

## Low genetic diversity of the reintroduced bearded vulture (*Gypaetus barbatus*) population in the Alps calls for further releases

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### Abstract

Reintroductions are a valuable tool to reestablish locally extirpated species. A difficult decision facing all reintroduction projects is when to stop further releases, both from a demographic and genetic viewpoint. Here we address this question in the case of the reintroduction of bearded vultures (*Gypaetus barbatus*) in the Alps, which were exterminated in the early 20th century mainly due to human hunting. To investigate the genetic diversity present in today's reintroduced Alpine bearded vulture population, we reconstructed a pedigree, spanning the entire reintroduction program since the beginning of the captive breeding (1973-2010). We found that not every founder bird was equally well represented in the wild population and that founder genome equivalents were low (13). Moreover, wildborn bearded vultures showed a relatively high mean inbreeding coefficient compared to the captive birds and the effective population size was estimated to be only 28. Overall, this suggests that there is not enough genetic diversity in the wild Alpine bearded vulture population to ensure its long-term sustainability. Therefore further releases are recommended.

### Keywords

Bearded vulture, conservation, genetics, reintroduction, pedigree, captive breeding

### Introduction

With the current rate of population extinction, reintroductions are becoming an increasingly important conservation tool (IUCN 1996). A difficult decision facing every reintroduction programme is when to call the project a success and stop further releases. Quantitative demographic evaluations of the success of reintroduction projects are rare (EVANS et al. 2009; MERETSKY et al. 2000; SARRAZIN & BARBAULT 1996), but exist (e.g. SCHAUB et al. 2009). Even more scarce are combinations of demographic and genetic evaluations of the reintroduction success in a species (ALEXANDRE et al. 2007; LANDE 1988).

The reintroduction of the Alpine bearded vulture (*Gypaetus barbatus*) offers an excellent opportunity to investigate both demographic and genetic aspects of the reintroduction and to ask when to stop the release of individuals from captivity into the wild. Bearded vultures are large scavenging raptors which lived in mountain areas throughout Europe, Asia and Africa. They went extinct in the Alps in the early 20<sup>th</sup> century. Population size decreased due to changes in agricultural management, and reduced numbers of wild ungulates. Human hunting and poisoning were additional factors leading to the total extirpation in the whole Alpine range (MINGOZZI & ESTEVE 1997).

In 1978, an international reintroduction project was launched with the aim to rebuild a self-sustainable population of bearded vultures in the Alps. In 1986 the first bearded vultures were released from a captive breeding program into the wild. Until 2010 a total of 179 birds were released in four Alpine countries (Austria, France, Italy, and Switzerland). In 1997 the first wild pair consisting of reintroduced birds bred successfully in the French Alps. Until 2010, 69 wildborn birds fledged in the Alps, with the population estimated at 150-160 individuals in 2010 (IBM 2010).

Given the current population size and the reproductive success in the wild it is time to ask when to stop the release of individuals from captivity into the wild. SCHAUB and colleagues (2009) performed a demographic analysis, which suggested that it would be possible to stop the release at once and shift the reintroduction effort to other regions and populations outside the Alps. However, no assessment from a genetic point of view has been carried out. Demographic and genetic considerations need not to lead to the same conclusion because demographic self-sustainability does not automatically mean long-term persistence (SEDDON 1999). For a population to persist in the long-term, both a positive population growth and adequate genetic diversity is crucial.

Here, we analyse the genetic diversity present in the Alpine bearded vulture population using pedigree data and ask how much genetic diversity has already been transferred from captivity into the wild. Furthermore, we will discuss how the different management possibilities have influenced inbreeding, founder contribution, and founder genome equivalents in the captive population, where managers have control of matings and in the wild populations where managers can only influence the number and composition of released birds.

