The Majorero camel (*Camelus dromedarius)* breed

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Summary

A brief historical, morphologic and behavioural review of the camel Fuerteventura breed (Majorero) is presented. Genetic variability within the breed was analysed (n = 10) using 11 microsatellite markers, neutral to the selection, and compared with an African camel population (n = 37). In spite of the fact that there are significantly fewer Majorero camels than African, the level of inbreeding, measured by means of the statistic F_{IS}, is almost 3 times higher in the African camel, (3.2 versus 8.7). The set of markers used shows significant differences between the two populations, (F_{ST} = 3.1%) and provides sufficient discrimination (> 99%) to carry out a proper control of parentage in the studbook. Nevertheless, the molecular information available does not manage to assign the individuals into clusters corresponding to its population.

Resumen

Se presenta una revisión breve de la historia, morfología y comportamiento de la raza de camello de Fuerteventura (Majorero). La variabilidad genética dentro de la raza fue analizada (n=10) utilizando 11 marcadores de microsatélites, neutros en la selección, y comparando con la población africana de camellos (n=37). A pesar del hecho que existan de forma significativa menos ejemplares de camellos Majorero en comparación con los africanos, el nivel de consanguinidad, medido utilizando el sistema de estadística FIS, es por lo menos 3 veces mayor en el camello africano (3,2 frente a 8,7). El grupo de marcadores utilizado muestra diferencias significativas entre las dos poblaciones (FST = 3,1%) y provee suficiente discriminación (> 99%) para llevar a cabo un control de parentesco en el libro genealógico. En todo caso, la información molecular disponible no basta para colocar cada individuo dentro los clusters correspondientes a su población.

Keywords*: Canary Islands, Physical characteristics, Behaviour, Microsatellite markers, Genetic differentiation.*

Introduction

History of the breed

The camel was first introduced to Fuerteventura island from the nearby African coast in the 15th century. From those days on it was used to power agricultural implements such as ploughs and watermills, as a riding animal, as a pack animal and also for wheeled transport. The dry and barren climate of the eastern Canary Islands, (Lanzarote and Fuerteventura), proved to be very favourable for this species. In addition, the vegetation of these islands provided an excellent nutritional source since there are available, among various other minerals, significant sources of sodium chloride, which is essential to the vital functions of camels.

Dromedaries became a basic necessity for every household and much of the colloquial language refers to this quadruped. Its excellent adaptation to the local environment made the camel almost indispensable in the

