true for the red panda (*Ailurus fulgens*), a charismatic but threatened flagship species. Conservation breeding of this remarkable mammal species started in the early 1960s and is nowadays coordinated through a Global Species Management Plan (GSMP). In Europe the Endangered Species Programme (EEP) forms the biggest regional breeding group of red pandas (subspecies *A. f. fulgens*). Little is known about the true origin of the 23 founders of the EEP and how their unknown relationships may affect their living descendants now and in the future. Studbook data on 1350 individuals were analysed for different assumptions about founder relationships and compared with each other. We provide studbook-based evidence that the annual change in genetic parameters (gene diversity and mean inbreeding) are not affected in long-term by the original founder relationship within strongly intermixed zoo populations managed for mean kinship. Our results point out the importance of good genetic management in the early years of breeding programmes. These early years are crucial in implementing knowledge of founder relationships in the studbook, since a population becomes genetically equalised within the first decades and new information obtained later has no significant effects on subsequent genetic trends.

Introduction

Studbooks are the major tool used for the scientific management of ex-situ populations organised in breeding programmes (Glatston 1986). As many captive breeding programmes suffer from small number of founders, optimal breeding management is necessary to ensure long term viability and genetic diversity (Frankham et al. 2010). Traditionally, management of most breeding programmes aims to minimise the overall kinship in a population (Ballou and Lacy 1995). Kinship (*f*) for two individuals is defined as the probability that two randomly drawn alleles at a given locus within these individuals are identical by descent from a common ancestor (Falconer 1981). The average of kinships between a specific individual and all living individuals within a population (itself included) is defined as an individual's mean kinship (*mk*) (Ballou and Lacy 1995). Breeding management recommendations are regularly given by calculating these mean kinship values (Ballou and Foose 1995) for all individuals under the assumption that all founders were unrelated. Individuals with a low *mk* value are prioritised breeders as they are less represented within the population. Individuals with similar *mk* values are paired to avoid linking rare and common founder lineages in offspring, thereby making it impossible to independently increase the representation of the under-represented founders (Saura et al. 2008). By doing so, the founders representation within the population can be equalised and the risk of losing unique alleles due to genetic drift can be minimised (Fienieg and Galbusera 2013).

A classic example of a species managed via studbook at an international scale is the red panda (*Ailurus fulgens*), a charismatic and beautiful but threatened flagship species contending with Himalayan conservation issues (Glatston 2011a). In 2015 the conservation status of red pandas in the IUCN Red List was changed from vulnerable to endangered, due to a population decline of 50% over the last three generations (Glatston et al. 2015). Beside in-situ conservation efforts within the natural range and habitat of the species, red pandas in captivity can represent a backup population for possible future reintroductions and conservation translocations, as well as having an educational role. That ex-situ management of wild species has potential to contribute to conservation purposes is widely recognised (IUCN/SSC, 2014). Regional management of red pandas in zoos started in the early 1960s and was, in contrast to many other species, shifted to a global scale in the early 1990s (Glatston and Princée 1993). The red panda studbook is one of the first in which population genetic studies based on simulation models were applied at an early stage (Princée 1988, 1989). Today, breeding of red pandas in captivity is organised through Global Species Management Plans (GSMP) and regional breeding programmes in Australasia, Europe, India, Japan, North America and South Africa. In total 632 living animals in 233 institutions are registered in the GSMP database. With 380 animals in 155 institutions (see Figure 1), the European Endangered Breeding Programme (EEP) maintains nearly 60% of the global red panda population (subspecies *Ailurus f. fulgens*) (Glatston 2015).

The keystone of a breeding programme is the inclusion of a suitable number of founders: wild-caught animals, providing the genetic information of the species which should be preserved within the programme. To capture 97.5% of the original genetic diversity at least 20 unrelated wild individuals are necessary (Leus and Lacy 2010). To capture a higher amount of gene diversity many more founders would be necessary. About 20 unrelated founders are considered to be an adequate compromise between gene diversity captured and the number of individuals needed to be removed from the (potentially endangered) wild population (Crow and Kimura 2009). However, as the term founder can only be used for those wild-caught animals which produced surviving descendants within the captive population, the necessary number of animals taken from the wild can be much higher than only 20 individuals. The zoo population of the red panda subspecies *Ailurus f. fulgens* can be traced back to 23 individuals imported from the wild between 1964 and 1974 and in 1984 (Glatston 2011b). Little is known about their origin, but recognising that they came into captivity in large groups, it is highly unlikely that all animals are completely unrelated.

