

Conservation genetics of Iberian raptors

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Abstract

Conservation genetics of Iberian raptors.— In this paper I provide an overview of conservation genetics and describe the management actions in the wild that can benefit from conservation genetic studies. I describe the genetic factors of risk for the survival of wild species, the consequences of loss of genetic diversity, inbreeding and outbreeding depression, and the use of genetic tools to delimitate units of conservation. Then I introduce the most common applications of conservation genetics in the management of wild populations. In a second part of the paper I review the conservation genetic studies carried on the Iberian raptors. I introduce several studies on the Spanish imperial eagle, the bearded vulture, the black vulture and the red kite that were carried out using autosomal microsatellite markers and mitochondrial DNA (mtDNA) sequencing. I describe studies on the lesser kestrel and Egyptian vulture that additionally applied major histocompatibility complex (MHC) markers, with the purpose of incorporating the study of non–neutral variation. For every species I explain how these studies can be and/or are applied in the strategy of conservation in the wild.

Key words: Conservation genetics, Conservation genomics, Molecular markers, Iberian raptors, Management of threatened populations.

Resumen

Genética de la conservación de rapaces ibéricas.— En este artículo se da una visión global de lo que es la genética de la conservación y cuáles son las acciones de manejo en la naturaleza que pueden beneficiarse de los estudios genéticos. Se presentan en primer lugar los factores genéticos de riesgo para la supervivencia de las especies y cuáles son las consecuencias de la pérdida de diversidad genética y de la depresión tanto por endogamia como por exogamia. Se explica el uso de las herramientas genéticas en la delimitación de las unidades de conservación. Tras ello se explica cuáles son las aplicaciones más comunes de la genética de la conservación en el manejo de poblaciones silvestres. En una segunda parte del artículo se hace una revisión de los estudios en genética de la conservación llevados a cabo en rapaces ibéricas. Se explican varios estudios llevados a cabo sobre el águila imperial ibérica, el quebrantahuesos, el buitre negro y el milano real usando marcadores en microsatélites autosomales y secuencias de ADN mitocondrial (mtDNA). Se describen estudios sobre el cernícalo primilla y el alimoche que han utilizado adicionalmente marcadores en el complejo de histocompatibilidad mayor (MHC) con el propósito de incorporar el estudio de variación no neutral. Para cada una de las especies se explica cómo estos estudios se pueden aplicar y/o se aplican en las estrategias de conservación de dichas especies en la naturaleza.

Palabras clave: Genética de la conservación, Genómica de la conservación, Marcadores moleculares, Rapaces ibéricas, Manejo de poblaciones amenazadas.

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What is conservation genetics?

Population genetics theory predicts that when a population goes through a steep and maintained decline drift will become the preponderant evolutionary force in detriment of selection. Under genetic drift, allele frequencies will change arbitrarily from one generation to another, with some alleles getting fixed and others getting lost, thus decreasing genetic diversity and increasing inbreeding in the population. Conservation genetics is a relatively novel discipline dealing with these issues by describing genetic patterns and assessing evolutionary processes in endangered species.

A loss of genetic diversity could undermine the adaptive potential of the population, and it has been shown that the adaptive diversity of a population is most of the times already present in the population and not created anew when it is exposed to an environmental change or a new environment (Barrett & Schluter, 2008). The consequences of a limited genetic diversity when facing an environmental perturbation are clearly exemplified in the response of hosts to novel pathogen infections (O'Brien & Evermann, 1988). Otherwise, some long-lived species show intrinsic low levels of diversity and perform as well as their sister species with higher diversity levels (e.g. Milot et al., 2007), but this seems to be more the exception than the rule.

In addition, from generation to generation, the inheritance of identical copies of the same allele will increase even under a random mating system. At the same time, recessive deleterious alleles that purifying selection maintains at low frequencies, and mostly in heterozygosity, might evolve under drift as neutral variants and eventually increase in frequency. Both factors contribute to the inbreeding depression of the population, compromising the persistence of the species or population in the long term. This has been documented extensively in the wild and for multiple taxa (Crnokrak & Roff, 1999; Keller & Waller, 2002). For instance, inbreeding depression has been invoked as the origin of the lethal coronary disease and low sperm quality in the Florida panther (Roelke et al., 1993), the mortality due to viruses in dolphins (Valsecchi et al., 2004), and the decrease of sperm quality in rabbits (Gage et al., 2006). Although desirable, predicting whether and to what degree a population will suffer from inbreeding depression is almost impossible due to the stochastic nature of drift, but some patterns have been recognized experimentally (Reed & Frankham, 2003; Reed et al., 2003; Armbruster & Reed, 2005).

The combination of reduced genetic diversity and inbreeding depression are expected to precipitate the extinction of endangered populations through their effects on fitness components. Even though some authors have argued that the extinction of a population will happen long before the genetic factors become sufficiently important to provoke it (Lande & Shannon, 1996), the existence of lower levels of genetic diversity in endangered species than in their non-endangered sister species argues against this view (e.g. Frankham, 1996). Furthermore, inbreeding depression has been shown to severely affect extinction probabilities in simulations (Tanaka, 2000; O'Grady et al., 2006) and empirical studies (Saccheri et al., 1998; Vilas et al., 2006; Reed et al., 2007).

