Title: **Intensive management and natural genetic variation in red deer (*Cervus elaphus*)**

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Running Title: Genetic diversity in fenced vs wild deer populations

Abstract

The current magnitude of big-game hunting has outpaced the natural growth of populations, making artificial breeding necessary to rapidly boost hunted populations. In this study we evaluated if the rapid increase of red deer (*Cervus elaphus*) abundance, caused by the growing popularity of big-game hunting, has impacted the natural genetic diversity of the species. We compared several genetic diversity metrics between 37 fenced populations subject to intensive management and 21 wild free-ranging populations. We also included a historically protected population from a national park as a baseline for comparisons. Contrary to expectations, our results showed no significant differences in genetic diversity between wild and fenced populations. Relatively lower genetic diversity was observed in the protected population, although differences were not significant in most cases. Bottlenecks were detected in both wild and fenced populations, as well as in the protected population. Assignment tests identified individuals that did not belong to their population of origin, indicating anthropogenic movement. We discuss the most likely processes, which could have led to the observed high levels of genetic variability and lack of differentiation between wild and fenced populations and suggest cautionary points for future conservation. We illustrate our comparative approach in red deer. However, our results and interpretations can be largely applicable to most ungulates subject to big-game hunting as most of them share a common exploitation-recovery history as well as many ecological traits.

Keywords: Hunting states, Microsatellites, Habitat fragmentation, translocations, big-game.

Introduction

Hunting for large mammals has long being part of human history (Fletcher 2011; Olivieri *et al.* 2014). Deer species in particular have been subject to intensive hunting during the 20th century, mainly by subsistence poaching, causing severe declines of many populations worldwide (Hoglund *et al.* 2013; Milner *et al.* 2006). During the past few decades, however, the economic development experienced in most regions has turned deer hunting into a highly lucrative activity, complementing and sometimes replacing, traditional livestock rearing and agriculture in rural areas (Mbaiwa 2004; Newey *et al.* 2010; Papaspyropoulos *et al.* 2012). Deer hunting also holds associated environmental benefits by conserving the species' natural habitat. However, the current magnitude of big-game hunting has outpaced the natural growth of populations, making artificial breeding (or big-game ranching) necessary to rapidly augment populations. Thus, current deer abundances are been boosted from intensively managed populations with an economic interest.

While various studies have evaluated the consequences of deer population declines (i.e. bottlenecks) (Goodman *et al.* 2001; Haanes *et al.* 2011), as well as some the recovery actions taken, such as re-introductions (i.e. founder effects) (Conard *et al.* 2010; Hajji *et al.* 2008; Hundertmark & Van Daele 2010), translocations and non-native introductions (i.e. hybridization) (Biedrzycka *et al.* 2012; Fernández-García *et al.* 2014; Perez-Espona *et al.* 2013; Senn *et al.* 2010; Smith *et al.* 2014; Torres *et al.* 2016), and range expansions (Haanes *et al.* 2010; Pérez-Barbería *et al.* 2013; Ryckman *et al.* 2010), it is unknown how rapid population increases and intensive management have impacted the natural genetic composition of the species.

Here we present a study case from Andalusia, southern Spain, where we conducted a large-scale genetic survey of the Iberian red deer (*Cervus elaphus hispanicus*), which was hunted almost to extinction during the first half of the 20th century (De Leyva 2002), and whose populations are now being recovered mainly for commercial hunting. In this region, hunting estates have experienced an unprecedented growth fueled by the economic development in the 1960s and the application of the hunting law of 1970 (Soriguer *et al.* 1994). Currently, 75% of the hunting area is fenced, owned mainly by private states (Landete-Castillejos *et al.* 2010), but some wild populations under governmental management still remain as free-ranging (Supplementary material S1). In addition, a few historically protected populations still exist within natural reserves and national parks (Galarza *et al.* 2015).

Only two previous studies have specifically compared genetic diversity between wild and managed red deer populations in Spain, and they have found incongruent results. In the first study, Martinez *et al.* (2002) did not find genetic differences between wild and managed populations, whereas in a later study Queiros *et al.* (2013) found the opposite. The relatively small number of populations analyzed in both studies (16 in Martinez *et al*. 2002; 4 in Queiros *et al.* 2013) makes it difficult to draw conclusive statements about patterns of genetic diversity between populations under varying levels of anthropogenic influence. A systematic comparison with large sample sizes, both in terms of number of populations and number of individuals is therefore needed to better understand the impact of management in genetic diversity.

In this study, we ask a basic, but yet largely unaddressed question; how does genetic diversity from fenced populations compares to that of wild populations?. On the one hand,

genetic diversity may be increased in fenced populations because management is often aimed to maintain diversity of certain phenotypic traits relevant to hunting practices. On the other hand, fenced populations may have reduced genetic diversity through drift and mutational processes because the number of breeders may be restricted, and because gene flow is suppressed by obstructing natural dispersion. Specifically, we test if i) wild (open hereafter) populations posses higher levels of genetic variability than fenced (closed hereafter) populations, if ii) closed populations are more genetically structured than open populations, and if iii) closed populations have experienced more bottlenecks due to confinement. As a reference for our comparisons, we included a historically protected population from a national park.

