

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

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## Tools and Technology

# A Method for Improving the Reliability of Sound Broadcast Systems Used in Ecological Research and Management

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**ABSTRACT** Automated sound broadcast systems have been used to address a variety of ecological questions, and show great potential as a management tool. Such systems need to be reliable because treatments are often applied in the absence of a human observer and system failure can cause methodological ambiguity. During the breeding seasons of 2012 and 2013, we used a sound broadcast system previously described by Farrell and Campomizzi (2011) in an experiment evaluating the use of post-breeding song in forest-bird habitat selection in southern Indiana, USA. This system incorporates a portable compact disc (CD) player where the play button is permanently depressed using manual compression so that when a timer connects an electrical current to the unit, the CD player automatically starts. Despite exhaustive efforts to find a reliable way to manually compress the play button on numerous CD player models, play button failure was the most significant source of broadcast system failure (88%) in 2012. We attempted to resolve this problem in 2013 by removing the need for manual compression and soldering the play button contact poles on each CD players' integrated circuit boards. Though we did experience broadcast system failures during <5% of treatment periods in 2013, none of those were attributable to play button failure. By removing all possibility of failure from manual play button compression we improved our system reliability. Thus, soldering the CD player play button on such broadcast systems represents a methodological improvement that can be used by researchers and managers interested in sound broadcast. © 2014 The Wildlife Society.

**KEY WORDS** bird song, CD player, momentary action pushbutton switch, play button depression mechanism, sound broadcast system.

Sound broadcast systems serve a variety of purposes in ecological research. They have been used to answer questions about perceived predation risk on reproductive performance (Eggers et al. 2006, Zanette et al. 2011), movement ecology in fragmented landscapes (Sieving et al. 1996, 2000, 2004; Desrochers and Hannon 1997; Bélisle and Desrochers 2002), habitat selection (Doligez et al. 2002, Ward and Schlossberg 2004, Nocera et al. 2006, Fletcher 2007, Betts et al. 2008), heterospecific interactions (Diego-Rasilla and Luengo 2004, Fletcher 2007, Pupin et al. 2007), impacts of noise pollution (Bee and Swanson 2007), and to explore mechanisms driving species distribution patterns (Fletcher 2009). Additionally, audio broadcasts can be incorporated into survey protocols to improve detectability for some organisms (Gibbs and Melvin 1993, Conway and Simon 2003, Kubel and Yahner 2007, Ichikawa et al. 2009), and manipulation of species responses

to conspecific attraction shows great promise as a management tool (Ward and Schlossberg 2004, Ahlering and Faaborg 2006).

In many instances, application of auditory experimental or management treatments is done most efficiently using automated systems that can be programmed to operate without a human present (Ward and Schlossberg 2004, Fletcher 2007, 2009; Betts et al. 2008; Zanette et al. 2011). Drawing correct and useful conclusions from such studies is contingent on broadcast treatments being applied correctly, making the reliability of automated systems a critical consideration. System failures lead to heterogeneity and ambiguity in treatment applications, and unreliable systems need to be visited with greater frequency, resulting in a loss of money and man hours.

Farrell and Campomizzi (2011) presented a description of a sound broadcast system that incorporated a portable compact disc (CD) player on which the play button was permanently depressed so that when a timer switch connected an electrical current to the unit, the CD player automatically started. These authors describe using “a combination of adhesive

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tape, wooden dowels, and rubber stoppers to set the play button in the on position” (Farrell and Campomizzi 2011:463). During field tests with comparable broadcast systems, we attempted to use similar tools for our play button depression mechanisms (PBDM), yet we were unable to develop a reliable method of securing the play buttons in the on position using compression. As such, PBDM failure was by far our greatest source of system failure, and we sought to develop a more reliable method of initiating playback for our experiments by bypassing the need for a PBDM on the CD player component. As part of a broader experiment evaluating the use of post-breeding song in breeding habitat selection by wood thrush (*Hylocichla mustelina*) and Kentucky warblers (*Oporornis formosus*) in forest tracts of southern Indiana, USA, we evaluated whether soldering the electrical play button connection would be a more reliable method of initiating playback in broadcast systems regulated by a timer than consistently applying pressure to the play button.

## STUDY AREA

Our study area encompassed approximately 750,000 ha of land in the central hardwoods region of southern Indiana, specifically within Big Oaks National Wildlife Refuge, Naval Surface Warfare Center Crane, Martin State Forest, and the Lost River Tract of Hoosier National Forest. This region was dominated by corn and soybean agriculture and remnant tracts of temperate broadleaf and mixed forests. The mean annual rainfall in southern Indiana was approximately 119 cm (Indiana State Climate Office 2002), and mean annual temperatures ranged from 6° C in winter to 18° C in summer (National Climatic Data Center 2011).

