Conserv Genet (2015) 16:289–300
DOI 10.1007/s10592-014-0658-0

RESEARCH ARTICLE

Signatures of natural and unnatural selection: evidence from an immune system gene in African buffalo

K. E. Lane-deGraaf · S. J. Amish · F. Gardipee · A. Jolles · G. Luikart · V. O. Ezenwa

Received: 30 September 2013 / Accepted: 15 September 2014 / Published online: 16 October 2014

Abstract Pathogens often have negative effects on wildlife populations, and disease management strategies are important for mitigating opportunities for pathogen transmission. Bovine tuberculosis (*Mycobacterium bovis*; BTB) is widespread among African buffalo (*Syncerus caffer*) populations in southern Africa, and strategies for managing this disease vary. In two high profile parks, Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HIP), BTB is either not actively managed (KNP) or managed using a test-and-cull program (HIP). Exploiting this variation in management tactics, we investigated potential evolutionary consequences of BTB and BTB management on buffalo by examining genetic diversity at IFNG, a locus which codes for interferon gamma, a signaling molecule vital in the immune response to BTB. Both heterozygosity and allelic richness were significantly and positively correlated with chromosomal distance from IFNG in KNP, suggesting that directional selection is acting on IFNG among buffalo in this park. While we did not see the same reduction in genetic variation around IFNG in HIP, we found evidence of a recent bottleneck, which might have eroded this signature due to genome-wide reductions in diversity. In KNP, alleles at IFNG were in significant gametic disequilibrium at both short and long chromosomal distances, but no statistically significant gametic disequilibrium was associated with IFNG in HIP. When, we compared genetic diversity between culled and non-culled subsets of HIP animals, we also found that individuals in the culled group had more rare alleles than those in the non-culled group, and that these rare alleles occurred at higher frequency. The observed excess of rare alleles in culled buffalo and the patterns of gametic disequilibrium in HIP suggest that management may be eroding immunogenetic diversity, disrupting haplotype associations in this population. Taken together, our results suggest that both infectious diseases and disease management strategies can influence host genetic diversity with important evolutionary consequences.

Keywords Parasite-mediated selection · *Syncerus caffer* · Bovine tuberculosis · IFNG · Disease management · Genetic diversity · Gametic disequilibrium

K. E. Lane-deGraaf and S. J. Amish have contributed equally to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s10592-014-0658-0) contains supplementary material, which is available to authorized users.

K. E. Lane-deGraaf (✉) · V. O. Ezenwa (✉)
Odum School of Ecology and Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA
e-mail: lanedegraaf.kelly@gmail.com

V. O. Ezenwa
e-mail: vezenwa@uga.edu

S. J. Amish · G. Luikart
Fish & Wildlife Genomics Group, Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

F. Gardipee
U.S. Fish and Wildlife Service, Sacramento, CA 59825, USA

A. Jolles
College of Veterinary Medicine and Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA

Introduction

Parasites, including helminths, protozoa, bacteria, and viruses, can act as strong selective forces on host

populations driving evolutionary change (Smith et al. 2009; Koskella et al. 2012; Leung et al. 2012). Selection by parasites may have particularly potent impacts on regions of the genome that are directly involved in immune defense. Immune system genes often show much greater adaptive evolution than genes not directly involved in immune function, and this pattern is often interpreted as a signature of intense co-evolutionary interactions between hosts and parasites (McTaggart et al. 2012). Parasite-mediated selection on immune genes can occur directly when parasites reduce individual survival or depress fecundity, increasing the frequency of resistant genotypes (Altizer et al. 2003; Thrall and Burdon 2003), and also indirectly via management responses to infectious disease outbreaks (Shim and Galvani 2009). In the latter case, strategies used to control parasite spread, such as culling or vaccination, may accelerate the rate and intensity of observable selection or drive cryptic evolutionary change.

Parasites interact with the host immune system in different ways, resulting in distinct forms of selection acting on immunity. Although immune system genes are expected to experience strong selection as a group, the mode and degree of selection can be highly variable across loci. Balancing selection was once viewed as a dominant form of selection acting on immune loci in wildlife populations in particular, due in part to a historical focus of research on major histocompatibility (MHC) genes (Acevedo-Whitehouse and Cunningham 2006). However, more recent work on a broader set of immune function genes has expanded this view (e.g. Downing et al. 2009; Tonteri et al. 2010; Tschirren et al. 2011; Llewellyn et al. 2012). In the last five years, more than one thousand studies have described evidence of selection on immune genes in mammals (excluding humans); of these studies, almost half found evidence of directional selection, while less than a tenth found evidence of balancing selection, and fewer still found evidence of diversifying selection highlighting the level of variability in the mode of selection acting on immune genes. For example, in a study of 18 microsatellites linked to immune genes in Atlantic salmon (*Salmo salar*), Tonteri et al. (2010) found that genes for interleukin1 and calmodulin production, were under strong directional selection. By contrast, a study of five different immune genes in the greater prairie-chicken (*Tympanachus cupido*), including interleukin 2 and transforming growth factor β3, found evidence of directional selection at only one of five genes (inhibitor of apoptosis protein-1), while selection at the remaining loci was more consistent with balancing selection (Bollmer et al. 2011). Finally, in an analysis of the interleukin 4 (IL-4) locus, thought to be under selective pressure from helminth parasites in mammals, diversifying selection was found to play a role in maintaining genetic diversity at fifteen IL-4 residues involved in receptor binding. This pattern of diversifying selection was maintained across multiple mammalian genomes, ranging from mice to primates, carnivores and ungulates (Koyanagi et al. 2010). These case studies suggest that interactions between parasites and the host immune system result not only in distinct signatures of selection on different immune genes, but also that the form of selection can vary across species and populations.

The environmental context of a particular host-parasite interaction can play a key role in determining the strength and form of selection acting on any immune gene. In recent years, disease management has emerged as an important factor potentially driving selection on immune loci (Altizer et al. 2003; McCallum 2008). Management strategies for controlling infectious diseases in natural populations are becoming increasingly common, and as such, understanding how management can shape the evolution of host immune defenses is critical (Woodroffe 1999; Cross et al. 2009; Joseph et al. 2013). Culling, in particular, is one disease management strategy used in wildlife that has enormous potential to drive selection on hosts, particularly when specific groups of individuals are targeted for removal (Myerstud and Bischof 2010; Myerstud 2011; White et al. 2011). While a number of studies have documented unintended ecological effects of culling (Donnelly et al. 2006; Woodroffe et al. 2006, 2009a, b), empirical evidence of evolutionary consequences are still very limited (Smith et al. 2009). However, culling has the potential to erode host evolutionary potential by facilitating the loss of genetic diversity via reductions in rare alleles (Sackett et al. 2013), impeding the evolution of resistance (Shim and Galvani 2009), and even driving broad evolutionary changes in the ecological community if disease management for one species changes the demography and ecology of non-target species (Chauvenet et al. 2011).