For practical reasons all founders of a breeding programme are usually treated as unrelated (Ballou et al. 2010). Consequently all calculations of genetic values, calculated by special breeding management software, rely on this assumption (Traylor-Holzer 2011). However, this “studbook assumption” is an extreme simplification. It is most likely that animals taken at the same time and from the same place are related to each other in some way and belong to the same source population, whereas animals from other places may belong to different source populations.

Today, the red panda EEP population offers the unique opportunity to study both short and long term impact of original founder relationships on the changing genetic and demographic parameters in a captive population managed and selected for mean kinship. As a result of continued research on historical data, the studbook coordinators were able to add missing or incomplete information and validate the entire studbook for the time frame from 1977 until 2015 (Glatston, personal communication). This led to a database spanning 38 years of management and completely known founder lines. A hundred percent of the living red panda population’s ancestry is known, allowing the best possible genetic evaluation by population software.

There is little evidence on how different levels of founder relationships change the quality of a breeding programme and its ability to preserve genetic diversity over time, although various researchers have used molecular methods to estimate the relationship between founders in the past for conservation purposes (Geyer et al. 1993; Haig et al. 1994; Russello and Amato 2004). Rudnick and Lacy (2008) tested computer simulated populations with different numbers of founders and compared the benefit of knowing the genetic relationships among founders, with applying the traditional “studbook assumption” of zero founder relatedness. They concluded that different founder assumptions do strongly affect the level of inbreeding in the earlier generations of a simulated population, as well as cumulative inbreeding. After several time steps, however, inbreeding declines to an approximately steady value and the benefit of knowing founder relationships is lost.

Based on this theoretical background we aimed to identify the influence of founder relationships on the living population within the red panda EEP. We wanted to know whether the assumed degree of founder relationship affects the annual change in genetic parameters (gene diversity retained, inbreeding) and whether the general pattern of changing influence of founder relationships described by Rudnick and Lacy (2008) can be verified for an existing studbook population.

Methods

We used the red panda international studbook (*Ailurus fulgens*) current to 31 December 2015 provided by Rotterdam Zoo (Glatston 2015). The studbook data was analysed using PMx (Version 1.3.20150713, Ballou et al. 2014) a specialised program that provides a range of tools for the demographic and genetic management of wildlife populations kept with the primary goal of conserving their genetic diversity (Lacy et al. 2012). Studbook data were cleaned up and validated for major logical problems using SPARKS Version 1.66 (ISIS 2015). Data on 1350 individuals were exported from a time span of 01.01.1977 to 31.12.2015 and subspecies *Ailurus f. fulgens* selected. Some institutions within Europe do not officially belong to the European Association of Zoos and Aquaria (EAZA), but they breed or keep red pandas which are managed within the EEP. Therefore the two criteria “geographic area” and “associations” were exported separately and merged together to get the most precise overview of red pandas in Europe. While Rudnick and Lacy (2008) referred to generalised time steps, we carried out our research based on years.

To outline both the short and long term impact of founder relationships on the genetic management of red pandas five different scenarios were tested and compared to each other for the EEP subpopulation. The original dataset was modified for the different scenarios by adding new founder individuals and changing

Table 1. Founder relationship scenarios for red pandas managed in the EEP population.

Scenario	Description
1	All 23 founders are completely unrelated to each other (traditional studbook assumption)
2	Founders clustered in six groups, on the basis of their arrival into captivity (see supplementary material)
3	Founders clustered in eight groups, on the basis of the region/country into which they were imported (see supplementary material)
4	Complex founder relationships (see supplementary material)
5	The early 21 founders (1964–1974) are full siblings. The other two founders added in the 1980s are also full siblings but completely unrelated to the first founders.
6	All founders are related in the first generation (full siblings)

Figure 1. Map of European zoos participating in the red panda EEP (n=155).

the genetic relationship (dam, sire) of the original founders (see Table 1). Detailed information on the different scenarios, as well as census data for the EEP population are compiled and presented in the appendix.