## Materials & Methods

To investigate the genetic diversity present in today's bearded vulture population, we reconstructed a pedigree spanning the entire reintroduction program from 1973 to 2010. We divided the whole Alpine population into two different groups that differ in the options available to the managers: the captive birds, which were born in captivity and remained there, and the wild group, which consists of the released and the wildborn birds.

We quantified genetic diversity using a variety of pedigree-based measures: effective population size ( $N_e$ ), founder contribution, and founder genome equivalents ( $f_{ge}$ ). Bearded vultures have overlapping generations, therefore we used the increase in coancestry to calculate the effective population size (CERVANTES et al. 2011). Founder genome equivalents are “the number of equally represented founders that would produce the same level of gene diversity as that observed in the current population, assuming no loss of alleles” (LACY 1989, p.115). Both unequal founder contribution and genetic drift reduce  $f_{ge}$  and are reflected in both the number of alleles and the heterozygosity present in the descendant population. Founder contribution is defined as the proportion of genes derived from each founder and present in the living descendants. It is calculated with Monte Carlo methods following the “gene drop” method from MACCLUER et al. (1986). For all calculations we used ENDOG 4.8 (GUTIERREZ & GOYACHE 2005).

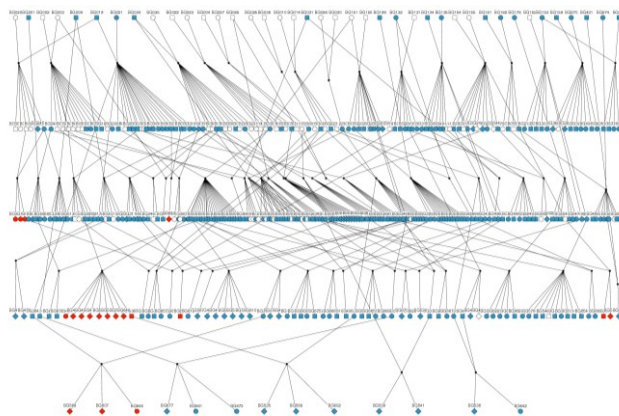


Figure 1: Pedigree of all bearded vultures in the Alpine reintroduction project. Circles are females, squares males, and diamonds are birds of unknown sex. In blue are the birds which are still alive, in white the ones that already died, and in red those which are inbred. At the top are all founder birds which reproduced.

## Results

The whole pedigree of the bearded vultures includes five generations (Fig. 1). Although the number of individuals increased steadily since the beginning of the captive breeding and the reintroduction project, this is not the case for genetic diversity (Fig. 2). Both effective population size ( $N_e$ ) and founder genome equivalents ( $f_{ge}$ ) level off around 2004, although the total number of birds alive continued to increase. The temporal pattern observed in the development of  $f_{ge}$  is similar to the one in  $N_e$  (Fig. 2), reflecting the effects of genetic drift on both measures. Founder genome equivalents in 2010 were low (maximum of 21.2 in the captive and 14 in the wild group) and the effective population size was estimated to be only 37.9 in captivity and 28.7 in the wild.

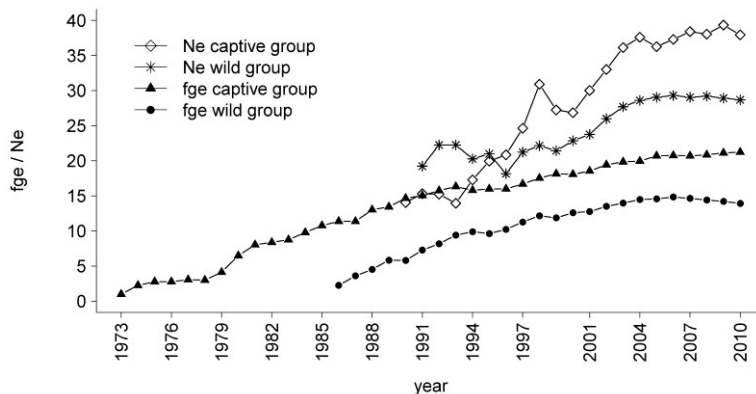


Figure 2: Founder genome equivalents ( $f_{ge}$ ) and effective population size ( $N_e$ ) of the captive and the wild bearded vulture group between 1973 and 2010.