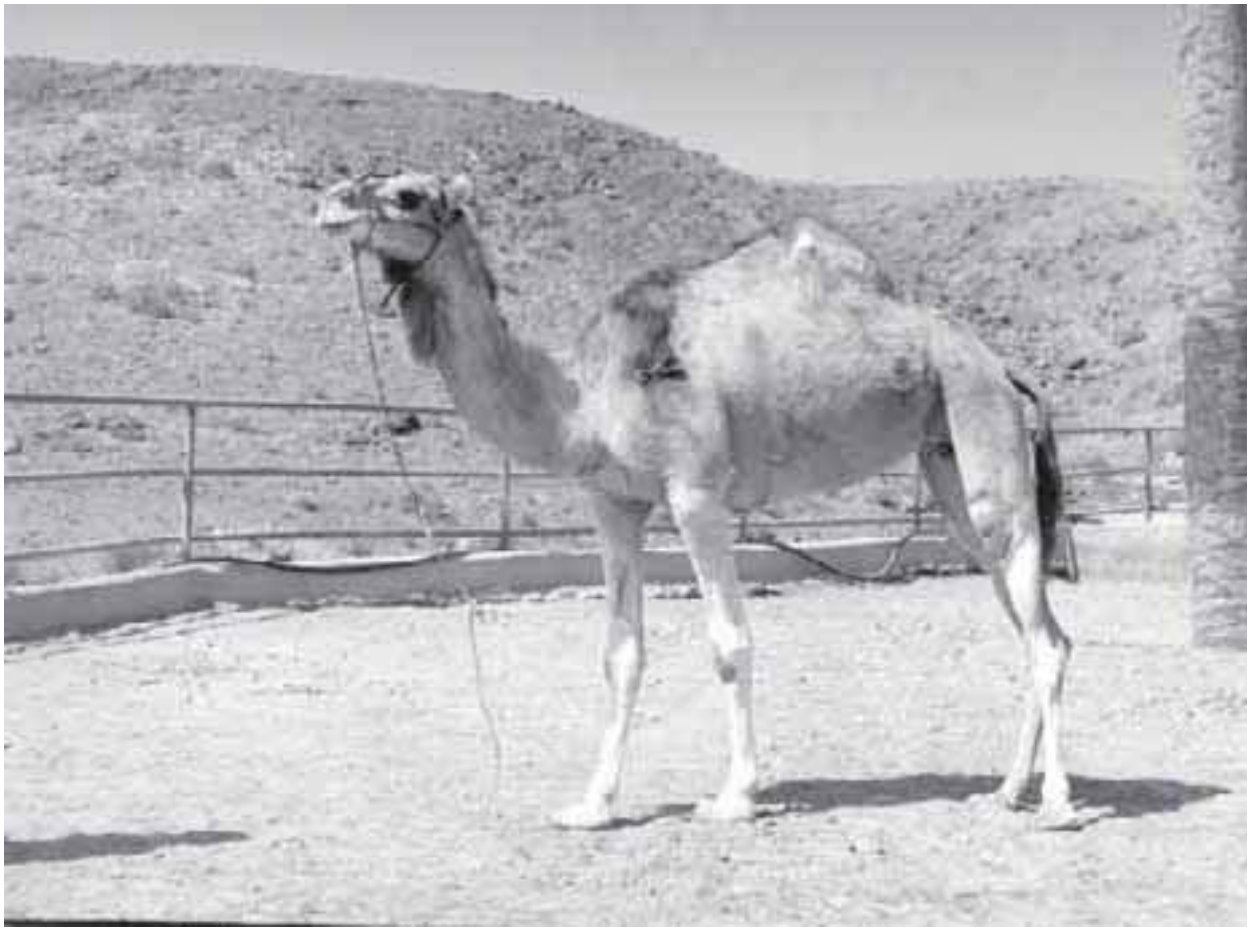

Figure 1. Female of the Majorero dromedary with a slim body constitution, but a strong and sturdy skeleton. The hump of these camels is smaller and shorter. The wool is always straight and the cream colour is basically homogenous.

countryside. It can survive on food of low nutritious value (at least as well as goats can), which is the kind of food available on the island. In addition, the camel is gifted with an extraordinary disease resistance. There used to be more than 4 000 camels on Fuerteventura alone.

Morphology

The colour of the Majorero camel coat is very homogenous among animals, beige and sometimes light reddish, which can vary a little towards the abdomen, taking into consideration the fact that the tone will vary during the animals' lifetime and that colouration also depends on the feeding it receives (Figure 1). The skin and mucous are usually dark coloured. The hair is straight and coarse, never frizzy or curly, which distinguishes them very clearly from the African camel.

The cranial muscles of the pelvic limbs are not very developed as well as the caudal muscles (*m. semitendinosus* and *m. semimembranosus*), even for well trained animals, compared to many African camels (Figure 2), which are very muscular, (*m. vistas laterals*, *m. rectos femora's*). The frontal part of the trunk is slightly more developed than the hind end, causing the triangular form. The hump of this camel breed is very characteristic because it is always short from front to back, surpassing neither the shoulder blades nor the sixth lumbar vertebra. It is half moon shaped and forms a very pronounced angle at the back of

the lumbar region. It is not known if this shape was selected intentionally, but in any case it is extremely well adapted to carry the kind of saddles that are used on this island. This is one of the fundamental differences between the Majorero and the African camels (Schulz, data not published).

Behaviour

Generally the Majorero camels are animals of pleasant character and very docile, once tamed. They are governed by habits and as long as they lead a regular life and have an established timetable, will accept different tasks and activities with ease, even though some might be unpleasant. This makes them good lead camels and very trustworthy, for once they have learned something, they will be constant in performing their task correctly.

