
Relationship between Coefficient of Inbreeding and Parasite Burden in Endangered Gazelles

JORGE CASSINELLO,* MONTSERRAT GOMENDIO, AND EDUARDO R. S. ROLDAN

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales (CSIC), C/José Gutiérrez Abascal 2, 28006-Madrid, Spain

Abstract: *We studied the effects of inbreeding depression on parasite infection in three species of endangered gazelles: Gazella cuvieri, G. dama, and G. dorcas. Coefficients of inbreeding were calculated for all individuals because complete genealogies were available. The levels of inbreeding differ both intra- and interspecifically. We collected samples of feces and determined nematode infection by counting nematode eggs in the samples. At the interspecific level, the species with the highest mean levels of inbreeding (G. cuvieri) had the highest levels of gastrointestinal parasites. Analyses done at the intraspecific level revealed a positive relationship between individual coefficient of inbreeding and parasite infection in G. cuvieri, but not in the species with the intermediate and lowest levels of inbreeding. Our findings suggest that high levels of inbreeding may make individuals more susceptible to parasitism, even under favorable environmental conditions, so this factor should be taken into account by those managing endangered species.*

Relación entre el Coeficiente de Consanguinidad y la Carga Parasitaria en Ungulados en Peligro de Extinción

Resumen: *Estudiamos los efectos ocasionados por la consanguinidad sobre el grado de infestación parasitaria en tres especies de gacelas en peligro de extinción: Gazella cuvieri, G. dama y G. dorcas. Los coeficientes de consanguinidad fueron calculados para todos los individuos debido a que se encontraban disponibles genealogías completas. Los niveles de consanguinidad difieren tanto intra como interespecíficamente. Muestras de heces fueron colectadas y la infección por nemátodos se determinó por conteo de huevos en las muestras. A nivel interespecífico, la especie que posee el nivel promedio de consanguinidad más elevado (G. cuvieri) tuvo los niveles más altos de parásitos intestinales. Los análisis efectuados a nivel intraespecífico revelaron la existencia de una relación positiva entre el nivel de consanguinidad individual y la infestación por parásitos en G. cuvieri, pero no en las especies con niveles de consanguinidad medio y bajo. Nuestros resultados muestran que niveles elevados de consanguinidad podrían dar origen a una mayor susceptibilidad a los parásitos en los individuos, incluso bajo condiciones ambientales favorables, por lo que este factor deberá ser tomado en cuenta para el manejo de especies en peligro de extinción.*

Introduction

Understanding the consequences of inbreeding is a key issue in conservation biology. Despite considerable debate over the evolutionary significance of inbreeding in natural populations (Lande 1988, 1993; Harcourt 1991; Shields 1993; Caro & Laurenson 1994; Caughley 1994; Frankham 1995a, 1995b), recent studies that have incor-

porated molecular techniques suggest that low levels of heterozygosity in natural populations may be related to reduced fitness (Coulson et al. 1998). Evidence from a butterfly metapopulation suggests that inbreeding may lead to the extinction of natural populations (Saccheri et al. 1998).

One of the most important effects of inbreeding could be a decrease in resistance to parasites. Inbreeding could increase vulnerability to pathogens in two ways. First, heterozygotes at loci involved in pathogen resistance could be at an advantage because of the greater range of parasites recognized by the immune system. In verte-

*email cass@mncn.csic.es

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brates growing evidence suggests that such genetic diversity is particularly important at the level of the major histocompatibility complex because its gene products play an important role in immune function (Thursz et al. 1997; Paterson 1998; Paterson et al. 1998; Carrington et al. 1999). Second, genome-wide effects could also influence vulnerability to pathogens, either through homozygosity of deleterious recessive alleles or overdominance (e.g., Ferguson & Drahushchak 1990). Recent data from a Soay sheep population (*Ovis aries*) revealed that less heterozygous individuals were more susceptible to parasitism by gastrointestinal nematodes and that this was the cause of greater mortality during harsh winters (Coltman et al. 1999). The authors suggest that inbreeding is the main cause of homozygosity, but further evidence is needed to clarify this issue, because pedigree information is incomplete.

Our goal was to determine whether individual coefficient of inbreeding is related to parasite resistance in captive populations of three related species of endangered gazelles. These populations offer a unique opportunity to study the effects of inbreeding because the presence of only one male in each group of females has allowed the determination of paternity for every offspring born, thus making it possible to reconstruct whole pedigrees. Because of differences in the size of founding populations between each species, average levels of inbreeding among the three species differ. More important, there is enough intraspecific variation in each species to allow us to determine whether differences in individual levels of inbreeding are related to susceptibility to parasitism.

Methods

Since 1971 the Estación Experimental de Zonas Áridas (CSIC) has developed a successful captive-breeding program for three endangered species: *Gazella dama mhorr*, *G. dorcas neglecta*, and *G. cuvieri*. These subspecies of dama gazelle and Cuvier's gazelle are thought to be extinct in the wild.

