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

2
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23
24 Running Title: Genetic diversity in fenced vs wild deer populations

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51 **Abstract**

52

53 The current magnitude of big-game hunting has outpaced the natural growth of populations,
54 making artificial breeding necessary to rapidly boost hunted populations. In this study we
55 evaluated if the rapid increase of red deer (*Cervus elaphus*) abundance, caused by the
56 growing popularity of big-game hunting, has impacted the natural genetic diversity of the
57 species. We compared several genetic diversity metrics between 37 fenced populations
58 subject to intensive management and 21 wild free-ranging populations. We also included a
59 historically protected population from a national park as a baseline for comparisons.

60 Contrary to expectations, our results showed no significant differences in genetic diversity
61 between wild and fenced populations. Relatively lower genetic diversity was observed in the
62 protected population, although differences were not significant in most cases. Bottlenecks
63 were detected in both wild and fenced populations, as well as in the protected population.

64 Assignment tests identified individuals that did not belong to their population of origin,
65 indicating anthropogenic movement. We discuss the most likely processes, which could
66 have led to the observed high levels of genetic variability and lack of differentiation between
67 wild and fenced populations and suggest cautionary points for future conservation. We
68 illustrate our comparative approach in red deer. However, our results and interpretations can
69 be largely applicable to most ungulates subject to big-game hunting as most of them share a
70 common exploitation-recovery history as well as many ecological traits.

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

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

116

117 **While various studies have evaluated the consequences of deer population**
118 **declines (i.e. bottlenecks) (Goodman *et al.* 2001; Haanes *et al.* 2011), as well as some the**
119 **recovery actions taken, such as re-introductions (i.e. founder effects) (Conard *et al.***
120 **2010; Hajji *et al.* 2008; Hundertmark & Van Daele 2010), translocations and non-**
121 **native introductions (i.e. hybridization) (Biedrzycka *et al.* 2012; Fernández-García *et***
122 ***al.* 2014; Perez-Espona *et al.* 2013; Senn *et al.* 2010; Smith *et al.* 2014; Torres *et al.***
123 **2016), and range expansions (Haanes *et al.* 2010; Pérez-Barbería *et al.* 2013; Ryckman**
124 ***et al.* 2010), it is unknown how rapid population increases and intensive management**
125 **have impacted the natural genetic composition of the species.**

126

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

138

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

149

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

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

162

163 **Materials and Methods**

164 *Samples collection.*-

165

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

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

178

179 *DNA extraction and microsatellite amplification.*-

180

181 Total genomic DNA was extracted from tongue tissue through a Hot Sodium and
182 Tris (HotSHOT) protocol (Truett *et al.* 2000) and from antler bone following a Silica
183 protocol (Milligan 1998). We genotyped all samples at 11 microsatellite loci previously
184 isolated from other ungulates: TGLA94 (Georges *et al.* 1992), OarFCB193, OarFCB304
185 (Buchanan & Crawford 1993), CSSM43 (Barendse *et al.* 1994), BM302, BM203 (Bishop *et*
186 *al.* 1994) RT1, RT13 (Wilson *et al.* 1997), NVHRT48, NVHRT73 (Røed & Midthjell
187 1998), MB25 (Vial *et al.* 2003). **Multiplexed PCRs were carried out according to**
188 **Sánchez-Fernández *et al.* (2008) in a PTC-100 Programmable Thermal Controller (MJ**
189 **Research Inc.) using the following conditions: an initial denaturation step at 95°C for**
190 **10min followed by 35 cycles of 30s at 94°C, 1 min at 54°C, 1 min 30s at 72°C and a last**
191 **extension of 10 min at 72°C. Multiplex setup and PCR labeling is described in table 1.**
192 Amplified products were resolved on an ABI Prism 3100 Genetic Analyser (Applied
193 Biosystems) and scored in GENEMAPPER v 3.7 software (Applied Biosystems) **using LIZ**
194 **labeled ladder (0-490bp) as size standard.**

195

196 Microsatellite analysis.-

197

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

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

205 

206 *Genetic diversity*

207

208 Genetic diversity within each population was characterized by calculating the mean
209 number of alleles per locus using GenAlEx v.6 (Peakall & Smouse 2006), as well as by
210 observed (H_O) and expected heterozygosities (H_E) calculated in Arlequin v.3.5.1.3 (Excoffier
211 & Lischer 2010). Inbreeding coefficients (F_{IS}) for each population were calculated in
212 GENEPOP software v.4.0 (Rousset 2008) according to Weir & Cockerham (1984). We used
213 FSTAT v. 2.9.3 (Goudet 1995) to determine the effective number of alleles (R_S) correcting
214 for sample size (i.e. allelic richness). The GenAlEx software v.6 (Peakall & Smouse 2006)
215 was used to detect private alleles, that is, alleles exclusive to only one population (Slatkin
216 1985).