In this study, we focus on bovine tuberculosis (BTB) as a potential agent of selection on immune genes in a wild reservoir host population. BTB is a chronic disease of wildlife and livestock, caused by *Mycobacterium bovis*. The disease has a global distribution and accounts for significant economic losses worldwide (Michel et al. 2010; Goodchild et al. 2011). In South Africa, African buffalo (*Syncerus caffer*) serve as the primary wildlife reservoir of BTB and are responsible for spillover into other wildlife populations (e.g. lions, cheetahs) and cattle (Renwick et al. 2007; Fitzgerald and Kaneene 2013; de Garine-Wichatitsky et al. 2013). As such, effective disease control in buffalo populations is of critical importance (de Garine-Wichatitsky et al. 2013). Management strategies for controlling BTB in South African buffalo range from passive surveillance to active test and cull programs (Michel et al. 2006). For instance, in Kruger National Park (KNP), where BTB was first detected in the buffalo population between

1950 and 1960 and the population is estimated to be between 23,000 and 25,000 individuals, BTB management has focused on surveillance, population monitoring, and research (Michel et al. 2006). By contrast, in Hluhluwe-iMfolozi Park (HIP) where the first BTB positive buffalo was detected in 1986 and the buffalo population is considerably smaller (est. 3,000 individuals), a test and cull program was initiated in the mid-1990s (Michel et al. 2006). Current estimates of BTB prevalence in both parks are between 5 and 45 % for buffalo herds in KNP (Cross et al. 2009), and 0–73 % for buffalo herds in HIP (Jolles et al. 2006). Since negative effects of BTB on survival and reproduction have been described for buffalo (Jolles et al. 2005), it is possible that this disease could act as a direct selective force on buffalo populations. Moreover, in HIP where the BTB control program culled approximately 700 buffalo testing positive for BTB (out of a total of 4,681 animals tested between 1999 and 2006; Jolles et al., unpublished data), selective culling could impose additional indirect selective pressure, particularly at loci involved in immune defense against the disease. Thus, the African buffalo-BTB system presents an ideal platform for studying potential direct and indirect effects of parasites on the evolution of immune genes in the wild.

Taking advantage of this unique study system, we tested for evidence of selection on the interferon gamma (IFNG) gene in populations of African buffalo in KNP and HIP. The IFNG locus codes for an immune signaling molecule of the same name that plays an important role in the response to *M. bovis* infection. IFNγ is a key cytokine involved in T helper (T_{H1}) cell responsiveness that is triggered by intracellular pathogens, including *M. bovis* (Waters et al. 2012). Given the critical role of IFNγ in pathogen defense generally, and the response to BTB specifically, variability in the IFNγ phenotype is likely to be associated with fitness in the face of infection. Importantly, BTB diagnosis in buffalo relies on IFNγ-based tests that measure the strength of an individual's immune response to *M. bovis* antigen challenge (Wood et al. 1991; Ryan et al. 2000; Cousins and Florisson 2005), thus culling based on variation in this response could impose strong selection on the IFNG locus.

To explore the possibility that BTB, BTB-related culling, or both could be driving selection at IFNG, and to identify the form of selection that might be acting, we quantified genetic diversity (heterozygosity and allelic richness) and gametic (linkage) disequilibrium (GD) across neutral loci flanking IFNG to examine how diversity and GD change with distance around a locus putatively under selection. First, we investigated patterns of genetic diversity and GD around IFNG in the total population at both parks to evaluate how intracellular pathogens in general (and BTB specifically) may be driving selection at immune genes, irrespective of management strategy. Second, we explored whether culling had additional effects on IFNG by examining whether the number and frequency of rare alleles at IFNG and surrounding loci were directly impacted by culling. We predicted that parasite-mediated selection would result in a distinct signature of selection at IFNG and nearby loci in both parks. Specifically, we expected that if balancing selection is occurring, we would see higher levels of diversity at IFNG relative to surrounding loci. By contrast, if directional selection is occurring, we would see lower levels of diversity at IFNG compared to flanking loci. With respect to GD, we expected that directional selection might result in stronger patterns of gametic disequilibrium involving IFNG versus flanking loci. Finally, we also predicted that if disease management contributes to selection at IFNG this would be evident as a stronger selection signature in the HIP population where culling takes place. Alternatively, culling might produce relatively cryptic genetic changes in this population, disrupting patterns genetic diversity and GD around IFNG.

Methods

Sample collection

We sampled buffalo at two sites, Hluhluwe-iMfolozi Park (HIP) and Kruger National Park (KNP) in South Africa. In HIP, males and females were captured in the Masinda section of the park as part a Bovine Tuberculosis Control Program in 2005 and 2006. In KNP, female buffalo were captured in the Lower Sabie and Crocodile Bridge regions in 2008 as part of a research study. Captures in HIP were carried out by park management using a helicopter and funnel system to drive herds into a capture corral. In KNP, animals were darted from a helicopter by the South Africa National Parks Veterinary Wildlife Services. Whole blood from HIP buffalo ($n = 83$) was preserved on FTA cards (Whatman® Inc, Clifton, NJ, USA), and dried cards were stored at room temperature for one year until DNA extraction. In KNP, tissue samples were collected from buffalo ($n = 209$) and stored in 2 ml tubes with silica gel at room temperature for up to 24 months prior to DNA extraction.

In HIP, all captured buffalo were tested for bovine tuberculosis using a tuberculin skin test. Briefly, individual buffalo were injected with bovine tuberculin intra-dermally, and a localized swelling response measured 72 h later (Ryan et al. 2000). Animals were considered BTB + if the swelling response was greater than 2 mm. Skin test-positive buffalo were culled as part of the BTB control program.

Molecular methods

We focused on the IFNG gene which codes for IFNγ, a protein critical in the immune response to a variety of intracellular pathogens, including *M. bovis* (Bradley et al. 1996, Bream et al. 2000, Pollock et al. 2008). Twelve flanking microsatellite loci were chosen based on their proximity to IFNG, and range in distance from 3.1 (BMS1617) to 28.4 (KERA) cM on either side of IFNG (See Table S1). Studies of cattle and sheep suggest that all but one of these 12 flanking loci are neutral, or functionally neutral, genes. BL4 has been associated with immunity and disease resistance in sheep and African buffalo (Coltman et al. 1999, 2001, Ezenwa et al. 2010); while the following six loci have been reported to be neutral: BMS1617 (Kappes et al. 1997), RM154 (Maddox et al. 2001), BR2936 (Kappes et al. 1997), KRT2 (Maddox et al. 2001), ILSTS22 (Kappes et al. 1997), AGLA293 (Kappes et al. 1997). We considered five additional loci to be functionally neutral based on their involvement or proximity to genes with functions not related to immunity or disease resistance, including: CSSM34, which codes for a retinoic acid receptor important in Vitamin A absorption (Barendse 2002); GLYCAM1, KERA, and TEX15, which code for proteins important in lactation, corneal development, and chromosomal synapsis and meiotic recombination, respectively (Groenen et al. 1995; Tocyap et al. 2006; Yang et al. 2008); and IGF, which codes for insulin-growth factor, which modulates cell growth and is linked with increased tumor development (Renehan et al. 2004).

DNA was extracted from tissue samples using the QIAGEN Blood & Tissue Kit (Valencia, CA) following the manufacturer's protocol; for FTA cards, a modified protocol was used (Lisette Waits, pers. comm). Multiplex PCRs were optimized, and 10ul reactions were performed in MJR PTC200 thermocyclers. Each reaction contained: 1ul of template DNA, 4.5 ul of QIA multiplex mix (Qiagen), and 1 ul of 2 pM forward and reverse primers. Two different touchdown profiles with 35–40 cycles were used, one with an initial annealing temperature of 64 °C stepping down to 59 °C, and another starting at 58 °C and stepping down to 53 °C. Fluorescently-labeled DNA fragments were visualized on an ABI3130xl automated capillary sequencer (Applied Biosystems). Allele sizes were determined using the ABI GS600LIZ ladder (Applied Biosystems). Chromatograms were analyzed and confirmed by two independent technicians using GeneMapper software v3.7 (Applied Biosystems).

