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RESEARCH ARTICLE

# Genetic variation in a network of natural and reintroduced populations of Griffon vulture (*Gyps fulvus*) in Europe

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**Abstract** It is generally considered that limiting the loss of genetic diversity in reintroduced populations is essential to optimize the chances of success of population restoration. Indeed, to counter founder effect in a reintroduced population we should maximize the genetic variability within the founding group but also take into account networks of natural populations in the choice of the reintroduction area. However, assessment of relevant reintroduction strategies requires long-term post-release genetic monitoring. In this study, we analyzed genetic data from a network of native and reintroduced Griffon vulture (*Gyps fulvus*) populations successfully restored in Southern Europe. Using microsatellite markers, we characterized the level of genetic diversity and degree of genetic structure within and among three native colonies, four captive founding groups and one long-term monitored reintroduced population. We also used Bayesian assignment analysis to examine recent genetic connections between the reintroduced population and the other populations. We aimed to assess the level of fragmentation among native populations, the effectiveness of random choice of founders to retain genetic variability of the species, the loss of genetic diversity in the reintroduced population and the effect of gene flow on this founder effect. Our results indicate that genetic diversity was similar in all populations but we detected signs of recent isolation for one native population. The reintroduced population showed a high immigration rate that limited loss of genetic diversity. Genetic investigations performed in native populations and post-released genetic monitoring have direct implications for founder choice and release design.

**Keywords** Reintroduction · Griffon vulture · Genetic diversity · Gene flow

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## Introduction

The importance of genetic processes for the success of population restoration through reintroduction or transloca-

tion has been largely discussed but accurate data remain scarce (Sarrazin and Barbault 1996; Frankham et al. 2002; Goossens et al. 2002). One of the main concerns of species reintroduction program is to conserve genetic variability in newly founded populations. Indeed post-reintroduction genetic drift, with similar consequences as founder effect, often results in a reduction of genetic diversity (Nei et al. 1975; Robichaux et al. 1997). One way to limit negative effects of foundation during reintroductions is to maximize the genetic diversity of the founding group, which can be achieved via a pre-release genetic monitoring (Earnhardt 1999). Indeed, in any reintroduction program, avoiding inbreeding in the creation of reintroduced stock increases the long-term reintroduction success (Gautschi et al. 2003a). The choice of sufficiently genetically diverse founders is thus crucial for reintroduction success and depends on the genetic variability of remnant captive or native populations. The historical structure of the former populations must also be taken into account to better define appropriate sources of founders. To avoid outbreeding depression, genetic structure among available source populations must be studied to assess whether individuals from various populations can be mixed (Friar et al. 2001). Moreover, for endangered species that have recently undergone a fragmentation event modifying their inter- and intra-population genetic structure, reintroduction programs should aim to recover past genetic structure. Knowledge of the genetic diversity and historical events of gene flow among remnant populations is essential to optimize the choice of released founders (Godoy et al. 2004).

Another way to restrain the loss of genetic diversity in a newly founded population is to aim for re-establishment of the population in a network of populations that have the potential to exchange individuals (Latch and Rhodes 2005). In theory, a relatively small effective number of migrants (one per generation; Newman and Tallmon 2001) can stop the loss of genetic diversity in small populations (Keller et al. 2001) and decrease the deleterious effects of inbreeding in fragmented populations (Couvet 2002). Theoretical (Lubow 1996), empirical studies (Madsen et al. 1999) and experimental reinforcements confirmed the genetic and demographic ''rescue effects'' of immigration on population persistence. In that case, we must evaluate the impact of the potential gene flows between reintroduced populations and remnant ones on the genetic restoration of the former (Hedrick 2005). Thus, in restored or reintroduced populations, genetic monitoring is useful to evaluate founder effects, estimate gene flows between reintroduced and native populations and assess the long-term restoration of genetic diversity (e.g., DeYoung et al. 2003; Vernesi et al. 2003). In return, such information can be used to optimize reintroduction strategies in terms of origin, number, location of releases, etc.

However, the assessment of founder effects, restoration of genetic diversity and gene flow patterns ideally requires long-term intensive demographic and genetic monitoring. This probably explains why relevant population genetics studies primarily concern plant reintroductions (Falk and Holsinger 1991) and are seldom conducted on the restoration of long lived animals (but see Hedrick 1995; Ralls and Ballou 2004). Here, we present the analysis of genetic diversity and structure of an exceptional network of restored Griffon vulture (*Gyps fulvus*) populations in Southern Europe, including both natural and reintroduced populations.