217

218 *Genetic structure*

219

220 To evaluate genetic structuring we implemented a Bayesian clustering algorithm
221 using STRUCTURE v.2.3 (Pritchard *et al.* 2000). This method assigns individuals to
222 populations according to their posterior probability of membership to each of the populations
223 given the individual's multilocus genotype. Inference was performed using the correlated
224 allele frequency model, with no prior information about individual's geographic origin or
225 population-type (open-closed) specified. We set the number of populations (K) from one to
226 58, and ran three independent iterations consisting of a burn-in step of 300,000 MCMC

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

233

234 *Comparing genetic diversity between open and closed populations.-*

235

236 To examine if significant genetic differences exist between open and closed
237 populations, we compared estimates of R_s , H_E , and F_{IS} for each locus. Statistical significance
238 for differences between the estimates was attained through a Mann–Whitney test performed
239 in MATLAB v.7. (Mathworks). Furthermore, the software STRUCTURE v.2.3 (Pritchard *et*
240 *al.* 2000) was used in two different ways. First, to assess differences at the population level,
241 we grouped the populations as open or closed, and set $K=2$ using the correlated frequencies
242 model. Second, to infer possible gene flow (i.e. individual translocations), we set the
243 *USEPOPINFO* model to pre-specify that all individuals originate from their respective
244 population. The number of generations backwards (*GENSBACK*) was set to 1 and K was fixed
245 to the total number of populations ($K=58$). When using these parameters, miss-assignments
246 reflect individuals with recent ancestry in a population other than where it was sampled.
247 Both runs ($K=2$ and $K=58$) consisted of 1,000,000 MCMC repeats after 300,000 MCMC
248 burn-in period. Finally, to evaluate whether closed and/or open populations have
249 experienced recent bottlenecks, we used the software BOTTLENECK (Cornuet & Luikart
250 1997) setting 10000 replicates of the two-phased model (TPM) with 70% of the mutations
251 following a step-wise mutation model (SMM) and 30% following an infinite alleles model

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

254

255 **RESULTS**

256 *Microsatellite analyses.-*

257

258 **Our results showed significant deviations from HWE at locus RT13 across all**
259 **populations after Bonferroni correction. Likewise, significant stuttering was indicated**
260 **by MICROCHECKER for locus CSSM43. Therefore, both loci were removed from**
261 **further analyses. Mean null allele frequency within populations varied between 0.002**
262 **at locus TGLA94 to 0.193 in locus BM203 (Table 1). Previous studies have shown the**
263 **influence of null alleles to be negligible at low frequencies (<0.2) (Dakin & Avise 2004).**
264 **Thus, the rest of loci were kept for downstream analyses. We found no linkage**
265 **disequilibrium between any locus pair.** The observed measures of genetic diversity
266 calculated from allele frequency distributions were high overall (Table 2). Relatively high
267 levels of allelic richness (range 5.3 - 8.5) and average expected heterozygosity (range 0.69 –
268 0.82) were found across all populations (Table 2). The associated F_{IS} estimates for each
269 population ranged between -0.010 and 0.127, displaying high positive values for both open
270 (Cr, Cs, Cu, Cz, Ng, Nh, Ns, Pl, Re) and closed populations (Cd, Hl, Jt, No, Sd, Sn, Tj, Vz).
271 Eight populations (Al, Jt, Tj, Oz, Br, Cu, Ti, Tm) displayed private alleles, accounting for a
272 5.59 % of the overall allelic diversity.