Their high degree of perception allows them to feel strong emotions towards their masters in certain situations. Female camels can get extremely jealous if their master pays more attention to any other camel. On the other hand they are nosy animals, very interested in the people around them. The way in which humans treat them plays a major role in the development of their characters. If they remain within a herd of camels all day long, they may acquire bad habits, like rocking from one leg to the other,

Figure 2. Female of the African dromedary, which, compared to the Majorero specimen, normally have a greater development of the striated muscle tissue and of the lipid tissue as well. The wool of the North African camels is softer, either straight or curly and the colour varies from nearly white or cream to dark red or brown. Frequently we can observe a decrease of the colour intensity towards distal points of the extremities and dark mane hairs.

Table 1. References, primer sequences, average number of alleles and experimental parameters for 14 microsatellite markers.

Locus	Reference	Primer sequences (5'- 3')	Nº of alleles	T (ºC)	Mg Cl_2 (mM)	Size range (pb)
YWLL02	(Lang *et al.*, 1996)	GTGCACTCAGATACCTTCACA	4[1]	60	2	298-318[1]
		TACATCTGCAATGATCGACCC	6			278-290
YWLL44	(Lang *et al.*, 1996)	CTCAACAATGCTAGACCTTGG	3	55	2	104-108
		GAGAACACAGGCTGGTGAATA	11			86-120
LCA 18	(Penedo *et al.*,1998)	TCCACCCATTTAGACACAAGC	4	55	2	224-230
		TAGGAAGCTCCAAGAAGAAAAGAC	9			221-241
VOLP 10	(Obreque *et al.*, 1998)	CTTTCTCCTTTCCTCCCTACT	5	58	2	236-246
		CGTCCACTTCCTTCATTTC	6			278-290
VOLP67	(Obreque *et al.*, 1998)	TTAGAGGGTCTATCCAGTTTC	11	55	2	152-182
		TGGACCTAAAAGAGTGGAG	5			158-170
YWLL08	(Ray *et al.*, 1996)	ATCAAGTTTGAGGTGCTTTCC	18	58	2	164-204
		CCATGGCATTGTGTTGAAGAC	13			135-177
LCA90	(Penedo *et al.*,1998)	TATAACCCTGGTCTCGCCAA	5	58	2	237-248
		CCAAGTAGTATTCCATTATGCG	14			229-263
LCA37	(Penedo *et al.*,1998)	AAACCTAATTACCTCCCCCA	3	58	2	162-166
		CCATGTAGTTGCAGGACACG	18			128-180
LCA19	(Penedo *et al.*,1998)	TAAGTCCAGCCCCACACTCA	1	58	2	80
		GGTGAAGGGGCTTGATCTTC	14			84-118
LCA65	(Penedo *et al.*,1998)	TTTTTCCCCTGTGGTTGAAT	1	58	1.5	
		AACTCAGCTGTTGTCAGGGG	14			165-191
LCA33	(Penedo *et al.*,1998)	GAGCACAGGGAAGGATATTCA	5	55	2.5	147-167
		ACAGCAAAGTGATTCCATAATACA	5			122-130
LCA66	(Penedo *et al.*,1998)	GTGCAGCGTCCAAATAGTCA				
		CCAGCATCGTCCAGTATTCA	24			220-262
VOLP77	(Obreque *et al.*, 1999)	TATTTGGTGGTGACATT				
		CATCACTGTACATATGAAGG	11			144-168
VOLP03	(Obreque *et al.*, 1998)	AGACGGTTGGGAAGGTGGTA				
		CGACAGCAAGGCACAGGA	3			129-169

[1]First row refers to the results obtained in this paper, the second row to the results obtained into the South America cameliade.

raising their front legs into the air or showing lack of attention toward the other animals of the herd (Schulz, unpublished data).