Griffon vultures are social scavengers and colonial cliff nesters. They are long lived birds with a generation time up to 11 years (Ferrière et al. 1996). They become sexually mature at 4 years; females lay one egg per year. Griffon vultures are generally philopatric, with breeders being faithful to their area of first breeding (Sarrazin et al. 1996). Nevertheless, erratic behavior of young birds is frequent (Bernis 1983). Movement capacities of Griffon vulture are high, e.g., a study on seasonal movements of marked birds in Croatia showed that annual average distance out of their natal area was between 400 and 600 km (Susic 2000). Griffon vultures were widespread around the Mediterranean Sea but underwent a severe demographic decline between the end of the 19th century and the beginning of the 20th century, mainly due to direct and indirect human persecutions (Donázar 1993). In France, they went extinct in the Alps at the end of the 19th century and in the Massif Central in 1945. In the 1970s, ban on poisoning and management of feeding places favored an increase in Griffon vulture populations in the western part of the species range, with the largest population settling in Spain (18,000 pairs in 1999, Del Moral and Martí 2001). In contrast, in the Balkans and in Northern Africa, the number of pairs remained low and declining and local extinctions are still topical (Garrido et al. 2005). In France, five Griffon vulture reintroduction programs have taken place since the 1980s, into a matrix of spatially fragmented native populations. For all reintroduction programs, founders coming from natural populations were randomly sampled among birds from rescue centers and zoos. Release stocks were built without genetic information.

The demography of native and reintroduced Griffon vulture populations has been intensively monitored since 1970. Such surveys have provided valuable information regarding survival (Sarrazin et al. 1994), breeding biology (Sarrazin et al. 1996; Fernandez et al. 1998), and movement (Susic 2000, Bosé et al. (in press)). However, they are not sufficient to infer some important demographic processes, such as effective dispersal. To our knowledge no genetic study has yet been undertaken on Griffon vulture populations. Indeed, as the main population in Spain always contained more than 3,000 breeding pairs (Camiña

2004) and potentially sent individuals to other native populations, we do not think that the demographic bottleneck in Western native populations affected their genetic diversity. Moreover, taking into account the high dispersal ability of the Griffon vulture and the fact that in the recent past, the distribution of the Griffon vulture was more or less continuous over its range, low genetic structure is expected among native populations. On the contrary, due to the current decline and extinction events in native populations east of the French Alps, the genetic diversity and structure of these populations and the possibility of gene exchange with Western populations are still questioned. Because of the heterogeneity in resightings of banded breeders among Griffon vulture populations, estimating gene flow using banded birds monitoring is difficult. The importance of recent genetic connections between newly established and remnant native populations can however be studied with genetic approaches. A genetic monitoring of Griffon vulture populations was thus required to evaluate (1) recent genetic diversity in Eastern Griffon vulture populations and (2) current patterns of differentiation and gene flow among populations, particularly between reintroduced and native populations.

We used ten microsatellite markers to investigate genetic diversity and structure of three native populations (one successfully reintroduced population two generations after its release and four captive founding stocks) and to assess recent gene flows among established populations. We tested (1) whether fragmentation existed among native populations, (2) whether the random founder sampling strategy used for the creation of release stocks was effective with regards to genetic diversity and structure of native populations, (3) whether the genetic diversity of the reintroduced population was affected by founder effect and (4) whether genetic connections between reintroduced and native populations were restored.

## Materials and methods

### Study areas

We studied three native colonies in Israel, Croatia and in the French Pyrenees (Ossau), one settled reintroduced colony in southern Massif Central in France (Causses) and four captive founding groups of reintroduction programs (one in Navacelles and three in the southern French Alps), in which we sampled and analyzed a total of 363 Griffon vultures (Fig. 1).

#### *Geographical and historical situation*

The native populations we studied exhibit contrasting demographic histories. Israel and French Pyrenees populations have undergone a demographic decline but are now increasing. In Israel, the estimated number of breeding pairs was 68 in 1978, which was three to five times smaller than in 1950 (Mendelssohn and Leshem 1983) but it had reached 140 by 2002 (Slotta-Bachmayr et al. 2004). In the French Pyrenees, only seven pairs had been observed in 1970 in the Ossau Valley but the population increased up to 117 pairs in 2002. In contrast, the Croatian population is still declining: Griffon vultures were formerly widespread over all the Dalmatian coast and Adriatic Islands in Croatia but they now breed only on the Kvarner Islands (Slotta-Bachmayr et al. 2004). In the 1980s the number of Croatian Griffon pairs was about 150. Due to the prevalence of poisoning, the Croatian population declined and the number of pairs was estimated at 90 in 2002 (Slotta-Bachmayr et al. 2004).

