International Journal for Parasitology xxx (xxxx) xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Contrasting study designs reveal nuance in heterozygosity-parasite associations in the wild

Katherine E.L. Worsley-Tonks [a,b,*], Stacey L. Lance [c], Vanessa O. Ezenwa [b,d,*]

[a] Institut Pasteur, Lyssavirus Epidemiology and Neuropathology Unit, Paris, France
[b] Odum School of Ecology, University of Georgia, Athens, GA, USA
[c] Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, USA
[d] Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

ARTICLE INFO

Article history:
Received 7 March 2025
Received in revised form 17 December 2025
Accepted 19 December 2025
Available online xxxx

Keywords:
Age
Cross-sectional
Experiment
Heterozygosity-fitness correlation
Longitudinal
Parasite
Season
Sex

ABSTRACT

Heterozygosity-fitness correlations (HFCs) are widely used to explore the effects of inbreeding in wild populations. However, the biological significance of HFCs has been the subject of intense debate, and it has been suggested that the magnitude and direction of these correlations may be context-dependent (e.g., vary with different host characteristics or environmental conditions). We tested this hypothesis in a free-ranging population of Grant's gazelles (*Nanger granti*). Specifically, we tested for associations between standardized multilocus heterozygosity (sMLH) and endoparasite infections, and examined how these relationships varied with animal age, sex and environmental context (e.g., seasonality). We used three approaches: a cross-sectional approach focusing on 103 individuals sampled at a single time point, a longitudinal approach focusing on 25 naturally infected individuals sampled over 12 months, and an experimental approach in which 15 individuals were cleared of their parasites and parasite re-accumulation was tracked over 12 months. We found that the presence of heterozygosity-parasite associations varied with study design and context. Cross-sectional patterns varied with environmental context, whereas the longitudinal analysis revealed host trait-specific HFCs, and the experiment established a causal link between heterozygosity and parasitism. Overall, our longer-term study approaches indicated that higher levels of heterozygosity are associated with lower parasite burdens, underscoring the value of longitudinal and experimental approaches for detecting HFCs in wild populations.

1. Introduction

Across a wide range of organisms, traits related to performance or fitness such as reproductive success, birth rate, body size, rate of development, survival, and parasite susceptibility have all been linked to measures of individual genetic variability, or heterozygosity (Acevedo-Whitehouse et al., 2005; Buzan et al., 2020; Charpentier et al., 2008; Coltman et al., 1999; Haanes et al., 2013; Ryder et al., 2010; Slate et al., 2000; Slate and Pemberton, 2002). These so-called heterozygosity-fitness correlations (HFCs) can arise for three main reasons. First, in instances where HFCs are driven by processes such as inbreeding, the measure of genetic variability is expected to reflect a reduction in genome-wide heterozygosity and an increase in the expression of deleterious alleles (general effect hypothesis, (Hansson and Westerberg, 2008; Szulkin et al., 2010)). Alternatively, HFCs may occur if the chosen markers are in genes that have direct effects on fitness (direct effect hypothesis, (David, 1998)), or if they are linked with genes under selection (local effect hypothesis, (David, 1998; Hansson and Westerberg, 2008; Lynch et al., 1995)). Because HFCs can reflect patterns of selection in nature, it is essential to understand how frequently they occur and what mechanisms drive observed patterns in natural populations.

While our understanding of the underlying mechanisms causing HFCs has improved (Acevedo-Whitehouse et al., 2005; Brambilla et al., 2018; Mitchell et al., 2017a; Portanier et al., 2019; Szulkin et al., 2010), the biological significance of these patterns is still under intense debate, in part, because the detectability

* Corresponding authors at: Institut Pasteur, Lyssavirus Epidemiology and Neuropathology Unit, Paris, France and Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA.

E-mail addresses: katherine.worsley-tonks@pasteur.fr (K.E.L. Worsley-Tonks), vanessa.ezenwa@yale.edu (V.O. Ezenwa).

https://doi.org/10.1016/j.ijpara.2025.104763
0020-7519/

of HFCs is notoriously inconsistent (Hulse et al., 2023; Martin et al., 2021). For example, a meta-analysis of HFCs in wild and domestic animal populations found that although correlations arise frequently, the associations tend to be weak and often differ from one population or taxon to the next (Chapman et al., 2009). The authors of the study suggested that discrepancies in detecting HFCs might relate to the context under which individuals are sampled, such as differences in the environment. In support of this hypothesis, several studies suggest that environmental context is an important determinant of the strength and detectability of HFCs, however, the type of environment necessary to detect associations is highly inconsistent. For example, in some instances HFCs were only detected under stressful conditions (e.g. limited resources, habitat disturbance) (Brock et al., 2015; Ferrer et al., 2016; Lesbarrères et al., 2005; Marr et al., 2006), while in others, HFCs were observed when conditions were favorable (Annavi et al., 2014; Harrison et al., 2011). In addition to environmental context, non-genetic characteristics of individuals, or life history context, might also influence the detectability of HFCs. For example, some studies suggest that HFCs might only be detected for individuals of a certain age (Judson et al., 2018) or sex (Arct et al., 2017; Rioux-Paquette et al., 2011). Study design can also affect detectability of HFCs, if for example, the sample size is small or few genetic markers are used to quantify heterozygosity (Chapman et al., 2009). These findings emphasize that a focus on both environmental and life history context may be central to understanding which mechanisms influence the occurrence of HFCs in natural populations, and that study design may also be influential in detecting patterns.

Context-dependency and study design might be particularly important for understanding heterozygosity-parasite correlations (HPCs). Parasites often negatively impact the fitness of their hosts by reducing growth and development rates, body mass, condition, survival and reproduction (Hillegass et al., 2010; Hurtrez-Boussès et al., 1997; Sperry et al., 2009; Ujvari and Madsen, 2006), and as such, have been the target of many HFC studies (e.g., Acevedo-Whitehouse et al., 2003; Coltman et al., 1999; Ferrer et al., 2016; Hawley et al., 2005; Rijks et al., 2008; Voegeli et al., 2012). However, variable outcomes are common in HPC studies. For example, across a range of host-parasite systems, some studies report no correlation between heterozygosity and different metrics of parasitism (Kubacka et al., 2020; Vallender et al., 2012), whereas other studies report negative (e.g., Brambilla et al., 2018; Budischak et al., 2023; Ferrer et al., 2014; Hoffman et al., 2014; Mitchell et al., 2017b; Townsend et al., 2018) or positive (e.g., Ferrer et al., 2016; Sutton et al., 2016) correlations. Indeed, even within the same host population, HPCs have been reported for some parasites but not others (Charpentier et al., 2008; Ruiz-López et al., 2012a; Townsend et al., 2018). Given that fitness consequences of infection can vary depending on traits of the parasite (Sweeny et al., 2022) and host (Gkafas et al., 2020; Shaner et al., 2013), as well as dynamically over time and across environmental conditions (Lesbarrères et al., 2005; Sweeny et al., 2022), fluctuations in the strength of HPCs in wild populations may be the norm rather than the exception.

In this study, we tested for context-dependent HPCs using three complementary study designs (cross-sectional, longitudinal, and experimental) that allowed us to simultaneously examine the occurrence, persistence, and causal basis of HPCs. Focusing on a wild population of Grant's gazelles (*Nanger granti*) in Central Kenya and a group of endoparasites (helminths, protozoa) that commonly occur in this population (Ezenwa, 2003; Ezenwa et al., 2012), we first tested for the occurrence of HPCs in gazelles using samples collected from 103 individuals sampled at a single time point (cross-sectional analysis). Next, we examined the persistence of these correlations through time using data from 25 individuals that were sampled repeatedly for parasites over a 12-month period (longitudinal analysis). In both cases, we examined whether HPCs depended on host life-history (sex, age) and environmental (seasonality) context. Finally, we evaluated whether there was a causal link between individual heterozygosity and parasitism by testing for an effect of individual heterozygosity on the propensity for individuals to re-acquire parasites after experimental clearance using data from 15 individuals (experimental analysis). In general, we predicted that individual heterozygosity would correlate negatively with parasite infection, with more genetically diverse individuals hosting fewer parasites or showing slower rates of parasite re-accumulation. However, we also expected that the strength of these relationships would vary depending on host and environmental context as well as study design.

2. Materials and methods

2.1. Study population and sampling

Grant's gazelles were captured and ear-tagged at the Mpala Research Centre (MRC), Laikipia, Kenya (0°17′N, 37°52′ E) in August 2009 and June 2011 as part of a long-term study of parasitism and host behavior (Ezenwa et al., 2012). At capture, animals were sexed and aged, and tissue and fecal samples were collected for genetic and parasitological analysis, respectively. To age males, we took dental impressions of the upper molars and used tooth wear criteria established by Stelfox et al. (Stelfox et al., 1985) to assign age. To age females, we measured horn length and then used an equation relating horn length to tooth-wear developed for a subset of animals from the same study population (Ezenwa, unpublished data). For animals captured in 2011, half (treated group) received a subcutaneous injection of the anthelmintic drug moxidectin (0.05 ml/kg of Cydectin Long-Acting Injection for Sheep, Virbac Animal Health) and the other half (control group) received saline injections. Animals were randomly assigned to the treated or control group based on the sequence of capture. Fecal samples were collected from all animals before treatment to determine pre-treatment parasite burdens. The moxidectin treatment provided protection against various strongyle nematodes for ~120 days in both male and female gazelles (Ezenwa and Snider, 2016; Worsley-Tonks and Ezenwa, 2015).