Materials and Methods

Samples collection.-

A total of 1270 tongue and 39 antler bone samples were collected from adult individuals shot over three consecutive hunting seasons (2003-2006) throughout Andalusia (Fig. 1). Individual samples originate from 21 open (N=498) open and 37 closed (N=811) populations with a mean of 22.6 samples/population. When available, the area (in hectares) and the census size data were collected (Table 2). Open populations consist of free-ranging herds whose natural dispersion is not affected by fencing and their management is minimal. Closed populations on the other hand, refer to herds within fenced areas with intensive management for commercial hunting purposes. The reference population from Doñana national park (Dn) is one of the few that persisted in Andalusia during the decline and has been protected ever since, with a strict conservation-only management (Soriguer *et al.* 2001).

The names of all sampling locations are not available and thus, we used two letters to identify them (Table 2).

DNA extraction and microsatellite amplification.-

Total genomic DNA was extracted from tongue tissue through a Hot Sodium and Tris (HotSHOT) protocol (Truett *et al.* 2000) and from antler bone following a Silica protocol (Milligan 1998). We genotyped all samples at 11 microsatellite loci previously isolated from other ungulates: TGLA94 (Georges *et al.* 1992), OarFCB193, OarFCB304 (Buchanan & Crawford 1993), CSSM43 (Barendse *et al.* 1994), BM302, BM203 (Bishop *et al.* 1994) RT1, RT13 (Wilson *et al.* 1997), NVHRT48, NVHRT73 (Røed & Midthjell 1998), MB25 (Vial *et al.* 2003). **Multiplexed PCRs were carried out according to Sánchez-Fernández *et al.* (2008) in a PTC-100 Programmable Thermal Controller (MJ Research Inc.) using the following conditions: an initial denaturation step at 95°C for 10min followed by 35 cycles of 30s at 94°C, 1 min at 54°C, 1 min 30s at 72°C and a last extension of 10 min at 72°C. Multiplex setup and PCR labeling is described in table 1.** Amplified products were resolved on an ABI Prism 3100 Genetic Analyser (Applied Biosystems) and scored in GENEMAPPER v 3.7 software (Applied Biosystems) **using LIZ labeled ladder (0-490bp) as size standard.**

Microsatellite analysis.-

Deviations from Hardy–Weinberg expectations (HWE) and linkage disequilibrium were estimated according to the level of significance determined by means of 10 000 MCMC iterations executed in GENEPOP v.4.0 (Rousset 2008). Significance was determined by applying a Bonferroni correction setting 5% threshold level (Rice 1989). The software MICROCHECKER (van Oosterhout *et al.* 2004) was used

to predict the most likely causes of departures from HWE (i.e, large allele dropouts or stutter bands). **Null allele frequencies for each locus and population were estimated using FREENA (Chapuis & Estoup 2007) with the EM algorithm.**

Genetic diversity

Genetic diversity within each population was characterized by calculating the mean number of alleles per locus using GenAlEx v.6 (Peakall & Smouse 2006), as well as by observed (H_O) and expected heterozygosities (H_E) calculated in Arlequin v.3.5.1.3 (Excoffier & Lischer 2010). Inbreeding coefficients (F_{IS}) for each population were calculated in GENEPOP software v.4.0 (Rousset 2008) according to Weir & Cockerham (1984). We used FSTAT v. 2.9.3 (Goudet 1995) to determine the effective number of alleles (R_S) correcting for sample size (i.e. allelic richness). The GenAlEx software v.6 (Peakall & Smouse 2006) was used to detect private alleles, that is, alleles exclusive to only one population (Slatkin 1985).

Genetic structure

To evaluate genetic structuring we implemented a Bayesian clustering algorithm using STRUCTURE v.2.3 (Pritchard *et al.* 2000). This method assigns individuals to populations according to their posterior probability of membership to each of the populations given the individual's multilocus genotype. Inference was performed using the correlated allele frequency model, with no prior information about individual's geographic origin or population-type (open-closed) specified. We set the number of populations (*K*) form one to 58, and ran three independent iterations consisting of a burn-in step of 300,000 MCMC

chains and 1,000,000 MCMC repeats after burn-in. We then used STRUCTURE HARVERSTER (Earl & vonHoldt 2012) to assess the likelihood of the different *Ks* according to the Evanno *et al.* (2005) method. Finally, we used CLUMMP v.1.1.2 (Jakobsson & Rosenberg 2007) to evaluate the consistency of the results across the iterations using the full-search algorithm. The software DISTRUCT v.1.1 (Rosenberg 2004) was used to graphically display the results.