Individuals were selected so that the sample would cover the whole range of coefficients of inbreeding present in the current population; all were males ranged from 2 to 15 years of age. We took samples from as many sexually mature individuals as possible. Individual coefficients of inbreeding were assigned following the additive relationship method (Wright 1922; Ballou 1983).

Samples of feces were collected directly from the rectum while the individuals were anesthetized, following a routine used for the collection of samples in the study populations (Cassinello et al. 1998). All the samples were collected during the autumn of 1996 and 1997 from 17 Cuvier's gazelles, 26 dama gazelles, and 24 dorcas gazelles. This season was chosen because of the

mating activities of Cuvier's gazelle peak at this time (Olmedo et al. 1985); the other two species do not follow a seasonal pattern.

We used fecal egg counts (FEC) or eggs per gram of feces (epg) as a measure of the intensity of nematode parasitism and parasite resistance, following methods of recent studies on ungulates (e.g., Gulland et al. 1993; Cabaret et al. 1998). Furthermore, FEC is a noninvasive measure that reflects worm burden and fecundity, which are both influenced by the immune state of the host (e.g., Stear et al. 1996, 1997). In the analyses, two nematode families were distinguished: Trichostrongylidae (several species, among which is *Nematodirus* sp.), which is especially common and pathogenic in grazing ruminants and usually located in the abomasum and small intestine, and Trichuridae (*Trichuris* sp.), also common in ruminants and located mainly in the large intestine (caecum and colon). We used the MacMaster dilution egg-counting technique to analyze egg shedding (e.g., Kaufmann 1996). This technique presents several variations, but it comes originally from the work of Stoll (1923, 1930) and Gordon and Whitlock (1939). Despite some controversy (e.g., Bowman 1995; Kaufmann 1996), this technique has been considered a valid method of evaluating worm burdens in several ungulates (e.g., Gulland et al. 1993; Cabaret et al. 1998; Coltman et al. 1999).

Some individuals received an antiparasitic treatment 8–9 months before the samples were collected, but a comparison between individuals that had received treatment and those that had not, showed no statistical differences in FEC, indicating that any effects of the treatment had disappeared by the time we collected the samples. No differences were found between males under different housing conditions, and age and year of sampling had no effect. We used analysis of variance and simple regressions; log transformations were applied to the variables that did not conform to normality (Zar 1984).

Results

Inbreeding

Founding population sizes for the three species were 3 males and 9 females for *G. dama*, 11 males and 13 females for *G. dorcas*, and 2 males and 2 females for *G. cuvieri*. The highest levels of inbreeding were found in Cuvier's gazelle (0.15 ± 0.016), followed by the dama gazelle with intermediate levels (0.104 ± 0.012) and then by the dorcas gazelle with the lowest levels (0.020 ± 0.005). The overall difference was significant, as were all the differences between each pair of species (analysis of variance: $F_{2,68} = 45.15$, $p < 0.0001$, with all pairwise comparisons being significant [Fisher's post-hoc test]).

Parasitism

The three species differed in FEC (Cuvier's 192.32 ± 59.15 epg; dama, 14.27 ± 4.37 epg; dorcas, 19.20 ± 5.44 epg; $F_{2,68} = 15.03$, $p < 0.0001$), with Cuvier's gazelle significantly higher in FEC than the other two (both Fisher's PLSD post-hoc tests $p < 0.0001$). When trichostrongylids and *Trichuris* sp. were distinguished the results followed a similar trend, Cuvier's males again having significantly higher values (trichostrongylids: $F_{2,68} = 5.14$, $p = 0.01$; post-hoc tests, Cuvier's-dama $p = 0.01$; Cuvier's-dorcas $p = 0.003$) (*Trichuris* sp.: $F_{2,68} = 16.18$, $p < 0.0001$; both post-hoc tests $p < 0.0001$).

The range of individual inbreeding coefficients present within each species differed: In dorcas gazelles the values ranged from 0 to 0.08, in dama gazelles they ranged from 0 to 0.31, and in Cuvier's gazelles they varied from 0.06 to 0.31.

Intraspecific analyses showed a positive relationship between FEC and individual coefficient of inbreeding in Cuvier's gazelles ($n = 17$, $R^2 = 0.26$, $p = 0.03$; Fig. 1a) but not in the other two species (dama: $n = 26$, $R^2 = 0.001$, $p = 0.85$; dorcas: $n = 24$, $R^2 = 0.10$, $p = 0.14$). This relationship was due primarily to the effects of individual coefficient of inbreeding on Trichostrongylid egg shedding ($n = 17$, $R^2 = 0.42$, $p = 0.005$; Fig. 1b).