Locus descriptions

We tested for the presence of null alleles at all loci using Micro-Checker v. 2.2.3 (van Oosterhout et al. 2004), and for deviations from Hardy–Weinberg proportions (HWP) using GENEPOP v. 4.1 (Raymond and Rousset 1995). No null alleles were found at any locus. All loci were in HWP except for AGLA293 ($p < 0.001$ in both populations; Table 1), which had a heterozygote deficit, so further analyses were run both with and without this locus. Since inclusion of AGLA293 did not qualitatively change our results, we only report the results including all loci. We quantified the magnitude of deviation from HWP by computing F_{IS} in each population. We also calculated F_{ST} values to evaluate the degree of genetic differentiation between the two study populations. Both F_{IS} and F_{ST} values were calculated using Fstat v. 2.9.3 (Goudet 2001). Finally, mean population relatedness was calculated in Genalex using the Lynch & Ritland estimator multiplied by two so that it scales from zero to one with full sibs sharing half their alleles ($r = 0.5$).

Indices of genetic diversity and GD

To evaluate whether selection has affected the IFNG locus, we calculated two measures of genetic diversity for IFNG and the 13 flanking loci. First, we calculated allelic richness (A_R) at each locus using Fstat v. 2.9.3 (Goudet 2001). Next, we estimated the heterozygosity at each locus by calculating both observed and expected heterozygosity under Hardy–Weinberg proportions. Observed and expected heterozygosities (H_O and H_E, respectively) were calculated in Arlequin v. 3.1 (Schneider et al. 2000), but subsequent analyses are limited to H_E as it more accurately reflects genetic variation within the population as a whole (Nei 1987).

We calculated pairwise gametic disequilibrium, a measure of non-independence between loci due to either proximity or function. GD was expressed as D′, a derivative of D which is the deviation in the frequency of co-occurrence between multiple alleles (i.e. haplotypes) due to gametic disequilibrium (Lewontin and Kojima 1960). D′ is defined as $D' = D/D_{max}$ where D_{max} is the maximum value of D, given a set of allele frequencies, and $D' = 1$ is complete gametic disequilibrium (Lewontin 1964). In addition to D′, we also calculated GD as r^2, which is a measure of GD that is influenced by allele frequencies. However, results for r^2 were not quantitatively distinct from D′, and as such, we report only the results of D′. D′ and r^2 values were calculated using PowerMarker (Liu and Muse 2005). The significance of pairwise D′ estimates were evaluated using Genepop (v4.2), and accepted at $p \leq 0.0002$.

Patterns of selection

To test for a signature of selection within each study population, we examined the association between genetic diversity at each locus and its chromosomal distance (the absolute value in cM) from the putative gene under selection (IFNG).

Table 1 Population structure statistics for HIP and KNP

Locus	cM from IFNG	HWP		F_{IS}		F_{ST}	A_R		H_O		H_E	
		HIP	KNP	HIP	KNP		HIP	KNP	HIP	KNP	HIP	KNP
TEX15	−26	0.19	0.06	0.057	0.023	0.048*	5	8.97	0.638	0.796	0.675	0.815
IGF	−19.4	0.99	0.91	0.023	0.052	0.149*	3	6	0.627	0.711	0.641	0.750
RM154	−15.6	0.70	0.12	−0.029	0.029	0.115*	7.81	15.36	0.795	0.871	0.773	0.897
BR2936	−7.1	0.24	0.56	−0.090	0.027	0.071*	5	7.95	0.866	0.731	0.795	0.752
BMS1617	−3.1	0.29	0.21	−0.155	−0.102	0.010	2	2	0.361	0.253	0.313	0.229
IFNG	0	1.00	0.08	−0.043	−0.071	0.056*	2	4.72	0.096	0.368	0.091	0.344
BL4	+3.6	0.32	0.40	0.097	−0.021	0.089*	7	8.59	0.676	0.836	0.749	0.819
CSSM34	+10.9	0.79	0.99	−0.038	0.037	0.050*	4.99	8.02	0.687	0.699	0.662	0.716
KRT2	+15.2	0.59	0.76	−0.056	−0.015	0.080*	4.82	8.20	0.771	0.754	0.730	0.743
ILSTS22	+19.1	0.48	0.31	−0.056	−0.013	0.032*	3	7.47	0.542	0.638	0.514	0.632
GLYCAM1	+19.7	0.74	0.56	−0.085	−0.046	0.095*	4.97	10.90	0.795	0.909	0.733	0.869
AGLA293	+21.3	0.00*	0.00*	0.357	0.183	0.180*	6.66	13.78	0.415	0.701	0.643	0.868
KERA	+28.4	0.41	0.41	−0.033	0.011	0.062*	7.98	11.57	0.867	0.839	0.839	0.848

+ and − signs denote chromosomal distance from IFNG in centimorgans (cM). * denote significant ($p \leq 0.05$) deviations from Hardy–Weinberg proportions (HWP) and significant genetic structure (pairwise F_{ST}). Also reported are F_{IS} values, allelic richness (A_R), observed and expected heterozygosity (H_O, H_E)

This use of multiple loci across the gene region helps control for genome-wide (e.g. demographic) variations and has been previously used to identify loci under selection and selective sweeps (Ihle et al. 2006; Makinen et al. 2008). We tested for correlations between distance and allelic richness and heterozygosity using Spearman rank tests. Since differences in chromosomal distance between loci should reflect differences in the rate of recombination and/or selection, we also tested for associations between statistically significant values of pairwise D′ and the distance (cM) between locus pairs. Linear regression tests were used to evaluate whether levels of GD decayed with increasing distance between loci.

Genetic diversity in HIP

We compared genetic diversity between the two study populations using Wilcoxon paired sign rank tests. In HIP, where overall genetic diversity was much reduced, we tested for evidence of potential bottlenecks using heterozygosity excess and deficiency tests in Bottleneck (v. 1.2). We used a two-phase model of microsatellite evolution, a biologically appropriate model that captures the evolution of microsatellites (Cornuet and Luikart 1996; Luikart and Cornuet 1998). We parameterized the model by defining 80 % of mutations as conforming to a stepwise mutation model (Kimura and Ohta 1978) and 20 % to a multistep model, assuming a variance of 12 for the geometric distribution of number of repeat units per multi-step mutation. Mode shift tests were used to assess bottleneck strength (Cornuet and Luikart 1996).

To identify potential effects of culling on genetic diversity in HIP, we compared our measures of diversity (allelic richness and heterozygosity) between two subsets of the HIP population: animals that were culled as a result of a positive tuberculin skin test (BTB positive, $n = 11$), and animals that were poor reactors on the skin test and were not culled (BTB negative, $n = 64$). Comparisons between population subsets were done using Wilcoxon paired signed rank tests. We also examined the occurrence of rare alleles in the culled and non-culled population subsets of HIP to test if rare alleles are being removed disproportionately as a result of culling, potentially contributing to an overall erosion of genetic diversity in the park. To do this, for each population subset, we calculated the number and frequency of alleles at each locus with a frequency of less than 0.1. We then tested for locus-specific differences in the number and frequency of these rare alleles in the two population subsets using Wilcoxon paired signed rank tests.

