Brief report
Evaluation of genetic variability in introduced populations of red deer (*Cervus elaphus*) using DNA fingerprinting

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Wild populations of large mammals are exposed to the effects of human activity that directly or indirectly disturb them and lead to population size reduction and occasionally to local extinctions. Consequently, the need for specific programmes to conserve them is progressively increasing (O'BRIEN 1998). Among the practices in conservation biology is the translocation of foreign individuals to increase genetic variability, or to reintroduce species in historically occupied ranges. The success of translocations is highly variable and dependent on species and founder size among other factors (GRIFFITH et al. 1989). Population genetics predicts that the maintenance of variability is important for population survival since its reduction could lead to inbreeding depression and low adaptation capability (FALCONER and MACKAY 1996). Hence, it is important to know the effects of translocations on genetic variability. Some studies have shown a reduction of genetic variability after translocation (SCRIBNER and STÜWE 1993; LEBERG et al. 1994; FITZSIMMONS et al. 1997), but experimental work on this subject is still scarce.

The red deer (*Cervus elaphus*) is widely distributed in Europe. In Spain its distribution has narrowed drastically in the first half of the 20th century. Native populations of the northern half of the Iberian Peninsula became extinct due to large-scale hunting, and were later reintroduced in virtually all their historical range (BRAZA et al. 1989).

The aim of this work is to study the success of reintroductions in maintaining genetic diversity. We apply DNA fingerprinting, revealed as a powerful technique in the study of the genetic structure of populations (ARMOUR and JEFFREYS 1992), to study Cantabrian populations of red deer which have quickly expanded after introduction, and compare them with their populations of origin regarding genetic variability.

MATERIALS AND METHODS

The three reintroduced populations studied (Caso, Somiedo and Sueve) are located in the region of Asturias, in the northern side of the Cantabrian Mountains, in three areas that are isolated from each other by roads, railways and densely populated zones. Founder animals were from two reserves, Quintos de Mora (Toledo) and Lugar Nuevo (Jaén) in the main red deer native range of distribution (Fig. 1). Each of these reserves is at present enclosed with wire fences and maintain a large population size above 2000 individuals (ALVAREZ 1988; ORTUÑO and DE LA PEÑA 1979). Individuals from these two reserves were introduced in Asturias between the years 1950 and 1972 (NORES and VAZQUEZ 1987): 98 individuals (sex ratio unknown) were released in Caso in 1952–56, 126 individuals (33 males, 63 females and 30 unknown) were introduced in Somiedo in 1969–72 and 14 individuals (7 males and 7 females) were released in Sueve in 1970. Populations increased in number quickly after introduction, reaching at the time of this study approximate population sizes of 4000, 1000 and 80, respectively.

Muscle samples were taken from a total of 41 red deer shot during the hunting season of 1995–96. These came from the three repopulated areas in Asturias (12 from Caso, 13 from Somiedo and 4 from Sueve) and the two native areas (6 from Jaén and 6 from Toledo) from which reintroduced individuals came.

Probes 33.15 and 33.6 (JEFFREYS et al. 1985a; JEFFREYS et al. 1985b) were used to obtain fingerprints. All the samples were run in the same gel, dispersing samples from the same locality across separate lanes. Genomic DNA extraction, restriction with *Hae* III, Southern blotting, hybridization and analysis of fingerprints were performed as described (PÉREZ et al. 1996).