For this study, we genotyped 103 animals sampled across the 2009 and 2011 capture events. First, we paired these genotypes with fecal samples collected at both capture events to perform a cross-sectional HPC analysis (n = 103; females = 55, males = 48). Next, we used study animals captured in June 2011, that were repeatedly re-sampled for feces over a 12-month period, for longitudinal and experimental analyses. The longitudinal analyses involved control individuals only (n = 25; females = 17, males = 8) and the experimental analysis involved treated individuals only (n = 16; females = 9, males = 7). To conduct repeated sampling, we monitored and collected fecal samples from individually identifiable animals 2–3 times per month starting in the weeks following capture until June 2012. Cross-sectional fecal samples were collected directly from the rectum of captured animals. Other than the initial (capture) fecal sample, all samples used for the longitudinal and experimental analyses were collected by observing individuals defecate and then collecting the sample off the ground within 10 min of the defecation event. Given this sampling design, some samples were used in multiple analyses. Capture samples collected from control individuals and used for cross-sectional analyses also contributed to the longitudinal analyses. Similarly, capture samples collected from treated individuals and used for cross-sectional analyses also contributed to the experimental analyses.

2.2. Parasitological analyses

Parasite infections were assessed from fecal samples. We used fecal parasite counts as a proxy for the impact of parasites on hosts because the shedding of parasite propagules in host feces typically reflects a combination of the number, size, and fecundity of the parasite population within a host (Gasbarre et al., 2001). Moreover, for Grant's gazelles our prior work has linked fecal parasite counts to various aspects of individual performance (Ezenwa et al., 2012; Ezenwa and Snider, 2016; Sabey et al., 2024; Worsley-Tonks and Ezenwa, 2015). We screened fecal samples for five different parasite taxa that commonly infect Grant's gazelle: strongyle nematodes (Trichostrongylidae), *Trichuris* spp. (Trichuridae), *Strongyloides* spp. (Strongyloididae), coccidia (Eimeriidae), and lungworms (Trichostrongyloidea). Lungworms were further distinguished into three morphotypes based on tail morphology (Ezenwa et al., 2012). For all taxa except lungworms, we used a modification of the McMaster egg counting technique to quantify parasite egg or oocyst output in feces (Ezenwa, 2003). For lungworms, we used a modified Baermann method to quantify first stage larvae output (Forrester and Lankester, 1997). We estimated parasite abundance for two dominant parasite types (strongyles and lungworms) as the number of eggs or lungworm larvae per gram feces. We estimated parasite richness as the number of different parasite taxa (and morphological types for lungworms) present in a single sample.

2.3. DNA extraction and genotyping

We genotyped study animals at 20 microsatellite loci developed from individuals in the same population (Worsley-Tonks et al., 2015). Ear tissue samples collected at capture were stored in 95 % ethanol until DNA extraction. Whole genomic DNA was extracted from tissue using DNeasy Tissue Kits (QIAGEN Inc, Valencia, CA, USA) according to the manufacturer's instructions. Microsatellites were amplified using standard polymerase chain reactions (PCR) using a three-primer nested reaction including a universal fluorescently labeled primer to label all reactions (modified from (Schuelke, 2000)). PCRs were performed in a volume of 12.5 µl containing 5–10 ng of genomic DNA, 2 µg bovine serum albumin, 1.5 mM $MgCl_2$, 1x PCR gold buffer (Applied Biosystems), 0.36 µM universal dye-labeled primer, 0.04 µM tag labeled primer, 0.4 µM unlabeled primer, 0.8 mM dNTPs, 4.99 µl sterile double-distilled water, and 0.3 units AmpliTaq Gold® Polymerase (Applied Biosystems). All PCR amplifications were performed using an Applied Biosystems GeneAmp 9700, and PCR products were then run on an ABI-3130xl sequencer (Applied Biosystems) and sized with Naurox size standard prepared as described in DeWoody (DeWoody, 2005) except that unlabeled primers started with GTTT. Alleles were scored and manually verified using GeneMapper v.3.7 (Applied Biosystems).

Observed and expected heterozygosity at each microsatellite locus were calculated using GeneAlEx v. 6.4 (Smouse et al., 2008). We evaluated each locus for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium using GENEPOP v. 4.2 and accounted for multiple testing using Bonferroni correction. We used the program Micro-Checker (Van Oosterhout et al., 2004) to test for null alleles. Of the 20 microsatellite loci initially screened, 8 showed null alleles and were excluded from further analysis.

We calculated three measures of heterozygosity: standardized multilocus heterozygosity (sMLH, (Coltman et al., 1999)), internal relatedness (IR, (Amos et al., 2001)), and homozygosity weighted by locus (HL, (Aparicio et al., 2006)) using the program IRmacroN4 (Amos et al., 2001). Since all three metrics were strongly correlated (Pearson's correlation: $P < 0.0001$), we focus on sMLH as it quantifies an individual's multilocus heterozygosity relative to the population average across the typed loci (Coltman et al., 1999), and is widely used in wildlife HFC studies (Brambilla et al., 2018; Gkafas et al., 2020; Mitchell et al., 2017b). Finally, given the demographic structure of Grant's gazelles in Kenya and potential for extensive reproductive isolation among populations (Lorenzen et al., 2008), we estimated identity disequilibrium across our microsatellite markers to understand whether our measures of heterozygosity reflected potential inbreeding effects (Miller and Coltman, 2014). We did this by calculating the g_2 statistic using the program RMES (David et al., 2007). The g_2 depends on the variance of inbreeding of the population and not locus specific characteristics and when there is no variance in inbreeding ($g_2 = 0$), HFCs are not expected to arise (David et al., 2007; Szulkin et al., 2010).

2.4. Statistical analysis

For the cross-sectional analyses, we tested patterns of association between individual heterozygosity (sMLH) and parasite infection and examined whether these associations depended on host or environmental context using data from individuals sampled at a single time point (see Table 1 for sample sizes). To do this, we ran three models with strongyle abundance, lungworm abundance, and parasite richness as separate response variables, and in all instances, included sMLH, age, sex, capture year (2009 or 2011), and two-way interactions between sMLH and age, sex, and capture year as the predictor variables. Capture year was used as a proxy for environmental context since rainfall conditions in the months preceding the two capture events varied substantially (June–Aug 2009: mean monthly rainfall = 6.03 mm, range = 5.4–7.3 mm vs. April–June 2011: mean = 79.57 mm, range = 69–92.4 mm), and prior rainfall has been shown to have strong effects on gazelle parasite burdens at the study site (Shearer and Ezenwa, 2020). Thus, in our analysis, capture year served as an environmental stratum given the pronounced rainfall contrasts between periods. We modelled strongyle and lungworm abundance using generalized linear

Table 1
Sample sizes for nominal host and environmental predictor variables. For the longitudinal and experimental designs, sample sizes are reported as the number of unique individuals and the total number of observations.

Variable	Cross-sectional (n = 103)	Longitudinal (n = 25 individuals and 269 observations)	Experimental (n = 15 individuals and 340 observations)
Sex	• female (55) • male (48)	• female (17 individuals; 164 observations) • male (8 individuals, 105 observations)	N/A
Capture year/ season	• 2009 (47) • 2011 (56)	• dry (14 individuals, 82 observations) • wet (25 individuals, 187 observations)	N/A
Social status	N/A	N/A	• bachelor (2 individuals; 20 observations) • nursery (8 individuals; 149 observations)* • territorial (5 individuals; 171 observations)

* One individual was removed as it was identified as an outlier by diagnostic tests.

models (GLMs) with negative binomial distributions and modelled parasite richness using a linear model (LM). To allow for potential non-linear relationships, we inspected residual diagnostic plots using the "DHARMa" R package (Hartig and Hartig, 2017) and when curvature was evident, replaced linear terms with natural splines for the relevant predictors (specifically age for the cross-sectional and longitudinal analyses and number of days post treatment for the experimental analysis of parasite abundance). Finally, since our microsatellite locus screening tests identified 3 out of 12 loci that deviated from HWE (see Results), and such loci can influence the strength or direction of HFCs (Luikart et al., 2008), we estimated sMLH with and without HWE-deviant loci and re-ran our models with these different estimates to understand the influence of inclusion vs. exclusion of deviant loci on our results. Overall, exclusion of HWE-deviant loci had only minor effects on the results across analyses. We report models based on all 12 loci in the main text and results for the inclusion/exclusion tests are shown in Tables S1.

For the longitudinal analyses testing for longer-term persistence of HPCs, we used the same model structure as in the cross-sectional analyses except that we fitted generalized (strongyle and lungworm abundance) or linear (parasite richness) mixed-effect models (G/LMMs) including animal ID as a random effect to account for repeated sampling of individuals through time (see Table 1 for sample sizes). Also, instead of using year as a proxy for variation in environmental context, we used the season (wet vs. dry) during which a fecal sample was collected. Sampling months were coded as wet or dry based on rainfall records from the study site (wet: mean monthly rainfall = 136.4 mm, range = 77.5–239.7 mm [July-Nov 2011, April-May 2012]; dry: mean = 79.44 mm, range = 0–39.9 mm [Dec 2011-March 2012, June 2012]). We used a similar inclusion/exclusion analysis approach to evaluate the effects of HWE-deviant loci on longitudinal model results (see Table S2).