Materials and Methods

Forty seven camels, 10 Majorero and 37 African, were used in this study. Although between 35-50 animals are usually required to study the variability within a population, difficulties in identifying other Majorero animals and the restriction of unrelatenedness between individuals analysed meant it was not possible to include more than these 10 animals. Consequently the result presented should be considered as a preliminary study.

A set of *14* microsatellite markers that had been located in camelidae of South America (lamas and alpacas) was used. Primers and technical PCR conditions are presented in Table 1. Microsatellite genotyping was carried out by an automated ABI PRISM 3100 DNA sequencer (Applied Biosystems) and DNA fragments were analysed using the Genescan 3.7.1®.

Software used included FSTAT (Goudet, 1995) to calculate the genetic parameters of the population such as the values of the F statistics of Wright, (Wright, 1965), GENETIX (Belkhir *et al*., 1996) to estimate allelic frequencies and heterozygosity values and MICROSAT (Minch *et al*., 1995) to estimate the genetic distances based on the Malécot index (Malécot, 1948). The genetic distances thus obtained were transformed into measures of distance with ultrametric properties, (Weitzman, 1992, 1993), using software developed by the authors (Garcia *et al*., 2000; Garcia y Cañón, 2001).
The hypotheses of the anonymous sample belonging to either of the two populations were tested, and a significance test was obtained empirically. Given a set of *L* loci and the allelic frequencies in each population, if $p_{l_a}^{(M)}$ denote the allelic frequency of allele *a* of locus *l* in population *M*, then, assuming H-W and linkage equilibrium, the probability of observing the genotype of individual *I* when this belongs to population *M* is

$$P[I \mid M] = \prod_{l=1}^{L} (2 - \delta(Il_1, Il_2)) p_{Il_1}^{(M)} p_{Il_2}^{(M)}$$

where Il_1 and Il_2 are the alleles carried by *I* at locus *l*, and δ (*a*,*b*) is the Kronecker delta, which equals 1 if *a* = *b* and 0 otherwise. Now, if $H_0 = I \in$ Pop1 and $H_1 = I \in$ Pop2, then a statistic for this test can be built as

$$LS = \log_{10} \frac{P[I \mid Pop1]}{P[I \mid Pop2]}$$

Since finding a theoretical distribution for *LS* would be much too complicated, empirical p-values and power must be considered. Given a value of *LS* for a certain individual *I*, a number of random individuals from population in H_0 is simulated from the allelic frequencies, and *LS* is computed for each of them. When an individual belongs to the population in H_1, *LS* is low, as opposed to that for individuals in H_0, so the empirical p-value is calculated as the proportion of the simulated genotypes with *LS* values lower than that of individual *I*.

A similar procedure is followed to calculate the empirical power of the test. Given a certain Type I error α, a number of genotypes from H_0 are simulated and their *LS* value calculated. The *LS* values are then ordered and the first value for which a proportion of α of the total are lower than itself is stored as the rejection threshold. Next, a number of genotypes from H_1 are simulated and their *LS* value calculated. The power of the test is calculated as the proportion of these values which are lower than the rejection threshold.

Results and Discussion

From the 14 microsatellite markers selected, based on the results obtained from the New World camelids, 11 were amplified under chosen technical specifications and 9 of those were polymorphic in the populations included in the present study, providing a

Figure 3. Distribution of the allele frequencies for 9 microsatellites within the African ▬ *and Majorera* ▭ *populations.*