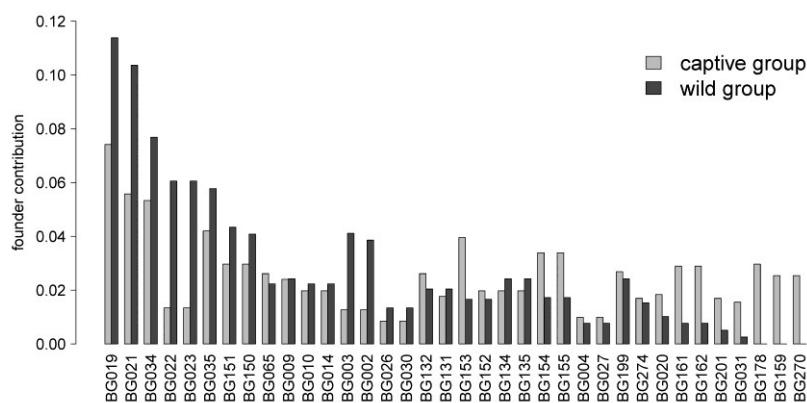


Figure 3: Contribution of the 37 founders to the two bearded vulture groups in 2010. The different founder individuals are listed on the X-axis and the Y-axis represents their relative contribution to the captive (light grey) and the wild (black bars) groups. Additional 10 founders lived in the captive population which did not reproduce so far.

The current population was founded by 37 individuals (Fig. 3). The three most successful founders in all four groups were BG019, BG021, and BG034. Together they contribute 18.3% to the captive and 29.4% to the wild individuals (Fig. 3). In both groups the founder contribution was skewed, but it was more skewed in the wild group. Four founders were only represented in the captive birds, and an ten additional birds lived in captivity but did not reproduce so far.

Among both, the captive and the wild pairs, mating of relatives occurred. In captivity the kinship coefficient under random mating was larger than the kinship coefficient of the existing pairs ( $p = 0.05$ ) while the opposite was true among wild pairs, albeit not significantly ( $p = 0.44$ ). This means, that inbreeding was avoided in the captive group. In the wild neither inbreeding avoidance nor preference was observed.

Only five inbred ( $F > 0.125$ ) birds fledged in captivity, two of them remained in the captive breeding program, three were released into the wild. From the wild breeding pairs 16 inbred offspring fledged ( $F > 0.0625$ ). Both the kinship coefficient and the inbreeding coefficient were higher in the wild than in the captive group. In 2010 the parents of the five fledglings with sufficient pedigree information were on average more related to each other than first-cousins (mean inbreeding coefficient  $> 0.07$ ).

Additionally we found some evidence for inbreeding depression among pairs breeding in the wild: the reproductive success of pairs with a kinship coefficient of 0.25 was on average reduced by 57% compared to pairs with a kinship coefficient of zero ( $p=0.03$ ). However, this result was strongly affected by one breeding pair with very poor breeding success.

## Discussion

We showed that, compared to the captive breeding population, there is little genetic variation and substantial inbreeding in the wild Alpine bearded vultures. In fact, the effective population size in the wild is so small, that levels of genetic variation have stopped to increase since 2004 despite continued releases (Fig. 2). This suggests that there is not enough genetic diversity in the wild Alpine bearded vulture population to justify stopping the release of birds from captivity. However, as there is still more genetic variation and a very low level of inbreeding in captivity, it is possible to increase the genetic variation and decrease inbreeding in the wild with further releases of birds from captivity.