Comparing genetic diversity between open and closed populations.-

To examine if significant genetic differences exist between open and closed populations, we compared estimates of R_S, H_E, and F_{IS} for each locus. Statistical significance for differences between the estimates was attained through a Mann–Whitney test performed in MATLAB v.7. (Mathworks). Furthermore, the software STRUCTURE v.2.3 (Pritchard *et al.* 2000) was used in two different ways. First, to assess differences at the population level, we grouped the populations as open or closed, and set K=2 using the correlated frequencies model. Second, to infer possible gene flow (i.e. individual translocations), we set the *USEPOPINFO* model to pre-specify that all individuals originate from their respective population. The number of generations backwards (*GENSBACK*) was set to 1 and K was fixed to the total number of populations (K =58). When using these parameters, miss-assignments reflect individuals with recent ancestry in a population other than where it was sampled. Both runs (K=2 and K=58) consisted of 1,000,000 MCMC repeats after 300,000 MCMC burn-in period. Finally, to evaluate whether closed and/or open populations have experienced recent bottlenecks, we used the software BOTTLENECK (Cornuet & Luikart 1997) setting 10000 replicates of the two-phased model (TPM) with 70% of the mutations following a step-wise mutation model (SMM) and 30% following an infinite alleles model

(IAM). A one-tailed Wilcoxon test was used to determine the significance of the resulting values.

RESULTS

Microsatellite analyses.-

Our results showed significant deviations from HWE at locus RT13 across all populations after Bonferroni correction. Likewise, significant stuttering was indicated by MICROCHECKER for locus CSSM43. Therefore, both loci were removed from further analyses. Mean null allele frequency within populations varied between 0.002 at locus TGLA94 to 0.193 in locus BM203 (Table 1). Previous studies have shown the influence of null alleles to be negligible at low frequencies (<0.2) (Dakin & Avise 2004). Thus, the rest of loci were kept for downstream analyses. We found no linkage disequilibrium between any locus pair. The observed measures of genetic diversity calculated from allele frequency distributions were high overall (Table 2). Relatively high levels of allelic richness (range 5.3 - 8.5) and average expected heterozygosity (range 0.69 – 0.82) were found across all populations (Table 2). The associated F_{IS} estimates for each population ranged between -0.010 and 0.127, displaying high positive values for both open (Cr, Cs, Cu, Cz, Ng, Nh, Ns, Pl, Re) and closed populations (Cd, Hl, Jt, No, Sd, Sn,Tj, Vz). Eight populations (Al, Jt, Tj, Oz, Br, Cu, Ti, Tm) displayed private alleles, accounting for a 5.59 % of the overall allelic diversity.

Genetic structure.-

The Bayesian clustering method implemented by STRUCTURE showed that the mean probability of the log-likelihood values (LK) saturated at $K = 8$ (Supplementary material S2). However, the *ad-hoc* method of Evanno *et al.* (2005), which is based on the rate of change of the log-likelihood probabilities (DK), indicated that $K = 5$ (Supplementary material S2).

Comparing genetic diversity between open and closed populations.-

Overall, genetic diversity as measured by heterozygosity, allelic richness, and F_{IS} estimates did not show significant differences (all P values > 0.05) between open and closed populations for any locus (Fig. 2). In the protected population, however, four loci showed lower heterozygosity values relative to open-closed populations (Fig. 2). A similar trend was observed in F_{IS} values, being overall smaller in the protected population, although the majority of values lied within the 25th and 75th percentiles observed for open-closed populations. Allelic richness was higher only in two loci from the protected population, whereas no differences were observed between open and closed populations at any locus (Fig. 2). The Bottleneck tests revealed evidence of recent bottlenecks in 14 populations, which represent a 24% of all populations analyzed. Among these, nine occurred in open populations (Ad, Ag, Cr, Cu, Dn, Fr, Ms, Nh, Pl), and five in closed populations (Ab, Ay, Nb, No, Pi).

The Bayesian approach showed no structuring when the samples were grouped into open and closed populations. The results were consistent across all three iterations (Supplementary material S3). However, when the individuals were pre-assigned to their own population, the Bayesian analysis identified six individuals that showed evidence of recent

ancestry in a different population, presumably as a result of translocations (Fig. 3; Supplementary Material S4). The majority occurred from population Al (open) to Cs (open), Jt (closed), Ng (open), and Sn (closed) populations. But also from Jn (closed) to Pt (closed), and from Br (closed) to Tj (closed) populations (Fig. 3; Supplementary Material S4). All assignment *Q*-values showed a high associated probability ($P < 0.001$).

DISCUSSION

In the present study we compared levels of genetic variability between wild and intensively managed fenced red deer populations. A historically protected population from a national park was also included as a baseline for comparisons. We did not find significant differences in genetic diversity between wild and fenced populations, and a high overall genetic variability was observed. We identified several individuals that were genetically assigned to other populations, indicating possible anthropogenic movement. Below we discuss the most likely processes, which could have led to the observed high levels of genetic variability and lack of differentiation between wild and fenced populations and suggest cautionary points for future conservation.

Conflicting results have been found by two previous studies that evaluated genetic variability in closed and open red deer populations. In the first study, Martinez *et al.* (2002) reported no differences, whereas Queiros *et al.* (2013) found the opposite in a later evaluation. Interestingly, the genetic variability from the protected population of Doñana was assessed by both studies. For this population, Queiros *et al.* (2014) found lower levels of variation, whereas Martinez *et al.* (2002) found a higher variation when compared to the other populations analyzed in their respective studies. Our results did

not show clear evidence supporting either a reduced or an enhanced genetic diversity in the protected population relative to the rest. Nonetheless, our results are in line with those of Martinez *et al.* (2002) in that no differences were observed between open and closed populations. It should be noted, however, that the open populations (n=8) analyzed by Martinez *et al.* (2002) were surrounded by fenced populations, making them effectively closed populations. In the present study we analyzed a larger number of open populations (n=21) that do not share borders with fenced populations. The two studies together suggest that fencing has a weak effect (but see below). Queiros *et al.* (2014) on the other hand, reported a higher genetic variability in the fenced population relative to other two populations that had a different management strategy.