Heterozygosity was estimated following STEPHENS et al. (1992) from band frequency. Similarity coefficient (S_{xy}) between each pair of individuals (x and y) was calculated as the number of common bands in their fingerprint profiles (n_{xy}) divided by the average number of bands scored for both individuals: $S_{xy} = 2n_{xy}/(n_x + n_y)$ (LYNCH 1990). Population subdivision was analyzed by studying the index of dissimilarity
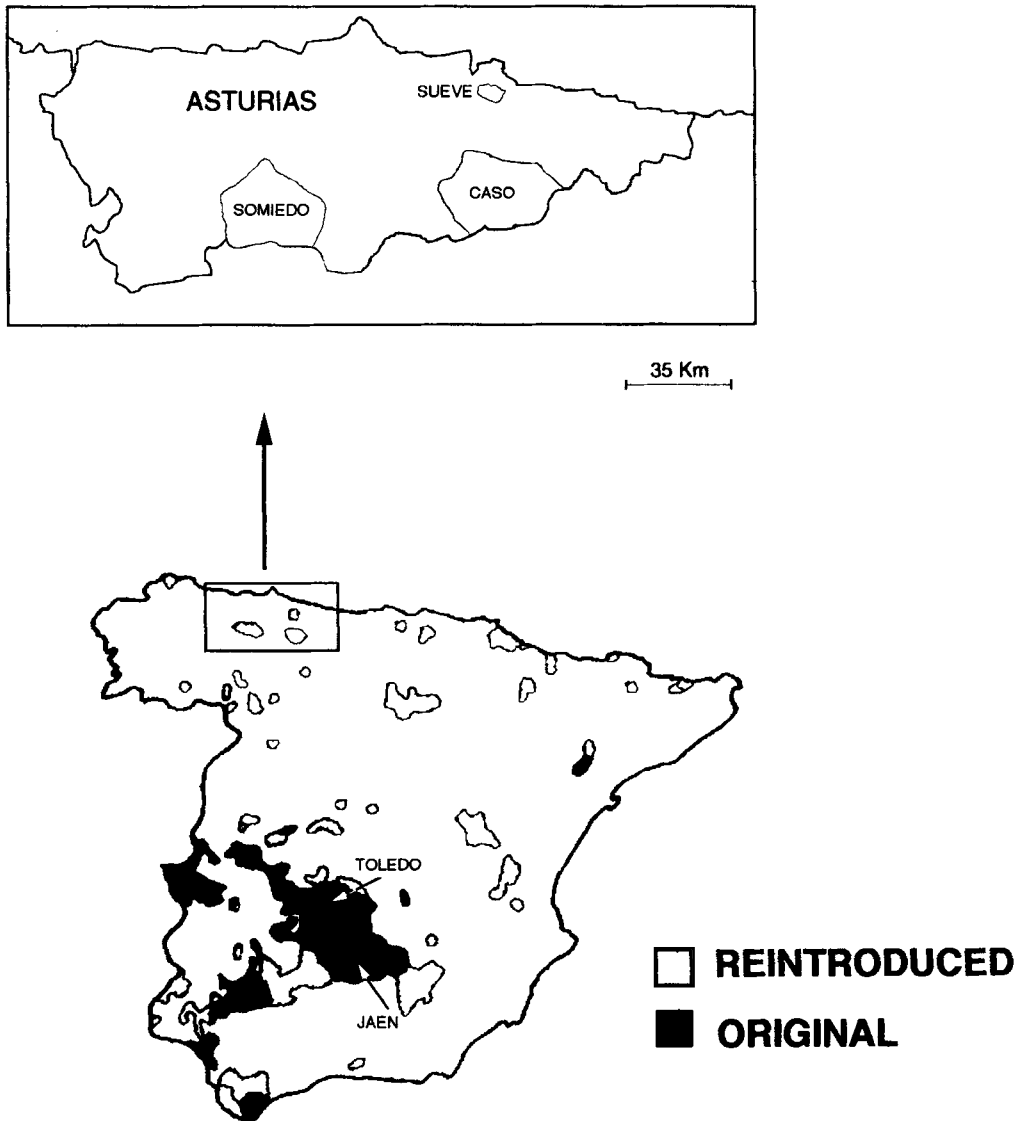
Fig. 1. Distribution of original and reintroduced red deer in Spain as depicted from (BRAZA et al. 1989).