The first reintroduction occurred in France, in the Causses area. Between 1980 and 1986, 61 birds coming from Spain, the French Pyrenees and several zoos were released. Currently, 116 pairs breed in Causses and the colony is still increasing. Fifty Griffon vultures, coming mainly from Spain and zoos, were then released in Navacelles between 1993 and 1997. This reintroduction failed and 22 of these birds breed at present in the colony of Causses. Finally, Griffon vultures were reintroduced in the southern French Alps between 1996 and 2005. In the Baronnies, 56 birds coming mainly from Spain, Causses, French Pyrenees and zoos were released between 1996 and 2001. In the Diois, 43 birds coming from Spain, Causses and French Pyrenees were released between 1999 and 2004. In Verdon Canyon, 90 birds coming from Spain and French Pyrenees were released between 1999 and 2005. For all reintroduction programs, founders were kept several years in captivity before release.

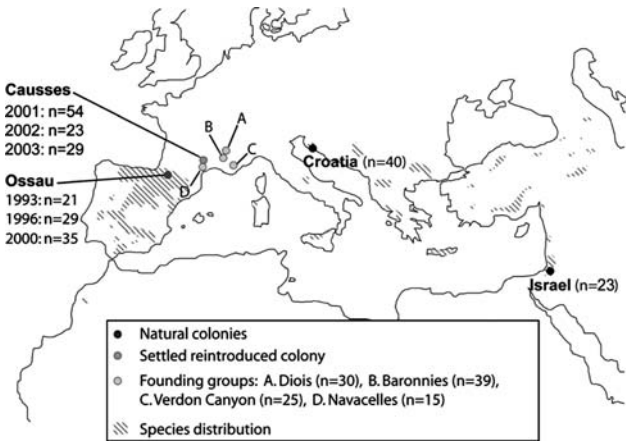


**Fig. 1** Current Griffon vulture distribution (adapted from Slotta-Bachmayr et al. 2004) and location of sampled colonies and founding groups (*n* number of individuals used for genetic analyses)

*Sampling*

In Israel, we collected 23 feathers plucked on adults captured in 2000 and preserved them in dry condition. In Croatia, we analyzed dry feathers of 40 chicks born on islands between 1997 and 2004. In Ossau and Causses, growing feathers were sampled each year on chicks and conserved in 70% ethanol. In Ossau, we analyzed 85 individuals hatched in 1993, 1996 and 2000. In Causses, we analyzed 106 chicks hatched in 2001, 2002 and 2003. The latter chicks hatched about two generation times after release. As successful breeding pairs were faithful to their nest across years, we kept one chick per nest over the 3 years in order to exclude siblings. All birds sampled in Israel, Croatia, Ossau and Causses were banded birds. Consequently, all samples come from distinct individuals, although some individuals could be related. All sampled individuals were either chicks or adults, thus pre-breeders or seasonal migrants were unlikely to be sampled.

For the captive founding groups, we sampled blood on captive individuals before release in Navacelles ($n$ = 15), Baronnies ($n$ = 39), Verdon Canyon ($n$ = 25) and Diois ($n$ = 30). Among the 94 individuals released in the French Alps sites, 64 were known to be wild caught in Spain and kept for some time in rescue centers (Navarre and Valence). We used these 64 individuals as Spanish sample for analyses on the genetic structure of native populations. We also tested if Ossau, considered as the Northern fringe of the Spanish population (Terrasse 1983), could be considered as a sample of the Spanish population for all other analyses.

Molecular techniques

DNA samples from Alpine sites, Navacelles, Causses (1993, 2000) and Ossau were obtained using the Chelex 100 (Ellegren 1994) and standard phenol/chloroform protocols (Sambrook et al. 1989). For all other feather samples, we extracted DNA from a 2 mm piece of calamus with the CTAB method (Kretzmann et al. 2003). We retained ten microsatellite loci for the quality of their amplification products and their polymorphism level on Griffon vulture. Five loci were chosen among the 13 characterized by Mira et al. (2002) on Griffon vulture and five among the ten characterized by Gautschi et al. (2000) on Bearded vulture (*Gypaetus barbatus*, Table 1). PCR mixtures (10 μL final volumes) contained approximately 2 ng of template DNA, 0.2 mM dNTP, 0.25 μM R-Primer, 0.25 μM L-Primer fluorescently labeled with one of 6-FAM, VIC, NED, PET (Applied Biosystems), 1 U *Taq* DNA polymerase and 1× buffer (Qbiogene). Cycling was performed in a MWG AG thermal cycler (Biotech) under the following conditions: 95°C for 5 min, five cycles of 1 min at 95°C, 30 s at the suitable annealing temperature (from 53 to 56°C) and 1 min at 72°C, then 20–30 cycles of 30 s at 94°C, 30 s at the annealing temperature, and 30 s at 72°C and a final step of 20 min at 72°C; annealing temperatures and cycle number depended on loci. PCR products were resolved on a 310 DNA sequencer and analyzed using GENESCAN software (Applied Biosystems).