Discussion

Our findings indicate that the species with the highest mean level of inbreeding (Cuvier's gazelle) is more susceptible to nematode infection than the other two species. More important, we show that at the individual level, coefficient of inbreeding is positively related to levels of parasitism in Cuvier's gazelle but not in the other two species. To our knowledge, this is the first study to show that individual levels of inbreeding, rather than other indirect measures, are related to vulnerability to parasites.

In our study populations, all animals were kept under identical environmental conditions, samples were taken during the same months in 2 consecutive years, and only males were included. Thus, habitat, season, diet, and sex were excluded as confounding factors. Under such conditions, correlations between individual coefficient of inbreeding and parasite burden most likely reveal a causal relationship.

An increase in inbreeding probably results in a decrease in heterozygosity, which makes individuals more vulnerable to the enormous diversity of rapidly evolving parasites. This decrease in heterozygosity may increase susceptibility to parasitism either through genome-wide effects or through its effects on specific loci related to pathogen resistance, such as MHC loci. The available data do not allow us to distinguish between these two alternatives.

Although the species with the highest level of inbreed-

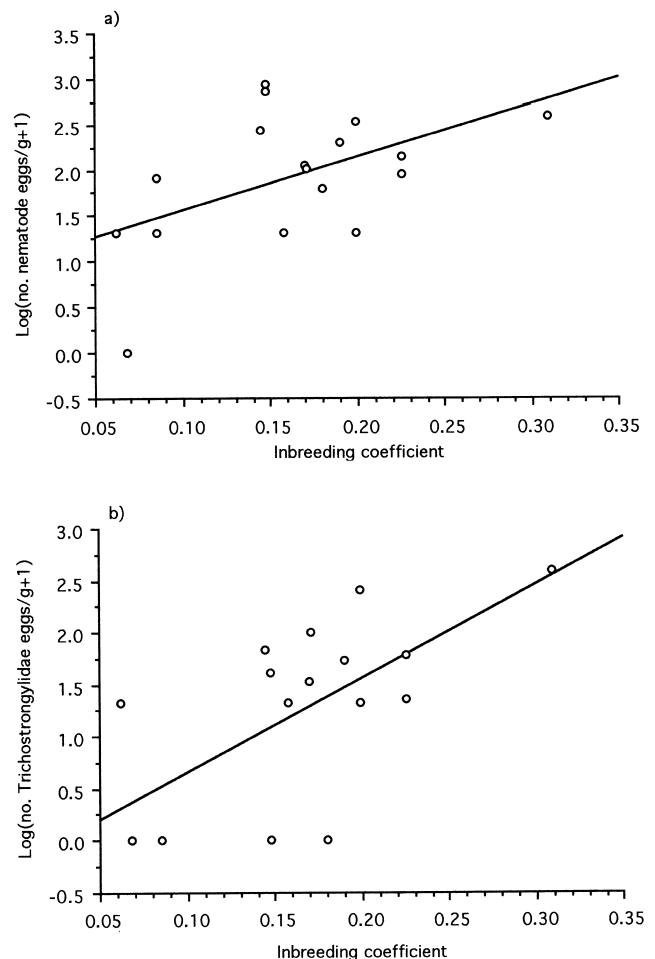


Figure 1. Relationship between the frequency of (a) nematode egg shedding and the coefficient of inbreeding in Cuvier's gazelles ($n = 16$) and of (b) Trichostrongylidae egg shedding and the coefficient of inbreeding in Cuvier's gazelles.

ing (Cuvier's gazelle) shows the highest level of parasite infection and a clear correlation between inbreeding and parasite burden at the individual level, the relationship is not as clear when the other two species are considered. Thus, the dama and dorcas gazelles have intermediate and low levels of inbreeding, respectively, yet the two show low levels of parasite infection, and neither show a relationship between nematode infection and individual coefficient of inbreeding. There could be several reasons for these findings. First, inbreeding may not have deleterious effects until a threshold is crossed, and both dama and dorcas gazelles may be below those critical levels. Second, inbreeding may affect different traits in different populations, depending on the genetic load present in their founding populations (Lacy et al. 1996). Third, Cuvier's gazelle is the only species of the three with a seasonal pattern of reproduction, and samples were collected during the breeding season. Thus, male Cuvier's gazelles may have been under greater stress

than males of the other two species, which may be why the effects of inbreeding became more pronounced.

We have also studied the effects of inbreeding on semen quality in these three species and found that it has a strong negative effect in Cuvier's gazelle (Cassinello et al. 1998; Roldan et al. 1998; Gomendio et al. 2000). Thus, inbreeding depression not only influences juvenile survival but also seems to have a strong negative influence on other traits that may be crucial to the future of endangered species. Furthermore, although early juvenile mortality is easy to detect, effects on semen quality or parasite resistance may be less apparent but may become crucial under harsh ecological conditions. These findings should therefore be taken into account in the design of reintroduction plans, and selection of noninbred individuals should be a priority.

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