

GENETIC VARIABILITY INFLUENCES PHYSICAL CONDITION IN MALE ROE DEER

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ABSTRACT

Understanding the relation between genetic variability, individual fitness and the viability of populations is crucial to ensure their adequate conservation. The most frequently used approach when tackling this question in natural populations is to analyze the correlation between heterozygosity, measured using molecular markers, and traits related to fitness, such as growth, survival, fecundity and developmental stability. Roe deer (*Capreolus capreolus*) is a species of great importance in Spain that has undergone recent geographical expansion. Understanding all the variables that affect its population dynamics is fundamental to regulate its management and improve its conservation. We examined the relationship between physical condition and individual genetic variability using 30 males captured between May and June (2002) in the Sierra de la Demanda (Burgos). We analysed 11 microsatellites, and individual genetic variability was calculated using *standardised individual heterozygosity* and mean d^2 . Relative body weight (residuals of body weight versus body size) is positively correlated with mean d^2 . High values of this variable are considered to represent high genetic variability since there are greater differences between the alleles inherited from the father and the mother. Thus, our results indicate that individuals with higher genetic variability accumulate more weight for a given size. Previous studies indicate that males in better physical condition are able to defend a larger territory and therefore have access to a greater number of females.

Key words: genetic variability, heterozygosity, physical condition, roe deer.

RESUMEN

La variabilidad genética influye sobre la condición física en machos de corzo

Entender la relación entre variabilidad genética, eficacia biológica individual y viabilidad de las poblaciones es vital para la conservación de éstas. La aproximación más frecuente al estudio de esta cuestión en poblaciones naturales es analizar la correlación existente entre la heterocigosis medida con marcadores moleculares y rasgos relacionados con la eficacia biológica (crecimiento, supervivencia, fecundidad o estabilidad en el desarrollo). El corzo (*Capreolus capreolus*) es una

especie de gran importancia en España, que ha experimentado además una reciente expansión en su territorio. Entender todas las variables que afectan su dinámica poblacional es fundamental para regular su manejo y promover su conservación adecuadamente. Se estudió la relación entre condición física y variabilidad genética individual en 30 machos cazados entre mayo y julio de 2002 en la Sierra de la Demanda (Burgos). Se analizaron 11 microsatélites, y se calculó la variabilidad genética individual como *heterocigosis individual estándar* y d^2 *media*. El peso corporal relativo (residuos del peso frente al tamaño del animal) está positivamente relacionada con la d^2 *media*. Mayores valores de esta variable se consideran indicadores de mayor variabilidad genética porque existe una mayor diferencia entre los alelos heredados del padre y de la madre. En este caso, los resultados indican que aquellos individuos con mayor variabilidad genética son capaces de acumular más peso para un tamaño dado. Estudios anteriores indican que en machos una mejor condición física favorecería la defensa de un territorio mayor y por lo tanto acceso a más hembras.

Palabras clave: condición física, corzo, heterocigosis, variabilidad genética.

INTRODUCTION

The effect that genetic variability has on individual fitness and the viability of populations is a central question in evolutionary and conservation biology. In natural populations a common approach is to analyze the correlation between traits related to fitness (survival, growth rate, parasite resistance) and individual heterozygosity measured using molecular markers. Although initially markers under selection, such as allozymes, were used (David 1998), in recent years neutral markers, such as microsatellites, are more frequently used due to their high mutation rate and polymorphism levels. Heterozygosity derived measures, such as *mean d^2* , have also been developed to synthesize better the molecular information obtained (Coltman *et al.* 1999, Coulson *et al.* 1999). Positive heterozygosity-fitness correlations have been widely reported. Several studies show a relationship between individual genetic variability and birth weight (Coltman *et al.* 1998), neonatal and young mortality (Coltman *et al.* 1998, Coulson *et al.* 1998), susceptibility to parasites (Coltman *et al.* 1999) and reproductive success (Slate *et al.* 2000). The underlying mechanism invoked to explain these relations is often inbreeding depression (Coltman *et al.* 1999, Slate *et al.* 2000). Inbreeding is the mating between genetically related individuals which generates offspring with reduced heterozygosity. This heterozygosity depletion exposes recessive deleterious alleles, which has a negative impact upon traits related to fitness, a phenomenon known as “inbreeding depression”.