The introduction of new alleles from external populations can ameliorate the negative effects of inbreeding, a phenomenon that has been termed 'genetic rescue'. For instance, the arrival of a single individual increased the genetic diversity and population size of a wolf population in Scandinavia (Vila et al., 2003), and the introduction of eight puma females into the Florida panther population restored genetic diversity and reversed the negative demographic trend of the population (Johnson et al., 2010).

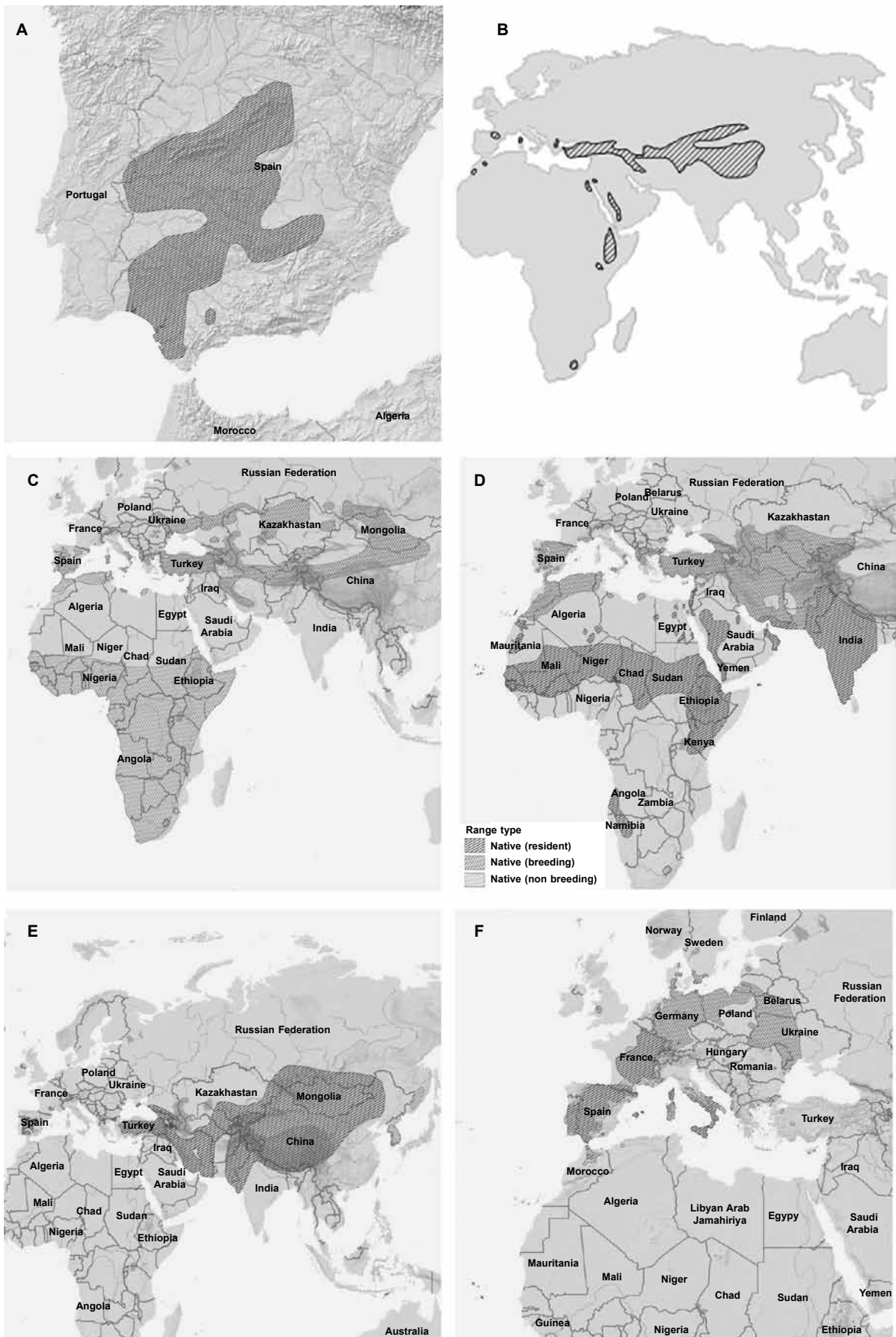
On the other hand, the mix of divergent gene pools may lead to the opposite problem, termed outbreeding depression, in which offspring or a second generation hybrids have a lower fitness than parents due to phenotypes not adapted to local conditions or the disruption of coadapted gene complexes by recombination (Lynch & Walsh, 1998). As in the case of inbreeding depression, evaluating the risks for outbreeding depression is very difficult.

In addition to measuring genetic diversity, molecular tools have been applied in conservation genetics to define conservation units (e.g., subpopulation, population, species) through the study of the evolutionary history of the population. The definition of these units relies on any genetic discontinuity requiring independent management and tries to answer the question of which populations to manage conjunctly or independently. Two main units of conservation have been proposed: ESUs (Evolutionary Significant Units) and MUs (Management Units, inside the ESUs).