Data analysis

We analyzed eight ''populations'' sorted into three groups: (1) native group: Israel, Croatia, Ossau (cohorts 1993, 1996 and 2000 pooled), (2) settled reintroduced group: Causses (cohorts 2001, 2002, 2003 pooled) and (3) captive founding group: Navacelles, Baronnies, Verdon Canyon and Diois. For analyses without comparison with captive groups, the Spanish population was added to the native group.

To assess whether the selected loci were sufficiently polymorphic to evaluate between-group structure, we calculated the probability of identity $P_{ID}$, which is the probability that two individuals drawn at random from a population have the same genotype at multiple loci (Taberlet and Luikart 1999). We computed the multilocus unbiased $P_{ID}$ among the 363 analyzed individuals using the Waits et al. (2001) formulae implemented in the FAMOZ software (Gerber et al. 2003).

In order to detect homozygous excess in populations, we tested departures from Hardy–Weinberg equilibrium (HWE), genotypic linkage disequilibrium among loci and over all ''populations'' by Fisher's exact test with GENEPOP v 3.4 (Raymond and Rousset 1995). A sequential Bonferroni correction (Rice 1989) was used for these tests.

*Assessment of fragmentation within the native group*

We first quantified the genetic variability within each native population by the expected and observed heterozygosity, the number of polymorphic loci and the mean number of alleles per locus using GENETIX 4.02 (Belkhir et al. 1996) and the mean allelic richness using FSTAT 2.9.3.2 (Goudet 1995). We also used Queller and Goodnight's (1989) estimator of relatedness ($r$) to calculate relatedness value among individuals of the native group. The expected relatedness values are 0 among unrelated individuals, 0.25 among half sibs and 0.5 among full-sibs. We chose this method to compare our results with those obtained in studies on genetic diversity of long lived raptor populations using microsatellite data.

We then used the following genetic differentiation tests (1) to estimate the level of fragmentation among native populations, (2) to confirm if Ossau and Spain could be considered as a single population. We estimated Wright's $F$-statistics according to Weir and Cockerham (1984) as

**Table 1** PCR conditions and polymorphism of ten microsatellite loci in European populations of Griffon vultures

| Locus[a] | Published in | PCR conditions | $A_m$ |
|---|---|---|---|
| GF8G1 | Mira et al. (2002) | 55°C, 35 cycles | 2.0 |
| GVBV17 | Gautschi et al. (2000) | 55°C, 35 cycles | 2.0 |
| GF3F3 | Mira et al. (2002) | 55°C, 35 cycles | 2.25 |
| GVBV13 | Gautschi et al. (2000) | 50°C, 30 cycles | 2.5 |
| GVBV20 | Gautschi et al. (2000) | 56°C, 30 cycles | 4.58 |
| GVBV11 | Gautschi et al. (2000) | 56°C, 30 cycles | 5.5 |
| GF3H3 | Mira et al. (2002) | 56°C, 30 cycles | 6.7 |
| GF11A4 | Mira et al. (2002) | 53°C, 30 cycles | 8.1 |
| GF9C1 | Mira et al. (2002) | 53°C, 30 cycles | 9.5 |
| GVBV12 | Gautschi et al. (2000) | 56°C, 30 cycles | 12.6 |

*Am* mean number of alleles per locus

[a] Loci are sorted by increasing mean number of alleles per locus

implemented in GENEPOP v 3.4. We estimated pairwise $F_{ST}$, and determined their statistical significance by 10, 000 permutations with MSA 3.12 (Dieringer and Schlötterer 2003). We then used the Mantel test (Mantel 1967) for correlation between $F_{ST}$ and geographical distance matrices based on 10, 000 permutations as implemented in GENEPOP v 3.4. This allowed us to evaluate whether there was generalized isolation by distance among native populations.