## METHODS

During the 2012 and 2013 breeding seasons, we deployed post-breeding song broadcast systems at point-count stations (34 in 2012 and 24 in 2013) previously unoccupied by target species (wood thrush and/or Kentucky warblers). Treatments were applied between 28 June and 23 August each year in order to experimentally test whether post-breeding song would attract future breeders. In ideal circumstances, treatment sites received between 48 and 56 hours of song broadcast (8 hr/day) in intervals of 6–7 days based on the life of the batteries (12 V 12 A Energy Power Absorbent Glass Mat battery; Energy Battery Group, Atlanta, GA). Our study design incorporated paired control sites, though information about those is tangential to our focus and will not be discussed further.

All broadcast systems contained 1 of 2 portable CD player models (INSIGNIA NS-P4112 or INSIGNIA NS-P113; INSIGNIA Products, Richfield, MN). The play buttons on both CD player models utilize a momentary action pushbutton switch that, when depressed, connects power required to play the CD to 4 contact poles on the integrated circuit board via a convex disk of flexible metal (Fig. 1). In 2012, we rotated 18 broadcast systems among treatment sites on a bi-weekly schedule, and each time a unit was placed at a treatment site, a trained technician used a combination of adhesive tape, rubber bands, metal pins, square keys, sticky



**Figure 1.** A close-up image of the exposed upper integrated circuit board of a CD player (INSIGNIA NS-P4112; INSIGNIA Products, Richfield, MN). When the 4 contact poles (indicated by red arrow) associated with the play button are connected by an electrically conductive medium, the unit begins playing.

tack, glue, and pieces of eraser to manually depress and secure the CD player play button. Methods for permanently depressing the play buttons varied temporally as we attempted to identify a reliable solution, and varied among CD players because of subtle differences in model design.

In summer of 2013, we increased the number of broadcast systems to 24 and did not rotate them among sites. To eliminate the need to apply manual pressure to CD player play buttons, we applied a thin layer of electrically conductive rosin soldering flux (RadioShack Corporation, Fort Worth, TX) to the contact poles and connected them with solder using a Weller soldering iron (Cooper Hand Tools, Apex, NC) prior to system deployment (for detailed instructions, see Supporting Information, available online at [www.onlinelibrary.wiley.com](http://www.onlinelibrary.wiley.com)). This connection allows a constant electrical current to flow among contact poles to create a closed contact system, which is electrically identical to having the play button depressed at all times (Eaton Corporation 2007). Both PBDMs and soldering ensure that the 4 poles are connected when the CD player receives power, but by soldering the connection we removed virtually all possibility of manual failure (e.g., play button deformation, PBDM dislodging, rubber bands stretching, adhesives failing, or human error in application). We then re-assembled the CD players to their original condition and incorporated them as components in the broadcast systems. All other components used in the treatments were identical for each year.

We visited treatment sites once every 6 or 7 days to change the batteries and rotate the units (2012 only), and considered each period between battery changes (treatment period) as the experimental unit for our analyses. This is a logical temporal unit because a broadcast system operator would ideally be able to deploy a system and be confident that the components would continue working until the battery was scheduled to expire. When convenient, we made additional visits to treatment sites to verify that the units were working as intended.

If a unit was visited before the battery was scheduled to expire, and the unit was not playing appropriately, a technician thoroughly assessed all components, documented the reason for failure, and fixed the source of failure. When units were visited after the battery had likely expired, the technician connected a new, charged battery, and then assessed the status of the unit. Any broadcast system malfunctions that could not be explained by a dead battery were also documented. If the playback unit or one of its components failed at least once during a treatment period, whereby bird song was not being broadcasted as intended, the treatment was considered a failure; whereas, if the unit continued broadcasting until the battery expired, it was considered a success. Prior to analyses, we eliminated all failures resulting from human error (i.e., avoidable situations in which the broadcast system was not properly assembled by field personnel) and limit our discussion to treatment periods that were disrupted by component failure.

## RESULTS

Our sample size was 100 treatment periods in 2012, and we experienced 8 treatment period failures, 7 (88%) of which were specifically attributable to PBDM failures. All PBDM failures occurred when rubber bands stretched or snapped, depression components moved slightly so that pressure was no longer being applied in the correct area, or the CD player became warped. The only other failure occurred when the micro Universal Serial Bus (USB) speaker charger stopped functioning in one unit. In 2013, our sample size was 170 treatment periods, and we experienced 8 total failures, none of which were attributable to the CD player play button. All failures were due to malfunction of other broadcast system components, including 1 speaker, 3 speaker chargers, 1 12-V direct current (DC) power outlet y-adaptor, and 3 CD player power jacks. Therefore, the soldering method had a 100% success rate while units were deployed in the field.