Results

Locus descriptions

Thirteen microsatellite loci spanning IFNG were genotyped successfully in a total of 292 individuals from two populations (KNP: $n = 209$; HIP: $n = 83$). Locus-specific FIS ranged from −0.155 to 0.357 in HIP and from −0.102 to 0.183 in KNP (Table 1). Low and/or negative FIS values found at 9 of 13 loci in HIP and 6 of 13 loci in KNP indicate that there is little to no cryptic subpopulation structuring occurring within these populations. Pairwise

FST values between populations ranged from 0.010 to 0.1804 among loci (Table 1). There was evidence of significant genetic differentiation between the two populations at all but one locus; BMS1617 was the only locus with a non-significant FST value (FST = 0.010; $p = 0.075$). This locus also had the lowest FIS value in both populations (FIS KNP = −0.155; FIS HIP = −0.102; Table 1). Mean relatedness among individuals in HIP was 0.185 and 0.019 in KNP.

Signature of selection: patterns of genetic diversity and GD

Overall, genetic diversity was lower at IFNG compared to other loci in both populations. A_R ranged from 2 to 7.97 alleles per locus in HIP and from 2 to 15.36 in KNP (Table 1). Excluding BMS1617 for which we found no significant evidence of genetic differentiation between the two parks, IFNG was the locus with the lowest A_R value in both populations, with only 2 alleles identified in HIP and 4.7 alleles in KNP. Similarly, expected heterozygosity was also lowest at IFNG in both populations (0.091 in HIP, 0.344 in KNP; Table 1). Observed heterozygosity showed similar patterns as expected heterozygosity.

By examining genetic variation across loci at increasing distances from IFNG, we found evidence of a signature of selection in one population but not the other. In KNP, both allelic richness and heterozygosity were significantly and positively correlated with distance from IFNG (Spearman rank correlation: A_R: rho = 0.637, $p = 0.019$; H_E: rho = 0.593, $p = 0.032$; Fig. 1a–b). Although the distance pattern observed for allelic richness and heterozygosity in HIP mirrored the pattern observed in KNP, no significant associations were detected between either measure of genetic diversity and distance from IFNG in the HIP population (A_R: rho = 0.463, $p = 0.111$; H_E: rho = 0.324, $p = 0.279$; Fig. 1 a–b). Given the reduced genetic diversity at BMS1617 relative to IFNG and other surrounding loci in KNP, we also examined whether the patterns we observed for IFNG could have been driven by BMS1617. To do this we re-ran the distance analyses (e.g., association tests) using BMS1617 as the target locus. We found no evidence of an association between chromosomal distance from BMS1617 and genetic diversity (A_R: rho = 0.545, $p = 0.066$; H_E: rho = 0.486, $p = 0.106$). This supports the supposition that IFNG, and not BMS1617, is the putative target of selection in the region under analysis.

Fifteen pairs of loci were in significant GD in HIP, with the average $D' = 0.63$; by contrast, seven pairs of loci were in significant GD in KNP, with the average $D' = 0.44$ (Table 2). The two populations shared only four of 22 significant locus pairs (Table 2), with two of these showing similar deviations in haplotype frequency across

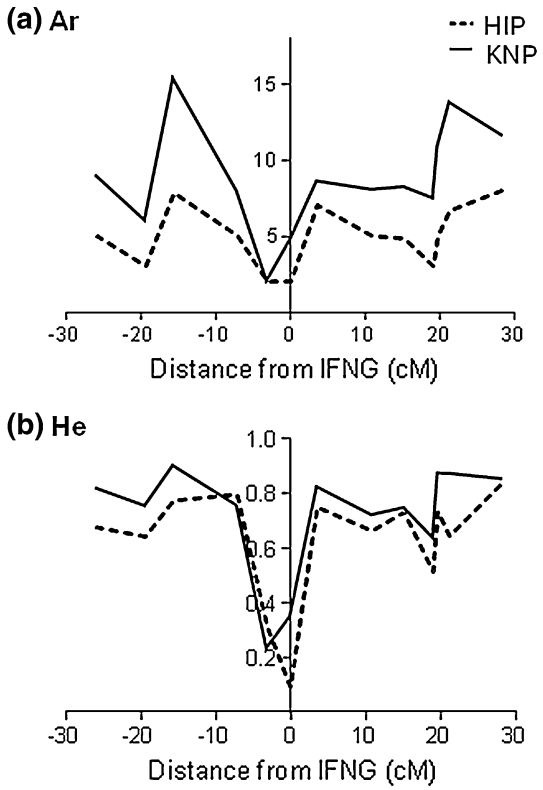

Fig. 1 Patterns of **a** allelic richness and **b** expected heterozygosity at increasing chromosomal distance (cM) from IFNG in KNP and HIP. HIP is represented by the *dashed line*, and KNP is represented by the *solid line*

populations (ILSTS22-AGLA293 and KRT2-AGLA293), and two showing higher levels of D′ in HIP (ILSTS22-GLYCAM1 and RM15-BR2936; Table 2). The median distance between loci with significant pairwise GD was 5.8 cM in HIP and 7.3 cM in KNP, with pairwise distances ranging from 0.6 to 19.7 cM (Table 2). In KNP, the strongest deviation in haplotype frequencies was between IFNG and BL4 ($D' = 0.572$; 3.6 cM), while the longest significant GD observed was between IFNG and GLYCAM1 (19.7 cM). In HIP, the strongest deviation in haplotype frequencies was between ILSTS22 and GLYCAM1 ($D' = 0.987$; 0.6 cM), and there was no significant pairwise GD at distances greater than 13.2 cM. When we tested for associations between GD and the chromosomal distance between loci, we found that GD declined significantly with pairwise distance in KNP ($n = 7$, $r^2 = 0.77$, $p = 0.0094$; Fig. 2), but not HIP ($n = 15$, $r^2 = 0.18$, $p = 0.1183$; Fig. 2).

Reduced genetic diversity in HIP

Two tests—the mode shift and heterozygosity-excess tests—revealed evidence of a recent bottleneck in HIP (mode shift: bimodal distribution; heterozygosity excess

Table 2 Locus pairs with significant levels of gametic disequilibrium (GD) in HIP and KNP

Population	Locus 1	Locus 2	CM	D′
HIP	ILSTS22	GLYCAM1	0.6	0.987
	GLYCAM1	AGLA293	1.6	0.634
	ILSTS22	AGLA293	2.2	0.418
	KRT2	ILSTS22	3.9	0.707
	BR2936	BMS1617	4	0.800
	CSSM34	KRT2	4.3	0.727
	KRT2	GLYCAM1	4.5	0.655
	KRT2	AGLA293	6.1	0.456
	TEX15	IGF	6.6	0.504
	AGLA293	KERA	6.9	0.670
	BL4	CSSM34	7.3	0.435
	RM154	BR2936	8.5	0.668
	GLYCAM1	KERA	8.7	0.587
	ILSTS22	KERA	9.3	0.620
	KRT2	KERA	13.2	0.528
KNP	ILSTS22	GLYCAM1	0.6	0.556
	ILSTS22	AGLA293	2.2	0.434
	IFNG	BL4	3.6	0.572
	KRT2	AGLA293	6.1	0.437
	RM154	BR2936	8.5	0.428
	CSSM34	AGLA293	10.4	0.359
	IFNG	GLYCAM1	19.7	0.276

Chromosomal distance between loci is listed in centimorgans (cM) and the strength of GD is measured as D′. The shortest distance between possible pairwise comparisons was 0.6 cM while the longest distance was 54.4 cM