*Assessment of the random sampling strategy of founders*

The random sampling strategy can be considered effective if the genetic diversity within founding groups is similar to that in the native populations. To assess the effectiveness of the random sampling strategy of founders, we compared the genetic variability between native and captive founding groups and we estimated the genetic differentiation between these groups. In all analyses, we excluded the Spanish population from the native group to avoid duplicate individuals. We quantified genetic variability within each captive founding stock using the same tests as for the native populations. We used the ''Comparison among groups of samples'' option of FSTAT 2.9.3.2 with 10, 000 permutations to assess the significance of differences in allele richness, observed heterozygosity and relatedness between native and captive founding groups. To assess genetic differentiation between native populations and captive founding stocks, we used pairwise $F_{ST}$ as before.

*Assessment of founder effect in the settled reintroduced population*

If a founder effect occurred in the settled reintroduced population (Causses), the genetic diversity is expected to be smaller than in native populations. We quantified the genetic diversity of the Causses population using GENETIX 4.02 (Belkhir et al. 1996) and we estimated Queller and Goodnight's (1989) estimator of relatedness among individuals. We then compared the allele richness, observed heterozygosity and relatedness between the native and reintroduced groups using the ''Comparison among groups of samples'' option of FSTAT 2.9.3.2 with 10, 000 permutations.

*Assessment of genetic connections between settled reintroduced and native populations*

We estimated recent migration rates among the native and the Causses populations using the Bayesian inference method based on Markov chain Monte Carlo approach (MCMC) implemented in BAYESASS 1.2 (Wilson and Rannala 2003). This method uses individual multilocus genotypes to estimate rates of recent immigration (last generation). It requires that all populations exchanging migrants have been sampled. We thus considered, for this analysis, the 64 individuals coming from Spanish rescue centre as a Spanish group. The use of the hatching cohorts two generations after release in Causses allowed us to limit the effect of origin of founders. To examine the strength of the information in the Griffon vulture microsatellite dataset, 95% confidence intervals were determined for migration rates and compared to a scenario where all proposed changes throughout the Markov chain are accepted. Thereby simulating the event where any information that may exist in the data is insufficient to affect the posterior distribution of migration rates. As recommended by the authors, we ran $3 \times 10^6$ MCMC iterations, with the first $10^6$ discarded as burn-in. Optimal delta values for migration rate ($m$), inbreeding coefficient ($F$) and allele frequencies ($p$) were fixed at $m = 0.10$, $F = 0.15$ and $p = 0.10$ according to the numerical convergence of MCMC algorithm assessment in three previous runs. We performed eight runs, rearranging the order of the populations in the input file to test the reliability of the results.

## Results

Except for the Croatian and Navacelles populations, for which GVBV13 was monomorphic, all loci were polymorphic in native populations, the settled reintroduced population and in founding stocks. The mean number of alleles per locus and per population over all studied individuals ranged from 2 to 12.6 (Table 1). No significant genotypic linkage disequilibrium was detected after Bonferroni corrections. The probability of identity for the ten-locus combination was less than 0.001, i.e., the ten loci used in this study were polymorphic enough to unambiguously characterize each individual by a unique multilocus genotype.

For all populations in each of the three groups, HWE at each locus was not rejected except for GF8G1 in Causses as well as GF3F3 and GVBV13 in Diois (Table 2). According to this test, each settled (either native or reintroduced) population could thus be considered as a panmictic pool of individuals. Moreover, as no Wahlund effect was detected in captive founding groups, the founding groups can be considered as random samples of a panmictic population.

### Fragmentation of native populations

The mean number of alleles per polymorphic locus, the allelic richness and rates of heterozygosity (both observed and expected) were similar in all native populations (Table 2).The estimator of relatedness among individuals in native populations was $r = -0.0071$ (±0.001). This value was similar to the one obtained in among wild born mated pairs of Eastern imperial eagle population (*Aquila heliaca*, $r = -0.0027 \pm 0.0367$, $n = 31$, Rudnick et al. 2005).

Global *F*-statistics among native populations were characterized by a low value of $F_{IT}$ (0.049), with most variance observed among colonies ($F_{ST} = 0.028$, $p < 0.001$). The $F_{IS}$ value (within-population variability) was 0.021. $F_{ST}$ estimated between population pairs were generally low (between 0 and 0.063) and were significantly different from 0 only between Croatia and all other groups and between Ossau and Israel (Table 3). Analyze of isolation by distance revealed no significant association between geographic distances and $F_{ST}$ ($r_{Mantel} = -1.4 \times 10^{-6}$, $p = 0.498$).

All genetic differentiation tests indicated that there was no statistical difference in allelic composition between Ossau and the set of 64 individuals coming from Spanish rescue centers that were released in the French Alps. Thus, we considered Ossau as a sub-sample of the Spanish population.

