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Research paper

Tuberculosis, genetic diversity and fitness in the red deer, *Cervus elaphus*

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ABSTRACT

Understanding how genetic diversity, infections and fitness interact in wild populations is a major challenge in ecology and management. These interactions were addressed through heterozygosity-fitness correlation analyses, by assessing the genetic diversity, tuberculosis (TB) and body size in adult red deer. Heterozygosity-fitness correlation models provided a better understanding of the link between genetic diversity and TB at individual and population levels. A single local effect was found for Ceh45 locus at individual level, enhancing the importance of its close functional genes in determining TB presence. At population level, the ability of the red deer to control TB progression correlated positively with population genetic diversity, indicating that inbred populations might represent more risk of deer TB severity. Statistical models also gained insights into the dynamics of multi-host interaction in natural environments. TB prevalence in neighbouring wild boar populations was positively associated with deer TB at both individual and population levels. Additionally, TB presence correlated positively with red deer body size, for which "general and local effect" hypotheses were found. Although body size might be correlated with age, an indirect genetic effect on TB presence could be implied. This study provides new insights towards understanding host-pathogen interactions in wild populations and their relation to fitness traits.

1. Introduction

Understanding how genetic diversity, infectious disease and fitness interact in wild populations has become a major challenge in ecology and management (Luikart et al., 2008). Fitness has long been recognized as the ability of organisms, populations or species to survive and reproduce in nature and therefore to contribute to passing specific genes on to the next generations (Barker, 2009; Orr, 2009). Various morphological, physiological and life-history traits, directly and indirectly related with fitness, have been studied using heterozygosity-fitness correlations (HFC) (Acevedo-Whitehouse et al., 2005; Brambilla et al., 2014; Brommer et al., 2015; Da Silva et al., 2009; Hansson and Westerberg, 2002; Olano-Marin et al., 2011; Pemberton, 2004; Pérez-González et al., 2010; Reed and Frankham, 2003; Slate et al., 2004). Two alternative hypotheses have been proposed to explain significant associations between neutral markers and fitness-related traits: the "general effect" and "local effect" hypotheses (reviewed in Hansson and Westerberg, 2002). Whereas the "general effect" hypothesis accounts for a genome-wide effect and requires identity disequilibrium (i.e. variation in the genome-wide diversity among individuals) within a population (Slate et al., 2004; Szulkin et al., 2010; Weir and Cockerham, 1973), the "local effect" hypothesis relies on a strong linkage disequilibrium between some neutral markers (i.e. microsatellites) and close loci displaying overdominance or carrying deleterious recessive alleles (Chapman et al., 2009; Szulkin et al., 2010). Despite these hypotheses have been largely investigated, few studies have assessed the interactions between genetic variation and infectious disease in natural environments (Acevedo-Whitehouse et al., 2005, 2003; Luikart et al., 2008). In particular, there are very few studies evaluating the relationships between genetic diversity and chronic infectious diseases (Amos and Acevedo-Whitehouse, 2009; Lyons et al., 2009).

The red deer, *Cervus elaphus,* has been widely used as a model species for HFC studies in the literature (Brommer et al., 2015; Coulson et al., 1998; Slate et al., 2000; Walling et al., 2014). It is one of the most widespread and important game species in Europe (Milner et al., 2006), especially in central-southern Spain where populations are managed for hunting in a semi-intensive management regime (Azorit et al., 2002). As in the populations of central Europe, the demographic history of Iberian red deer has been predominately shaped by recent anthropogenic impacts on the natural distribution patterns resulting from ancient bioclimatic events (i.e. glacial and interglacial periods of Pleistocene; Milner et al., 2006; Azorit et al., 2002; Queirós et al., 2014). Signs of a population bottleneck, admixture and inbreeding

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have been observed throughout Iberia, with marked differences among populations promoted mainly by the gamekeepers' estate-specific managing practices (Queiros et al., in revision). Associated with a semi-intensive production of ungulates, various disease problems have emerged (Gortázar et al., 2006), among them, animal tuberculosis (TB) (Martín-Hernando et al., 2007; Parra et al., 2005; Vicente et al., 2013).

Tuberculosis caused by *Mycobacterium bovis* and closely related members of the *Mycobacterium tuberculosis* complex (MTC) is a re-emergent zoonotic disease that affects a broad range of host species worldwide (Gortázar et al., 2012). In central-southern Spain, TB is considered endemic in wild ungulates (Vicente et al., 2013), with red deer and Eurasian wild boar (*Sus scrofa*) populations displaying a high prevalence of this disease (Gortazar et al., 2011; Vicente et al., 2013). It is recognized that the wild boar is the main reservoir of TB in the wild (Naranjo et al., 2008b), while the red deer, fallow deer (*Dama dama*) and badger (*Meles meles*) may be involved locally to maintain infection (Gortázar et al., 2012). Increasing evidence suggests that genetic and environmental factors (i.e. co-infections with parasitic diseases and viruses, climatic conditions, management practices) contribute to the pathogenesis and differences in susceptibility to TB in various species, including domestic animals (Allen et al., 2010; Flynn et al., 2009; Phillips et al., 2002), wildlife (Acevedo-Whitehouse et al., 2005; Beechler et al., 2015; Mackintosh et al., 2004; Trinkel et al., 2011; Vicente et al., 2013) and humans (de Wit et al., 2011; Li and Zhou, 2013; Lienhardt, 2001; Möller and Hoal, 2010). Previous studies on farmed red deer have revealed that the risk of MTC infection and disease transmission were dependent on individual genetic backgrounds (Griffin and Mackintosh, 2000), suggesting that TB resistance may be a heritable trait under farmed conditions (Mackintosh et al., 2000). In theory, individuals with a strong genetic background (i.e. greater genetic diversity) are more able to cope with infection and the progression of a disease than individuals with lower genetic variability (Howard and Lively, 1998).