The first and sixth scenarios involve the most extreme assumptions: the first (traditional) scenario treats all founders as completely unrelated, while the sixth treats all founders as related in the first generation, respectively as full siblings. Together both scenarios indicate the range of possibilities for the two genetic parameters measured. In the second and third scenarios, the original founders were clustered into either six source populations based on their arrival in captivity (scenario 2) or eight groups based on the region/country to which they were imported (scenario 3). All founders within a source population were treated as full siblings. The fourth scenario, based on a more complex pedigree, predicted a wide range of different genetic relationships between the original founders within four source populations (see supplementary material). In the fifth scenario all individuals were treated as full siblings except those two individuals brought into the population later in the 1980s. These two individuals were treated as full siblings to each other but completely unrelated to the other pandas. This scenario should indicate how the integration of new founders for supplementation purposes can affect the population’s mean inbreeding coefficient and may serve as a “genetic rescue” (Frankham 2015). Calculations for gene diversity (GD) retained and mean inbreeding (F) were run in PMx, calculated over time and compared between the scenarios (see Traylor-Holzer 2011). Annual increase in inbreeding and loss in genetic diversity were analysed by performing an analysis of covariance (ANCOVA).

Results

Six different founder relatedness assumptions were tested for the red panda. The first and sixth scenario points out the strongest possible effect of founder relationships: Only 68.8% gene diversity would have been retained within a population based on closely related founders within the 40 year span, whereas 91.1% would have been retained within a population based on completely unrelated founders (see Figure 2).

Despite the huge differences in assumed founder relationships, both the calculated mean inbreeding and genetic diversity in the second, third and fourth scenarios did not differ greatly over time. In all three scenarios inbreeding grew rapidly due to mating of siblings and close relatives in early years, but decreased again after 1989 (see Figure 3).

The more complex assumed relationship between the founders resulted in similar patterns to the simple ones: when founders were grouped into six source populations on the basis of their arrival in captivity (scenario 2), inbreeding initially increased but decreased again after 1990. When founders where grouped into eight source populations on the basis of the region or country to which they were imported (scenario 3), inbreeding initially increased rapidly, as individuals within a region were first bred with each other and exchange between regions was initiated on a regular basis. After 1985 this exchange between regions resulted in a decrease in inbreeding similar to scenario 2. The second, third and fourth scenarios all showed a decrease in inbreeding from 1988, followed by a small annual increase from 1998. Scenario 5 dramatically highlights the effect of supplementing genetically unrelated animals to an inbred population. Mean inbreeding was reduced by 8.84% compared to scenario 6. This was possibly due to a lot of breeding with the two new founders.

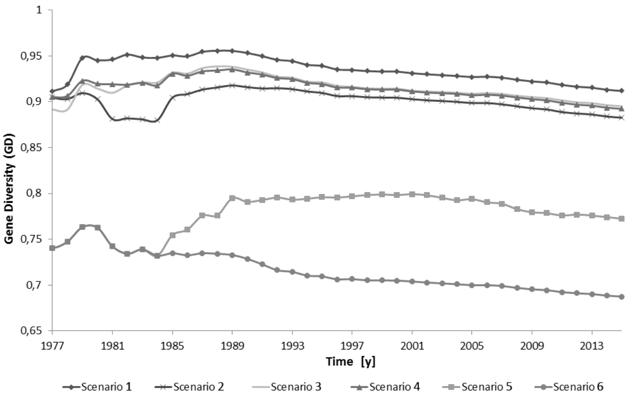

Figure 2. Genetic diversity (GD) that is retained in the EEP population of *Ailurus f. fulgens* under six different founder relationship scenarios calculated on basis of detailed pedigree information (n= 1350).