Although there are different definitions of ESU in the literature (e.g. Ryder, 1986; Moritz, 1994; Crandall et al., 2000), the main criteria for a synthetic definition encompass reproductive isolation, adaptive differentiation and concordance across genetic, morphological,

Fig. 1. Maps with the distribution ranges of the Spanish imperial eagle (A), bearded vulture (B), lesser kestrel (C), Egyptian vulture (D), black vulture (E), and red kite (F). For A, C, D, E, and F maps were taken from the IUCN (2011, IUCN Red List of Threatened Species, version 2011.1 <www.iucnredlist.org>), downloaded on 10 June 2011.

Fig. 1. Mapas con los rangos de distribución de águila imperial ibérica (A), quebrantahuesos (B), cernícalo primilla (C), alimoche (D), buitres negro (E) y milano real (F). Los mapas A, C, D, E y F se han tomado de IUCN (2011, Lista roja IUCN de las especies amenazadas, versión 2011.1 <www.iucnredlist.org>), descargados el 10 de junio 2011.



behavioural, geographical data types (Allendorf & Luikart, 2007). Information from sets of molecular markers evolving at different rates provides a good base for assessing the main criteria for the delimitation of ESUs. This is usually based on neutral variation only, but the new genomic approaches promise to overcome this limitation (e.g. Primmer, 2009).

Every ESU might be sub-divided into one or more Management Units (MUs), described as a population or group of populations that are demographically independent (Moritz, 1994, 2002). Genetic markers do help delimitate MUs through the analysis of panmixia, gene flow and genetic structure (Waples & Gaggiotti, 2006; Palsboll et al., 2007).

Application of conservation genetics in the management of wild populations

The most commonly practised actions in management for conservation of endangered populations that can benefit from genetic studies are captive breeding, reintroduction of individuals in the wild and translocations. All three kinds of actions are complementary to any other management action and may try to maximize the global genetic diversity while preserving local adaptations.

Any captive breeding program should aim at incorporating a good number of healthy founders representing the genetic diversity remaining in the wild and at minimizing effects of the unavoidable drift. Genetic management of captive breeding populations should be conducted after compiling basic pedigree and demographic data on the population (Ballou & Foose, 1996). In the usual situation where information on the relatedness of the founders is lacking, molecular markers could provide these estimates to optimize genetic management (Gautschi et al., 2003a; Russello & Amato, 2004). Deleterious alleles in captive populations might stochastically drift to high frequencies and their elimination may then enter into conflict with the preservation of overall genetic diversity. For instance, the hereditary chondrodystrophy, a Mendelian disease due to a single recessive allele, could be prevented in the Californian condor captive population by avoiding crossing breeders that would produce affected individuals, but the reproduction of carriers could not be prevented without a major impact on global genetic diversity (Ralls et al., 2000). Nonetheless, the success of the reintroduction of Californian condor to the wild attests to the success of the application of genetic criteria in the captive breeding program (Ralls & Ballou, 2004).

The second common management action considers the reintroduction of populations in the wild. Genetic tools may assist in the careful selection of the origin, number, and sex of the released individuals, and in the monitoring of the reintroduced population. Whenever possible, the best candidates to be reintroduced in a determined area would be individuals belonging to the same lineages of the extinct populations or from nearby populations in order to maximize adaptation (Frankham et al., 2002).

In the short term, inbreeding depression and loss of genetic diversity can be counteracted by the translocation of individuals from a different population (the genetic rescue effect mentioned above). In this case, and in order to minimize the possible effects of outbreeding depression, individuals should come from nearby populations and the number of them should be the minimum needed to revert to the normal situation (Kleiman & Stanley, 1994).

Conservation genetics on Iberian raptors

Avian biodiversity in the Iberian peninsula is one of the highest in Europe. Due to its geographical location it is an important stopover for migratory birds on their way from northern Europe to Africa or viceversa. But Iberia is not only a crossing point as millions of birds migrate directly to the Iberian estuaries and wetlands to winter. Additionally, the high diversity of biotopes in Iberia harbours a large number of resident species.

Despite their ecological relevance, many birds of prey are threatened to different degrees due to a long history of direct human persecution and anthropogenic disturbances, such as electrocution on power lines, decreases in prey populations, poisoning, direct shooting, and landscape fragmentation. Many raptor populations have recently declined and become fragmented, some of them being currently confined to small and geographically isolated patches (see below). The genetic consequences of such processes and how genetics can assist raptor conservation are issues that have received much attention during the last decades. We present here what has been done in the field of conservation genetics as it applies to Iberian raptors.

The vast majority of works on this subject were carried out at the Molecular Ecology Laboratory (LEM) of Doñana Biological Station in Sevilla, Spain (on Spanish imperial eagles, Egyptian vultures, some of the bearded vulture studies, works on lesser kestrel as well as red kites) or elsewhere in collaboration with Doñana researchers (studies on bearded vultures by Gautschi et al., 2003). The creation of LEM in the mid 1990s boosted conservation–genetics–driven projects not only in raptors but also in many other non–model organisms, disentangling the genetic processes and patterns occurring in the wild and how they affect and can be used in the conservation of wild species.