The main aim of this study was to identify the determinants of TB presence and severity (i.e. measure of disease progression) in red deer, both at individual and population levels, as well as its impacts on fitness. To achieve this goal, HFC models were created integrating genetic, biological, demographical and management components. Thoroughly understanding the role of genetic mechanisms and the effects of ecological factors on wild populations suffering from a chronic infectious disease, such as TB, is potentially useful for developing more appropriate management and conservation strategies, as well as for designing control measures for TB in natural environments.

2. Material and methods

2.1. Study populations

The deer populations sampled in this study are located in the central-southern region of Spain, where the presence of TB in wild ungulates has been reported since the 1980s (Gortazar et al., 2011; Vicente et al., 2013; Vizcaíno et al., 1990). The sampling sites comprised twelve populations representative of the two most traditional management regimes found in this region: fenced estates, where populations, enclosed by high fences, are artificially fed and managed for hunting by gamekeepers; and protected areas, which represent natural populations where intensive management is not performed (just population control; Table 1). The demographic history of these populations was assessed in previous studies and revealed a strong influence of recent anthropogenic effects on the natural distribution patterns resulting from past glacial and interglacial cycles of Pleistocene. Over the last century, historical records and genetic data showed a reduction in the distribution of red deer populations across Iberia, as a consequence of intensive hunting, that was followed by a population recover after the middle of the last century, mainly promoted by hunting proposes. Management practices implemented during the last decades have contributed to a great variety of genetic diversity patterns among populations, although the phylogeographic scenario resulted from the Last Glacial Maximum still persist across Iberia (Queirós et al., 2014, Queiros et al., in revision).

2.2. Data collection

Samples were collected from randomly hunted individuals during the regular hunting season or control programs (between 2005/2006 and 2010/2011 - October to February). Although hunted animals may not be representative of overall population, the traditional hunting scheme carried out in the sampled populations and known as "batidas/montarias" has been shown to be the less biased procedure to obtain data from hunted red deer (Martinez et al., 2005; Pérez-González et al., 2010). A detailed necropsy protocol was performed for each individual in the field. Briefly, sex, age class and morphometric measurements (i.e. body length and thoracic perimeter)

Table 1

Detailed characterization of red deer and the neighbouring wild boar populations in central-southern Spain.

			Red deer					Wild boar[a]		Red deer population genetic diversity and demography				HFC models			
			Density (ind/km2)		TB (%)		"TB severity"(%)	TB (%)						TB and BS			"TB severity"
Population	Sampled hunting seasons	Management regime	Mean	SE	Mean	SE		Mean	SE	n	F_{IS}	AR	Bottleneck	n	Male	Female	n
MT6	2008–2010	Fenced	0.21	<0.00	8.6	0.6	–	31.2	0.6	27	0.05	4.15	Yes	12	4	8	0
PNC	2007–2009	Protected	0.27	<0.00	24.9	8.2	33.3	69.1	2.4	24	0.02	4.14	No	11	7	4	3
PND	2005–2007	Protected	0.22	<0.00	31.5	3.4	100.0	70.7	0.0	30	0.18	2.93	Yes	22	13	9	4
MT4	2009–2011	Fenced	0.14	<0.00	27.7	15.2	0.0	84.0	16.0	34	0.04	3.94	No	22	16	6	2
MT7	2008–2010	Fenced	0.60	<0.00	44.8	3.1	50.0	77.4	12.7	53	0.01	4.07	Yes	21	13	8	6
SM4	2008–2010	Fenced	0.22	<0.00	4.6	4.6	0.0	22.7	0.5	31	0.06	4.09	No	25	14	11	1
QM	2008–2011	Protected	0.35	0.03	26.2	5.3	22.2	81.9	1.6	44	0.00	4.03	Yes	30	14	16	1
MT1	2008–2010	Fenced	0.15	<0.00	6.7	6.7	50.0	42.9	3.3	20	0.00	3.92	No	15	5	10	9
MT2	2009–2011	Fenced	0.36	0.15	3.0	3.0	100.0	35.5	5.5	27	0.06	3.82	No	24	15	9	2
MT5	2009–2010	Fenced	0.33	–	20.0	–	50.0	60.0	–	27	0.00	4.13	Yes	10	0	10	2
SM5	2006–2007	Fenced	0.27	–	4.2	–	–	54.2	–	22	0.04	3.83	No	20	8	12	0
SM1	2006–2007	Fenced	0.21	–	4.5	–	0.0	72.7	–	26	0.00	4.06	No	16	8	8	1
Total										365				228	117	111	31

Legend: ind – individuals; Fenced - populations enclosed by high fences, which are artificially fed and managed for hunting by gamekeepers; Protected - populations belonging to natural preserves where intensive management is not performed, just population control; TB – tuberculosis; "TB severity" – the presence of TB compatible lesions in more than one region/cavity of organism (i.e. TB lesions in lymph node/tissues from head region, cavities thoracic and/or abdominal); HFC - samples included on heterozygosity-fitness correlation tests; BS - body size; SE – standard error of the mean; F_{IS} – population coefficient of inbreeding; AR – population allelic richness.

[a] Prevalence of TB in the neighbouring wild boar populations was recompiled from Vicente et al. (2013).