The low effective population size ( $N_e$ ) in the wild means that inbreeding will increase and heterozygosity decrease in the future. With no change in  $N_e$ , the expected mean inbreeding coefficient will increase to approximately 0.15 over the next 100 years and less than 90% of the heterozygosity will remain. One goal which is often defined for a long-term persistence of a population, is to maintain 90-95% of the initial heterozygosity for 100-200 years (e.g. MILLER et al. 2009; SOULE et al. 1986). Hence with no increase in  $N_e$  in the near future, the wild bearded vultures will fall below this limit.

Founder genome equivalents ( $f_{ge}$ ) are a direct measure of founder contribution and the effects of genetic drift a population experienced. A population with 20  $f_{ge}$  would contain about 97.5% of the genetic variability initially present in the population from where the founders were captured (LACY 1989). This is the case in the captive bearded vultures where  $f_{ge}$  exceeded 20. The 13  $f_{ge}$  observed in wild individuals in 2010 (Fig. 2) indicate that the genetic diversity is equal to one of a population founded by 13 equally represented founders with no loss of alleles due to genetic drift. Both a more equal founder contribution and an increase in population size would enhance  $f_{ge}$  (LACY 1989). This could be achieved with the 14 birds already living in captivity but not reproducing so far.

A highly skewed founder contribution, as it was observed in both the captive and the wild group of the Alpine bearded vulture (Fig. 3), reduces genetic diversity. Furthermore, the risk to lose alleles of underrepresented founders is high, because some founders are only represented in just a small number of birds. A more equal founder representation would enlarge the genetic variation and is therefore worth pursuing. This skewed founder representation is due to differences in reproductive success and survival among founders or their descendants. The underlying cause may be biological but it may also reflect differences in management between breeding

facilities. It might be possible to reduce the differences in reproductive success among the facilities with translocations of birds from underrepresented founder lines to facilities where birds have on average a higher reproductive output.

The smaller kinship coefficient among the captive pairs compared to the random mating expectations is a consequence of the good management of the captive population, which in all but six pairs avoided mating of relatives. Hence, compared to other studies, the frequency of close to moderate inbreeding in the captive birds is relatively small (8.8%). However, in the wild no clear pattern for preference or avoidance of mating with relatives was apparent, leading to much more inbreeding in the wild. The observed kinship coefficient was even higher in the wild pairs, but not significantly so.

The reintroduction project of bearded vultures in the Alps is on its way to be successful. The first wildborn offspring hatched in 1997 and since then the number of fledglings increased steadily. Further even without the release of further young birds from captivity the population would have a positive population growth rate (SCHAUB et al. 2009). Nevertheless we showed that the genetic diversity present today in the wild is still low. Therefore, to ensure the long-term persistence of this species it would be wise to increase the genetic variability in the wild population (LOERCHER 2011). There are several ways to achieve this goal: This could be realised by specific releases of juveniles from underrepresented founder lines. Thereby, the pairing of descendants from two underrepresented founders should be preferred over the mating of descendants between under- and overrepresented founders in the breeding stock. With this management strategy it will be possible to favour an underrepresented founder without increasing the contribution of an already overrepresented founder at the same time (BALLOU & FOOSE 1996). Furthermore, it is recommended to recruit new founder birds from other populations. However, this might have negative consequences for the source populations. Therefore, careful evaluation of these consequences and the potential for the Alpine bearded vultures should be performed prior to action. Additionally connecting the Alpine and the Pyrenean population to a metapopulation would substantially improve the genetic diversity.

To summarize, we recommend continuing the release of birds in the Alps for now, and recommend releasing these birds in the near future (Fig. 4). Birds for release should be chosen carefully with respect to their genetic lineages. We recommend continuing the releases of birds from captivity until approximately 20 founder genome equivalents are present in the wild population.



Figure 4: A young Bearded vulture (BG665) who was released in 2011 in Switzerland. This individual originates from a rare genetic line and was deliberately chosen to increase the genetic diversity in the reintroduced Alpine population. © Franziska Lörcher

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