The Spanish imperial eagle, *Aquila adalberti*

The Spanish imperial eagle is one of the most iconic species of the Iberian fauna. With around 200 pairs in the wild distributed in several breeding nuclei in the southern quadrant of the Iberian peninsula (fig. 1A) (Birdlife international), the Spanish imperial eagle is one of the most endangered raptors in the world (Collar & Andrew, 1988; Birdlife International, 2004). The species must have been relatively abundant until the mid–20th century with a distribution area that comprised most of the Iberian peninsula and

the north of Africa (Gonzalez et al., 1989). By the end of the 19th century and during most of the 20th century, the species went through a steep decline and population fragmentation due to human pressures (Collar & Andrew, 1988), mainly direct prosecution and collection (Gonzalez et al., 1989), poisoning (Ferrer, 2001), electrocution on power lines (Ferrer & Hiraldo, 1992), and two severe viral epidemics of its main prey, the rabbit *Oryctolagus cuniculus* (Villafuerte et al., 1995). Many traditional breeding areas were lost and the population that had previously been settled in humanized areas such as forested plains and fluvial valleys moved to non-humanized mountainous areas, probably as a response to human prosecution (Ferrer, 2001). The 1974 breeding survey revealed only 38 couples in the wild, although the populations started to recover in the 1980s. By the 1990s the species reached around 130 couples (Ferrer, 2001), and thanks to the conservation actions the population has been increasing since 2000, reaching 282 pairs in the wild in 2010 (Spanish Ministry of Environment, unpublished report). However, the population is still classified as vulnerable by the IUCN (International Union for Conservation of Nature) due to its small population size and because it still relies on human actions for its survival (IUCN, 2011).

To understand whether the demographic history of decline and fragmentation of the Spanish imperial eagle had affected the genetic variation of the species, a study was carried out using an extensive sampling of the present population covering the whole distribution range, and of the historical population, for both mitochondrial sequences and nuclear microsatellite markers (Martinez-Cruz et al., 2004, 2007). The historical population was represented by museum individuals covering the historical distribution range from 1853 to 1904 when the population was abundant and not endangered. The origin and evolution of the Spanish imperial eagle were further investigated by analysing its divergence from its sister species, the Eastern imperial eagle (Martinez-Cruz & Godoy, 2007), that numbers over 5000 breeding pairs (Ferrer & Negro, 2004) and is not globally threatened.

Only three different mitochondrial control region haplotypes differing in one or two base pairs were found in the Spanish imperial eagle, resulting in very low levels of contemporary haplotype diversity ($H = 0.322$), although levels were already low in the historical population ($H = 0.498$) in comparison with the higher diversity found in the Eastern imperial eagle ($H = 0.779$). In contrast, diversity in the nuclear genome seemed to have been unaffected by the demographic decline, and levels of diversity measured by heterozygosity between either current vs. historical populations (0.603 and 0.627, respectively) or between Spanish vs. Eastern imperial eagle populations (0.549 and 0.627, respectively) were similar. Nonetheless, the breeding nucleus of Doñana, in South Western Andalusia, showed lower levels of diversity and significant genetic differentiation from the other breeding nuclei. These effects were observed even though genetic and field monitoring data suggested some gene flow and dispersal from and into the population, which indicates extremely low effective

population sizes. Indeed, the number of breeding pairs in Doñana National Park is very low, there being only nine in 2009 (López-López et al., 2011), and it is the most geographically isolated nuclei. Historical genetic data indicate a globally panmictic population in the past, so the currently observed genetic structure is the direct consequence of the demographic decline and the fragmentation of the population. Under these circumstances, the authors indicated that conservation management actions should aim to restore the historical situation of the Spanish imperial eagle by managing the population as a whole as a single ESU and aimed to connect the genetically isolated nucleus of Doñana to the rest of the population. This could be done in the short term by the translocation of individuals, and in the long term by the recovery of populations that could serve as stepping stones to connect Doñana with the remaining nuclei. Since 2002, a reintroduction project in Cádiz has released about 45 individuals into the wild by hacking. In 2010, the project finally succeeded with the first reproduction attempt of a reintroduced couple (Junta de Andalucía, Counsel of Environment, www.juntadeandalucia.es/medioambiente). On the other hand, the continuous efforts to breed the Spanish imperial eagle in captivity have been unsuccessful so far, precluding the development of an *ex situ* conservation program for the species. Nonetheless, thanks to assisted reproduction techniques, the first imperial eagle chick born in captivity hatched in May 2011 (<http://www.jccm.es/web/en/CastillaLaMancha/index/notaPrensa1212703217441np/index.html>), but there is still a long way to go to develop a successful captive breeding program for the species.

Based on a recent divergence date and lack of complete isolation in the past estimated through coalescent-based analyses of genetic data (Martinez-Cruz & Godoy, 2007), the possibility of introducing individuals from the sister Eastern imperial eagle might not be completely ruled out if the situation turned critical.

The bearded vulture, *Gypaetus barbatus*

The bearded vulture is a large scavenger that mainly feeds on bones of medium-sized ungulates (Hiraldo et al., 1979). The species inhabits high-altitude mountain ranges in Eurasia and Africa; it is relatively abundant in Asia, from Anatolia to the Tian Shan and the Himalaya Mountains, and in Africa, with populations in South Africa, Lesotho, the Rift Valley and Morocco (Fig. 1B) (IUCN 2011), although very few individuals have been observed in Morocco in recent years (Godino et al., 2003, 2004). While in the past the species was widely distributed in Southern Europe, during the 20th century the population declined dramatically, becoming extinct in the Alps, the Balkans, Southern Spain and continental Greece. By the end of last century the European population was confined to the Pyrenees (with around 100 breeding pairs, Heredia & Heredia, 1991), and two small and highly endangered populations in Crete and Corsica (with less than 10 breeding couples on each island, Thibault et al., 1992; Xirouchakis & Nikolakakis, 2002; Schaub et al., 2009).