$\bar{D}_{ij} = (\bar{S}_i + \bar{S}_j)/2 - \bar{S}'_{ij}$, where $\bar{S}_i$ is the average similarity of individuals within population i and $\bar{S}'_{ij}$ is the average similarity between random pairs of individuals across populations i and j (LYNCH 1990, 1991). The expected value for this index lies between 0, when there is no subdivision between populations, and 1 when populations are fixed for different alleles. Sampling variances for mean similarity indices were estimated by the formulae of LYNCH (1990, 1991). All coefficients and errors were calculated using a computer program written for this purpose in BASIC (available on request). Following LYNCH (1990), indices were compared by a z-test. Significance was corrected following the sequential comparison method of Bonferroni (RICE 1989) to amend for non-independent multiple comparisons.

An almost unbiased estimate of F_{ST}, Wright's index of population subdivision, was estimated following LYNCH (1990): $F'_{ST} = D_b/(D_w + D_b)$ where D_b is the average value of $\bar{D}_{ij}$ over all i,j and D_w is the average value of $1 - \bar{S}_i$ over all i.

RESULTS

All bands obtained with either probe were polymorphic and none was population specific. The two probes gave alike similarity and heterozygosity coefficients (Table 1). Band number ranged between 14 and 30 with a mean of 21.49 for probe 33.15 and between 7 and 16 with a mean of 10.32 for probe 33.6. The approximate number of loci studied (equal to $n(4 - S)/4(2 - S)$, LYNCH 1990, 1991) was larger for probe 33.5. Consequently, the individual identifi-

Table 1. *Data from DNA fingerprints with probes 33.15 and 33.6. Total number of different bands scored, average band frequency ± standard error (S.E.), mean number of bands (n) ± standard error (S.E.), heterozygosity, mean similarity (S), approximated number of loci and individualisation power (Pf)*

Probe	n° of bands	Frequency ± S.E.	n ± S.E.	Heterozygosity	S	n° loci	Pf
33.15	96	0.21 ± 0.01	21.49 ± 3.55	0.85	0.25	11.61	2.69E-18
33.6	61	0.17 ± 0.02	10.32 ± 2.39	0.88	0.21	5.49	2.28E-09

cation power, of probe 33.15 was higher. The probability that any two profiles would be identical by chance (equal to $(1 - 2S + 2S^2)^{n/s}$, (JONES et al. 1991)) is 2.69×10^{-18} for probe 33.15 and 2.28×10^{-9} for probe 33.6. Accordingly, the use of these probes would enable individual identification and paternity assignation.

Mean similarity indices for all pairwise comparisons within and across populations are presented in Table 2. Within population similarities ($\bar{S}i$) were equal except for the Sueve population that showed a greater similarity (p < 0.001) than Somiedo, Toledo and Jaén, which denotes a lower level of heterozygosity.

The dissimilarity indices (Table 2) allow us to test whether there is less similarity between populations than expected on the basis of within population similarity. The dissimilarity index was significantly higher than zero for every comparison involving the Sueve population while it was equal to zero for every other comparison. Thus the Sueve population has been differentiated from the rest that are homogeneous. Mean F'_{ST} estimated for the pairs involving Sueve and each of the other populations was 0.07. It is also possible to obtain an estimate of distance from F_{ST}, the coancestry distance $d = -\ln(1 - F_{ST})$. This distance is appropriate for divergence due to drift only (WEIR 1996) and hence it is the parameter of choice for the present situation. Mean estimated distance between Sueve and the rest of the populations was 0.07.

DISCUSSION

Theoretical considerations predict that the use of a limited number of founder individuals for introductions can lead to differentiation among populations and to a decrease of within population variability. These predictions have been shown to occur in reintroductions of mammals such as the alpine ibex (SCRIBNER and STÜWE 1993) or bighorn sheep (FITZSIMMONS et al. 1997). Our study shows these effects for the population of Sueve founded from a small number of individuals. The small sample size available from this population could influence this result, particularly if by chance some of the individu-

als sampled resulted to be close relatives. We can exclude this possibility by noting that the larger similarity index within this population is 0.35. This value is far lower than expected for first degree relatives that, with a mean similarity for non-related individuals of 0.24 (excluding the population from Sueve), is $0.24 + (1 - 0.24)/2 = 0.62$. In addition, analysing a large number of loci per individual compensates for the low number of individuals in some samples. If we assume that DNA profiles obtained with probes 33.15 and 33.6 detect independent sets of variable DNA as in humans (JEFFREYS et al. 1986), by combining both probes we are studying a sample of 17 independent loci (Table 1).