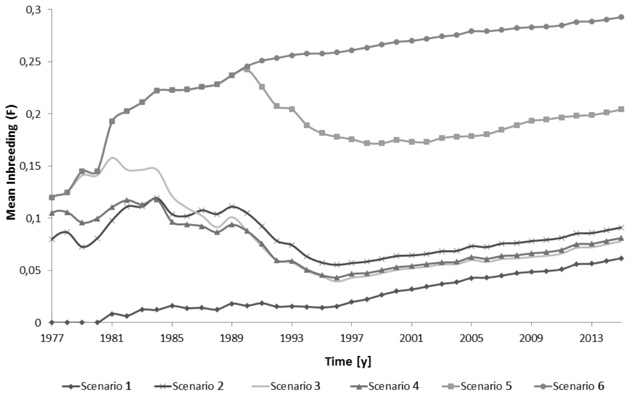

Figure 3. Mean inbreeding (F) in the EEP population of *Ailurus f. fulgens* under six different founder relationship scenarios calculated on basis of detailed pedigree information (n= 1350).

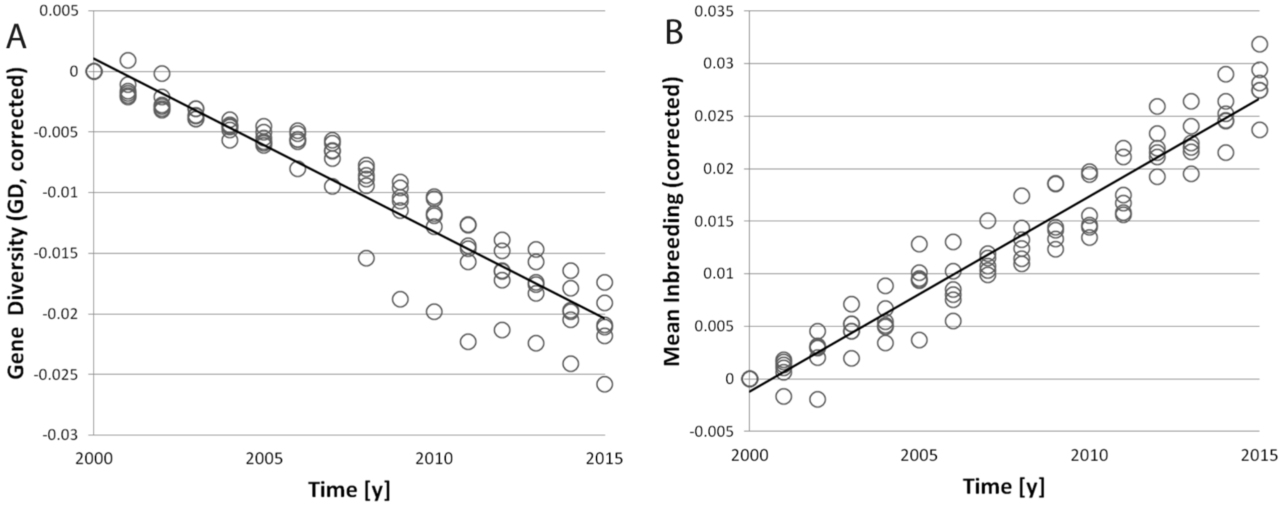

Figure 4. Linear regressions of gene diversity (A) and mean inbreeding (B) for all six founder relationship scenarios. Values were corrected by the scenario-specific intercept and indicate the linear annual change in both genetic parameters after 2000 independent of the underlying scenario.

From 2000 all six scenarios show a similar pattern of growth and a small increase in inbreeding over time. A linear regression analysis (ANCOVA) was performed to characterise the differences between the slopes of the scenarios in the time frame from 2000 to 2015. Two ANCOVA models were executed. In Model 1, inbreeding was modelled as the dependent variable with the six scenarios as factors and time as covariate. Modelling showed a highly significant effect of the scenario ($p<0.001$) and a significant effect of time ($p=0.0416$), but no significant interaction between the two, suggesting that the slope between time and inbreeding is similar for all founder relationship scenarios. Removing interactions between time and the scenarios in a second model showed that the scenario has an important effect on inbreeding. This can be interpreted as a significant difference in the intercepts between the regression lines of the different scenarios. Removing the interaction did not significantly affect the fit of the model ($F=0.0082$, $p=0.928$). The same analysis was done with the calculated genetic diversity retained. Modelling showed a highly significant effect of the scenario ($p<0.001$). Removing interactions between time and the scenarios showed that the scenario had a highly significant effect on genetic diversity. Removing the interaction did not affect the fit of the model ($F=0.0011$, $p=0.9741$).