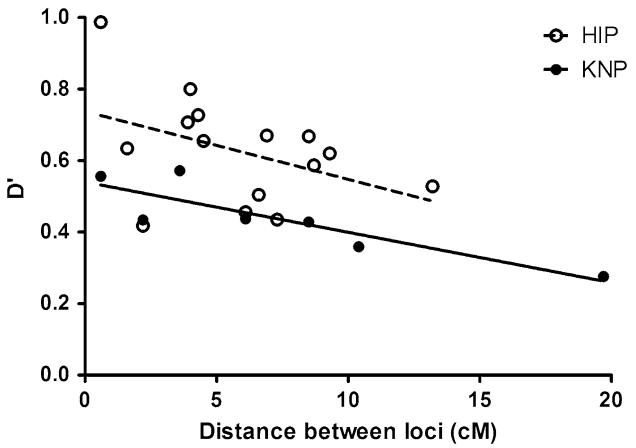

Fig. 2 Relationship between pairwise gametic disequilibrium (GD) and chromosomal distance (cM) in KNP and HIP. HIP is represented by the *dashed line with open circles,* and KNP is represented by the *solid line with filled circles*

test: $p < 0.05$; see Table S2). Furthermore, a comparison of genetic diversity between the HIP and KNP populations indicated that diversity is reduced in HIP compared to KNP. Both allelic richness and expected heterozygosity were significantly lower in HIP compared to KNP (Wilcoxon paired signed rank test: A_R, $S = 39.0$, $p = 0.0005$; H_E, $S = 36.5$, $p = 0.0081$).

While the bottleneck in HIP may account for the pattern of eroded genetic diversity in this population, BTB management (i.e. culling) could also contribute to the overall reduction in genetic diversity. To explore this possibility, we tested for differences in genetic diversity between culled (C) and non-culled (NC) groups of individuals from HIP. Although there was no difference between population subsets in either diversity index (Wilcoxon signed rank test: A_R: $S = -18.0$, $p = 0.229$; H_E: $S = -5.0$, $p = 0.734$), the culled group had no heterozygosity at IFNG, possibly indicating strong selection acting on this locus.

Focusing on rare alleles, we found that individuals in the culled group had a significantly higher number of rare alleles at loci surrounding IFNG ($C = 26$; $NC = 22$; $S = 13.5$, $p = 0.0358$; Table 3). Moreover, for those rare alleles present in both the culled and non-culled groups, over 70 % (10 out of 14) occurred at a higher frequency in the culled group (Table 3). Rarity is influenced by the number of alleles per locus and therefore could vary between population subsets simply because of the greater number of alleles available to sample in larger populations. However, even when we considered only the seven rare alleles that were present in both population sub-groups, and that occurred at loci where all alleles were shared between groups, over 80 % (5 out of 6) occurred at a higher frequency in the culled group (Table 3), suggesting that rare alleles were overrepresented in the culled subset of the population and that culling may be disproportionately eliminating these alleles.

Discussion

Our findings suggest that directional selection is acting on and around the IFNG locus in the buffalo population of Kruger National Park. In the Hluhluwe-iMfolozi Park population, reduced genetic diversity due to recent bottleneck events may have masked any signature of directional selection driven by disease. However, we found evidence that disease management may be compounding the loss of genetic diversity in the IFNG gene region. In particular, culling to reduce bovine tuberculosis (BTB) may be selectively removing rare alleles from the HIP buffalo population, prolonging genetic recovery from a recent reduction in population size. Overall, our results suggest that disease can directly or indirectly drive selection at immune loci in wild populations, and that disease management might result in unintended evolutionary

Table 3 Number and frequency of rare alleles in HIP, parsed by culled (C, $n = 11$) and non-culled (NC, $n = 64$) population segments

Loci	N_A			Rare N_A			Rare alleles	Frequency		
	Total	NC	C	Total	NC	C		T	NC	C
IFNG	2	2	1	1	1	0	IFNG allele1	0.045	0.052	0
BMS1617	2	2	2	0	0	0	NA			
BL4	7	7	7	3	3	4	BL4 allele1	0.032	0.027^S	0.063^S
							BL4 allele2	0.063	0.064^S	0.063^S
							BL4 allele3	0.024	0.018^S	0.063^S
BR2936	5	5	5	0	0	1	NA			
CSSM34	5	5	4	2	2	1	CSSM allele1	0.086	0.077	0.136
							CSSM allele2	0.026	0.031	0
KRT2	5	5	4	1	1	1	KRT2 allele1	0.007	0.008	0
RM154	8	8	6	5	5	6	RM154 allele1	0.086	0.085^S	0.091^S
							RM154 allele2	0.086	0.092^S	0.045^S
							RM154 allele3	0.007	0.008	0
							RM154 allele4	0.059	0.054^S	0.091^S
							RM154 allele5	0.013	0.015	0
ILSTS22	3	3	3	1	0	1	ILSTS allele1	0.099	0.1	0.091
IGF	3	3	3	0	0	1	NA			
GLYCAM1	5	5	4	1	1	2	GLYCAM allele1	0.013	0.015	0
AGLA293	7	7	5	4	4	4	AGLA allele1	0.093	0.094^S	0.091^S
							AGLA allele2	0.007	0.008	0
							AGLA allele3	0.02	0.016^S	0.045^S
							AGLA allele4	0.007	0.008	0
TEX15	5	5	5	2	2	2	TEX allele1	0.075	0.073	0.091
							TEX allele2	0.055	0.04	0.136
KERA	8	8	7	3	3	3	KERA allele1	0.086	0.069	0.182
							KERA allele2	0.02	0.015^S	0.045^S
							KERA allele3	0.007	0.008	0

Zeros represent alleles that are absent from the associated population segment

S shared rare alleles

consequences by exacerbating underlying population demographic effects.

Evidence of selection in KNP

Our conclusion that selection is acting on IFNG in buffalo comes from several lines of evidence. By examining 12 loci flanking IFNG, we uncovered a significant, positive relationship between chromosomal distance from IFNG and both allelic richness and heterozygosity in the KNP population. The positive relationship between genetic diversity and chromosomal distance from IFNG suggests that directional selection (e.g. a selective sweep) is occurring at this locus. In addition, we observed significant gametic disequilibrium (GD) between IFNG and BL4 which is likely the effect of directional selection at IFNG and genetic hitchhiking at BL4. While there was a significant association between pairwise chromosomal distance between loci and GD in KNP, the level of GD between IFNG and BL4 was higher than for any other pair of loci, irrespective of distance. This suggests that in this region of the chromosome, selection may be generating a non-random association between alleles at these two loci which is stronger than that expected based on distance alone. Interestingly, despite this signature of directional selection, several low frequency alleles remain at IFNG in the KNP population (three alleles at <2 % frequency), and the long distance GD observed between IFNG and GLYCAM1 (19.7 cM apart) suggests that haplotypes involving these low frequency alleles are being maintained in KNP.