Among the different factors affecting individual fitness, physical condition is one of the most important and most variable, particularly among males. There are several indicators of physical condition, but the most commonly used is body mass. Body mass is a key determinant of fitness components in many organisms. In ungulates it determines young and adult survival (Gaillard *et al.* 1997) and is related with parasite resistance (Coltman *et al.* 2001). It also affects reproductive success in females (Hewison 1996) and in males it determines mating success (Kruuk *et al.* 1999). However, in spite of having direct consequences on fitness, most studies have examined offspring weight, and few studies have addressed the factors that influence inter-individual variation in adult body mass. The effects of population density at birth and of habitat quality have been previously studied (Pettorelli *et al.* 2002). However, no studies have been carried out in ungulates considering the effect of genetic variability on body condition in adults.

In roe deer (*Capreolus capreolus* (Linnaeus, 1758)), female weight determines litter size (Hewison 1996), as well as age of first breeding (Gaillard *et al.* 1992). In this species, males in better physical condition are able to defend larger territories and therefore have sexual access to a greater number of females (Vanpé *et al.* 2005). In this work we use physical condition as an indicator of phenotypic quality, and examine the effect that genetic variability has on it. Given that there is a strong relation between body weight and body size, we use relative body weight as an indicator of physical condition.

MATERIAL AND METHODS

a) Study species

Roe deer (*Capreolus capreolus*), is a medium sized ungulate (around 25 kg) which is widely distributed in the North of the Iberian Peninsula. Its range is currently expanding mainly due to the reduction of agricultural land and to its value for hunters, which favours its protection. Its expansion has been especially marked in the areas of the Sistemas Ibérico and Central (San José 2002). This species shows a high morphological and ecological plasticity, occurring in a wide variety of habitats including natural forest (deciduous, coniferous and Mediterranean forests, shrublands, moorlands and marshes) as well as man-made habitats (plantation forest, mixed forest, farmland mosaics and agricultural plains). It shows moderate polygyny, which has favoured its monomorphism,

with males only exceeding by 10% the weight of females (Andersen *et al.* 1998). In the Iberian Peninsula three subspecies have been described: *C. c. decorus*, *C. c. canus* y *C. c. garganta*. Individuals of the subspecies *C. c. capreolus*, which is distributed in the rest of Europe, have also been introduced (San José 2002).

b) Morphometric measures

Our study sample consists of 30 males hunted from May to June (2002) in Sierra de La Demanda (Burgos). Measures of body size and weight were taken in the field, and a muscle sample was also obtained to carry out genetic analysis.

Morphometric variables measured were: (i) body weight: including viscera and blood (ii) body length: measured along the dorsal area of the body, from the tip of the snout to the tail (iii) height at shoulder: straight line distance from the base of the hoofs to the upper edge of the shoulder (iv) hind limb length: length along the inner side of the hind limb from the base of the hoof to the point where it joins the trunk.

Means were calculated for each variable; in all cases mean values were well within the range of values reported for Spanish populations.

c) Genetic analysis

Each individual was genotyped at eleven dinucleotide microsatellites: CSSM39, IDVGA8, BM1706, HUI1177, BMC1009, OarFCB304, BM848, NVHRT48, CSSM41, BM757, CSSM43. All of them have been successfully used in this species before and are polymorphic (Galan *et al.* 2002).

Two variables were calculated for each individual from the microsatellite data to obtain measures of individual genetic variability, *standardised individual heterozygosity* and *mean d^2* . *Standardised individual heterozygosity* is the ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed. *Mean d^2* is calculated as the squared difference in repeat units between the two alleles at a locus averaged over all loci at which an individual was scored, i.e.,

$$d^2 = \sum_{i=1}^n \frac{n (i_a - i_b)^2}{n}$$

Where i_a and i_b are the lengths in repeat units of alleles a and b at locus i and n is the number of typed loci for an individual.