### Founder sampling strategy

The allelic richness and rates of heterozygosity (both observed and expected) of the captive founding groups were similar to that in native populations (Table 2). The estimator of relatedness among individuals in founding group was similar to the one in native populations and it was near 0 ($r = -0.009 \pm 0.0027$). It was lower than the one obtained in captive Bearded vulture population which was significantly different from 0 ($r = -0.051 \pm 0.007$, $n = 702$, Gautschi et al. 2003a). Comparisons among native and captive founding groups revealed no difference in allele richness ($p = 0.32$), observed heterozygosity ($p = 0.38$) or relatedness ($p = 0.45$). No genetic structure was observed among captive founding groups or between founding groups and native populations, except with Croatia (Table 4).

### Founder effect in the settled reintroduced population

The mean number of alleles per polymorphic locus in the settled reintroduced population was high (7.2, Table 2).

**Table 2** Genetic variability over all ten loci for the different groups of European Griffon vultures

| Group | Populations | *N* | *A* | *R* | *P* | $H_o$ | $H_e$ | Departure from HW |
|---|---|---|---|---|---|---|---|---|
| Native | Ossau | 85 | 5.9 | 3.7 | 100 | 0.588 (0.243) | 0.587 (0.223) | – |
| | Israel | 23 | 5.6 | 3.9 | 90 | 0.545 (0.314) | 0.524 (0.306) | – |
| | Croatia | 40 | 5.6 | 3.8 | 90 | 0.594 (0.291) | 0.604 (0.263) | – |
| Settled reintroduced | Causses | 106 | 7.2 | 3.9 | 100 | 0.580 (0.253) | 0.592 (0.239) | GF8G1 |
| Captive founding | Baronnies | 39 | 5.7 | 3.7 | 100 | 0.588 (0.254) | 0.573 (0.226) | – |
| | Diois | 30 | 5.7 | 3.8 | 100 | 0.565 (0.267) | 0.609 (0.191) | GF3F3, GVBV13 |
| | Verdon Canyon | 25 | 5.3 | 3.6 | 100 | 0.549 (0.291) | 0.561 (0.241) | – |
| | Navacelles | 15 | 4.8 | 3.7 | 90 | 0.533 (0.303) | 0.530 (0.298) | – |

Native and settled populations are given on the upper part; founding groups of reintroduction programs on the lower part

*N* sample size, *A* mean number of alleles per polymorph locus, *R* allelic richness *P* percentage of polymorph loci (95% threshold), $H_o$ observed heterozygosity, $H_e$ expected heterozygosity at Hardy–Weinberg equilibrium, *Departure from HW* loci showing a departure from Hardy–Weinberg equilibrium

**Table 3** $F_{ST}$ values (above the diagonal) between pairs of native populations and their statistical significance (calculated with MSA 3.12, below the diagonal)

| | Spain | Ossau | Croatia | Israel |
|---|---|---|---|---|
| Spain | – | 0 | 0.063[a] | 0.021 |
| Ossau | n.s. | – | 0.057[a] | 0.025 |
| Croatia | 0.0006 | 0.0006 | – | 0.055[a] |
| Israel | n.s. | 0.003 | 0.0006 | – |

[a] Significant $F_{ST}$ after Bonferroni correction

Moreover, the relatedness estimate among individuals in this group was as low as the one among individuals in native populations ($r$ = –0.0068 ± 0.003). The other indices of genetic variability also had the same range as those in native populations (Table 2). Comparisons with native populations revealed no decrease in allele richness ($p$ = 0.18), observed heterozygosity ($p$ = 0.97) or relatedness ($p$ = 0.43).

Genetic connections between settled reintroduced and native populations

The mean posterior probabilities and 95% confidence intervals for migration rates between Causses and native populations are reported in Table 5. We pooled samples from Ossau and Spain because of their low genetic differentiation. When all proposed changes were accepted in the Markov chain, which is equivalent to using no information from the data to estimate migration rates, we obtained a 95% confidence interval of approximately (0.67–0.99) for nonmigration rates and of (0–0.21) for migration rates. When the Griffon vulture data set was used to estimate migration, the confidence intervals were considerably smaller than those obtained from the above scenario, suggesting that our microsatellite data set contained an appreciable amount of information to estimate migration rates and other parameters of interest. Self recruitment was low in Israel and Causses, reflecting higher immigration rates in these populations (Table 5). Self recruitment was higher in Spain, which was the biggest population. We found higher migration rates from Spain to Israel than from Israel to Spain (Table 5). On the contrary, migration into and from Croatia was limited (Table 5). The migration rate estimated from Spain into Causses colony was very high (Table 5). Very few exchanges were detected between Causses and Croatia or Israel (Table 5). Rearranging the order of populations in the input files never affected the ‘‘source’’ status of Spain.