Several explanations may be put forward in understanding previous results and ours. A combination of factors can give rise to a lack of genetic differentiation between open and closed populations. Firstly, for closed populations, a high genetic diversity observed could be due to a highly variable genome inherent to red deer. Other studies have also found high genetic diversity in red deer supporting this notion (Kuehn *et al.* 2003; Niedziałkowska *et al.* 2011; Pérez-Espona *et al.* 2009; Skog *et al.* 2008). Recently, a comprehensive study using microsatellites showed that red deer posses high levels of genetic variation throughout Europe (Zachos *et al.* 2016). A high genetic variation in closed populations may also be the result of a large effective population size at the time of fencing. Evaluating levels of genetic diversity before and after the creation of enclosures could help distinguish between these hypotheses. It has been shown that time-series analyses can reveal increases/decreases of genetic diversity in red deer and that these correlate well with management policies (Hoffmann *et al.* 2016). Unfortunately, analyses of this sort are not possible in our case since no historical red deer samples are available from our sampling area.

Secondly, it is possible that the effect of fencing in genetic diversity is not yet detectable. Other studies that have made similar comparisons to ours, have found no differentiation between wild and managed populations. For instance, introduced red deer in the island of Corsica showed no signs of reduced genetic variation compared to its Sardinian source after 20 years of the introduction (Hajji *et al.* 2008). Similarly, genetic variability did not differ significantly between domesticated and wild deer populations from North America, despite a domestication process of over 24 years (Cronin *et al.* 2009). The same result of no differentiation was observed in populations that had been isolated for more than 20 years between the German and Czech border (Fickel *et al.* 2012). **In our case, all of the closed populations were established after 1990 (Soriguer *et al.* 1994) when a law (Decreto 146/1998 de la Junta de Andalucía referente a la Ordenación Cinegética) allowed for their creation. Therefore, and inline with previous evidence, erosion of genetic diversity by drift and isolation, is probably not yet obvious within the timeframe of our study (≈ 25 years).**

Thirdly, our results show that undocumented translocations within Andalusia are not uncommon, and they are known to be widespread throughout Europe (Apollonio *et al.* 2014; Frantz *et al.* 2006; Skog *et al.* 2008) and North America (Williams *et al.* 2002) as well. In this respect, incoming breeders of different genetic background can quickly mask deleterious effects of drift and inbreeding (Vilà *et al.* 2003), and thus, maintaining genetic variation high in closed populations. This has been suggested by previous studies where unexpectedly high genetic diversity was observed in managed and presumably closed deer populations (De Garine-Wichatitsky *et al.* 2009; Queiros *et al.* 2013). This could also be a contributing factor to the trend of high genetic diversity reported in studies where translocations have been

identified (Karaiskou *et al.* 2014; Niedziałkowska *et al.* 2011; Pérez-Espona *et al.* 2009; Skog *et al.* 2008). Thus, anthropogenic movement of individuals into closed populations could help explain the comparable levels of diversity with their wild counterparts.

Contrary to expectations, we found genetic bottlenecks to be less common in closed populations. These results should be treated with caution, as many simultaneous factors may be causative. For instance, an initially large effective population size and/or high gene flow from neighbouring populations before fencing could explain the absence of a bottleneck in closed populations. On the other hand, for open populations, the genetic bottlenecks observed may not be necessarily attributed to reductions in population size only. Natural range expansion of a small number of breeders (i.e. founder effect) can be also reflected as a genetic bottleneck. Likewise, it should be noted that bottlenecks might go undetected if population abundance increases rapidly from a few founder individuals. This is best exemplified by a previous study that failed to detect a genetic bottleneck associated with a known demographic reduction of red deer populations (Hundertmark & Van Daele 2010). Contrasting results have also been found when different methods are applied to test for genetic bottlenecks (Queiros *et al.* 2013). Finally, as mentioned above, the red deer suffered a severe demographic decline throughout Spain, and its current genetic diversity represents that of the few relict populations that remained (Galarza *et al.* 2015). Thus, it is not possible to identify with certainty the process(es) underlying the bottleneck signal (or its lack of). However, our results are illustrative in that the theoretical expectation of enhanced genetic drift in closed isolated populations is not always met.

Our study provides comparative framework to address the potential implications that intensive large-scale management could have in a species' genetic

diversity. Monitoring genetic diversity is particularly important when a species has suffered a severe decline and is rapidly replenish by anthropogenic means outpacing its natural growth rate. We illustrate our comparative framework on red deer, but it can be largely applicable to most ungulates subject to big-game hunting as most of them share a common exploitation-recovery history, as well as many biological and ecological traits. In our case, we see no immediate reason for concern about loss of genetic variation. However, constant monitoring on genetic diversity should be carried out, particularly in closed populations. In addition, our set of markers is thought to be representative of neutral genetic variation. Future studies should also consider the monitoring of fitness-related genes to ensure population persistence.