Finally, for the experimental analyses exploring whether individual heterozygosity was a driver of variation in parasitism, we tested for an effect of sMLH on parasite re-accumulation and absolute parasite abundance after anthelmintic treatment. For this set of analyses, we focused solely on strongyle nematodes given the targeted effect of the drug treatment we employed and evidence of strong effects of treatment on this group of parasites (Ezenwa and Snider, 2016; Worsley-Tonks and Ezenwa, 2015). To capture the speed at which an individual experienced parasite recovery after treatment, we used parasite re-accumulation quantified as the difference between an individual's strongyle egg count at each observation and its original egg count at capture as a response variable. To capture absolute changes in parasite abundance over time we used strongyle abundance as a response variable. We modelled parasite re-accumulation using a LMM and parasite abundance using a GLMM. For both models, sMLH, time since treatment (in days), and the interaction between sMLH and time since treatment were included as key predictor variables. We also included group size and social status (female, bachelor male, or territorial male) as covariates in the model since these factors have been previously shown to affect the re-accumulation of strongyle nematodes in gazelles (Ezenwa and Snider, 2016; Ezenwa and Worsley-Tonks, 2018). Model diagnostics identified one individual as a significant outlier in both the re-accumulation and abundance models, so this individual was dropped from the models which changed the sample size from 365 observations on 16 unique individuals to 340 observations on 15 unique individuals. Models with the outlier included and results from HWE-deviant loci inclusion/exclusion tests are shown in Tables S3 and S4.

All statistical analyses were performed in R version 4.3.1, and results were considered significant at $P \leq 0.05$. GLMs and GLMMs were run using the "*glmmTMB*" package (Brooks et al., 2017) and LMs and LMMs were run using the "*lme4*" package (Bates, 2007; R Core Team, 2021). Post-hoc analyses were performed using 'emtrends' and 'contrasts' functions in the "*lsmeans*" R package (Lenth, 2016). All model diagnostics, including outlier analyses, were performed using the "*DHARMa*" package.

3. Results

3.1. Heterozygosity patterns

Heterozygosity was calculated for 103 gazelles. Of 20 microsatellites screened, 8 had null alleles and were excluded from HPC analyses. After Bonferroni correction, three of the twelve remaining loci (i.e., *Nagr29*, *Nagr33*, and *Nagr35*) deviated significantly from HWE (Table 2). Only one pair of loci showed significant linkage disequilibrium (*Nagr44-Nagr48*), and both loci were retained in downstream analyses (Table 2). Average observed and expected heterozygosity were 0.644 (SD = 0.183) and 0.641 (SD = 0.181), respectively. There was evidence of identity disequilibrium, with g_2 significantly different from 0 ($g_2 \pm SD = 0.0325 \pm 0.015668$), indicating the presence of variance in inbreeding.

3.2. Heterozygosity and parasite infection

3.2.1. Cross-sectional analysis

Parasite infections were common in the study population and individual heterozygosity explained significant variation in cross-sectional patterns of parasitism. Of 103 gazelles sampled in 2009 and 2011, 100 % were infected with strongyles, 96 % were infected with lungworms, and on average, individuals were infected with 4 (range: 1–6) different parasite taxa. Our models testing for associations between parasitism and individual heterozygosity revealed a main effect of sMLH on parasite richness in which parasite richness declined with sMLH (Fig. 1A), and significant sMLH × capture year interaction for strongyle and lungworm abundance (Table 3). For strongyles, parasite abundance decreased with sMLH in the wet year (2011: estimate = −0.25, $z = -1.94$, $p = 0.05$), while there was no relationship between sMLH and parasite abundance in the dry year (2009: estimate = 0.18, $z = 1.51$, $p = 0.13$; Fig. 1B). For lungworms the pattern was the opposite; parasite abundance decreased with sMLH in the dry year (2009: estimate = −0.45, $z = -2.08$, $p = 0.04$), while there was no relationship in the wet year (2011: estimate = 0.16, $z = 0.82$, $p = 0.41$; Fig. 1C).

3.2.2. Longitudinal analysis

Using the same underlying model structure as the cross-sectional analyses but focusing on 25 individual gazelles sampled repeatedly over a 12-month period (mean [range] number of samples per individual = 11 [1–25]), we found that individual heterozygosity interacted with host traits to predict significant variation in parasite abundance, but not parasite richness (Table 4). First, sMLH interacted with sex to predict strongyle abundance, with abundance decreasing with increasing sMLH in males (estimate = −0.71, $z = -7.29$, $p < 0.001$), but not females (estimate = 0.07, $z = 0.95$, $p = 0.34$; Fig. 2A). Second, sMLH interacted with age to predict variation in both strongyle and lungworm abundance (Table 4). For strongyles, patterns of association between sMLH and age differed by age class, however, none of these relationships were statistically significant (age group 6.75 years: estimate = 0.1, $z = 1.08$, $p = 0.28$; age group 5.67 years: estimate = 0.02, $z = 0.29$, $p = 0.77$; age group 4.6 years: estimate = −0.04, $z = -0.45$, $p = 0.65$; Fig. 2B). For lungworms, abundance decreased with increasing sMLH in older individuals (age group 6.75 years: estimate = −1.18, $z = -4.1$, $p < 0.001$; age group 5.67 years: estimate = −0.5, $z = -2.24$, $p = 0.03$; Fig. 2C), but did

Table 2
Characteristics of the 12 microsatellite loci used to calculate heterozygosity-parasite correlations. **Na**, number of alleles; **Ho**, observed heterozygosity; **He**, expected heterozygosity; **HWE**, Hardy-Weinberg equilibrium. The frequencies of null alleles were estimated using the Brookfield 1 estimator in Micro-Checker.

Locus	N	Na	Ho	He	HWE	Null alleles
Nagr 12	102	13	0.775	0.84	0.09	0.03
Nagr 13	102	4	0.235	0.227	1	0.01
Nagr 20	103	8	0.476	0.501	0.26	0.03
Nagr 23	101	6	0.624	0.675	0.77	0.03
Nagr 27	103	15	0.883	0.876	0.6	−0.01
Nagr 29	102	10	0.784	0.813	<0.05	0.02
Nagr 32	102	5	0.627	0.662	0.04	0.03
Nagr 33	103	4	0.553	0.473	<0.05	−0.05
Nagr 35	102	6	0.902	0.703	<0.05	−0.12
Nagr 36	103	5	0.583	0.561	0.14	−0.01
Nagr 44	97	7	0.639	0.709	0.75	0.04
Nagr 48	103	4	0.65	0.648	0.71	−0.003

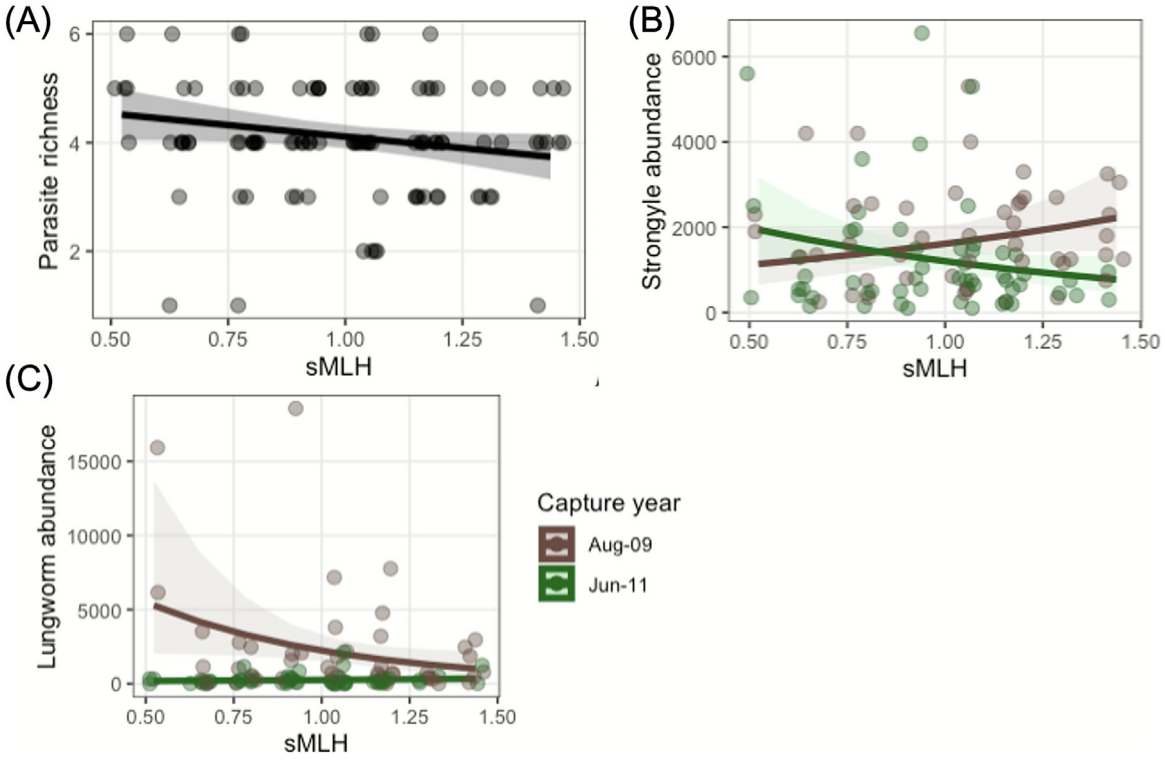

Fig. 1. Relationship between standardized multilocus heterozygosity (sMLH) across 12 microsatellite loci and measures of parasitism in a cross-sectional sampling of Grant's gazelles: **(A)** parasite richness by sMLH; **(B)** strongyle abundance by sMLH and capture year (brown line: dry year (2009), green line: wet year (2011)); and **(C)** lungworm abundance by sMLH and capture year (brown line: dry year (2009), green line: wet year (2011)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

not change with sMLH in younger individuals (age group 4.6 years: estimate = −0.33, z = −1.15, p = 0.23; Fig. 2C).