In a second step all values were subtracted by the scenario-specific intercept (value of genetic parameter in year 2000). A single linear regression was calculated based on the corrected values (see Figure 4). Mean inbreeding (F) increased on average by 0.19% per year ($R^2 = 0.936$), while gene diversity (GD) decreased on average by 0.14% per year ($R^2 = 0.8966$).

In summary the annual increase in inbreeding and therefore the annual loss of gene diversity was not significantly dependant on the different scenarios within the last fifteen years. The different assumptions about the degree of founder relationship did not affect the annual rate of change in genetic parameters.

Discussion

Our analyses examined the short and long term influence of different founder relationship assumptions on genetic status while applying the traditional assumption of no founder relationship in the EEP population of *Ailurus f. fulgens*. Between 2000 and 2015 both the annual increase in inbreeding (*F*) and the loss of gene diversity (GD) were almost identical across all assumed scenarios of founder relationships. Furthermore, the red panda EEP population impressively demonstrates the patterns described by Rudnick and Lacy (2008): while affecting inbreeding and gene diversity in the early years of the breeding programme, founder relationship level becomes less influential over time, and finally shows no significant effect on annual change in genetic parameters.

For 30 founders, Rudnick and Lacy (2008) observed that the difference in inbreeding initially increased across all scenarios, then decreased before becoming stable around the tenth time step (five generations). In the case of the red panda, inbreeding was initially increased across scenarios 2, 3 and 4, then decreased and became stable after approximately 20 years (approximately 12 years after the last supplementation of wild caught animals). With an average generation length of 5.2 years (based on studbook data) this means that the red panda population reached stability after four generations. From 2000 on, there is no evidence for an influence of founder relationships on the genetic parameters assessed. However, it is important to point out that the different founder assumptions did strongly affect the level of inbreeding in the earlier generations, as well as the cumulative inbreeding. Consequently, knowing the true relationships between founders is important for short-term genetic management and the successful establishment of long term-breeding programmes.

However, it must be noted that our study does not replicate the study by Rudnick and Lacy (2008) as their simulation examined the impact of founder relationships and how knowledge about it can be used to improve a breeding programme. Our results illustrate the impact of alternative assumptions about founder relationships on the calculated patterns of gene diversity and inbreeding in the early generations, when the population is continually managed without knowing the true founder relationship (as most populations are managed today).

In the case of the red panda, decisions about which animals to breed were led by availability of new individuals and geographical restrictions, and were based on the “no relationship” assumption. Individuals acquired from the wild were often bred with each other first and later spread between institutions. Organised breeding management started with the first international red panda studbook published in 1980. As management on a global scale started in the early 1990s, the higher cumulative inbreeding seen in the scenarios with more inter-related founders, might not have been avoidable after this time and knowledge of those relationships might not have led to (much) improved management of the population.

Our research emphasises that the first years in a breeding programme (or the period after a supplementation event) are a crucial time, as this lays the foundation for later generations. The computer simulations by Rudnick and Lacy (2008) indicate that change in annual parameters becomes stable after a certain time. The red panda EEP provides studbook-based evidence for these theoretical findings. The development of genetic parameters in the red panda may be a general pattern that can be observed in species populations after a longer management period without supplementations with new founders.