Both variables are continuous and normality was checked before including them in any of the parametric statistical analyses performed. *Standardised individual heterozygosity* showed a normal distribution ($p > 0.20$ Kolmogorov-Smirnov test), however *mean d^2* was log-transformed to attain a normal distribution ($p > 0.20$ Kolmogorov-Smirnov test).

d) Statistical analyses

To integrate all the variables measuring different aspects of body size (body length, height at shoulder and hind foot length) into one “overall body size” variable, these variables were included in a principal component analysis (PCA). All variables showed a normal distribution ($p > 0.2$ Kolmogorov-Smirnov test). We use relative body weight (i.e. the residuals of the relation between body weight and “overall body size”) as an indicator of physical condition.

Since genetic variables are continuous a simple linear regression was used to study the relation between individual genetic variability and physical condition including as the dependent variable relative body weight and as the independent both *standardised individual heterozygosity* and *mean d^2* .

All statistical analyses were carried out using the program STATISTICA 6.0 (Statsoft 2001), and the level of statistical significance for all tests was 0.05.

We chose the casewise deletion technique to perform every analysis. This technique excludes from the analyses those individuals with one or more missing values from the respective datasets. Thus in subsequent analysis the missing values of each analysis add together resulting in a reduced but still significant sample size of 17 individuals.

RESULTS

The PCA performed with body size measures rendered a significant factor score which explained 69% of the variance with a correspondent eigenvalue of 2.0. The three variables (body length, height at shoulder and hind limb length) showed high factor loadings and were highly correlated with the extracted factor (Table 1). This factor, transformed to be positive, integrates all body size measurements into one “overall body size” variable, which was used in subsequent analyses.

Body weight was positively related to “overall body size” ($F(1,15) = 42.23$; $p < 0.00001$; $R^2 = 0.74$). To correct body weight for body size, “relative body weight” was calculated as the residuals obtained from this relation (Figure 1).

TABLE 1

Body size: Factor loadings of the three body size measures obtained with the PCA.
The correlations among the original variables and the factor obtained as
“overall body size” are labelled as follows: *** < 0.0001

*Tamaño del animal: Factores de carga de las tres medidas de tamaño corporal obtenidas con el PCA.
Las correlaciones entre las variables originales y el factor obtenido como indicador
del tamaño corporal se marcan de la siguiente forma: *** < 0,0001*

Body size variables	Factor loadings	p
Body length	-0.920026	***
Height at shoulder	-0.736458	***
Hind limb length	-0.827493	***
Eigenvalue	2.073564	
Explained variance	0.691188	

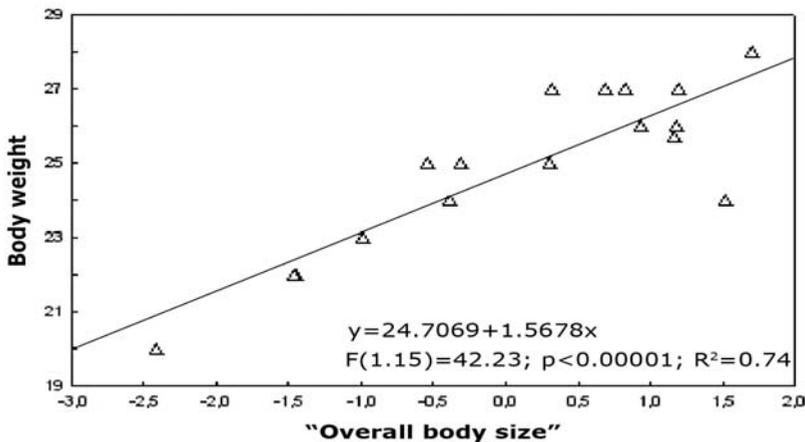


Figure 1. Relationship between body weight and “Overall body size” (n= 17).

Relación entre el peso y el indicador del tamaño corporal (n= 17).

When analysing the relation between individual genetic variability and relative body weight different results were obtained depending on the genetic variable used. No relation was found between *standardised individual heterozygosity* and relative body weight. In contrast, there was a significant and positive relationship between relative body weight and *mean d²* (F(1,15)= 4.58; p< 0.05; R²= 0.23) (Figure 2).