3.2.3. Experimental analysis

Finally, we used an anthelmintic drug treatment experiment to explore the role of individual heterozygosity as a driver of parasite re-infection risk. Focusing on 15 gazelles who received treatment and were sampled over 12-months (mean [range] of samples collected per individual = 23 [3–43]), we found that heterozygosity was a significant predictor of both the speed at which individuals re-accumulated parasites and absolute parasite abundance after treatment (Table 5). Specifically, sMLH interacted with time since treatment such that individuals with lower sMLH values re-accumulated strongyles faster than those with higher sMLH values (sMLH 0.51: estimate = 0.6, t = 14.14, p < 0.001; sMLH 0.91: estimate = 0.34, t = 12.82, p < 0.001; sMLH 1.18: estimate = 0.17, t = 4.1, p < 0.001; Fig. 3A). Likewise, there was a main effect of sMLH on strongyle abundance in which individuals with higher sMLH values maintained lower strongyle abundances following treatment (Fig. 3B). Similar results were found with the larger dataset with the outlier included (Table S4).

4. Discussion

HFCs are widely used to explore the effects of inbreeding in natural populations, however, across studies, observed patterns of association are highly inconsistent (Acevedo-Whitehouse et al., 2006; Fox and Reed, 2011; Martin et al., 2021). One primary hypothesis for these inconsistencies is that correlations are generally weak (Chapman et al., 2009), but other reasons may be differences in the fitness-related traits examined, study subject characteristics, environmental conditions, or study design

Table 3
Generalized linear (strongyle abundance, lungworm abundance) and linear (parasite richness) models showing main and interaction effects of sMLH (standardized multilocus heterozygosity) on three measures of parasite infection assessed in Grant's gazelles sampled at a single time point.

Response variable	*Predictor variable*	*Cross-sectional analysis n = 103 individuals*			
		Est.	*SE*	*z/t*	*P*
Strongyle abundance	(intercept)	7.45	0.15	51.23	–
	sMLH	0.26	0.16	1.66	0.53
	age	**0.17**	**0.08**	**2.12**	**0.03**
	sex (male)	−0.13	0.16	−0.81	0.42
	capture year (2011)	−0.29	0.16	−1.78	0.08
	sMLH × age	−0.15	0.1	−1.55	0.12
	sMLH × sex (male)	−0.16	0.17	−0.92	0.36
	sMLH × capture year (2011)	**−0.43**	**0.17**	**−2.49**	**0.01**
Lungworm abundance	(intercept)	7.79	0.24	32.28	–
	sMLH	−0.5	0.29	−1.69	0.6
	age	**0.56**	**0.2**	**2.85**	**0.008**
	sex (male)	−0.17	0.26	−0.67	0.48
	capture year (2011)	**−2.16**	**0.26**	**−8.37**	**<0.001**
	sMLH × sex (male)	0.1	0.29	0.35	0.73
	sMLH × age	0.18	0.18	1.01	0.31
	sMLH × capture year (2011)	**0.61**	**0.27**	**2.24**	**0.03**
Parasite richness	(intercept)	4.03	0.19	21.18	–
	sMLH	**−0.005**	**0.19**	**−0.02**	**0.04**
	age	**0.21**	**0.11**	**2.03**	**0.04**
	sex (male)	0.25	0.21	1.16	0.26
	capture year	−0.09	0.21	−0.45	0.62
	sMLH × sex (male)	−0.16	0.21	−0.78	0.44
	sMLH × age	−0.1	0.11	−0.91	0.37
	sMLH × capture year (2011)	−0.25	0.22	−1.16	0.25

Table 4
Generalized (strongyle abundance, lungworm abundance) and linear (parasite richness) mixed models showing main and interaction effects of sMLH (standardized multilocus heterozygosity) on three measures of parasite infection assessed in Grant's gazelles sampled repeatedly over 12 months.

Response variable	*Predictor variable*	*Longitudinal analysis n = 25 individuals and 269 parasite count observations*			
		Est.	*SE*	*z/t*	*P*
Strongyle abundance	(intercept)	5.19	0.33	15.66	–
	sMLH	**−0.04**	**0.23**	**−0.19**	**<0.001**
	age (spline, df = 2)	–	–	**77.76**	**<0.001**
	sex (male)	−0.09	0.11	−0.84	0.08
	season (wet)	−0.009	0.09	−0.1	0.9
	sMLH × age (spline, df = 2)	–	–	**6.91**	**0.03**
	sMLH × sex (male)	**−0.78**	**0.12**	**−6.42**	**<0.001**
	sMLH × season (wet)	−0.07	0.1	−0.81	0.42
Lungworm abundance	(intercept)	7.16	1.3	5.48	–
	sMLH	**2.5**	**0.84**	**2.99**	**0.001**
	age (spline, df = 4)	–	–	6.42	0.17
	sex (male)	**1.09**	**0.38**	**2.89**	**0.001**
	season (wet)	**0.43**	**0.1**	**4.43**	**<0.001**
	sMLH × sex (male)	0.26	0.4	0.64	0.52
	sMLH × age (spline, df = 4)	–	–	**21.99**	**<0.001**
	sMLH × season (wet)	0.007	0.1	0.07	0.94
Parasite richness	(intercept)	3.66	0.16	22.35	–
	sMLH	−0.19	0.17	−1.13	0.68
	age	0.03	0.09	0.31	0.95
	sex (male)	0.42	0.25	1.68	0.08
	season (wet)	**0.34**	**0.1**	**3.42**	**<0.001**
	sMLH × sex (male)	0.1	0.24	0.43	0.66
	sMLH × age	0.05	0.07	0.76	0.45
	sMLH × season (wet)	0.13	0.1	1.28	0.2

(Annavi et al., 2014; Chapman et al., 2009; Ferrer et al., 2016; Pujolar et al., 2006; Shaner et al., 2013; Szulkin et al., 2007). In our study, we combined cross-sectional, longitudinal, and experimental approaches to explore the occurrence and drivers of HPCs in a wild population of Grant's gazelles. While we found evidence of relationships between heterozygosity and parasite burdens, these patterns varied depending on environmental context, host traits, and study design. In the cross-sectional analyses, environmental context emerged as an important modifier of HPCs, whereas for the longitudinal analyses host traits were key modifiers. Nevertheless, by experimentally removing strongyle nematodes, we established a causal link between individual