In conclusion, our results suggest that fenced hunting enclosures are not a determinant factor towards genetic erosion as it could be expected. However, we wish to emphasize that the populations analyzed here have been managed for a relatively short time (≈ 25 years). Hence, the apparent high genetic diversity within closed populations does not imply that a detrimental effect cannot be ongoing or has the potential to arise. It has been shown that a loss of genetic diversity can gradually occur each generation when deer populations remain small and isolated for long periods (c.a. 130 years), resulting in strong inbreeding depression, which can have visible effects even in the phenotype (Zachos *et al.* 2007). In light of the rapidly increasing pace of management practices worldwide, we advise to carefully evaluate the genetic background of breeders in order to avoid both, outbreeding and inbreeding depression, whilst maintaining the autochthonous genetic diversity of the species.

Data Availability

Microsatellite primer sequences can be found in Sanchez-Fernandez *et al.*, (2008): DOI: 10.1111/j.1755-0998.2007.02034.x. GeneBank accession numbers of original sequences containing microsatellite are: G18774, L01533, L01535, AB204988, AF068214, AF068218, U90737, AF288204, U90743, U03824.

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Figure Captions

Figure 1. Study area in Andalusia showing 58 red deer sampling sites.

Figure 2. Comparison locus by locus of genetic diversity, Expected Heterozigosity (H_E), allelic richness (R_S), and F_{IS} between open (white boxes) and closed (grey boxes) red deer populations. The central mark in the box shows the median, the edges represent the 25th and 75th percentiles, while the whiskers extend to the most extreme data points not considered outliers. The continuous horizontal line indicates the value observed in the protected population (Dn).

Figure 3. Red deer estimated probabilities of population membership inferred by multilocus microsatellite genotypes. Each sample is represented by a vertical bar. Colours represent the population being assigned to**.** Only populations with individuals assigned to other populations are shown.

Supplementary material S1. Official hunting statistics in Andalucía (2006-2011) showing the number of private hunting states, governmental hunting states, and their respective areas in hectares. (www.magrama.gob.es/es/desarrollo-rural/estadisticas/Est_Anual_Caza.aspx).

Supplementary material S2. (A) Maximum rate of change in estimating the number of red deer populations (*K*) as inferred by STRUCTURE. (B) Mean logarithmic likelihood values for each *K* tested.

Supplementary material S3. Red deer estimated probabilities of population membership inferred by multilocus microsatellite genotypes when *K*=2. Each sample is represented by a vertical bar. Colours represent the population being assigned to**.**

Supplementary material S4. Red deer estimated probabilities of population membership inferred by multilocus microsatellite genotypes when *K*=58. Each sample is represented by a vertical bar. Colours represent the population being assigned to**.**

Table 1. PCR multiplex setup indicating the dye used for labeling, the amount of each primer used and the mean proportion of null alleles predicted for each locus within populations.

PCR Label Dye	Locus	Primer (µl)	Mean %Null Alleles/Population
PCR1	TGLA94	0.2	0.002
FAM	RT1	0.25	0.014
	RT13	0.35	0.003
PCR2	OarFCB193	0.25	0.034
NED	MB25	0.15	0.156
	CSSM43	0.6	0.079
PCR3	NVHRT48	0.1	0.138
PET	BM302	0.25	0.098
	NVHRT73	0.25	0.095
PCR4	OarFCB304	0.15	0.153
VIC	BM203	0.4	0.193

Table 2. Population ID, number of individuals genotyped, type of system, mean number of alleles (A), allelic richness (R_S), expected heterozygosity (H_E), and F_{IS} values averaged over 8 loci and 58 red deer population sampled in Andalusia region during three hunting seasons (2003-2006). Also shown are the area (in hectares) and census sizes of populations. Asterisk represents $P < 0.005$ after Bonferroni correction

Population	Ind Genotyped	System	A	R_S	H_E	F_{IS}	Area (ha)	Census Size
Aa	20	Closed	7.44	6.85	0.783	0.048	6253	790
Ab	25	Closed	6.88	6.14	0.767	0	2372	995
Ac	20	Closed	8.22	7.48	0.82	0.009		
Ae	22	Closed	7.22	6.612	0.765	0.067	1971	225
Aj	16	Closed	6.33	6.113	0.716	0.051	1545	300
Am	25	Closed	7	6.202	0.732	-0.043*	14131	657
Au	15	Closed	7	6.864	0.788	0.065	1190	450
Ay	26	Closed	6.77	6.054	0.769	0.002	1100	302
Br	23	Closed	8.77	8.16	0.799	-0.041	1860	209
Ca	15	Closed	6.33	6.226	0.743	0.024		
Cd	25	Closed	8.44	7.369	0.801	0.114*	1800	163
Ch	20	Closed	7.77	7.021	0.734	0.071	5305	515
Co	23	Closed	7.55	6.656	0.779	0.014		
Cq	23	Closed	8.88	6.986	0.798	0.035		
En	20	Closed	8	7.314	0.808	0.028		
Fn	27	Closed	8.55	7.132	0.788	0.004	3660	168
Ft	20	Closed	7.66	6.51	0.751	0.109	735	173
Gt	25	Closed	7.33	6.453	0.771	0.054		
Hl	18	Closed	8.22	7.524	0.798	0.051*		