## Discussion

The measured genetic diversity was similar in all native and successfully reintroduced populations, as well as in captive founding groups of Griffon vulture. Moreover, the levels of genetic diversity in the Griffon vulture populations were higher than those estimated for other species of Vulture in Europe (*G. barbatus*, Gautschi et al. 2003b and *Neophron percnopterus*, Kretzmann et al. 2003). Low $F_{ST}$ among native populations confirmed the past existence of high dispersal rates among populations. However, the population of Croatia was significantly differentiated from all other Western and Eastern populations, although pairwise $F_{ST}$ estimate were low. This genetic divergence, which was not explained by isolation by distance, could be due to limited immigration into Croatia as we revealed with Bayesian methods. Restricted gene flow into Croatia is worrying because this population is small, unlike the Spanish one in which immigration is also low. Low immigration rates and small population size may quickly lead to genetic differentiation. We suggest that the present genetic structure is due to the recent isolation of Croatia from other populations. Indeed, in the Alps, geographically intermediate populations between Croatia and Ossau disappeared at the end of 19th century. Extinctions of intermediate populations between Croatia and Israel, in the Balkans, occurred along the 20th century. These extinction

**Table 4** $F_{ST}$ values (above the diagonal) between pairs of natives, settled reintroduced and captive founding populations and their statistical significance (calculated with MSA 3.12, below the diagonal)

| | Ossau | Croatia | Israel | Baronnies | Verdon Canyon | Diois | Navacelles |
|---|---|---|---|---|---|---|---|
| Ossau | – | 0.057[a] | 0.025[a] | 0 | 0 | 0.004 | 0 |
| Croatia | 0.002 | – | 0.055[a] | 0.055[a] | 0.069[a] | 0.067[a] | 0.043[a] |
| Israel | 0.01 | 0.002 | – | 0.017 | 0.024[a] | 0.032[a] | 0.020 |
| Baronnies | n.s. | 0.002 | n.s. | – | 0.002 | 0.004 | 0 |
| Verdon Canyon | n.s. | 0.002 | 0.01 | n.s. | – | 0.006 | 0.004 |
| Diois | n.s. | 0.002 | 0.002 | n.s. | n.s. | – | 0.018 |
| Navacelles | n.s. | 0.002 | n.s. | n.s. | n.s. | n.s. | – |

[a] Significant $F_{ST}$ after Bonferroni correction

**Table 5** Mean values and 95% confidence intervals of the posterior distributions for migration rates among native and settled reintroduced Griffon vulture populations

| To | Rate from | | | |
|---|---|---|---|---|
| | Spain + Ossau | Croatia | Israel | Causses |
| Spain + Ossau | 0.995 (0.98–0.99) | 0.001 (0–0.007) | 0.001 (0–0.007) | 0.002 (0–0.01) |
| Croatia | 0.04 (0–0.14) | 0.943 (0.83–0.99) | 0.009 (0–0.04) | 0.009 (0–0.04) |
| Israel | 0.142 (0.007–0.17) | 0.09 (0.004–0.13) | 0.747 (0.67–0.88) | 0.01 (0–0.06) |
| Causses | 0.29 (0.26–0.32) | 0.003 (0–0.01) | 0.004 (0–0.017) | 0.693 (0.67–0.72) |

Source populations are given in columns, recipient populations in rows. Values along diagonal are self-recruitment rates in each generation for each colony

events, which occurred only about seven Griffon vulture generations ago, may have isolated Croatia from others colonies. The same pattern of genetic differentiation was observed for a long lived raptor, the Spanish imperial eagle, *Aquila adalberti*, in a similar demographic situation and after only five to seven generations of isolation (Martinez-Cruz et al. 2004). Therefore limited dispersal rate may increase quickly genetic differentiation in a formerly widespread and common species. The same genetic pattern that we observed in our study could be expected for the Indian Gyps species which declined about 90% in the last 10 years (Prakash et al. 2003). Moreover, theoretical and empirical studies showed that fragmentation can rapidly affect the genetic differentiation among populations, whereas measures of within-population genetic diversity decrease more slowly (Keyghobadi et al. 2005). According to these studies, the combination of significant genetic differentiation and maintenance of within-population genetic diversity in the Croatian population may be explained by a recent isolation of this population from others populations. Unfortunately, banded breeders were rarely identified in Griffon vulture populations. This prevented us to combine genetic and demographic data to understand a possible nonequilibrium population structure in recently fragmented population as Tallmon et al. (2002) suggested. However, we propose that, together with measures of eradication of major threats such as poisoning, immigration of foreign birds into Croatia should be favored to optimize population viability, by reducing the loss of genetic diversity and preventing the loss of adaptive variants (Hedrick 2005). The risks associated with outbreeding are suspected to be low due to low $F_{ST}$ but should still be monitored through crossing in captivity of individuals from Croatia and other colonies before choosing individuals for reinforcement.