In contrast to the genetic diversity-chromosomal distance pattern we observed in KNP, no such pattern was evident in HIP. However, an overall reduction in genetic diversity in the IFNG gene region in the HIP population could have masked any distance effect. In the mid-1950s the HIP buffalo population dropped to as few as 800 individuals followed by rapid population growth thereafter (Jolles 2007); this event and the elevated levels of relatedness among HIP individuals corroborates our evidence for a recent bottleneck in the population. Bottlenecks and founder events can drastically reduce the amount of genetic diversity in populations (Maruyama and Fuerst 1984),

making signatures of selection difficult to detect (Frere et al. 2011). Nevertheless, characteristics of individual loci hint that selection on IFNG could be occurring in HIP, as in KNP. Specifically, in both populations, heterozygosity at IFNG was at or close to the lowest values recorded across all loci (KNP = 0.344, range: 0.229–0.897; HIP = 0.091, range: 0.091–0.839, Table 1), a pattern suggestive of directional selection acting on IFNG in both populations.

Evidence of directional selection acting on immune loci in response to bacterial pathogens, including *M. bovis*, has been reported in livestock, suggesting that disease, possibly BTB, could be driving the pattern of selection we observed at IFNG. For example, BTB has been implicated as a potential force driving directional selection of the disease resistance gene, NRAMP1, in African Zebu cattle (Kadarmideen et al. 2011). Evidence of directional selection has also been found at the porcine TLR-4 gene in response to gram-negative bacterial pathogens (Palermo et al. 2009). Thus, it is possible that BTB infection acts to reduce genetic diversity at the IFNG locus in buffalo, resulting in the conservation of only those alleles important in mounting an effective immune response, as has been shown in for the PRNP gene in cervids in North America in response to chronic wasting disease (Robinson et al. 2012).

Management-driven selection in HIP

Effects of disease management on genetic variation are poorly understood, rarely assessed, but potentially strong (Allendorf and Hard 2009). In HIP, we exploited a rare opportunity to assess the role of disease management in driving indirect selection. Although there was no clear signature of selection in this population, we did find evidence that disease management (i.e. culling) might affect diversity at immune system genes. Specifically, we found that although there were no differences in allelic richness or heterozygosity between culled and non-culled segments of the HIP population, the culled segment had a significantly greater number and frequency of rare alleles suggesting that these alleles are being lost from the population via culling. A comparison of GD patterns in the two populations further suggests that culling may have disrupted the pairwise distance-GD relationship in HIP by eliminating haplotypes that are being maintained between IFNG and surrounding loci (e.g. GLYCAM1) in KNP.

Evidence that IFNG haplotypes being maintained in KNP have been lost from HIP suggests that the rare allele loss observed in HIP may have important fitness consequences. Rare allele advantage can be an important component of a population's ability to respond to infectious disease agents (Spurgin and Richardson 2010; Lankau and Strauss 2010). Thus, the reduction in rare alleles in HIP could have implications for population level responses to future infectious disease threats. Given the small sample size we used for the rare alleles comparison (11 vs. 64), we recognize that these results need to be interpreted with caution. Nevertheless, based on the strong preliminary patterns we report here and in light of work showing that rare alleles can confer fitness advantages, particularly with respect to response to pathogens (Koskella and Lively 2009; Sommer 2005), the potential loss of rare alleles due to culling in the buffalo-BTB system deserves further investigation.

The bottleneck in the mid-1950s, and subsequent rapid growth of the HIP population, likely resulted in the founder event and reduction in overall genetic diversity that we observed in the park. Culling could be prolonging this bottleneck event by eliminating rare alleles from the population and reducing heterozygosity at IFNG. Disease management in this population has had effects on the population ecology of buffalo, with rapid population declines and reduced population growth rates typically following culling events (Jolles 2007). In addition to these ecological effects, our results suggest that culling is also affecting buffalo population genetics and long-term evolutionary potential. Management strategies for controlling invasive and emerging diseases in wildlife will continue to be important due to the risk of disease spillover into livestock, wildlife, and humans (Michel et al. 2006; Smith et al. 2009). For buffalo in HIP, however, culling for disease management may also be sustaining the effects of a historical population bottleneck. More generally, our findings suggest that there is real potential for unforeseen evolutionary consequences to arise from disease management in wildlife populations.

There are several notable examples of unintended ecological consequences of culling (Singleton et al. 2007; Bowen 2013). One well-known example is badger culling in the UK, which was intended to limit spread of BTB to cattle, but was instead linked to increases in transmission (Donnelly et al. 2003, 2006; Pope et al. 2007; Woodroffe et al. 2006, 2009a, b). Far less is known about the evolutionary consequence of culling as a disease management strategy. Sport hunting, which in some instances has the same characteristics as culling, has been shown to have a variety of unintended outcomes, including altering gene flow between populations and, most importantly, selectively removing individuals with desired traits (Harris et al. 2002; Allendorf and Hard 2009). For example, hunter selection, in conjunction with deteriorating environmental conditions, led to a significant reduction in horn size among bighorn sheep rams in Arizona (Hedrick 2011). Because BTB-culling programs that use IFNγ-based diagnostic tests will selectively remove individuals that produce high levels of IFNγ in response to an antigen challenge (Wood et al. 1991; Ryan et al. 2000; Cousins and

Florisson 2005), an analogous potential consequence of BTB-culling could be the selective removal of individuals capable of mounting strong immune responses to BTB infection. This selection could lead to significant population biases in immune profiles. Future research is needed to examine whether genetic differences in culled versus non-culled buffalo translate into observable phenotypic differences in immune responses to BTB.

Conclusions

Opportunities for disease transmission between wildlife, livestock, and humans are growing. However, the effects of disease and disease management on evolution in wildlife populations remain poorly understood. In particular, strategies used to control disease might unwittingly lead to cryptic evolutionary change (Shim and Galvani 2009). We found that in an African buffalo population where BTB has been present for over 50 years, and where there is no active disease management, there was reduced diversity at (and near) IFNG, a locus involved in mounting an immune response to pathogens like tuberculosis. By contrast, in a population where disease-based culling occurs, reduced genetic diversity may be masking a signature of selection. Furthermore, preliminary patterns show that management actions, and thus unnatural selection, could be removing rare alleles from the population, potentially driving cryptic evolutionary change. While disease is widely recognized as a potentially powerful force of selection, our results suggest that disease management strategies can also have important evolutionary consequences.

Acknowledgments We thank Kwazulu-Natal Wildlife Service, Kwazulu-Natal State Veterinary Service, and South Africa National Parks Veterinary Services for facilitating this research. In particular, D. Cooper, P. Buss, and M. Hofmeyr provided invaluable assistance. We thank Matt Gruber for his assistance in the lab. Animal protocols used in this study were approved by the University of Georgia, the University of Montana and Oregon State University Animal Care and Use Committees. This work was supported by the National Science Foundation Ecology of Infectious Diseases Program (DEB-1102493/EF-0723928, EF-0723918), an NSF Small Grant for Exploratory Research (DEB-0541762, DEB-0541981) and a University of Montana Faculty Research Grant.