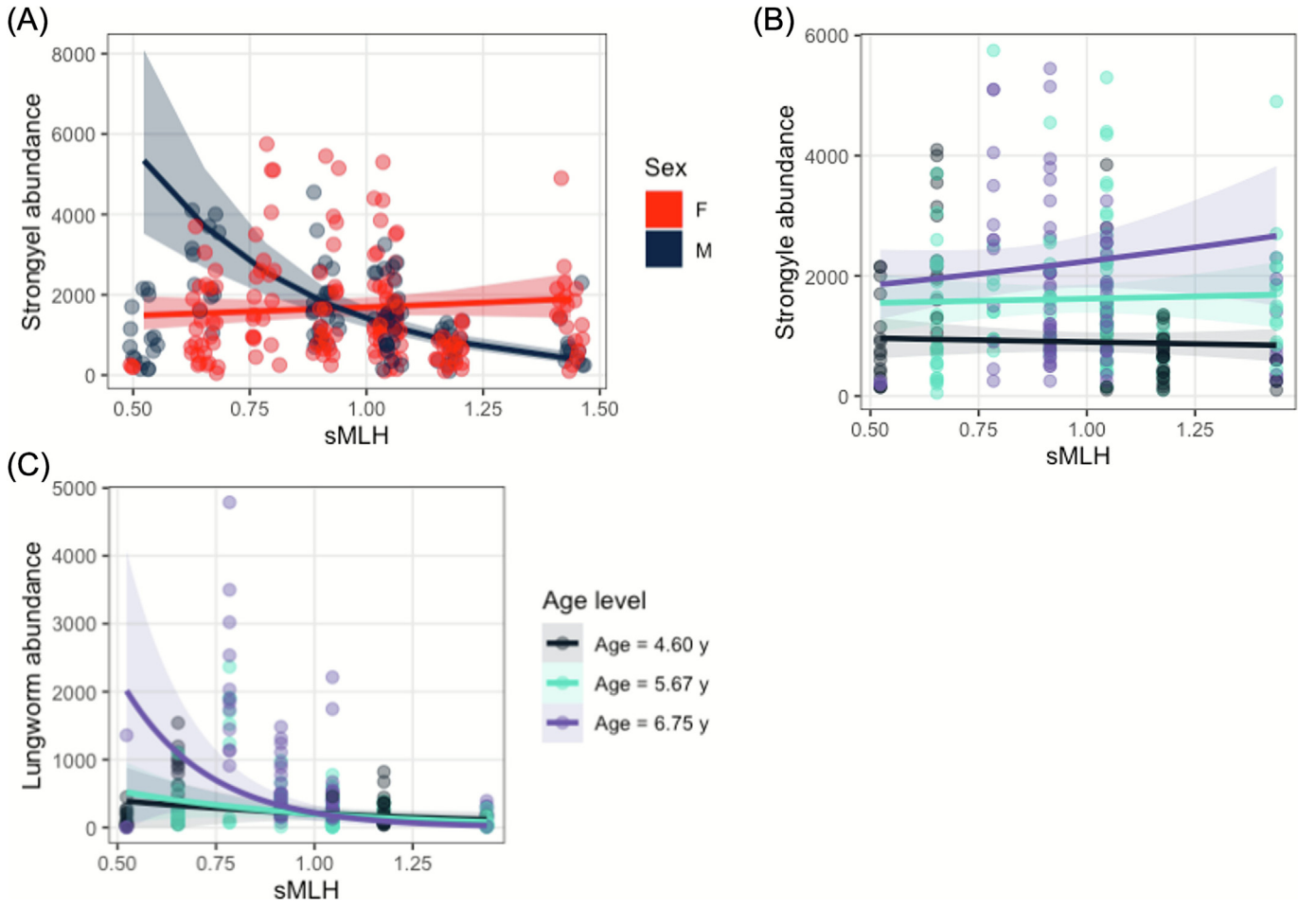

Fig. 2. Relationship between standardized multilocus heterozygosity (sMLH) and measures of parasitism in a longitudinal sampling of Grant's gazelles: **(A)** strongyle abundance by sMLH and sex (red line: females, blue line: males); **(B)** strongyle abundance by sMLH and age; **(C)** lungworm abundance by sMLH and age. For panels (B) and (C), the three age lines represent low, medium, and high age quantiles (i.e., the 15th, 50th, and 85th percentiles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Linear mixed models showing main and interaction effects of sMLH (standardized multilocus heterozygosity) on strongyle re-accumulation and abundance in Grant's gazelles sampled repeatedly for 12 months following an anthelmintic treatment.

Response variable	*Predictor variable*	*Experimental analysis 15 individuals and 340 parasite count observations*			
		Est.	*SE*	*T/z*	*P*
Strongyle re-accumulation	(intercept)	–0.1	0.44	–0.22	–
	number of days post-treatment	**0.36**	**0.03**	**13.84**	**<0.001**
	social status (N)	**0.65**	**0.49**	**1.31**	–
	social status (T)	**–0.27**	**0.51**	**–0.53**	**0.05**
	sMLH	0.36	0.17	2.14	0.06
	group size	0.003	0.03	0.08	0.93
	sMLH × number of days post-treatment	**–0.16**	**0.02**	**–6.8**	**<0.001**
Strongyle abundance	(intercept)	2.05	0.64	3.21	–
	sMLH	**0.21**	**0.4**	**0.53**	**0.04**
	number of days post-treatment (spline, df = 4)	–	–	–	**<0.001**
	social status (N)	–0.46	0.53	–0.85	–
	social status (T)	–0.32	0.54	–0.6	0.67
	group size	–0.02	0.04	–0.52	0.6
	sMLH × number of days post-treatment (spline, df = 4)	–	–	2.57	0.63

heterozygosity and parasite infection risk, showing that more heterozygous individuals acquired fewer strongyles, at a slower rate, after treatment. In combination, our findings highlight the important, but complex role of individual heterozygosity in modulating parasite burdens in wildlife. Furthermore, because we found significant variance in inbreeding as measured by identity disequilibrium, our estimates of heterozygosity and the observed HPCs may reflect genome-wide effects (Miller and Coltman 2014).

One clear pattern that emerged from our comparison of study designs was that cross-sectional analyses were more likely to yield

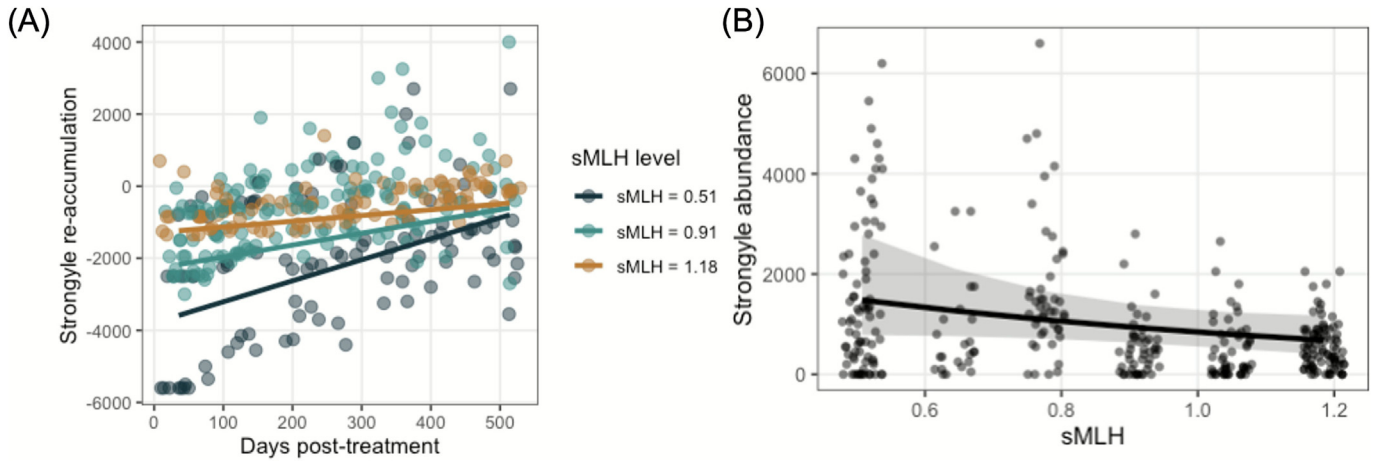

Fig. 3. Relationship between **(A)** strongyle re-accumulation over time (in days); and **(B)** strongyle abundance and standardized multilocus heterozygosity (sMLH). For panel (A), the three sMLH lines represent low, medium, and high sMLH quantiles (i.e., the 15th, 50th, and 85th percentiles).

environmentally stratified HPCs, with slopes differing between dry (2009) and wet (2011) years. This is consistent with other cross-sectional HFC studies exploring environmental effects (Annavi et al., 2014; Lesbarrères et al., 2005). By contrast, longitudinal analyses, which capture both within and among-individual variation in parasite loads revealed host trait-specific HPCs (sex and age) that were not detected in the cross-sectional analyses. These findings suggest that given well-known environmental variation in host-parasite interactions (Gorsich et al., 2014; Paull and Johnson, 2018), cross-sectional HPC studies are more sensitive to the timing of parasite sampling, while longitudinal studies, which are able to average over the environmental variability, are better at revealing the possible role of life-history context in shaping HPCs. This conclusion is in line with ecological research showing that cross-sectional 'snapshot' samples are highly contingent on environmental conditions at the time of sampling, whereas sampling of individuals through time better separates within-individual life-history effects from among-individual and environmental variation (Clutton-Brock and Sheldon, 2010).

The cross-sectional HPCs observed in our study were all negative in direction. Parasite richness was negatively associated with heterozygosity, suggesting that more heterozygous individuals support few parasites at any given time, a pattern that has been detected in other wildlife systems (e.g., raccoons *Procyon lotor* (Ruiz-López et al., 2012b)). Strongyle and lungworm abundance were also negatively correlated with heterozygosity but these patterns were modified by rainfall seasonality. Strongyles decreased with heterozygosity in the wet year but not the dry year, and vice versa for lungworms. Rainfall is known to affect gazelle parasite dynamics at our site (Ezenwa, 2004b; Shearer and Ezenwa, 2020), thus it is not surprising that differences in rainfall between years modulated HPCs. The difference between parasite taxa in whether HPCs were detected in the dry year vs. the wet year likely reflects differences in the ecology of these parasites. Lungworms have gastropod intermediate hosts (Shearer and Ezenwa 2020) which are typically more concentrated on high quality vegetation, which during dry periods may also be favored by gazelles, increasing their exposure to lungworms in the environment. Under such conditions, individuals with lower heterozygosity may be less able to control infections yielding a stronger negative HPC in the dry season. This idea is consistent with the "greater stress" hypothesis, which posits that harsh or energetically demanding conditions (e.g., drought) magnify inbreeding-depression phenotypes, so individuals with lower heterozygosity are less able to mount and sustain effective anti-parasite defenses, possibly making negative HFCs more evident under stressful conditions (Ferrer et al., 2016; Forcada and Hoffman, 2014; Szulkin and Sheldon, 2007). By contrast, strongyles have direct life cycles and the persistence and dispersal of free-living stages are enhanced in moister conditions (O'Connor et al., 2006; Shearer and Ezenwa, 2020; Stromberg, 1997). Consequently, gazelle contact rates with strongyles may be higher during the wet season and heterozygosity-linked differences in infection rates may be more apparent in the wet season.

The HPCs emerging from our longitudinal analyses were also negative in direction and mediated by both host age and sex. First, lungworm abundance was negatively correlated with heterozygosity in older but not younger individuals. Negative HPCs among older individuals may simply reflect patterns of acquired immunity to nematode parasites. Younger individuals are broadly susceptible to nematode parasites, whereas more variation may exist in older individuals due to greater variation in immunity and resistance to infection (Hayward et al., 2011; Stear et al., 1999). Thus, HPCs linked to differential susceptibility may be more detectable in older age classes because variance in parasite immunity is greater. Second, strongyle abundance was negatively correlated with heterozygosity in males but not females. Sex-dependent HPCs observed in other studies have been explained in the context of sex-biases in parasite vulnerability associated with periods of stress. For example, in a study of striped dolphins (*Stenella coeruleoalba*) where a negative correlation between lungworm burden and heterozygosity was observed in females but not males, the authors suggested that sex-specific stressors such as parturition or nursing might have uncovered the effects of low heterozygosity and inbreeding (Gkafas et al., 2020). In gazelles, the negative heterozygosity-strongyle correlation we detected in males and not females may also reflect sex-specific stressors that increased the variance in parasite vulnerability among males. In this system, territorial males tend to have higher strongyle nematode burdens than females (Ezenwa, 2004a), and aspects of male reproductive behavior and physiology that accompany reproductive investment are linked to variation in immune responses and parasite burdens (Ezenwa et al., 2012; Ezenwa and Snider, 2016).