The genetic diversity of Griffon vulture founding stocks, assembled from randomly chosen individuals, was similar to that of native populations. The random choice of founders among growing populations is thus effective to retain genetic variability of native populations. Moreover, excepting Croatia, all settled populations of Griffon vulture were not differentiated. This limited the risks of outbreeding depression in founding stocks. It thus appears that the creation of highly diversified release stocks does not require elaborate sampling strategies for this species: all founders can be sampled in one population or in several populations depending on logistic constraints. In addition, maximizing the number of founders would decrease the impacts of post-reintroduction genetic drift on the genetic diversity of the newly founded populations (Frankham et al. 2002). The effectiveness of the random sampling strategy has direct implications for the management of other Griffon vulture reintroduction projects currently performed in the Balkan region, but also for the management of other formerly widespread and abundant species experiencing recent demographic decline. Nevertheless, the random choice of founders, although successful for the Griffon vulture, may prove riskier for species with a more critical conservation status, due to lower genetic diversity. For example, Haig et al. (1990) demonstrated that the random choice of founders performed worst to preserve the genetic diversity of inbred captive populations of Guam rails (*Rallus owstoni*). In such cases, an active genetic management of captive populations is needed prior to the creation of founding groups to prevent founder effects (Earnhardt 1999). Besides, Montalvo and Ellstrand (2000) have shown that, regardless of the sampling strategy, it may be difficult to achieve restoration of the species evolutionary potential by gathering individuals from several populations if genetics are ignored. The relative risks associated with a given release strategy could be evaluated in the pre-release phase of reintroduction via a combination of genetic analyses and demo-genetic modeling (Robert et al. 2004).

No loss of genetic diversity was observed in the Causses population. The high genetic variability of founders could explain the absence of founder effect in this successfully reintroduced population. Immigration into the newly founded population could also alleviate the potential negative effects associated with small population sizes and

genetic drift following a reintroduction event (Allendorf 1983). Indeed, we estimated a high migration rate from Spain into Causses. Immigration of Spanish individuals into Causses is supported by the observations of unbanded breeders suspected to be exogenous birds. In this case, our results revealed asymmetrical gene flow between native and reintroduced populations. The persistence of asymmetrical migration rates could have consequences on local adaptation (Kawecki and Holt 2002) and deleterious effects on metapopulation viability (Bouchy et al. 2005). Thus, future investigations on the causes of asymmetrical migration between native and reintroduced populations would be relevant to long-term management of the reintroduced population. However, the high migration estimates could also be due indirectly to immigration of individuals released in Navacelles that failed to settle in their release area and settled in Causses. As most of individuals released in Navacelles came from Spanish rescue centers, they could be considered Spanish migrants. Thus, the reinforcement by a spatially close reintroduction failure in Navacelles probably ensured the maintenance of the genetic diversity of the Causses population. Despite high genetic diversity in the founding stock of the Navacelles program, the birds failed to settle and most of them were attracted into Causses. The potential downside of dispersal should be evaluated in regard to the spatial distribution of release points. Our empirical study suggests that reintroduction programs in the Alps should benefit from immigration, thanks to their geographical location between Western and Eastern populations. This is supported by the frequent observation of individuals from Croatia, Ossau, Spain and Causses in newly settled reintroduced colonies of the Alps. Using a simulation approach Robert et al. (2003) showed that the genetic and demographic dynamics of a restored metapopulation is in fact influenced by the release design (e.g., one vs. several patches, isolated vs. connected to other settled populations), influencing the dispersal pattern between release sites. Overall, the attraction of foreign birds seemed to be an important factor for the success of Causses reintroduction. For animals that are not limited by their dispersal abilities, habitat selection behavior appears to condition the genetic success of the reintroduction program. Indeed, a study on genetic structure and migration in native and reintroduced Gray wolf (*Canis lupus*) populations in US (Forbes and Boyd 1997) showed that genetic variation of reintroduced populations could be retained if migration from native populations occurs, which is likely if habitat is not impacted by human activities. There is thus a need for a better understanding of dispersal behavior and for a modeling of its consequences on the viability of restored metapopulations, which will help managers define reintroduction strategies. Specifically, translocations are likely to enhance or create asymmetrical flux, whose consequences on long-term viability of native and restored populations should be monitored.

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