References

Acevedo-Whitehouse K, Cunningham A (2006) Is MHC enough for understanding wildlife immunogenetics? TREE 21(8):433–438

Allendorf FW, Hard JJ (2009) Human-induced evolution caused by unnatural selection through harvest of wild animals. PNAS 106:9987–9994

Altizer S, Harvell D, Freidle E (2003) Rapid evolutionary dynamics and disease threats to biodiversity. TREE 18(11):589–596

Barendse W (2002) Development and commercialization of a genetic marker for marbling of beef in cattle: a case study. Intellectual property rights animal breeding and genetics., pp 197–212

Bollmer JL, Ruder EA, Johnson JA, Eimes JA, Dunn PO (2011) Drift and selection influence geographic variation at immune loci of prairie-chickens. Mol Ecol 20:4695–4706

Bowen WD (2013) Marine mammal culling programs: review of effects on predator and prey populations. Mamm Rev 43:207–220

Bradley LM, Dalton DK, Croft M (1996) A direct role of IFN-gamma in regulation of T_H1 cell development. J Immunol 157(4):1350–1358

Bream JH, Carrington M, O'Toole S, Dean M, Gerrard B, Shin HD, Kosack D, Modi W, Young HA, Smith MW (2000) Polymorphisms of the human IFNG gene noncoding regions. Immunogenetics 51(1):50–58

Chauvenet ALM, Durant SM, Hilborn R, Pettorelli N (2011) Unintended consequences of conservation actions: managing diseases in complex ecosystems. PLoS One. doi:10.1371/journal.pone.0028671

Coltman DW, Bowen WD, Wright JM (1999) A multivariate analysis of phenotype and paternity in male harbor seals, *Phoca vitulina*, at Sable Island, Nova Scotia. Behav Ecol 10:169–177

Coltman DW, Wilson K, Pilkington JG, Stear MJ, Pemberton JM (2001) A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally-parasitized population of Soay sheep. Parasitology 122(5):571–582

Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144(4):2001–2014

Cousins DV, Florisson N (2005) A review of tests available for use in the diagnosis of tuberculosis in nonbovine species. Rev Sci Tech Off Int Epiz 24(3):1039–1059

Cross PC, Drewe J, Patrek V, Pearce G, Samuel MD, Delahay RJ (2009) Host population structure and implications for disease management. In: Delahay RJ, Smith GC, Hutchings MR (eds) Management of disease in wild mammals. Springer, Tokyo, pp 9–30

de Garine-Wichatitsky M, Caron A, Kock R, Tschopp R, Munyeme M, Hofmeyr M, Michel A (2013) A review of bovine tuberculosis at the wildlife-livestock-human interface in sub-Saharan Africa. Epidemiol Infect 141:1342–1356

Donnelly CA, Woodroffe R, Cox DR, Bourne FJ, Gettinby G, LeFevre AM, McInerney JP, Morrison WI (2003) Impact of localized badger culling on TB incidence in British cattle. Nature 426:834–837

Donnelly CA, Woodroffe R, Cox DR, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, Wei G, Gettinby G, Gilks P, Jenkins H, Johnston WT, LeFevre AM, McInerney JP, Morrison WI (2006) Positive and negative effects of widespread culling on tuberculosis on cattle. Nature 439(16):843–846

Downing T, Cormican P, O'Farrelly C, Bradley D, Lloyd A (2009) Evidence of the adaptive evolution of immune genes in chicken. BMC Res Notes 2(1):254

Ezenwa VO, Etienne RS, Luikart G, Beja-Pereira A, Jolles AE (2010) Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. Am Nat 176(5):619–625

Fitzgerald SD, Kaneene JB (2013) Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. Vet Pathol 50(3):488–499

Frere CH, Prentis PJ, Gilding EK, Mudge AM, Cruickshank A, Godwin ID (2011) Lack of low frequency variants masks patterns of non-neutral evolution following domestication. PLoS One 8:e23041

Goodchild M, Sahu S, Wares F, Dewan P, Shukla RS, Chauhan LS, Floyd K (2011) A cost-benfit analysis of scaling up tuberculosis control in India. Int J Tuberc Lung Dis 15(3):358–362

Goudet J (2001) FSTAT: a computer program to calculate F-statistics. J Hered 86(6):485–486

Groenen MAM, Dijkhof RJM, ban der Peol JJ (1995) Characterization of a GlyCAM1-like gene (glycosylation-dependent cell adhesion molecule 1) which is highly and specifically expressed in the lactating bovine mammary gland. Gene 158(2):189–195

Harris RB, Wall WA, Allendorf FW (2002) Genetic consequences of hunting: what do we know and what should we do? Wildl Soc B 30(2):634–643

Hedrick PW (2011) Rapid decrease in horn size of bighorn sheep: environmental decline, inbreeding depression, or evolutionary response to trophy hunting? J Hered 2011:1–12

Ihle S, Ravaoarimanana I, Thomas M, Tautz D (2006) An analysis of signatures of selective sweeps in natural populations of the house mouse. Mol Biol Evol 23(4):790–797

Jolles AE (2007) Population biology of African buffalo (*Syncerus caffer*) at Hluhluwe-iMfolozi Park, South Africa. Afr J Ecol 45:398–406

Jolles AE, Cooper DV, Levin SA (2005) Hidden effects of chronic tuberculosis in African buffalo. Ecology 86(9):2258–2264

Jolles AE, Etienne RS, Olff H (2006) Independent and competing disease risks: implications for host populations in variable environments. Am Nat 167(5):745–757

Jolles AE, Ezenwa VO, Etienne RS, Turner WC, Olff H (unpublished) Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo

Joseph MB, Mihaljevic JR, Arellano AL, Kueneman JG, Preston DL, Cross PC, Johnson PTJ (2013) Taming wildlife disease: bridging the gap between science and management. J Appl Ecol 50(3): 702–712

Kadarmideen HN, Ali AA, Thomson PC, Muller B, Zinsstag J (2011) Polymorphisms of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. Anim Genet 42(6):656–658

Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TP, Lopez-Corrales NL, Beattie CW (1997) Genome Res 7(3):235–249

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. PNAS 75(6):2868–2872

Koskella B, Lively CM (2009) Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. Evolution 63(9):2213–2221

Koskella B, Lin DM, Buckling A, Thompson JN (2012) The costs of evolving resistance in heterogeneous parasite environments. Proc R Soc B 279(1735):1896–1903

Koyanagi M, Jerns JA, Chung L, Zhang Y, Moloveanu T, Malik HS, Bix M (2010) Diversifying selection and functional analysis of interleukin-4 suggests antagonism-driven evolution at receptor-binding interfaces. BMC Evol Biol 10:223–236

Lankau RA, Strauss SY (2010) Newly rare or newly common: evolutionary feedbacks through changes in population density and relative species abundance, and their management implications. Evol Appl 4:338–353

Leung TLF, King KC, Wolinska J (2012) Escape from the Red Queen: an overlooked scenario in coevolutionary studies. Oikos 121(5):641–645

Lewontin RC (1964) The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 49(1):49–67

Lewontin RC, Kojima K (1960) The evolutionary dynamics of complex polymorphisms. Evolution 14(4):458–472

Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21(9): 2128–2129

Llewellyn D, Thompson MB, Brown GP, Phillips BL, Shine R (2012) Reduced investment in immune function in invasion-front populations of the cane toad (*Rhinella marina*) in Australia. Biol Invasions 14:999–1008

Luikart G, Cornuet J-M (1998) Empirical evaluation of a test for detecting recent historical population bottlenecks. Conserv Biol 12:228–237

Maddox JF, Davies KP, Crawford AM, Hulme DJ, Vaiman D, Cribiu EP, Freking BA, Beh KJ, Cockett NE, Kang N, Riffkin CD, Drinkwater R, Moore SS, Dodds KG, Lumsden JM, van Stijn TC, Phua SH, Adelson DL, Burkin HR, Broom JE, Buitkamp J, Cambridge L, Cushwa WT, Gerard E, Galloway SM, Harrison B, Hawken RJ, Hiendleder S, Henry HM, Medrano JF, Paterson KA, Schibler L, Stone RT, van Hest B (2001) An enhanced linkage map of the sheep genome comprising more than 1000 loci. Genome Res 11(7):1275–1289