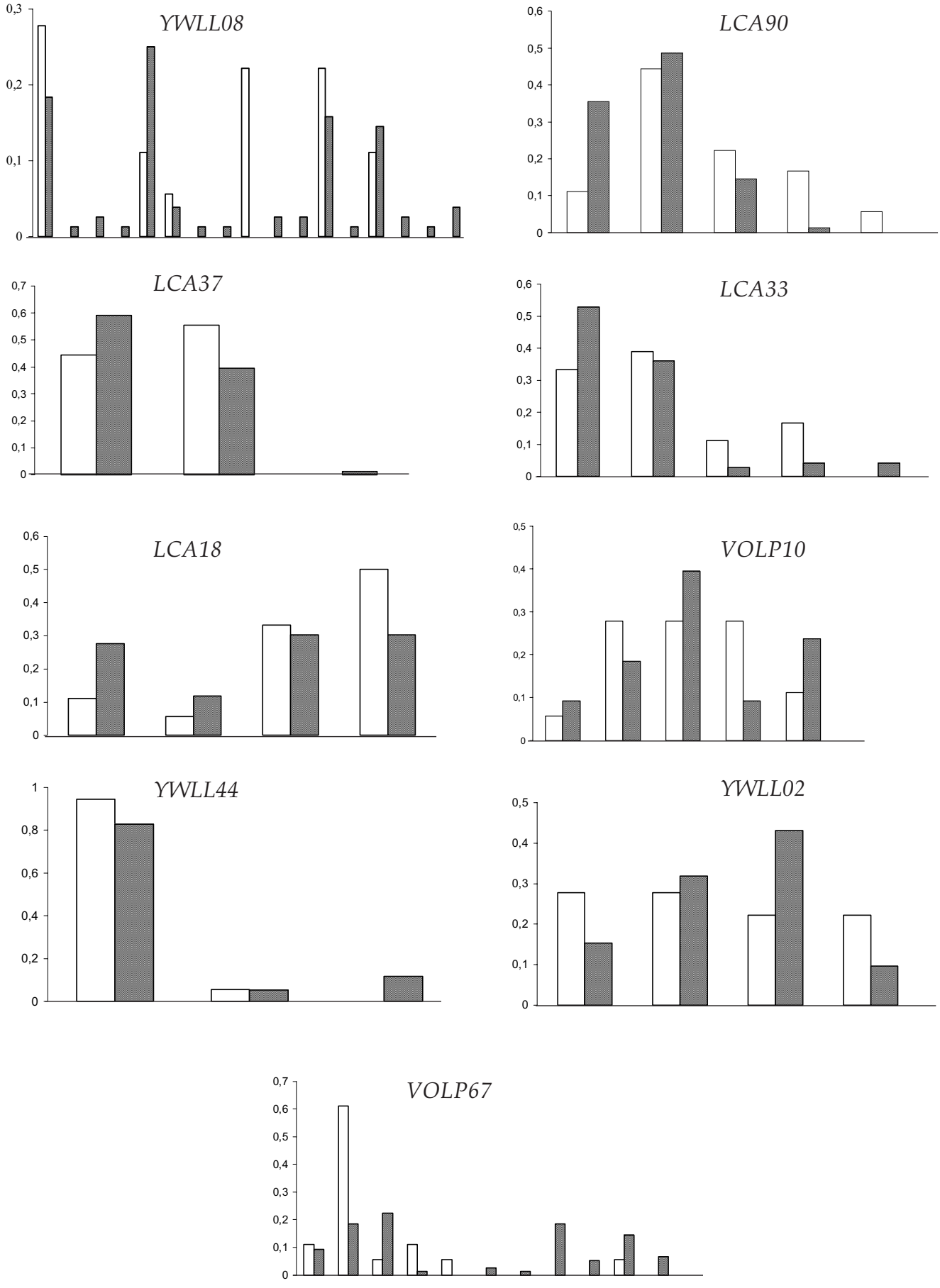

Table 2. Summary of the main descriptive statistics related to population genetic variability for 9 microsatellite markers: effective number of alleles, heterozygosity, probability of parentage exclusion and power of discrimination between sib-pairs

Locus	ENA	He	PE_2	PE_1	PES
YWLLO8	6.9	85.4	71.5	55.4	66.9
LCA90	2.8	64.8	38.6	22.7	53.0
LCA37	2.0	50.0	19.9	12.6	41.0
LCA33	2.6	61.9	35.1	20.7	50.5
LCA18	3.5	71.8	46.0	29.0	57.6
VOLP10	4.0	75.2	52.9	34.9	60.1
YWLL44	1.4	26.4	13.4	3.5	24.2
YWLL02	3.4	70.5	45.0	28.0	56.8
VOLP67	6.2	83.9	68.5	51.6	65.9
Average	3.6	65.5	99.7[1]	96.3	99.9

ENA: Effective number of alleles; He: Heterozygosis; PE2: Probability of parentage exclusion when both parents are known; PE1: Probability of parentage exclusion when only one parent is known; PES: Probability of discrimination between full-sib pair members.