273

274 *Genetic structure.-*

275

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

281

282 *Comparing genetic diversity between open and closed populations.-*

283

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

296

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

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

306

307 **DISCUSSION**

308

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

318

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

326 **not show clear evidence supporting either a reduced or an enhanced genetic diversity**
327 **in the protected population relative to the rest. Nonetheless, our results are in line with**
328 **those of Martinez *et al.* (2002) in that no differences were observed between open and**
329 **closed populations. It should be noted, however, that the open populations (n=8)**
330 **analyzed by Martinez *et al.* (2002) were surrounded by fenced populations, making**
331 **them effectively closed populations. In the present study we analyzed a larger number**
332 **of open populations (n=21) that do not share borders with fenced populations. The two**
333 **studies together suggest that fencing has a weak effect (but see below). Queiros *et al.***
334 **(2014) on the other hand, reported a higher genetic variability in the fenced population**
335 **relative to other two populations that had a different management strategy.**

336

337 Several explanations may be put forward in understanding previous results and ours.
338 A combination of factors can give rise to a lack of genetic differentiation between open and
339 closed populations. Firstly, for closed populations, a high genetic diversity observed could
340 be due to a highly variable genome inherent to red deer. Other studies have also found high
341 genetic diversity in red deer supporting this notion (Kuehn *et al.* 2003; Niedziałkowska *et al.*
342 2011; Pérez-Espona *et al.* 2009; Skog *et al.* 2008). Recently, a comprehensive study using
343 microsatellites showed that red deer possess high levels of genetic variation throughout
344 Europe (Zachos *et al.* 2016). A high genetic variation in closed populations may also be the
345 result of a large effective population size at the time of fencing. Evaluating levels of genetic
346 diversity before and after the creation of enclosures could help distinguish between these
347 hypotheses. It has been shown that time-series analyses can reveal increases/decreases of
348 genetic diversity in red deer and that these correlate well with management policies
349 (Hoffmann *et al.* 2016). Unfortunately, analyses of this sort are not possible in our case since
350 no historical red deer samples are available from our sampling area.

351

352 Secondly, it is possible that the effect of fencing in genetic diversity is not yet
353 detectable. Other studies that have made similar comparisons to ours, have found no
354 differentiation between wild and managed populations. For instance, introduced red deer in
355 the island of Corsica showed no signs of reduced genetic variation compared to its Sardinian
356 source after 20 years of the introduction (Hajji *et al.* 2008). Similarly, genetic variability did
357 not differ significantly between domesticated and wild deer populations from North
358 America, despite a domestication process of over 24 years (Cronin *et al.* 2009). The same
359 result of no differentiation was observed in populations that had been isolated for more than
360 20 years between the German and Czech border (Fickel *et al.* 2012). **In our case, all of the**
361 **closed populations were established after 1990 (Soriguer *et al.* 1994) when a law**
362 **(Decreto 146/1998 de la Junta de Andalucía referente a la Ordenación Cinegética)**
363 **allowed for their creation. Therefore, and inline with previous evidence, erosion of**
364 **genetic diversity by drift and isolation, is probably not yet obvious within the**
365 **timeframe of our study (\approx 25 years).**

366

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

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

379

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

398

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

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

411

412 **In conclusion, our results suggest that fenced hunting enclosures are not a**
413 **determinant factor towards genetic erosion as it could be expected. However, we wish**
414 **to emphasize that the populations analyzed here have been managed for a relatively**
415 **short time (≈ 25 years). Hence, the apparent high genetic diversity within closed**
416 **populations does not imply that a detrimental effect cannot be ongoing or has the**
417 **potential to arise. It has been shown that a loss of genetic diversity can gradually occur**
418 **each generation when deer populations remain small and isolated for long periods (c.a.**
419 **130 years), resulting in strong inbreeding depression, which can have visible effects**
420 **even in the phenotype (Zachos *et al.* 2007). In light of the rapidly increasing pace of**
421 **management practices worldwide, we advise to carefully evaluate the genetic**
422 **background of breeders in order to avoid both, outbreeding and inbreeding depression,**
423 **whilst maintaining the autochthonous genetic diversity of the species.**

424

425 **Data Availability**

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

430

431

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440 Jyväskylä.

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456 **Figure Captions**

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

458

459 **Figure 2.** Comparison locus by locus of genetic diversity, Expected Heterozygosity (H_E),
460 allelic richness (R_s), and F_{IS} between open (white boxes) and closed (grey boxes) red deer
461 populations. The central mark in the box shows the median, the edges represent the 25th and
462 75th percentiles, while the whiskers extend to the most extreme data points not considered
463 outliers. The continuous horizontal line indicates the value observed in the protected
464 population (D_n).

465

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

470

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

474

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

478

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

482

483

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

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

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PCR Label Dye	Locus	Primer (μ l)	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

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