In Central Europe a captive breeding program was established to serve as source population for the reintroduction of the bearded vulture in the Alps (Frey et al., 1995). The origin of founders was vaguely known, the majority coming from Central and South Asia and the Caucasus, and some of them from Europe (Frey et al., 1995). By 2005, 137 individuals had been released in the wild (Schaub et al., 2009) and at present there are almost 20 breeding territories and up to 15 incubating couples per year (Zink, 2009). After such success, a plan started for the reintroduction of the bearded vulture in the Cazorla Mountains, where the population had become extinct by the end of the 1970s (Hiraldo et al., 1979), and the first releases took place in 2006 (Simón et al., 2007) and continued until 2010. Additionally, many European countries started *in situ* conservation actions to preserve the remaining populations.

Although management actions were already on their way, it was recognized that application of molecular tools could help to improve the short and long term success of such actions (Negro & Torres, 1999; Gautschi et al., 2003b; Godoy et al., 2004; Simón et al., 2007). Additionally, the knowledge of the evolutionary history of the species would be crucial in the delimitation of conservation units for the optimal management of genetic variation in the species and to decide whether the design of the breeding program in the Alps, which included the mixing of geographically separated populations, might have inadvertently and inappropriately mixed divergent evolutionary lineages (Gautschi et al., 2003a; Godoy et al., 2004).

Gautschi et al. (2003a) used 14 nuclear microsatellite loci and sequence information on 268 bp of the mitochondrial control region to estimate kinship in the artificially built captive population, to test close kinship between founder pairs and to determine the unknown population of origin of some founders. After assessing the power and reliability of their dataset and the Queller & Goodnight (1989) kinship estimator to estimate known kinship within the captive population, and with the help of the scarce information on the origins of some individuals, the authors were able to exclude the existence of full-sib and parent-offspring relationships among most founders, but conservatively cautioned against the pairing of some founders. They concluded that unless the strategy of avoiding crosses among close relatives was abandoned and as long as the size of the captive population remained small, inbreeding was not a potential problem in the bearded vulture captive population.

Subsequently, Godoy et al. (2004) performed a study on the mitochondrial DNA variation in both the current and the historical population (before the process of decline of the European population) to assess patterns in population diversity and structure and to make an inference on the evolutionary or ecological processes that originated them. The study of the historical population was possible through the sampling of museum specimens covering the whole historical distribution range. Their analysis of 500 bp and 273 bp of the mitochondrial control region in the present and in the historical population, respectively, showed low levels of current diversity in Europe ($H = 0.40\text{--}0.90$)

in contrast with Asian levels ($H = 0.94$). The diversity in the Pyrenees was lower in the current population than in the historical population ($H = 0.40$ vs. $H = 0.72$). This decrease in diversity was due to the loss of some haplotypes and the increase in the frequency of the most frequent haplotype. Besides, historical bearded vulture populations showed a high level of genetic differentiation, with F_{ST} values higher than 0.25 even among close populations. This could be due to low rates of dispersal in both sexes as the same pattern has been found in nuclear microsatellite markers (Gautschi, 2001), or it could be the consequence of the recent isolation of populations and the consequent increase in genetic drift. Field observations support the highly philopatric dispersal behaviour hypothesis that is also consistent with the clear phylogeographic pattern observed. In any case, the marked genetic structure of bearded vulture populations indicated that the extinction of European populations during the last century resulted in the loss of a high proportion of the global genetic diversity of the species. Moreover, this process may continue in the small populations of Crete and Corsica as they approach extinction.

The phylogeographic analysis of the mitochondrial variation revealed the existence of two mitochondrial lineages with a mostly disjoint geographical distribution in Eastern (Asian and East African) and Western (European and North African) populations, but with some European populations exhibiting similar frequencies of both lineages (Godoy et al., 2004). The observed patterns suggested that the evolutionary history of the bearded vulture have been marked by a period of isolation in allopatry and a subsequent secondary admixture forming a hybrid zone in Europe and possibly in North Africa following the range expansion of the Eastern/African lineage. Furthermore, these results were not concordant with the delimitation of two subspecies, *G. b. barbatus* in Eurasia and North Africa and *G. b. meridionalis* in South and East Africa, previously proposed on the basis of morphological differences (Hiraldo et al., 1984).

The absence of reciprocal monophyly between the European and the Central Asian populations impeding the rejection of the hypothesis of genetic exchangeability, and the lack of evidence to reject ecological exchangeability argue against their delimitation as different ESU. The authors (Godoy et al., 2004) recommend managing all the population as a whole, validating the strategy of admixing Central Asian and European founders in the current captive breeding for posterior reintroduction programs in the Alps. In fact, the eventual dispersal of reintroduced individuals or their descendants into the remnant European populations could help counteract the effects of drift in these threatened populations.