Table 3. Average values for camel populations used in microsatellite marker analysis of population genetic variability.

								Mean heterozygosity	
Breed	ENA	F_{IS} (%)	PIC	PC	PE_2	PE_1	PES	H_o	H_e
Majorera	3.1	3.2	56.5	1.83E-07	99.1	92.5	99.8	0.63 (0.07)	0.65 (0.08)
Africana	3.6	8.7	59.6	3.80E-08	99.6	95.7	99.9	0.60 (0.05)	0.66 (0.06)

ENA: Effective number of alleles; F_{IS}: Inbreeding within breed; He: Heterozygosis; PIC: Polymorphic information content; PC: Probability of non-discrimination or probability of coincidence; PE_2: Probability of parentage exclusion when both parents are known; PE_1: Probability of parentage exclusion when only one parent is known; PES: Probability of discrimination between full-sib pair members; Ho: Observed and He: Expected heterozygosity, standard errors between brackets.

total of 58 alleles. The total number of alleles per locus ranged from 3 (LCA 37) to 18 (YWLL 08) (Table 1). The distribution of the allele frequencies is presented in Figure 3. It is remarkable that numerous alleles are present in only one population, often appearing in relatively high frequencies. For instance, the allele 184 of marker YWLLO8 only exists within the Majorero population with a relatively elevated frequency of 22%, while the allele 176 of the marker VOLP67 is only present in the African population with a frequency of 18.5%.

The average *ENA* (effective number of alleles) was 3.6, with no differences between populations found for this parameter when similar size for both populations was considered (Tables 2 and 3).

Parameter values for expected heterozygosity, polymorphic information content (*PIC*) and different discriminant power measurements by locus averaged over loci are presented in Tables 2 and 3 respectively. Although differences in expected and observed heterozygosities between populations were not significant, the level of inbreeding reflected by the F_{IS} statistic clearly shows a low genetic variation among African dromedaries probably due to genetic drift after migration from Africa. Nevertheless, this conclusion should be interpreted with caution, since other results

do not seem to confirm this tendency. For example, if the coefficient of kinship (Malécot, 1948), defined as the probability that one allele of a locus could be identical to the homologous in two individuals taken at random from one population was used, the Majorero population would present superior values, (0.34±0.014) to the African, (0.32±0.004).

The set of genetic markers used allows individual traceability, even within the Majorera population, since the probability of coincidence is very low, $1.8x10^{-7}$, and a high probability of parentage exclusion, greater of 99% when both parents are known, is possible. As can be seen, (Table 3), the values of discriminating power are somewhat reduced in the Majorero population due to the smaller size of the sample, only 10 animals, (as opposed to the 37 African animals available), which consequently causes a reduced number of alleles and therefore a reduced capacity for discrimination.

The use of theoretically neutral genetic markers to the selection can be applied, among other applications, to analyse the reproductive isolation of the two camel populations that are currently found on the Canary Islands. Population differentiation was examined by fixation indices F_{IS}, F_{IT}, and F_{ST}, for each locus and across all loci. Results of the *F*-statistics for each of the 9 analysed loci in both dromedary populations are shown in Table 4. As an average, a significant deficit of heterozygotes of 7.7% ($P < 0.001$) exists for each one of the analysed populations, the deficit in the whole population being 10.5 ($P< 0.001$). The deficit of heterozygotes, measured as F_{IS}, for each population is given in Table 5 where only the value for the African population of 8.7%, was statistically different from zero. It is not clear if inbreeding might be considered as the main cause of loss of heterozygotes since this deficit affects most of the loci in a similar manner and only two of them, YWLL02 and VOLP67, contribute to this loss. Other forces, such as the 'genetic hitchhiking' effect, or null alleles may be acting and contributing to the observed level of homozygosis.