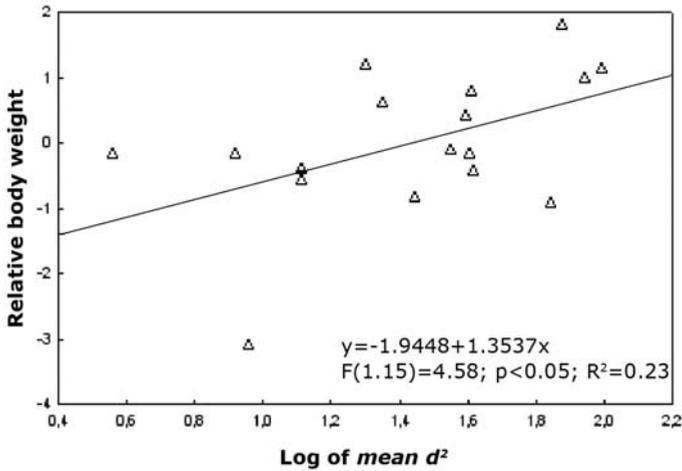


Figure 2. Relationship between *mean d^2* and relative body weight (n= 17).

Relación entre d^2 media y peso corporal relativo (n= 17).

DISCUSSION

Our results indicate that there is a positive relation between individual genetic variability, measured with the molecular parameter *mean d^2* , and relative body weight which is an indicator of physical body condition. High values of *mean d^2* are considered to represent high genetic variability since it indicates that there are greater differences between the alleles inherited from the father and the mother (Coulson *et al.* 1998). Thus, in our study population males with higher genetic variability gain more weight for a given size and, as a consequence, are in better physical condition. There is some evidence that higher values of heterozygosity are associated with enhanced metabolic performance and a more efficient use of the resources (Mitton 1993), suggesting a possible mechanism underlying the relationship between genetic variability and physical body condition.

The relation found between genetic variability and physical condition explains 23% of the variance. This percentage is high when compared to correlations between genetic variability (calculated with molecular markers) and other components of fitness which explain from 1 to 5% of the phenotypic variance observed (David 1998). Previous studies examining the factors that affect roe deer body weight explained percentages of variance slightly higher. Pettorelli *et al.* (2002), found for example that the year of birth and type of

habitat were acting together conditioning adult weight, and explained 40% of its variance. It is possible that there is an interaction between genetic variability and environmental factors that, considered together, would explain a higher percentage of the variation found in physical condition. Previous studies have shown that the deleterious effects of inbreeding increase under stressful conditions, such as a high density, low availability of food or adverse meteorological factors (Keller *et al.* 1994, 2002). The interaction between genetic and environmental factors deserves further attention.

The two genetic measures used behaved differently, since *mean d^2* was found to be associated to relative body weight, while *standardised individual heterozygosity* was not. This suggests that both variables convey information of a different kind. It has been suggested that *mean d^2* is more informative when used in populations that originate from a mixture of two very divergent populations (Hedrick *et al.* 2001, Tsitrone *et al.* 2001). If we take into account that our study population is an open one, and that roe deer populations in Spain became very fragmented before the recent expansion, it is likely that current populations are the result of a mixture of previously isolated and genetically divergent populations.

But, how does individual physical condition relate to fitness? In ungulates body condition influences male fighting ability and the capacity to defend territories or harems, which in turn determines mating success. Available evidence indicates that this is also the case among roe deer, where larger body size and enhanced physical condition would enable males to defend larger territories, gaining in this way sexual access to more females (Vanpé *et al.* 2005). Thus, high genetic variability eventually translates into higher male reproductive success, through its effects upon body condition.

Most of the previous studies where a relation was found between genetic variability and fitness were carried out in isolated populations and/or with a marked polygyny (Slate *et al.* 2000, Hoffman *et al.* 2004). This had led to the conclusion that it is only possible to find a correlation between genetic variability and traits related with fitness under very restrictive conditions, which include small size, a high substructure or extreme mating systems (highly polygynous) (Balloux *et al.* 2004). However, our study population is open and roe deer do not have a marked degree of polygyny. This means that levels of individual genetic variability may influence fitness under a greater diversity of scenarios than it has been previously envisaged.

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