Ht	18	Closed	6.66	6.169	0.696	-0.1	545	130
Jn	32	Closed	9.11	7.424	0.787	0.046	2362	710
Jt	25	Closed	8.11	6.783	0.736	0.127*	2021	165
Lc	30	Closed	7.33	6.383	0.764	0.04	1200	305
Mn	15	Closed	7.77	7.664	0.801	-0.016		
Nb	25	Closed	8.66	6.949	0.793	0.078		
No	25	Closed	8.77	7.509	0.811	0.090*		
Oz	20	Closed	7.77	7.086	0.788	0.042	865	375
Pi	17	Closed	7.11	6.815	0.8	0.001		
Pt	25	Closed	8	6.967	0.785	-0.010*	3546	855
Sd	20	Closed	7.66	7.047	0.798	0.022*	2256	328
Sm	21	Closed	7.33	6.469	0.745	0.049	990	57
Sn	21	Closed	9.55	8.513	0.804	0.087*	1027	520
St	25	Closed	7.22	6.414	0.767	0.007		
Ti	25	Closed	8.22	7.021	0.745	0.044	1206	415
Tj	24	Closed	7.66	6.717	0.758	0.121*	1110	145
Tm	16	Closed	6.77	6.555	0.776	0.023	1362	355
Vz	19	Closed	7	6.473	0.752	0.049*	5936	935
Ad	20	Open	6.11	5.768	0.773	0	1428	435
Ag	24	Open	7.66	5.967	0.735	0.052		
Al	32	Open	8.22	6.785	0.758	0.036	1145	283
Cc	25	Open	8.33	7.04	0.776	-0.011		
Cr	24	Open	8.33	7.348	0.814	0.110*	787	170
Cs	20	Open	9.66	8.576	0.826	0.086*	760	416
Cu	16	Open	8.55	8.198	0.829	0.099*	647	143

Cz	18	Open	7.44	6.917	0.771	0.089*		
Dn	52	Protected	6.55	5.873	0.745	0.038		
Fr	15	Open	5.55	5.532	0.766	0.033	1072	19
Gm	29	Open	8.22	6.901	0.77	0.069		
Ms	25	Open	7.44	6.711	0.781	0.03		
Ng	23	Open	7.88	6.948	0.769	0.093*	1131	225
Nh	25	Open	7.22	6.49	0.784	0.105*	1181	305
Ns	21	Open	7.88	7.293	0.807	0.028*		
Pa	23	Open	7.22	6.459	0.766	0.066		
Pd	25	Open	7.77	6.682	0.76	0.011	4371	450
Pl	19	Open	7	6.518	0.798	0.088*	613	170
Ps	16	Open	6.33	6.128	0.737	0.076	859	245
Rb	25	Open	7.11	6.174	0.735	-0.039	2342	113
Re	21	Open	7	6.48	0.765	0.120*	2433	455

References

Apollonio M, Scandura M, Sprem N (2014) Reintroductions as a management tool for European Ungulates. In: *Behaviour and management of European ungulates* (ed. R. Putman and M. Apollonio e), pp. 46-77. Whittles Publishing, Scotland.

Barendse W, Armitage SM, Kossarek LM, *et al.* (1994) A genetic linkage map of the bovine genome. *Nature Genetics* **6**, 227-235.

Biedrzycka A, Solarz W, Okarma H (2012) Hybridization between native and introduced species of deer in Eastern Europe. *Journal of Mammalogy* **93**, 1331-1341.

Bishop MD, Kappes SM, Keele JW, *et al.* (1994) A genetic linkage map for cattle. *Genetics* **136**, 619-639.

Buchanan FC, Crawford AM (1993) Ovine microsatellites at the OARFCB11, OARFCB128, OARFCB193, OARFCB266 and OARFCB304 loci. *Animal Genetics* **24**, 145-145.

Chapuis M-P, Estoup A (2007) Microsatellite Null Alleles and Estimation of Population Differentiation. *Molecular Biology and Evolution* **24**, 621–631.

Conard JM, Statham MJ, Gipson PS, Wisely SM (2010) The Influence of Translocation Strategy and Management Practices on the Genetic Variability of a Reestablished Elk (*Cervus elaphus*) Population. *Restoration Ecology* **18**, 85-93.

Cornuet JM, Luikart G (1997) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Cronin MA, Renecker LA, Patton JC (2009) Genetic variation in domestic and wild elk (*Cervus elaphus*). *Journal of Animal Science* **87**, 829-834.

Dakin E, Avise JC (2004) Microsatellite null alleles in parentage analysis. . *Heredity* **93**, 504-509.

De Garine-Wichatitsky M, De Meeus T, Chevillon C, *et al.* (2009) Population genetic structure of wild and farmed rusa deer (*Cervus timorensis russa*) in New-Caledonia inferred from polymorphic microsatellite loci. *Genetica* **137**, 313 - 323.