The lesser kestrel *Falco naumanni*

The lesser kestrel *Falco naumanni* is a small falcon that inhabits steppe and pseudo-steppe ecosystems (Cramp & Simmons, 1980). It is widely distributed in the western Palaearctic and mainly winters in sub-Saharan Africa (Fig. 1C; IUCN, 2011). Although the

global population outnumbers 100,000 individuals, the population was been considered vulnerable until 2011 by the IUCN after a decline of up to 95% in the Palearctic since 1950, mainly due to agriculture intensification, afforestation and urbanisation (Birdlife International, 2011). Many populations in Europe have since become extinct (Biber, 1990), resulting in the current patchy distribution. Recent surveys estimate 8,000 breeding pairs in Spain (IUCN, 2011). Various studies have shown the high natal and breeding philopatric behaviour of this species, as indicated by a steep negative relationship between effective dispersal and geographical distance (Negro et al., 1997; Serrano et al., 2001; Serrano & Tella, 2003; Serrano et al., 2003). Several studies have analysed populations in Spain, France, Italy, Greece, Israel and Kazakhstan in order to understand how the decline and fragmentation of the lesser kestrel population, together with the phylopatric behaviour of the species, has affected the genetics of European populations. For this purpose they have analysed both the neutral –autosomal microsatellite markers and mitochondrial control region sequences–, and the functional variation –the second exon of an MHC class II B locus (Alcaide et al., 2008a, 2008b, 2009a), that is involved in the immune response against parasites and bacterial infections. The contrast of both neutral and non-neutral systems was expected to reveal the extent of local adaptations in a species with low gene flow levels.

Both neutral and non-neutral markers showed that populations had a pattern of isolation by distance, probably increased by the fragmentation of the populations, as suggested by the authors (Alcaide et al., 2009a). However, the allelic distribution for the highly diverse MHC system across the distribution range (average heterozygosity = 0.98) was less uniform and many private alleles were observed, in contrast to neutral nuclear diversity, which may have been homogenised by relatively rare long-distance natal dispersal events (Prugnolle et al., 2003; Alcaide et al., 2009b). The MHC geographic pattern was more similar to that found for mtDNA, and the authors attributed the lower effects of drift observed at the MHC locus, in comparison to mtDNA, to balancing selection. They concluded that given that all lesser kestrel populations inhabit similar ecosystems their results would be explained by adaptation to local pathogen communities favouring the most locally efficient alleles although preserving a lot of genetic diversity to deal with a high array of pathogens. In contrast, the sister species, the Eurasian kestrel *F. tinnunculus* (that contrary to the lesser kestrel is panmictic, cosmopolitan, generalist and a long distance disperser) showed a higher level of MHC diversity in correlation with a higher burden of parasites and bacterial infections when populations of both species were compared, despite showing no higher levels of neutral microsatellite diversity in the continent ($H_o = 0.66$ for both species).

Among the management actions taken to recover European lesser kestrel populations, there are several ongoing captive breeding and reintroduction programs. The captive and some reintroduced populations were

genetically monitored through the study of genetic diversity and paternity analyses using the same eight microsatellites markers from the studies above (Alcaide et al., 2010). The genetic diversity in the captive breeding populations was similar to that in the wild and rejected a low genetic diversity as the cause of low hatching rates documented in captivity (Colás et al., 2002; Pomarol et al., 2004). However, levels in reintroduced populations were slightly lower than those of their source captive populations. This may have been caused by large variances in breeding performance of the reintroduced individuals: 28% of the breeders fathered 56% of the offspring, which would negatively affect the effective population size (N_e). Neither captive population differed genetically from its source populations. Neither did reintroduced populations differ from natural populations, suggesting that the captive breeding programs are not compromised by genetic factors, even in the absence of explicit genetic management. However, genetic monitoring was prudently advised in order to maximize the diversity of any reintroduced population in the wild, especially if this population was to be geographically isolated. It would also be very useful to monitor the evolution of the breeding success of the reintroduced individuals over time, as a main determinant of N_e and thus genetic diversity and inbreeding, and this could include not only neutral markers but also fitness-related markers as genes of the MHC.

In a nice example on how adaptive loci can help in conservation and management of wild populations, Rodríguez et al. (2011) applied MHC markers to infer the breeding origin of lesser kestrels in wintering quarters in sub-Saharan Africa. The genetic distances estimated confirmed the genetic connectivity of the western European populations to the wintering quarters in Senegal and rejected their connectivity to the South African populations, giving valuable information on the migration patterns of an endangered species.

The Egyptian vulture *Neophron percnopterus*

The Egyptian vulture is a long-lived, highly philopatric scavenger widely distributed around the Mediterranean Sea, the Middle East, Central/Southern Asia, the Indian sub-continent and sub-Saharan Africa (Fig. 1D). Considered as 'endangered' by the IUCN due to anthropogenic disturbances (IUCN, 2011), in the last decades the Egyptian vulture experienced a severe decline throughout its European distribution range (Tucker & Heath, 1994). Currently, the largest European population lives in the Iberian peninsula, with around 1,500 breeding pairs (Birdlife International) distributed throughout Navarra, Aragón, Cordillera Central, in the North, and Extremadura and Andalucía in the South (Perea et al., 1990), although the decrease during the 1980s reached 70% in some of these regions (IUCN, 2011). Additionally, there are two island populations in Spain, one in Menorca in the Balearic archipelago of the Mediterranean Sea, with 41 breeding pairs (De Pablo, 2000), and one in the Canary Islands in the Atlantic, where most of the population concentrates in Fuerteventura (around 30 breeding territories during the last decade, Agudo et

al., 2011b) and it is considered a different subspecies *N. p. majorensis* (Donazar et al., 2002b). Island populations have also suffered strong declines in the recent past (De Pablo, 2000; Palacios, 2000; Donazar et al., 2002b). Whereas the Iberian population is migratory (Brown and Amadon 1968), both island populations are non-migratory (De Pablo, 2000; Palacios, 2000). Under this circumstance, populations in Menorca and in the Canary Islands could be genetically differentiated from each other and from the mainland, and could be evolving in isolation, thus exacerbating potential genetic problems in such small populations.