Most HFC studies on parasites use cross-sectional or longitudinal study designs to investigate the links between host genetic diversity and parasitism. In contrast, experimental studies that manipulate infection patterns are rare. However, such experiments can provide essential insights. For example, in House finches (*Carpodacus mexicanus*) inoculated with the pathogen *Mycoplasma gallisepticum* under laboratory conditions, heterozygosity was associated with lower disease severity and stronger immune

responses suggesting that higher heterozygosity is associated with better parasite resistance (Hawley et al., 2005). Similarly, in natural settings, a study comparing nestling survival between broods of great tits (*Parus major*) experimentally infested versus non-infested with ectoparasites reported a significant association between heterozygosity and nestling survival in the infested broods but no association in non-infested broods, suggesting that parasites mediate HFCs and that low heterozygosity can magnify the severity of parasitism (Voegeli et al., 2012). Here, we found that after clearing individuals of their strongyle nematode infections, individual heterozygosity was an important predictor of parasite re-accumulation and abundance post-treatment. Specifically, individuals with lower heterozygosity re-acquired parasites faster and had higher absolute parasite abundances than individuals with higher heterozygosity, suggesting that heterozygosity was associated with variation in parasite exposure, susceptibility, or both. Our statistical model accounted for group size and social status, two factors that contribute to variation in exposure and susceptibility in gazelles (Ezenwa, 2004a; Ezenwa and Snider, 2016; Ezenwa and Worsley-Tonks, 2018), suggesting an independent contribution of heterozygosity. However, more work is needed to understand the mechanistic basis for how and why heterozygosity is linked to variation in parasitism in this system.

While this study advances our understanding of HPCs in the wild and underscores the value of exploring these associations across contexts and study designs, our work has some limitations. First, although we make comparisons across study designs, these comparisons are qualitative and are not intended to provide guidance on sampling approaches that should be used for exploring HPCs. This is especially true given that our sampling approaches were not independent in terms of sample composition (i.e., some of the same samples contributed to the cross-sectional and longitudinal and cross-sectional and experimental analyses, respectively). Rather our work highlights that different sampling approaches can reveal HPCs mediated by distinct environmental and host contexts. Second, one important area that requires further investigation is the contribution of different genetic markers to HPCs. Because identity disequilibrium was significant in our dataset ($g_2 > 0$), our heterozygosity results are consistent with a genome-wide ("general effects") signal of inbreeding. Moreover, the direction and significance of effects were qualitatively unchanged when HWE-deviant loci were excluded or included in our HPC models (Tables S1–S4), arguing against single or few loci driving observed patterns. Nevertheless, locus-specific effects ("local effects") cannot be ruled out and merit further exploration (Luikart et al., 2008).

Identifying the circumstances under which individual genetic diversity affects fitness is a critical step towards understanding how inbreeding can negatively affect natural populations. The role of parasitism in HFCs has attracted considerable attention, in part because of the threat parasites and infectious diseases can pose to declining wildlife species (Smith et al., 2009), however, our understanding of the frequency and strength with which HPCs occur in the wild is often challenged by issues of study design. By examining HPCs at a single time point, over the course of 12 months, and through experimental removal of parasites and by accounting for host and environmental context, our work demonstrates that the form of observed heterozygosity-parasite relationships can vary by study approach and context. Furthermore, our study suggests that experimental approaches can be a valuable tool for identifying the consequences of variation in individual genetic diversity for parasite infection risk in wild populations.

CRediT authorship contribution statement

Katherine E.L. Worsley-Tonks: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Stacey L. Lance:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Vanessa O. Ezenwa:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Acknowledgements

We would like to give a special thanks to M. Snider, J. Ewoi, Z. Chillag, A. Hines, A. Mwachanje, and M. Rajeev for assistance in the field. The Kenya Ministry of Science, Education and Technology and the Kenya Wildlife Service gave permission to conduct this work in Kenya, and animal capture protocols were approved by the University of Montana (#023-09VEDBS-051509) and the University of Georgia (#A2010 10-188) Animal Care and Use Committees. This work was supported by a National Science Foundation CAREER Award to VOE (IOS-1101836) and by the DOE under Award Number DE-FC09-07SR22506 to the University of Georgia Research Foundation.

Data availability

Zenodo (https://doi.org/10.5281/zenodo.18229305) Data are avaiable on Zenodo at https://doi.org/10.5281/zenodo.18229305.

Appendix A. Supplementary material

Tables S1–S4 can be found in the supplementary materials file. Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2025.104763.

References

Acevedo-Whitehouse, K., de la Cueva, H., Gulland, F.M.D., Aurioles-Gamboa, D., Arellano-Carbajal, F., Suarez-Güemes, F., 2003. Evidence of Leptospira interrogans infection in California sea lion pups from the Gulf of California. J. Wildl. Dis. 39 (1), 145–151. https://doi.org/10.7589/0090-3558-39.1.145.

Acevedo-Whitehouse, K., Spraker, T.R., Lyons, E., Melin, S.R., Gulland, F., Delong, R.L., Amos, W., 2006. Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups. Mol. Ecol. 15 (7), 1973–1982. https://doi.org/10.1111/j.1365-294X.2006.02903.x.

Acevedo-Whitehouse, K., Vicente, J., Gortazar, C., Hofle, U., Fernandez-De-Mera, I., Amos, W., 2005. Genetic resistance to bovine tuberculosis in the Iberian wild boar. Mol. Ecol. 14 (10), 3209–3217. https://doi.org/10.1111/j.1365-294X.2005.02656.x.

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T.M., Croxall, J.P., Bloch, D., Coulson, T., 2001. The influence of parental relatedness on reproductive success. Proc. R. Soc. Lond. Ser. B Biol Sci. 268 (1480), 2021–2027. https://doi.org/10.1098/rspb.2001.1751.

Annavi, G., Newman, C., Buesching, C.D., Macdonald, D.W., Burke, T., Dugdale, H.L., 2014. Heterozygosity–fitness correlations in a wild mammal population: accounting for parental and environmental effects. Ecol. Evol. 4 (12), 2594–2609. https://doi.org/10.1002/ece3.1112.

Aparicio, J.M., Ortego, J., Cordero, P.J., 2006. What should we weigh to estimate heterozygosity, alleles or loci? Mol. Ecol. 15 (14), 4659–4665. https://doi.org/10.1111/j.1365-294X.2006.03111.x.

Arct, A., Sudyka, J., Podmokła, E., Drobniak, S.M., Gustafsson, L., Cichoń, M., 2017. Heterozygosity–fitness correlations in blue tit nestlings (*Cyanistis caeruleus*) under contrasting rearing conditions. Evol. Ecol. 31 (5), 803–814. https://doi.org/10.1007/s10682-017-9911-6.

Bates, D.M., 2007. lme4: linear mixed-effects models using S4 classes. (No Title). https://cir.nii.ac.jp/crid/1370567187455717124.

Brambilla, A., Keller, L., Bassano, B., Grossen, C., 2018. Heterozygosity-fitness correlation at the major histocompatibility complex despite low variation in Alpine ibex (*Capra ibex*). Evol. Appl. 11 (5), 631–644. https://doi.org/10.1111/eva.12575.

Brock, P.M., Goodman, S.J., Hall, A.J., Cruz, M., Acevedo-Whitehouse, K., 2015. Context-dependent associations between heterozygosity and immune variation in a wild carnivore. BMC Evol. Biol. 15, 242. https://doi.org/10.1186/s12862-015-0519-6.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and

flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9 (2), 378–400. https://doi.org/10.32614/RJ-2017-066.
Budischak, S.A., Halvorsen, S., Finseth, F., 2023. Genomic heterozygosity is associated with parasite abundance, but the effects are not mediated by host condition. Evol. Ecol. 37 (1), 75–96. https://doi.org/10.1007/s10682-022-10175-8.
Buzan, E., Gerič, U., Potušek, S., Flajšman, K., Pokorny, B., 2020. First insights into the population genetic structure and heterozygosity–fitness relationship in roe deer inhabiting the area between the Alps and Dinaric mountains. Animals 10 (12). https://doi.org/10.3390/ani10122276.
Chapman, J.R., Nakagawa, S., Coltman, D.W., Slate, J., Sheldon, B.C., 2009. A quantitative review of heterozygosity–fitness correlations in animal populations. Mol. Ecol. 18 (13), 2746–2765. https://doi.org/10.1111/j.1365-294X.2009.04247.x.
Charpentier, M.J.E., Williams, C.V., Drea, C.M., 2008. Inbreeding depression in ring-tailed lemurs (*Lemur catta*): genetic diversity predicts parasitism, immunocompetence, and survivorship. Conserv. Genet. 9 (6), 1605–1615. https://doi.org/10.1007/s10592-007-9499-4.
Clutton-Brock, T., Sheldon, B.C., 2010. Individuals and populations: the role of long-term, individual-based studies of animals in ecology and evolutionary biology. Trends Ecol. Evol. 25 (10), 562–573. https://doi.org/10.1016/j.tree.2010.08.002.
Coltman, D.W., Pilkington, J.G., Smith, J.A., Pemberton, J.M., 1999. Parasite-mediated selection against inbred Soay sheep in a free-living island population. Evolution 53 (4), 1259–1267. https://doi.org/10.1111/j.1558-5646.1999.tb04538.x.
David, 1998. Heterozygosity–fitness correlations: new perspectives on old problems. Heredity 80 (5), 531–537. https://doi.org/10.1046/j.1365-2540.1998.00393.x.
David, P., Pujol, B., Viard, F., Castella, V., Goudet, J., 2007. Reliable selfing rate estimates from imperfect population genetic data. Mol. Ecol. 16 (12), 2474–2487. https://doi.org/10.1111/j.1365-294X.2007.03330.x.
DeWoody, J., 2005. Molecular approaches to the study of parentage, relatedness, and fitness: practical applications for wild animals. J. Wildl. Manag. 69 (4), 1400–1418. https://doi.org/10.2193/0022-541X(2005)69[1400:MATTSO]2.0.CO;2.
Ezenwa, V.O., 2003. Habitat overlap and gastrointestinal parasitism in sympatric African bovids. Parasitology 126 (4), 379–388. https://doi.org/10.1017/S0031182002002913.
Ezenwa, V.O., 2004a. Host social behavior and parasitic infection: a multifactorial approach. Behav. Ecol. 15 (3), 446–454. https://doi.org/10.1093/beheco/arh028.
Ezenwa, V.O., 2004b. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. Int. J. Parasitol. 34 (4), 535–542. https://doi.org/10.1016/j.ijpara.2003.11.012.
Ezenwa, V.O., Snider, M.H., 2016. Reciprocal relationships between behaviour and parasites suggest that negative feedback may drive flexibility in male reproductive behaviour. Proc. R. Soc. B Biol. Sci. 283 (1831), 20160423. https://doi.org/10.1098/rspb.2016.0423.
Ezenwa, V.O., Stefan Ekernas, L., Creel, S., 2012. Unravelling complex associations between testosterone and parasite infection in the wild. Funct. Ecol. 26 (1), 123–133. https://doi.org/10.1111/j.1365-2435.2011.01919.x.
Ezenwa, V.O., Worsley-Tonks, K.E.L., 2018. Social living simultaneously increases infection risk and decreases the cost of infection. Proc. Biol. Sci. 285 (1892). https://doi.org/10.1098/rspb.2018.2142.
Ferrer, E.S., García-Navas, V., Sanz, J.J., Ortego, J., 2014. Individual genetic diversity and probability of infection by avian malaria parasites in blue tits (*Cyanistes caeruleus*). J. Evol. Biol. 27 (11), 2468–2482. https://doi.org/10.1111/jeb.12489.
Ferrer, E.S., García-Navas, V., Sanz, J.J., Ortego, J., 2016. The strength of the association between heterozygosity and probability of interannual local recruitment increases with environmental harshness in blue tits. Ecol. Evol. 6 (24), 8857–8869. https://doi.org/10.1002/ece3.2591.
Forcada, J., Hoffman, J.I., 2014. Climate change selects for heterozygosity in a declining fur seal population. Nature 511 (7510), 462–465. https://doi.org/10.1038/nature13542.
Forrester, S.G., Lankester, M.W., 1997. Extracting protostrongylid nematode larvae from ungulate feces. J. Wildl. Dis. 33 (3), 511–516. https://doi.org/10.7589/0090-3558-33.3.511.
Fox, C.W., Reed, D.H., 2011. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65 (1), 246–258. https://doi.org/10.1111/j.1558-5646.2010.01108.x.
Gasbarre, L.C., Leighton, E.A., Sonstegard, T., 2001. Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. Vet. Parasitol. 98 (1–3), 51–64. https://doi.org/10.1016/s0304-4017(01)00423-x.
Gkafas, G.A., de Jong, M., Exadactylos, A., Raga, J.A., Aznar, F.J., Hoelzel, A.R., 2020. Sex-specific impact of inbreeding on pathogen load in the striped dolphin. Proc. R. Soc. B Biol. Sci. 287 (1922), 20200195. https://doi.org/10.1098/rspb.2020.0195.
Gorsich, E.E., Ezenwa, V.O., Jolles, A.E., 2014. Nematode–coccidia parasite co-infections in African buffalo: epidemiology and associations with host condition and pregnancy. Int. J. Parasitol. Parasit. Wildl. 3 (2), 124. https://doi.org/10.1016/j.ijppaw.2014.05.003.
Haanes, H., Markussen, S.S., Herfindal, I., Røed, K.H., Solberg, E.J., Heim, M., Midthjell, L., Sæther, B.-E., 2013. Effects of inbreeding on fitness-related traits in a small isolated moose population. Ecol. Evol. 3 (12), 4230–4242. https://doi.org/10.1002/ece3.819.
Hansson, B., Westerberg, L., 2008. Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects? Conserv. Genet. 9 (1), 73–83. https://doi.org/10.1007/s10592-007-9309-z.
Harrison, X.A., Bearhop, S., Inger, R., Colhoun, K., Gudmundsson, G.A., Hodgson, D., McELWAINE, G., Tregenza, T., 2011. Heterozygosity–fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects. Mol. Ecol. 20 (22), 4786–4795. https://doi.org/10.1111/j.1365-294X.2011.05283.x.
Hawley, D.M., Sydenstricker, K.V., Kollias, G.V., Dhondt, A.A., 2005. Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. Biol. Lett. 1 (3), 326–329. https://doi.org/10.1098/rsbl.2005.0303.
Hayward, A.D., Wilson, A.J., Pilkington, J.G., Clutton-brock, T.H., Pemberton, J.M., Kruuk, L.E.B., 2011. Natural selection on a measure of parasite resistance varies across ages and environmental conditions in a wild mammal. J. Evol. Biol. 24 (8), 1664–1676. https://doi.org/10.1111/j.1420-9101.2011.02300.x.
Hillegass, M.A., Waterman, J.M., Roth, J.D., 2010. Parasite removal increases reproductive success in a social African ground squirrel. Behav. Ecol. 21 (4), 696–700. https://doi.org/10.1093/beheco/arq041.
Hoffman, J.I., Simpson, F., David, P., Rijks, J.M., Kuiken, T., Thorne, M.A.S., Lacy, R.C., Dasmahapatra, K.K., 2014. High-throughput sequencing reveals inbreeding depression in a natural population. Proc. Natl. Acad. Sci. 111 (10), 3775–3780. https://doi.org/10.1073/pnas.1318945111.
Hulse, L. S., Thia, J. A., Schultz, B., Johnston, S. D., & Seddon, J. M. (2023). *Measures of inbreeding and heterozygosity-fitness correlations in koalas (Phascolarctos cinereus) from south-east Queensland, Australia*. https://doi.org/10.21203/rs.3.rs-3497287/v1.
Hurtrez-Boussès, S., Perret, P., Renaud, F., Blondel, J., 1997. High blowfly parasitic loads affect breeding success in a Mediterranean population of blue tits. Oecologia 112 (4), 514–517. https://doi.org/10.1007/s004420050339.
Judson, J.L.M., Knapp, C.R., Welch, M.E., 2018. Age-dependent, negative heterozygosity–fitness correlations and local effects in an endangered Caribbean reptile, *Iguana Delicatissima*. Ecol. Evol. 8 (4), 2088–2096. https://doi.org/10.1002/ece3.3826.
Kubacka, J., Podmokła, E., Korb, J., Dubiec, A., 2020. Heterozygosity and fitness in a threatened songbird: blood parasite infection is explained by single-locus but not genome-wide effects. J. Ornithol. 161 (3), 803–817. https://doi.org/10.1007/s10336-020-01753-0.
Lenth, R.V., 2016. Least-squares Means: the R package lsmeans. J. Stat. Softw. 69, 1–33. https://doi.org/10.18637/jss.v069.i01.
Lesbarrères, D., Primmer, C.R., Laurila, A., Merilä, J., 2005. Environmental and population dependency of genetic variability-fitness correlations in Rana temporaria. Mol. Ecol. 14 (1), 311–323. https://doi.org/10.1111/j.1365-294X.2004.02394.x.
Lorenzen, E.D., Arctander, P., Siegismund, H.R., 2008. Three reciprocally monophyletic mtDNA lineages elucidate the taxonomic status of Grant's gazelles. Conserv. Genet. 9 (3), 593–601. https://doi.org/10.1007/s10592-007-9375-2.
Luikart, G., Pilgrim, K., Visty, J., Ezenwa, V.O., Schwartz, M.K., 2008. Candidate gene microsatellite variation is associated with parasitism in wild bighorn sheep. Biol. Lett. 4 (2), 228–231. https://doi.org/10.1098/rsbl.2007.0633.
Lynch, M., Conery, J., Burger, R., 1995. Mutation accumulation and the extinction of small populations. Am. Nat. 146 (4), 489–518. https://doi.org/10.1086/285812.
Marr, A.B., Arcese, P., Hochachka, W.M., Reid, J.M., Keller, L.F., 2006. Interactive effects of environmental stress and inbreeding on reproductive traits in a wild bird population. J. Anim. Ecol. 75 (6), 1406–1415. https://doi.org/10.1111/j.1365-2656.2006.01165.x.
Martin, A.M., Cassirer, E.F., Waits, L.P., Plowright, R.K., Cross, P.C., Andrews, K.R., 2021. Genomic association with pathogen carriage in bighorn sheep (*Ovis canadensis*). Ecol. Evol. 11 (6), 2488–2502. https://doi.org/10.1002/ece3.7159.
Miller, J.M., Coltman, D.W., 2014. Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta-analysis. Mol. Ecol. 23 (8), 1899–1909. https://doi.org/10.1111/mec.12707.
Mitchell, J., Cant, M.A., Vitikainen, E.I.K., Nichols, H.J., 2017a. Smelling fit: scent marking exposes parasitic infection status in the banded mongoose. Curr. Zool. 63 (3), 237–247. https://doi.org/10.1093/cz/zox003.
Mitchell, J., Vitikainen, E.I.K., Wells, D.A., Cant, M.A., Nichols, H.J., 2017b. Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose. J. Zool. 302 (1), 32–39. https://doi.org/10.1111/jzo.12424.
O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. 142 (1), 1–15. https://doi.org/10.1016/j.vetpar.2006.08.035.
Paull, S.H., Johnson, P.T.J., 2018. How temperature, pond-drying, and nutrients influence parasite infection and pathology. EcoHealth 15 (2), 396–408. https://doi.org/10.1007/s10393-018-1320-y.
Portanier, E., Garel, M., Devillard, S., Maillard, D., Poissant, J., Galan, M., Benabed, S., Poirel, M.-T., Duhayer, J., Itty, C., Bourgoin, G., 2019. Both candidate gene and neutral genetic diversity correlate with parasite resistance in female Mediterranean mouflon. BMC Ecol. 19. https://doi.org/10.1186/s12898-019-0228-x.
Pujolar, J.M., Maes, G.E., Vancoillie, C., Volckaert, F.A.M., 2006. Environmental stress and life-stage dependence on the detection of heterozygosity–fitness correlations in the European eel, *Anguilla anguilla*. Genome 49 (11), 1428–1437. https://doi.org/10.1139/g06-104.
R Core Team, R. C, 2021. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. (No Title). https://cir.nii.ac.jp/crid/1370576118723163397.
Rijks, J.M., Hoffman, J.I., Kuiken, T., Osterhaus, A.D.M.E., Amos, W., 2008. Heterozygosity and lungworm burden in harbour seals (Phoca vitulina). Heredity 100 (6), 6. https://doi.org/10.1038/hdy.2008.18.