De Leyva E (2002) Caza mayor y ganadería extensiva. *Medio Ambiente (monograficos)* **41**, 18-20 [In Spanish].

Earl D, vonHoldt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359-361.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* **14**, 2611–2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

Fernández-García JL, Carranza J, Martínez JG, Randi E (2014) Mitochondrial D-loop phylogeny signals two native Iberian red deer (Cervus elaphus) Lineages genetically different to Western and Eastern European red deer and infers human-mediated translocations. *Biodiversity and Conservation* **23**, 537-554.

Fickel J, Bubliy OA, Stache A, *et al.* (2012) Crossing the border? Structure of the red deer (*Cervus elaphus*) population from the Bavarian–Bohemian forest ecosystem. *Mammalian Biology-Zeitschrift für Säugetierkunde* **77**, 211-220.

Fletcher J (2011) *Gardens of Earthly Delight* Oxford: Windgather Press.

Frantz AC, Pourtois JT, Heuertz M, *et al.* (2006) Genetic structure and assignment tests demonstrate illegal translocation of red deer (*Cervus elaphus*) into a continuous population. *Molecular Ecology* **15**, 3191-3203.

Galarza J, Sanchez-Fernandez B, Fandos P, Soriguer R (2015) The genetic landscape of the Iberian red deer (*Cervus elaphus hispanicus*) after 30 years of big-game hunting in southern Spain. *Journal of Wildlife Management and Wildlife Monographs* **79**, 500-504.

Georges M, Massey J, Polymorphic DNA (1992) markers in bovidae (World Intellectual Property Org Geneva). *Wo. Publi*.

Goodman SJ, Tamate HB, Wilson R, *et al.* (2001) Bottlenecks, drift and differentiation: the population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. *Molecular Ecology* **10**, 1357-1370.

Goudet J (1995) F_STAT (2.9.3) a program for IBM compatible PCs to calculate Weir and Cockerman's (1984) estimators of F-statistics. *Journal of Heredity* **86**, 485-486.

Haanes H, Røed K, Flagstad Ø, Rosef O (2010) Genetic structure in an expanding cervid population after population reduction. *Conservation Genetics* **11**, 11-20.

Haanes H, Røed KH, Perez-Espona S, Rosef O (2011) Low genetic variation support bottlenecks in Scandinavian red deer. *European Journal of Wildlife Research* **57**, 1137-1150.

Hajji GM, Charfi-Cheikrouha F, Lorenzini R, *et al.* (2008) Phylogeography and founder effect of the endangered Corsican red deer (*Cervus elaphus corsicanus*). *Biodiversity and Conservation* **17**, 659-673.

Hoffmann GS, Johannesen J, Griebeler EM (2016) Population dynamics of a natural red deer population over 200 years detected via substantial changes of genetic variation. *Ecology and evolution* **6**, 3146-3153.

Hoglund J, Cortazar-Chinarro M, Jarnemo A, Thulin C-G (2013) Genetic variation and structure in Scandinavian red deer (Cervus elaphus): influence of ancestry, past hunting, and restoration management. *Biological Journal of the Linnean Society* **109**, 43-53.

Hundertmark KJ, Van Daele LJ (2010) Founder effect and bottleneck signatures in an introduced, insular population of elk. *Conservation Genetics* **11**, 139-147.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**, 1801-1806.

Karaiskou N, Tsakogiannis A, Gkagkavouzis K, *et al.* (2014) Greece: A Balkan Subrefuge for a Remnant Red Deer (*Cervus Elaphus*) Population. *Journal of Heredity* **105**, 334-344.

Kuehn R, Schroeder W, Pirchner F, Rottmann O (2003) Genetic diversity, gene flow and drift in Bavarian red deer populations (*Cervus elaphus*). *Conservation Genetics* **4**, 157-166.

Landete-Castillejos T, Gallego L, Estévez JA, Garcia AJ, Fierro Y (2010) Fencing of game estates in Spain considered as management unit, 68-79.

Martinez JG, Carranza J, Fernández-García JL, Sánchez-Prieto CB (2002) Genetic variation of red deer populations under hunting exploitation in southwestern Spain. *The Journal of wildlife management*, 1273-1282.

Mbaiwa JE (2004) The socio-economic benefits and challenges of a community-based safari hunting tourism in the Okavango Delta, Botswana. *Journal of Tourism Studies* **15**, 37-50.

Milligan BG (1998) Total DNA isolation. In: *Molecular Genetic Analysis of Populations: A Practical Approach* (ed. Hoelzel A), pp. 50-52. IRL Press, Oxford, UK.

Milner JM, Bonenfant C, Mysterud A, *et al.* (2006) Temporal and spatial development of red deer harvesting in Europe: biological and cultural factors. *Journal of Applied Ecology* **43**, 721-734.