Whether these island populations are actually genetically differentiated and whether there is gene flow among them has been the object of several studies (Kretzmann et al., 2003; Agudo et al., 2010, 2011b). Kretzman et al. (2003) genotyped nine microsatellite nuclear markers and Agudo et al. (2010, 2011a) used twenty-two microsatellites in an extensive sampling from all the Spanish distribution range from both mainland and inland populations. Consistently, the population from the Canaries was found to have less genetic variability than Menorca and Iberian populations, with Menorca showing levels of diversity similar to those of the mainland, and both inland populations showing signals of a recent bottleneck (Kretzmann et al., 2003; Agudo et al., 2011b). Effective population sizes on the islands were low, with 38 individuals in Fuerteventura and 34 individuals in Menorca (Agudo et al., 2010), whereas they were higher but still low for the Iberian populations (45 individuals for the southern and 128 individuals for the northern). The analysis of population structure showed that both inland populations are genetically differentiated from the mainland populations ($F_{ST} = 0.078-0.095$ for Menorca and $F_{ST} = 0.103-0.114$ for Fuerteventura respectively), and that the level of differentiation between the two inland populations is even larger ($F_{ST} = 0.144$; Agudo et al., 2010). Still, some migrants from the peninsula and even from further populations have been found to reach the island populations and manage to reproduce (Agudo et al., 2010, 2011b). Nonetheless, as this level of gene flow is insufficient to prevent the primary role that drift is having over selection it might compromise the ability of the island populations to cope with new infectious diseases and thus may have important consequences for those populations in the long term (Agudo et al., 2011a). The study of MHC class II genes (Agudo et al., 2011a) in both mainland and inland populations suggests that even if drift is the main acting force and that diversity is reduced in inland and bottlenecked populations, the co-evolution of duplicate genes is counteracting the losses of genetic diversity in the MHC, thus maximizing the capacities of antigen recognition to face infections, which would be of crucial importance overall in these insular populations.

Although geography did not explain any genetic difference, mainland populations also showed moderate levels of structure, indicating that the population in Andalucía differs genetically from the rest, probably as a consequence of its recent fragmentation (Agudo et al., 2010). This Iberian structure was not found by

Kretzman et al. (2003) but this may be caused by their lower sampling size and the low number and heterospecific origin of the microsatellites used. The authors recommended that special attention should be paid to this southern population in order to prevent losses of genetic diversity and to facilitate conservation of the Iberian populations as a whole.

The black vulture *Aegypius monachus*

The distribution area of the black vulture has been severely reduced due to anthropogenic disturbances in the last century (Hiraldo, 1974; Donazar et al., 2002a) when many breeding areas across the European continent were lost (Cramp & Simmons, 1980; Meyburg & Meyburg, 1984). Currently, European populations are restricted to the Iberian peninsula, the Balkan and the Caucasus, extending to the less known Mongolian, Pakistan and Kazakhstan populations further east (fig. 1E) (IUCN, 2011). Field observations have failed to provide any evidence of contact between the Balkan and the Iberian populations since the disappearance of intermediate populations (Poulakakis et al., 2008). Poulakakis et al. (2008) carried out a genetic study across the whole distribution range in order to evaluate the current genetic status of the vulnerable Balkan and Iberian populations. By sequencing a fragment of the mitochondrial *cytochrome b* gene (*Cyt b*) and genotyping eight nuclear microsatellites in three European and the Mongolian populations they aimed to gain insights into the current and past processes shaping the genetic diversity in the species, and also to delineate management units for conservation.

While a lower mitochondrial diversity was found in both European populations than in Mongolia, no signals of genetic erosion were found at the nuclear level. The seven mitochondrial haplotypes found clustered in four deeply divergent and geographically separated lineages: A–Balkans, B–Iberia, C–Caucasus, D–Mongolia. Microsatellite analyses supported this phylogeographic distribution. The authors suggested a low level of gene flow due to the highly philopatric dispersal behaviour of the species and claim for the delimitation of different ESUs, based on reciprocal monophyly of the mtDNA clades and a high differentiation at microsatellite allele frequencies (Moritz, 1994, 1999). Nonetheless, when looking at the structure plot (figure 3 in Poulakakis et al., 2008) one can suspect the admixed ancestry of some individuals, at least in the Caucasus and the Mongolian populations, as well as the existence of one migrant in the Mongolian population. Given the low sample size of the population in the Caucasus, a better sampling could eventually reveal higher levels of migration in both directions. In that case, the delimitation as separate ESUs might not be warranted. Moreover, additional criteria should be taken into account in order to delimitate ESUs (Allendorf & Luikart, 2007).