## DISCUSSION

In 2012, when all of our playback systems were activated using manual depression (PBDMs), play button failure was by far the most significant source of treatment interruption. In 2013, we were able to completely eliminate this problem by soldering the contact poles that are connected when the play button is depressed, resulting in a much more reliable broadcast system.

We encountered 2 primary issues that made manual play button depression challenging. First, each CD player model (we experimented with several additional models before selecting those we used in the field) has a unique design that requires a unique solution for depressing the play button, meaning there is no ubiquitous methodology. Second, because the play button is designed to spring back after depression, applying too little pressure means the unit will not start playing as intended, and applying too much pressure can result in deformation of multiple pieces (e.g., the plastic play button, the convex metal disk, and the CD player shell itself) or physically prevent the CD from turning. In addition to the materials described above, we tried numerous other

methods for securing CD player play buttons in the laboratory (including manual clamps, glue, rubber stoppers, and weighted pressure), but were never able to generate an infallible solution until we eliminated the need for manual pressure altogether.

There are 3 explanations for why we saw a greater number of electronic component failures in the second season. First, it is possible that by tampering with the internal circuitry on the CD players, we interfered with other internal mechanisms, causing the DC power jacks to eventually fail on 3 of them. However, we believe that this scenario is very unlikely, given that every CD player that we disassembled, soldered, and put back together ( $n = 27$ ) worked as intended initially, and none of them failed until after they had been used in the field for several weeks. Second, our study region received more than twice as much precipitation in the summer of 2013 (approx. 32.8 cm) as in the summer of 2012 (approx. 16.4 cm; Indiana State Climate Office 2013). It seems likely that environmental moisture was responsible for many of the failures we endured with CD players and other electronic components in the second year, particularly given that others have identified moisture as a primary cause of failure in similar systems (Farrell and Campomizzi 2011). Third, approximately 75% of the playback unit components (including 18 of 27 CD players) we used in 2013 had been previously used in the field in 2012. As such, some may have simply failed from long, sustained periods of use in adverse environmental conditions (e.g., high heat and humidity).

Though we only used 2 different CD player models in our field tests, our experiences suggest that our method can be utilized in most portable CD player models, eliminating the need to design a unique PBDM in each case. For instance, we employed our soldering method on a third model (Memorex MD8151SL; Imation Corporation, Oakdale, MN) that utilizes a 4-pole momentary action pushbutton switch and on a fourth model (SONY D-EJ011; SONY Electronics, Inc., San Diego, CA) that utilizes a right-angle tactile switch. In each case, our method was successful at initiating playback, yet both of these latter models incorporated electronic volume buttons that are connected to the same integrated circuit board as the play button, rendering volume control unusable when the play button is activated (either by manual compression or electrical connection). We, therefore, chose to use the INSIGNIA models, which had a volume control dial not reliant on the integrated circuit board associated with the play button, and other experimenters should take this into consideration when designing their own systems.

Manipulating animal populations using broadcast systems for both experimental and management purposes holds great promise (Nocera et al. 2006, Betts et al. 2008, Ahlering et al. 2010, Farrell et al. 2012), yet can also be expensive, time-consuming, and logistically challenging to implement. Drawing correct and useful conclusions from manipulative experiments or management procedures that involve sound broadcast is contingent on broadcast treatments being applied correctly, often in the absence of a human observer.

In such instances when a broadcast system fails, there is no way to determine when the failure occurred, making it impossible to quantify the number of broadcast hours for that treatment period and thereby adding another source of variability to the experiment. In the systems we used, for instance, failure of any component could result in between 0 hours and 56 hours of lost playback time, depending on when during the treatment period the component failed. As such, any improvement in system reliability will, in turn, improve our ability to generate and accurately interpret results, and reduce the number of man hours required to maintain such experiments. We suggest that our method of soldering the CD player play button is a reliable complement to broadcast systems used in ecological research and for management practices that involve using sound broadcast.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Step-by-step instructions for soldering the electrical connection of a CD player play button that utilizes a tactile momentary action pushbutton switch.

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

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**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.

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.