The Sueve population was founded from 7 males and 7 females in 1970, three generations ago if we assume the generation time of 8.62 given by GAILLARD (1992), and expanded quickly to the approximate population size of 80 at present. Assuming an exponential growth in these first generations, the following population sizes can be estimated: $N_0 = 14$, $N_1 = 25$, $N_2 = 45$. Effective population size calculated as the harmonic mean of numbers in each generation is $N = 22$. The coancestry distance is related to Ne, and t, the time in generations since populations diverged, $d_t \sim t/2Ne$ (WEIR 1996). From this, a distance of 0.07 is expected, a value analogous to that observed. Populations of Somiedo and Caso, where the number of founders was around one hundred, have not been differentiated from the original populations as expected from considerations similar to the previous ones. It must be noted that those expectations are rather ideal ones. First, several factors reducing population size, and hence increasing expected distance, such as overlapping generations, differences in fertility or the polygynous nature of the species were not considered. Second, it is assumed that all released individuals survive and reproduce and that the population grows quickly and exponentially at a constant rate. The fact that observations fit expectations so well suggests that the later conditions must mostly be fulfilled.

Mean similarity index between non-related animals in the reduced population of Sueve was 0.30, a value close to the similarity index of 0.31 obtained by PEMBERTON et al. (1992) for an isolated red deer

Table 2. *Similarity and dissimilarity indices with their standard errors. Average similarity of individuals within population $(\bar{S}_i)$, on the diagonal. Average similarity between pairs of individuals across populations $(\bar{S}'_{ij})$, above the diagonal. Index of dissimilarity $(\bar{D}_{ij})$, below the diagonal. In parenthesis number of comparisons.* $**p < 0.01$; $***p < 0.001$

	Caso	Somiedo	Sueve	Toledo	Jaén
Caso	0.26 ± 0.01 (66)	0.24 ± 0.01 (156)	0.20 ± 0.01 (48)	0.22 ± 0.01 (72)	0.24 ± 0.01 (72)
Somiedo	0.01 ± 0.02	0.23 ± 0.01 (78)	0.24 ± 0.01 (52)	0.24 ± 0.01 (76)	0.22 ± 0.01 (76)
Sueve	0.07 ± 0.02***	0.03 ± 0.01**	0.30 ± 0.01 (6)	0.22 ± 0.01 (10)	0.22 ± 0.02 (10)
Toledo	0.03 ± 0.02	0.00 ± 0.01	0.06 ± 0.02**	0.24 ± 0.03 (15)	0.23 ± 0.01 (36)
Jaén	0.01 ± 0.02	0.01 ± 0.01	0.05 ± 0.01***	0.00 ± 0.01	0.24 ± 0.01 (15)

population of rather reduced size from the Isle of Run (Scotland). Mean similarities obtained for the other four populations (0.24) were in the order of values reported for human (JEFFREYS et al. 1985b) and natural populations (WETTON et al. 1987; BAKER et al. 1993; MÖRSCH and LEIBENGUTH 1994) and lower than the value of 0.44 recorded for the chamois (*Rupicapra rupicapra*) (PÉREZ et al. 1996) in the same area.

Our results provide evidence that reintroductions, even established with a relatively small number of individuals and dealing with a highly polygynous species, can reasonably maintain genetic variability. Comparable results were obtained from the analysis of protein polymorphism in enclosures of deer from Germany that originated from a few animals (HERZOG et al. 1991). The low or negligible drift effects observed in this work, can be related to the rapid increase in population number after reintroduction that, in turn can be connected with a number of factors known to increase the success of translocations (GRIFFITH et al. 1989) such as translocation into the species historical range of distribution, habitat quality and the herbivore nature of the species.

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