More information could be obtained through the analysis of the historical population using museum specimens, as well as a more informative set of genetic markers. In the case that the historical genetic and ecological exchangeability could not be rejected

(Crandall et al., 2000), the reintroduction of populations in Europe and the translocation of individuals could be envisaged as actions to increase the genetic diversity of the species.

The red kite *Milvus milvus*

With a geographical distribution limited to the western Palearctic (fig. 1F) the red kite is considered as near-threatened by the IUCN (IUCN, 2011). During the 20th century there was a strong decline in most of its distribution range. Some populations went extinct, as in the Canary Islands (Cramp & Simmons, 1980; Evans & Pienkowski, 1991), and the range became fragmented. This process of decline left two disjointed populations in Spain, Central and Southern Spain, and the process is still going on in southwest and eastern Europe (Viñuela et al., 1999). At present, the northwestern and central European populations are stable and recovering whereas the southern populations are not.

Roques & Negro (2005) made a genetic study covering the Central European (Germany, Switzerland, Luxemburg, and France) and the Southern European (Central Spain, Southern Spain, Italy, Mallorca and Menorca) populations by sequencing 357 bp of the mitochondrial control region. For comparison, they also typed one population of its successful sister species, the black kite *M. migrans* from Southern Spain. They tested the hypothesis that bottlenecks in the last century could have negatively affected the diversity of the species. Indeed they found very low levels of diversity, with two mitochondrial haplotypes dominating (82% in global) distributed in almost all populations. The lowest diversities were found in Southern Spain, Menorca and Mallorca (with a single haplotype; $H = 0.000-0.529$; $\pi = 0.0000-0.0016$), populations that are peripheral and in decline, and the highest diversities were found in central Europe (where the bulk of the population is; $H = 0.800$; $\pi = 0.0062$) and in central Spain ($H = 0.905$; $\pi = 0.0045$). The exception was Italy, with high levels of diversity ($H = 0.667$; $\pi = 0.0037$) despite a low breeding size that may still be reflecting pre-bottleneck levels of diversity. When comparing the southern Spanish red and black kite populations, levels of diversity were around half for the former. Both species depict two phylogenetic clades that would have diverged during the last glaciations of the Pleistocene, between 129 and 650 ky ago. Although no clear structure is found among the red kite populations probably due to the high dispersion capabilities of the species, a shallow separation exists between an eastern group (Germany, Central Europe and Italy) and a western group (Central Spain, Menorca and South Spain). The authors suggested that the genetic patterns found may have been shaped by the evolutionary history of the species and some contemporary events, including northward post-glacial expansions and recent successive bottlenecks and range contractions affecting more the peripheral southern populations. For these reasons they advise the reinforcement of those populations with individuals from healthier populations showing the closest genetic

relationship. For the impoverished Spanish populations, they suggest the central Spanish one as the source population of choice.

Conclusions and perspectives

Many species of Palearctic raptors have common recent histories of decline and fragmentation due to anthropogenic perturbations. Although Iberian raptors are capable of flying long distances, in most cases studies have shown common patterns of high genetic structure, in part due to low gene flow and low genetic diversity, which is exacerbated in the case of small and isolated populations. Studies on the populations predating the most recent anthropogenic disturbances show indeed that some of these patterns were already present historically, so one can hypothesize that the decline of the species could have started long ago. In other cases, however, the study of the historical population has revealed genetic erosion that may be directly attributed to the recent decline.

Similar processes may have different consequences for different species, implying that different management actions are needed. Genetics can help in adopting the adequate conservation measures, but most notably so when adequate markers are used and historical and contemporary patterns and processes are conjunctly investigated.

To date genetic tools have been successfully applied to conservation and a huge amount of insightful works have been performed in the very recent field of conservation genetics. However, many questions remain unresolved, in many cases due to technical limitations. The markers used have some limitations as the neutrality of mtDNA loci is not widely accepted (see Ballard & Witlock, 2004, for a review on the biology of this organelle) and microsatellite markers suffer from high homoplasy and back mutation rates (but see Estoup et al., 2002). Additionally, the use of a low number of markers has some limitations regarding accurateness in estimating parameters of interest to population genetics. Non-neutral markers have also been applied, but such studies are strongly biased towards MHC markers. Fortunately, with the recent development in genomic techniques such as next generation sequencing, whole genome scans and gene-expression pattern analyses, together with the decrease in costs of such techniques it is becoming possible to apply genomic approaches to non-model species of ecological and conservation relevance to overcome these eventualities. Such techniques allow us to tackle many of the unresolved issues in conservation genetics that could not be properly addressed before now. On one hand, the use of thousands of neutral genetic markers is increasing the accuracy in estimating population genetic parameters that are important for managing populations (e.g. kin relationships, inbreeding coefficients, effective population size, demographic bottlenecks, migration rates, etc), by identifying and eliminating loci under selection (outlier loci) that bias these estimates. On the other hand, using genomic tools might help in the identification of

variation with potential functional importance to study the genetic basis of local adaptation and inbreeding depression, highly relevant processes in conservation. In the last few years there have been some very interesting reviews on the high number of possibilities that genomics is opening to conservation studies (e.g. Primmer, 2009; Allendorf et al., 2010; Ouborg et al., 2010a, 2010b), and there is no doubt that genomic approaches will become routine in conservation in the very near future.

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