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

## 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  $\beta$ 3, 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. IFN $\gamma$  is a key cytokine involved in T helper (T<sub>H1</sub>) cell responsiveness that is triggered by intracellular pathogens, including *M. bovis* (Waters et al. 2012). Given the critical role of IFN $\gamma$  in pathogen defense generally, and the response to BTB specifically, variability in the IFN $\gamma$  phenotype is likely to be associated with fitness in the face of infection. Importantly, BTB diagnosis in buffalo relies on IFN $\gamma$ -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<sup>®</sup> 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 IFN $\gamma$ , 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 QIA-GEN 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 touch-down 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 <sub>IS</sub>		F <sub>ST</sub>	A <sub>R</sub>		H <sub>O</sub>		H <sub>E</sub>	
		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<sub>ST</sub>). Also reported are F<sub>IS</sub> values, allelic richness (A<sub>R</sub>), observed and expected heterozygosity (H<sub>O</sub>, H<sub>E</sub>)

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

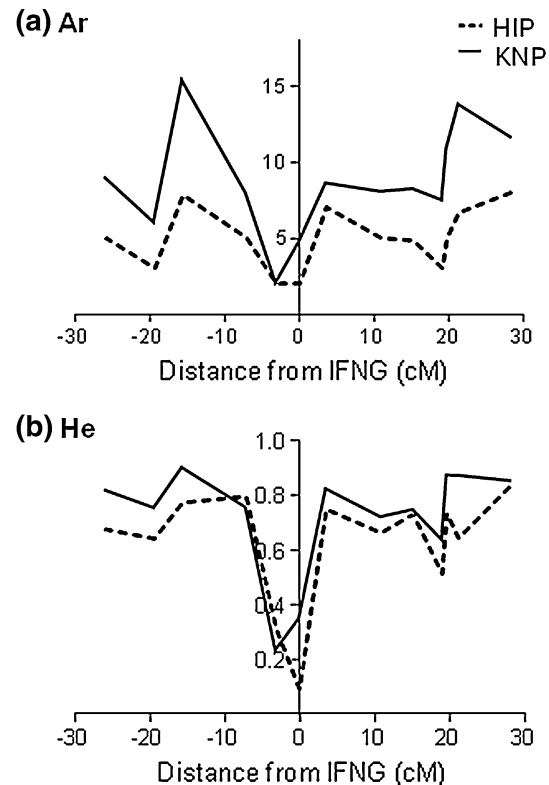
$F_{ST}$  values between populations ranged from 0.010 to 0.1804 among loci (Table 1). There was evidence of significant genetic differentiation between the two populations at all but one locus; BMS1617 was the only locus with a non-significant  $F_{ST}$  value ( $F_{ST} = 0.010$ ;  $p = 0.075$ ). This locus also had the lowest  $F_{IS}$  value in both populations ( $F_{IS}$  KNP =  $-0.155$ ;  $F_{IS}$  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).

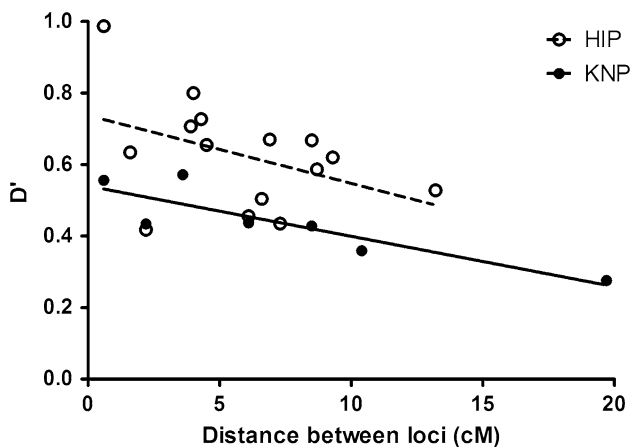
#### Reduced genetic diversity in HIP

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 <sub>A</sub>			Rare N <sub>A</sub>			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 <sup>S</sup>	0.063 <sup>S</sup>
							BL4 allele2	0.063	0.064 <sup>S</sup>	0.063 <sup>S</sup>
							BL4 allele3	0.024	0.018 <sup>S</sup>	0.063 <sup>S</sup>
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 <sup>S</sup>	0.091 <sup>S</sup>
							RM154 allele2	0.086	0.092 <sup>S</sup>	0.045 <sup>S</sup>
							RM154 allele3	0.007	0.008	0
							RM154 allele4	0.059	0.054 <sup>S</sup>	0.091 <sup>S</sup>
							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 <sup>S</sup>	0.091 <sup>S</sup>
							AGLA allele2	0.007	0.008	0
							AGLA allele3	0.02	0.016 <sup>S</sup>	0.045 <sup>S</sup>
							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 <sup>S</sup>	0.045 <sup>S</sup>
							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 IFN $\gamma$ -based diagnostic tests will selectively remove individuals that produce high levels of IFN $\gamma$  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.

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