The existence of genetic differences between the two populations is shown by the F_{ST} distance of 3.1 obtained, a value statistically different from 0 ($P<0.001$). This value means that a little more than 3% of the observed genetic variability of analysed animals is as a consequence of the genetic differences between the populations of the Majorero and the African camels. As systematic genetic migration between the two populations would certainly have played a role, the differences found can be explained by the genetic drift resulting from a certain degree of reproductive isolation. The magnitude of the genetic differentiation between the two populations, although apparently small, is similar to the values frequently found when comparing other domestic animals like, for instance, the Morucha *vs* the Asturiana de los Valles bovine breeds, (3.8%) (Cañón *et al*., 2001) or the Pottoka *vs* the Gallego equine celtic breeds, (2.6%), (Cañón *et al*., 2000). Recently, Mburu *et al*. (2003) found values of F_{ST} between dromedary population pairs ranging between 0.0013 and 0.162, and a value of 0.056 when Kenyan and non-Kenyan populations were compared.

The genetic differentiation between the two breeds allows for relatively high values of assignment of anonymous samples of their true population. For a Type I error of 0.01 the power getting with the set of genetic markers used in this study was 0.75, which means that with 100 anonymous samples coming from the African dromedary population, 75 will be rejected as to pertaining to the Majorero population (Figure 4).

Figure 5 shows an UPGMA tree based on individual pairwise genetic distances. It reflects a lack of clear grouping of the Majorero animals, which highlights the relatively reduced capacity for discrimination of the set of markers used. This, however, should not be interpreted as absence of a clear difference between both populations;

Table 4. F- statistic analysis for each of 9 microsatellite markers in two camel populations.

Locus	F_{IT}	F_{IS}	F_{ST}
YWLLO8	0.122*	0.110*	0.013
LCA90	0.05	0.014	0.036
LCA37	0.00	-0.013	0.013
LCA33	-0.122	-0.146	0.021
LCA18	-0.044	-0.062	0.017
VOLP10	0.139*	0.130	0.010
YWLL44	-0.025	-0.044	0.018
YWLL02	0.29**	0.284**	0.009
VOLP67	0.329**	0.246**	0.111**
Average	0.105**	0.077**	0.031**

*P<0.05; **P <0.01.

Table 5. Comparison of within-population inbreeding estimates (F_{IS}) in two camel breeds.

Locus/breed	Majorera	Africana
YWLLO8	0.082	0.116
LCA90	-0.037	0.030
LCA37	-0.297	0.053
LCA33	-0.057	-0.170
LCA18	-0.011	-0.074
VOLP10	0.172	0.121
YWLL44	0.000	-0.053
YWLL02	0.310	0.278
VOLP67	-0.067	0.296
Average	0.032	0.087*

*P<0.01.

the case is simply that the set of markers used is too small to be able to carry out an assured individual allocation with a high power.

Conclusions

Morphological and behavioural differences between the camels of African origin and those originating within the Majorero population can be identified.

In spite of the small number of animals used to represent the Majorero population, one can detect genetic differences when compared with the African population; it would be very interesting to be able to confirm this with a larger number of Majorero animals.

The set of genetic markers that were used can serve to identify individuals, traceability, and paternity relationship control.

The uniqueness of dealing with the only native European population of this species, which has had its roots firmly established in the Canary Islands for 600 years, is illustrated by the way in which isolation has allowed this population to differentiate itself through evolution and genetic drift, compared to the nearby African camel population. It constitutes a strong argument that the Majorero camel could be considered a distinct breed.