Makinen HS, Cano JM, Merila J (2008) Identifying footprints of directional and balancing selection in marine and freshwater three-spined stickleback (*Gasterosteus aculeatus*) populations. Mol Ecol 17(15):3565–3582

Maruyama T, Fuerst PA (1984) Population bottlenecks and nonequilibrium models in population genetics. I. Allele numbers when populations evolve from zero variability. Genetics 108(3):745–763

McCallum H (2008) Tasmanian devil facial tumour disease: lessons for conservation biology. TREE 23(11):631–637

McTaggart S, Obbard DJ, Conlon C, Little TJ (2012) Immune genes undergo more adaptive evolution than non-immune system genes in *Daphnia pulex*. BMC Evol Biol 12:63. doi:10.1186/1471-2148-12-63

Michel AL, Bengis RG, Keet DF, Hofmeyr M, deKlerk LM, Cross PC, Jolles AE, Cooper D, Whyte IJ, Buss P, Godfroid J (2006) Wildlife tuberculosis in South African conservation areas: implications and challenges. Vet Microbiol 112:91–100

Michel AL, Muller B, van Helden PD (2010) *Mycobacterium bovis* at the animal-human interface: a problem or not? Vet Microbiol 140:371–381

Myerstud A (2011) Selective harvesting of large mammals: how often does it result in directional selection? J Appl Ecol 48(4):827–834

Myerstud A, Bischof R (2010) Can compensatory culling offset undesirable evolutionary consequences of trophy hunting? J Anim Ecol 79(1):148–160

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York

Palermo S, Capra E, Torremorell M, Dolzan M, Davoli R, Haley CS, Giuffra E (2009) Toll-like receptor 4 genetic diversity among pig populations. Anim Genet 40(3):289–299

Pollock NR, Campos-Neta A, Kashino S, Napalitano D, Behar SM, Shin D, Sloutsky A, Joshi S, Guillet J, Wong M, Nardell E (2008) Discordant quantiFERON-TB Gold tests among US healthcare workers with increased risk of latent tuberculosis infection: a problem or solution? Infect Control Hosp Epidemiol 29(9):878–886

Pope LC, Butlin RK, Wilson GJ, Woodroffe R, Erven K, Conyers CM, Franklin T, Delahay RJ, Cheeseman CL, Burke T (2007) Genetic evidence that culling increases badger movement: implications for the spread of bovine tuberculosis. Mol Ecol 16(23):4919–4929

Raymond M, Rousset F (1995) GENEPOP: population genetics software for exact tests and ecumenicism. J Hered 86(3):248–249

Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M (2004) Insulin-like growth (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363(19):1346–1353

Renwick AR, White PCL, Bengis RG (2007) Bovine tuberculosis in southern African wildlife: multi-species host-pathogen system. Epidemiol Infect 135(4):529–540

Robinson SJ, Samuel MD, O'Rourke KI, Johnson CJ (2012) The role of genetics in chronic wasting disease of North American cervids. Prion 6(2):153–162

Ryan TJ, Buddle BM, de Lisle GW (2000) An evaluation of the gamma interferon test for detecting tuberculosis in cattle 8 to 28 days after tuberculin skin testing. Res Vet Sci 69:57–61

Sackett LC, Collinge SK, Martin AP (2013) Do pathogens reduce genetic diversity of their hosts? Variable effects of sylvatic plague in black-tailed prairie dogs. Mol Ecol 22(9):2441–2455

Schneider S, Roessli D, Excoffier L (2000) Arlequin: an integrated software package for population genetics data analysis. Evol Bioinform 1:47

Shim E, Galvani AP (2009) Evolutionary repercussions of avian culling on host resistance and influenza virulence. PLoS One 4(5):e5503

Singleton GR, Brown PR, Jacob J, Aplin KP, Sudaramaji (2007) Unwanted and unintended effects of culling: a case for ecologically-based rodent management. Integr Zool 2:247–257

Smith KF, Acevedo-Whitehouse K, Pederson AB (2009) The role of infectious diseases in biological conservation. Anim Conserv 12:1–12

Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Front Zool 2:16–34

Spurgin LG, Richardson DS (2010) How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proc R Soc B 277:979–988

Thrall PJ, Burdon JJ (2003) Evolution of virulence in a plant host-pathogen metapopulation. Science 299:1735–1737

Tocyap ML, Azar N, Chen T, Wiggs J (2006) Clinical and molecular characterization of a patient with an interstitial deletion of chromosome 12q15-q23 and peripheral corneal abnormalities. Am J Opthalmol 141(3):566–567

Tonteri A, Vasemagi A, Lumme J, Primmer C (2010) Beyond MHC: signals of elevated selection pressure on Atlantic salmon (*Salmo salar*) immune-relevant loci. Mol Ecol 19(7):1273–1282

Tschirren B, Raberg L, Westerdahl H (2011) Signatures of selection acting on the innate immunity gene Toll-like receptor 2 (TLR2) during the evolutionary history of rodents. J Evol Biol 24(6): 1232–1240

van Oosterhout C, Hutchinson W, Wills D, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4(3):535–538

Waters WR, Tacker TC, Nonnecke BJ, Palmer MV, Schiller I, Oesch B, Vordermeier HM, Silva E, Estes DM (2012) Evaluation of Gamma Interferon (IFN-γ)-Induced protein 10 responses for detection of cattle infected with Mycobaterium bovis: comparisons to IFN-γ responses. Clin Vaccine Immunol 19(3):346–351

White PJ, Wallen RL, Geremia C, Treanor JJ, Blanton DW (2011) Management of Yellowstone bison and brucellosis transmission risk—implications for conservation and restoration. Biol Conserv 144(5):1322–1334

Wood PR, Corner LA, Rotherl JS, Baldock C, Jones SL, Cousins DB, McCormick BS, Francis BR, Creeper J, Tweddle NE (1991) Field comparison of the interferon-gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. Aust Vet J 68(9):286–290

Woodroffe R (1999) Managing disease threats to wild mammals. Anim Conserv 2:185–193

Woodroffe R, Donnelly CA, Cox DR, Bourne FJ, Cheeseman CL, Delahay RJ, Gettinby G, McInerney JP, Morrison WI (2006) Effects of culling on badger *Meles meles* spatial organization: implications for the control of bovine tuberculosis. J Appl Ecol 43(1):1–10

Woodroffe R, Donnelly CA, Wei G, Cox DR, Bourne FJ, Burke T, Butlin RK, Cheeseman CL, Gettinby G, Gilks P, Heges S, Jenkins HE, Johnston WT, McInerney JP, Morrison WI, Pope LC (2009a) Social group size affects *Mycobacterium bovis* infection in European badgers (*Meles meles*). J Anim Ecol 78:818–827

Woodroffe R, Donnelly CA, Cox DR, Gilks P, Jenkins HE, Johnston WT, LeFevre AM, Bourne FJ, Cheeseman CL, Clifton-Hadley RS, Gettinby G, Hewinson RG, McInerney JP, Mitchell AP, Morrison WI, Watkins GH (2009b) Bovine tuberculosis in cattle and badgers in localized culling areas. J Wildl Dis 45(1):128–143

Yang F, Eckardt S, Leu NA, McLaughlin KJ, Wang PJ (2008) Mouse TEX15 is essential for double-strand break repair and chromosomal synapse during male meiosis. J Cell Biol 180(4):673–679