Newey S, Dahl F, Kurki S (2010) Game monitoring systems supporting the development of sustainable hunting tourism in Northern Europe: A review of current practises. University of Helsinki, Ruralia Institute, Helsinki, Finland.
Niedziałkowska M, Jędrzejewska B, Honnen AC, *et al.* (2011) Molecular biogeography of red deer *Cervus elaphus* from eastern Europe: insights from mitochondrial DNA sequences. *Acta Theriologica* **56**, 1-12.
Olivieri C, Marota I, Rizzi E, *et al.* (2014) Positioning the Red Deer (Cervus elaphus) Hunted by the Tyrolean Iceman into a Mitochondrial DNA Phylogeny. *PLoS ONE* **9**, e100136.
Papaspyropoulos KG, Sokos CK, Hasanagas ND, Birtsas PK (2012) Sustainability of recreational hunting tourism: a cluster analysis approach for woodcock hunting in Greece. In: *New Trends Towards Mediterranean Tourism Sustainability* (eds. Rosalino LM, Silva A, Abreu A), pp. 79-94. Science Publishers.
Peakall R, Smouse PE (2006) GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295.
Pérez-Barbería FJ, Hooper RJ, Gordon IJ (2013) Long-term density-dependent changes in habitat selection in red deer (Cervus elaphus). *Oecologia* **173**, 837-847.
Perez-Espona S, Hall RJ, Perez-Barberia FJ, *et al.* (2013) The Impact of Past Introductions on an Iconic and Economically Important Species, the Red Deer of Scotland. *Journal of Heredity* **104**, 14-22.
Pérez-Espona S, Pérez-Barbería F, Goodall-Copestake W, *et al.* (2009) Genetic diversity and population structure of Scottish Highland red deer (*Cervus elaphus*) populations: a mitochondrial survey. *Heredity* **102**, 199-210.
Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155**, 945–959.
Queiros J, Vicente J, Boadella M, Gortázar C, Alves PC (2013) The impact of management practices and past demographic history on the genetic diversity of red deer (*Cervus elaphus*): an assessment of population and individual fitness. *Biological Journal of the Linnean Society* **111**, 209-223.
Rice W (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223 - 225.
Røed KH, Midthjell L (1998) Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. *Molecular Ecology* **7**, 1773-1776.
Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**, 137-138.
Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* **8**, 103 - 106.
Ryckman MJ, Rosatte RC, McIntosh T, Hamr J, Jenkins D (2010) Postrelease Dispersal of Reintroduced Elk (*Cervus elaphus*) in Ontario, Canada. *Restoration Ecology* **18**, 173-180.
Sánchez-Fernández B, Soriguer R, Rico C (2008) Cross-species tests of 45 microsatellite loci isolated from different species of ungulates in the Iberian red deer (*Cervus elaphus hispanicus*) to generate a multiplex panel. *Molecular Ecology Resources* **8**, 1378-1381.
Senn HV, Barton NH, Goodman SJ, *et al.* (2010) Investigating temporal changes in hybridization and introgression in a predominantly bimodal hybridizing population of invasive sika (*Cervus nippon*) and native red deer (*C. elaphus*) on the Kintyre Peninsula, Scotland. *Molecular Ecology* **19**, 910-924.
Skog A, Zachos FE, Rueness EK, *et al.* (2008) Phylogeography of red deer (*Cervus elaphus*) in Europe. *Journal of Biogeography* **36**, 66-77.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16**, 393–430.
Smith SL, Carden RF, Coad B, Birkitt T, Pemberton JM (2014) A survey of the hybridisation status of Cervus deer species on the island of Ireland. *Conservation Genetics* **15**, 823-835.
Soriguer R, Rodríguez A, Domínguez L (2001) *Análisis de la incidencia de los grandes herbívoros en la Marisma y Vera del Parque Nacional de Doñana* Ministerio de medio Ambiente, Organismo Autónomo de Parques Nacionales, Spain.
Soriguer RC, Fandos P, Bernaldez E, Delibes JR (1994) El ciervo en Andalucía. Junta de Andalucía, Spain. [In Spanish].
Torres RT, Carvalho J, Fonseca C, Serrano E, López-Martín JM (2016) Long term assessment of roe deer reintroductions in North East Spain: A case of success. *Mammalian Biology-Zeitschrift für Säugetierkunde*.
Truett GE, Heeger P, Mynatt RL, *et al.* (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). *BioTechniques* **29**, 52-54.
van Oosterhout C, Hutchinson WF, Wills PM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.
Vial L, Maudet C, Luikart G (2003) Thirty-four polymorphic microsatellites for European roe deer. *Molecular Ecology Notes* **3**, 523-527.
Vilà C, Sundqvist A-K, Flagstad Ø, *et al.* (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society B: Biological Sciences* **270**, 91-97.
Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.
Williams CL, Serfass TL, Cogan R, Rhodes OEJ (2002) Microsatellite variation in the reintroduced Pennsylvania elk herd. *Molecular Ecology* **11**, 1299-1310.
Wilson GA, Strobeck C, Wu L, Coffin JW (1997) Characterization of microsatellite loci in caribou Ranginfer tarandus, and their use in other artiodactyla. *Molecular Ecology* **6**, 697–699.
Zachos F, Althoff C, Steynitz Yv, Eckert I, Hartl G (2007) Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. *European Journal of Wildlife Research* **53**, 61-67.
Zachos FE, Frantz AC, Kuehn R, *et al.* (2016) Genetic structure and effective population sizes in European red deer (Cervus elaphus) at a continental scale: insights from microsatellite DNA. *Journal of Heredity*, esw011.