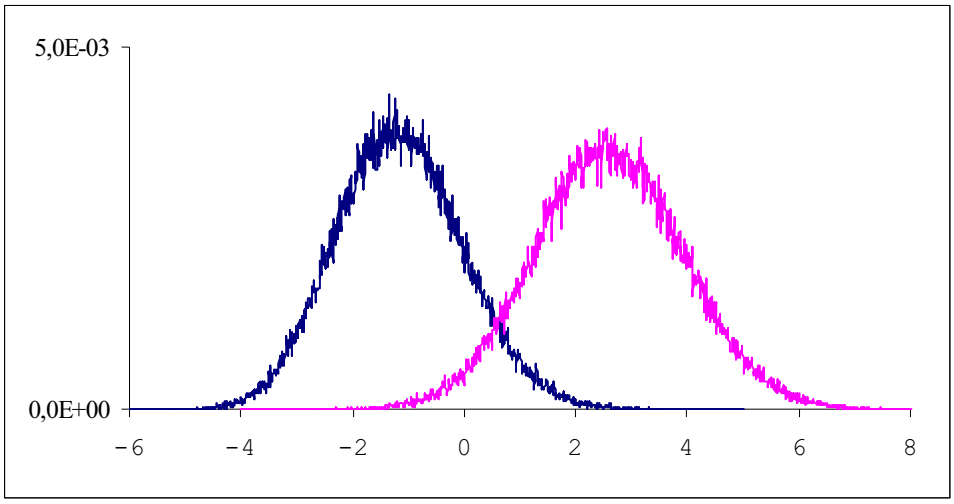

Figure 4. Empirical probability density functions of the $\log_{10}\frac{P[I \mid Pop1]}{P[I \mid Pop2]}$ *under the two hypothesis tested (null hypothesis on the left, see text). African (left) vs. Majorero (right).*

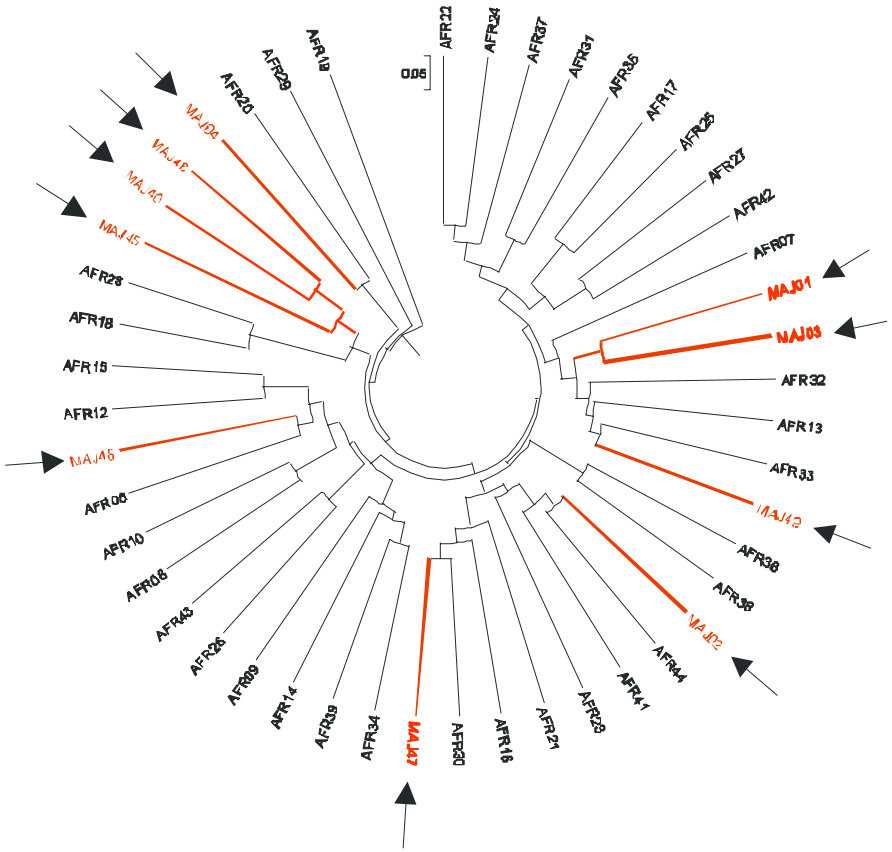

Figure 5. Dendrogram representing the relationships among the 47 animals. The arrows refers to animals belonging to the Majorera population.

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