The EEP population of *A. f. fulgens* comprises 23 founders. The last founding event within the red panda EEP was 32 years ago in 1984, when two wild born animals were brought to Spain. After this supplementation, only animals bred in other regional programmes (not new founders) were incorporated into the EEP population. The potential influence of two full siblings unrelated to all founders previously introduced into the captive population is illustrated in founder relationship scenario 4. Inbreeding was reduced by 9.4% within 17 years compared to the full-sibling scenario 5. After 1997, the effect flattened and from 2000 the annual increase in inbreeding was comparable to the other scenarios. However, the research by Rudnik and Lacy (2008) suggests that the number of founders does not necessarily affect the time until a population reaches stable growth/decrease in genetic parameters. Scenarios with 30 and 100 founders showed stable growth in inbreeding after 10 time steps.

Since the early years of the first red panda breeding plan, red pandas have been managed for mean kinship. Managing for *mk* means to genetically equalise a population over time. Since the last supplementation event, individuals within the EEP population have become more and more related to each other. Even so, founders were not equally represented within the red panda EEP. In 2015, the population was strongly intermixed and nearly all living individuals share the majority of founders. Rudnick and Lacy (2008) concluded that the most likely explanation when applying *mk* breeding strategies was that recent ancestry had a greater influence on the accumulation of inbreeding than deeper ancestry. Breeding selection for *mk*, while adding no new genetic supplementation to a captive population, seems to lead to the stable development of genetic parameters described. Ideally, increased inbreeding is reduced to a minimum, while genetic diversity is preserved for as long as possible.

After the founder genes have been spread across the whole population, with almost every animal descending from every founder, knowing the initial founder relationships has no significant effect on subsequent genetic trends. This could be important, as there is a huge range of applications for molecular methods in captive breeding (Fienieg and Galbusera 2013; Witzenberger and Hochkirch 2011), but using molecular estimates of founder relationships (for example with microsatellites or SNPs) might not lead to any great benefit many generations after the founding phase of a population.

Molecular analyses of founder relationships might be more important if used to guide genetic management in early generations, before all animals are descended from most founders, when founders of a population are still alive or when new founders are recruited into a population.

Wilcken and Lees (2012) highlight that the studbook assumption of zero founder relatedness may seem to be dangerous, but point out the greater importance of founders representing a random sample of the genetic variation found in the wild. They state that in that case, assuming genetic uniqueness of all founders is the most pragmatic way to manage a captive population to preserve the original genetic diversity. However, in the case of the red panda, only a small percentage of individuals brought into captivity bred successfully (Glatston 2011b) and built the founding base of the current captive population. It is not possible, either from studbook or historical data, to estimate genetic diversity or variation differences between imported wild-caught animals and the small number of individuals that ultimately managed to breed and act as founders for the current population. Therefore it is doubtful that these 23 founders (out of more than 200 individuals taken from the wild) are a representative and reasonable sample of the wild population. To clarify this, molecular methods can be used to assess the genetic composition of the living population and compare it to samples from the wild to assess future possibilities for conservation actions.

There are a huge number of studbooks for endangered species. In Europe alone EAZA is coordinating 201 EEPs and 199 ESBs (EAZA 2016). This paper provides the first studbook-based evidence that a captive population's recent ancestry has a bigger influence on the accumulation of inbreeding than its past ancestry when applying mean kinship (*mk*) breeding strategies. Further analysis of other studbooks will be required to generalise these observations. Thirty-two years since the last wild red panda entered the EEP population, the legacy of the 23 individuals who built the foundation of the recent population is ongoing. Although genetic relatedness between founders is no longer affecting current trends in genetic parameters, the genetic information from these individuals needs to be preserved for posterity. It is the duty of population managers to apply the best methods of breeding management to conserve the red panda in captivity.

Conclusions

1. The red panda EEP has been used to provide the first studbook based evidence that a population's recent ancestry has a bigger influence on the accumulation of inbreeding than the ancestry of founders when applying mean kinship breeding strategies.

2. In contrast to the early years of the breeding programme, the last 15 years show that the annual change in inbreeding and genetic diversity is not significantly dependent on founder relationship in red pandas.

3. In a population managed by mean kinship based on the assumption of no founder relationship, the change in annual genetic parameters becomes stable after 10–15 years (4-5 generations respectively). It is crucial in this time to implement knowledge of founder relationships. After the turning point the population has genetically equalised and new information obtained on founder relationships may have no significant effect on subsequent genetic trends.

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