Rioux-Paquette, E., Festa-Bianchet, M., Coltman, D.W., 2011. Sex-differential effects of inbreeding on overwinter survival, birth date and mass of bighorn lambs. J. Evol. Biol. 24 (1), 121–131. https://doi.org/10.1111/j.1420-9101.2010.02154.x.

Ruiz-López, M.J., Gañan, N., Godoy, J.A., Del Olmo, A., Garde, J., Espeso, G., Vargas, A., Martinez, F., Roldán, E.R.S., Gomendio, M., 2012a. Heterozygosity-fitness correlations and inbreeding depression in two critically endangered mammals. Conserv. Biol. 26 (6), 1121–1129. https://doi.org/10.1111/j.1523-1739.2012.01916.x.

Ruiz-López, M.J., Monello, R.J., Gompper, M.E., Eggert, L.S., 2012b. The effect and relative importance of neutral genetic diversity for predicting parasitism varies across parasite taxa. PLoS One 7 (9), e45404. https://doi.org/10.1371/journal.pone.0045404.

Ryder, T.B., Tori, W.P., Blake, J.G., Loiselle, B.A., Parker, P.G., 2010. Mate choice for genetic quality: a test of the heterozygosity and compatibility hypotheses in a lek-breeding bird. Behav. Ecol. 21 (2), 203–210. https://doi.org/10.1093/beheco/arp176.

Sabey, K.A., Castro, A., Song, S.J., Knight, R., Ezenwa, V.O., 2024. Anthelmintic treatment reveals sex-dependent worm–gut microbiota interactions. Parasite Immunol. 46 (12), e70000. https://doi.org/10.1111/pim.70000.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18 (2), 2. https://doi.org/10.1038/72708.

Shaner, P.-J.-L., Chen, Y.-R., Lin, J.-W., Kolbe, J.J., Lin, S.-M., 2013. Sex-specific correlations of individual heterozygosity, parasite load, and scalation asymmetry in a sexually dichromatic lizard. PLoS One 8 (2), e56720. https://doi.org/10.1371/journal.pone.0056720.

Shearer, C.L., Ezenwa, V.O., 2020. Rainfall as a driver of seasonality in parasitism. Int. J. Parasitol. Parasit. Wildl. 12, 8–12. https://doi.org/10.1016/j.ijppaw.2020.04.004.

Slate, J., Kruuk, L.E.B., Marshall, T.C., Pemberton, J.M., Clutton-Brock, T.H., 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proc. R. Soc. Lond. Ser. B Biol. Sci. 267 (1453), 1657–1662. https://doi.org/10.1098/rspb.2000.1192.

Slate, J., Pemberton, J.M., 2002. Comparing molecular measures for detecting inbreeding depression. J. Evol. Biol. 15 (1), 20–31. https://doi.org/10.1046/j.1420-9101.2002.00373.x.

Smith, K.F., Acevedo-Whitehouse, K., Pedersen, A.B., 2009. The role of infectious diseases in biological conservation. Anim. Conserv. 12 (1), 1–12. https://doi.org/10.1111/j.1469-1795.2008.00228.x.

Smouse, P.E., Peakall, R., Gonzales, E., 2008. A heterogeneity test for fine-scale genetic structure. Mol. Ecol. 17 (14), 3389–3400. https://doi.org/10.1111/j.1365-294X.2008.03839.x.

Sperry, J.H., Cimprich, D.A., Peak, R.G., Weatherhead, P.J., 2009. Is nest predation on two endangered bird species higher in habitats preferred by snakes? Ecoscience 16 (1), 111–118. https://doi.org/10.2980/16-1-3198.

Stear, M.J., Strain, S., Bishop, S.C., 1999. Mechanisms underlying resistance to nematode infection. Int. J. Parasitol. 29 (1), 51–56. https://doi.org/10.1016/S0020-7519(98)00179-9.

Stelfox, J.B., Hudson, R.J., Groer, N., 1985. Relationships among physical traits, age and social status in Thomson's and Grant's gazelles. Appl. Anim. Behav. Sci. 13 (4), 347–357. https://doi.org/10.1016/0168-1591(85)90014-0.

Stromberg, B.E., 1997. Environmental factors influencing transmission. Vet. Parasitol. 72 (3), 247–264. https://doi.org/10.1016/S0304-4017(97)00100-3.

Sutton, J.T., Castro, I., Robertson, B.C., Tompkins, D.M., Stanton, J.-A.-L., Jamieson, I. G., 2016. MHC genetic diversity and avian malaria prevalence in Mokoia Island saddlebacks. N. Z. J. Ecol. 40 (3), 351–360.

Sweeny, A.R., Corripio-Miyar, Y., Bal, X., Hayward, A.D., Pilkington, J.G., McNeilly, T. N., Nussey, D.H., Kenyon, F., 2022. Longitudinal dynamics of co-infecting gastrointestinal parasites in a wild sheep population. Parasitology 149 (5), 593–604. https://doi.org/10.1017/S0031182021001980.

Szulkin, M., Bierne, N., David, P., 2010. Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64 (5), 1202–1217. https://doi.org/10.1111/j.1558-5646.2010.00966.x.

Szulkin, M., Garant, D., Mccleery, R.H., Sheldon, B.C., 2007. Inbreeding depression along a life-history continuum in the great tit. J. Evol. Biol. 20 (4), 1531–1543. https://doi.org/10.1111/j.1420-9101.2007.01325.x.

Szulkin, M., Sheldon, B.C., 2007. The environmental dependence of inbreeding depression in a wild bird population. PLoS One 2 (10), e1027. https://doi.org/10.1371/journal.pone.0001027.

Townsend, A.K., Taff, C.C., Wheeler, S.S., Weis, A.M., Hinton, M.G., Jones, M.L., Logsdon, R.M., Reisen, W.K., Freund, D., Sehgal, R.N.M., Saberi, M., Suh, Y.H., Hurd, J., Boyce, W.M., 2018. Low heterozygosity is associated with vector-borne disease in crows. Ecosphere 9 (10), e02407. https://doi.org/10.1002/ecs2.2407.

Ujvari, B., Madsen, T., 2006. Age, parasites, and condition affect humoral immune response in tropical pythons. Behav. Ecol. 17 (1), 20–24. https://doi.org/10.1093/beheco/ari091.

Vallender, R., Bull, R.D., Moulton, L.L., Robertson, R.J., 2012. Blood parasite infection and heterozygosity in pure and genetic-hybrid golden-winged warblers (*Vermivora chrysoptera*) across Canada. Auk 129 (4), 716–724. https://doi.org/10.1525/auk.2012.12013.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4 (3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Voegeli, B., Saladin, V., Wegmann, M., Richner, H., 2012. Parasites as mediators of heterozygosity–fitness correlations in the Great Tit (Parus major). J. Evol. Biol. 25 (3), 584–590. https://doi.org/10.1111/j.1420-9101.2011.02445.x.

Worsley-Tonks, K.E.L., Ezenwa, V.O., 2015. Anthelmintic treatment affects behavioural time allocation in a free-ranging ungulate. Anim. Behav. 108, 47–54. https://doi.org/10.1016/j.anbehav.2015.07.018.

Worsley-Tonks, K.E.L., Lance, S.L., Beasley, R.R., Jones, K.L., Ezenwa, V.O., 2015. Development and characterization of 30 novel microsatellite markers for Grant's gazelle (Nanger granti). Conserv. Genet. Resour. 7 (1), 219–221. https://doi.org/10.1007/s12686-014-0339-9.

Hartig, F., Hartig, M.F., 2017. Package 'dharma'. R package 531, 532.