Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded mongoose

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Abstract
Inbreeding, reproduction between relatives, often impinges on the health and survival of resulting offspring. Such inbreeding depression may manifest itself through immunological costs as inbred individuals suffer increased propensity to disease, infection and parasites compared to outbred conspecifics. Here, we assess how the intestinal parasite loads of wild banded mongooses (Mungos mungo) vary with pedigree inbreeding coefficient (ƒ) and standardized multi-locus heterozygosity. We find a significant association between increased heterozygosity and lower parasite loads; however, this correlation does not stand when considering ƒ. Such findings may be explained by local genetic effects, linkage between genetic markers and genes influencing parasite burdens. Indeed, we find heterozygosity at certain loci to correlate with parasite load. Although these tentative local effects are lost following multiple test correction, they warrant future investigation to determine their strength and impact. We also suggest frequent inbreeding within banded mongooses may mean heterozygosity is a better predictor of inbreeding than pedigree ƒ. This is because inbreeding facilitates linkage disequilibrium, increasing the chances of neutral markers representing genome-wide heterozygosity. Finally, neither ƒ nor heterozygosity had a significant influence on the loads of two specific gastrointestinal parasites. Nevertheless, more heterozygous individuals benefited from reduced overall parasitic infection and genetic diversity appears to explain some variation in parasite burdens in the banded mongoose.

Introduction
Breeding between relatives often entails a fitness cost termed inbreeding depression, which is thought to result mainly from the unmasking of harmful recessive alleles (Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009). Detrimental effects of inbreeding have been documented both in captive (Jimenez et al., 1994; Meagher, Penn & Potts, 2000) and wild vertebrates, where they can lead to substantial reductions in offspring fitness (Coltman et al., 2001; Reid, Arcese & Keller, 2003; Whiteman et al., 2006; Ilmonen et al., 2008). One mechanism through which inbreeding depression may act is by negatively impacting on immune function. Inbred individuals can suffer immune suppression and increased susceptibility to pathogens and disease (Coltman et al., 1999, 2001; Reid et al., 2003; Charpentier, Williams & Drea, 2008). As the immune response to parasitic infection is under genetic control, increased diversity across the genome may correlate with immunity active against a greater diversity of parasites (Reid et al., 2003; Whiteman et al., 2006). Indeed, populations with reduced genetic diversity are more susceptible to disease (De Castro & Bolker, 2004). Parasite loads also appear higher in inbred or homozygous compared to outbred conspecifics (Coltman et al., 1999, 2001; Cassinello, Gomendio & Roldan, 2001; Acevedo-Whitehouse et al., 2003; Charpentier et al., 2008). Thus, within and between populations, genetic diversity and heterozygosity appear correlated with reduced parasitic infection. Inbreeding is a growing threat to wildlife, owing to human induced habitat change (Frankham, 2010), and may increase susceptibility to disease outbreak and parasites in small or fragmented populations (De Castro & Bolker, 2004). Understanding how these factors may interact is crucial, yet our knowledge on the relationship between parasites and inbreeding in wild systems is limited. This may reflect well-known problems of using marker heterozygosity to estimate fitness effects of inbreeding, instead of pedigree inbreeding coefficients (ƒ). Pedigrees contain ancestral genetic information and have the power to detect inbreeding in previous generations. Thus, ƒ is considered the most robust

and accurate estimate of inbreeding unless a large number of genetic markers are used (Pemberton, 2004; Slate et al., 2004). However, few wild systems have pedigree data and inbreeding is instead estimated by heterozygosity at neutral markers such as microsatellites or SNPs. Correlations between marker heterozygosity and fitness-related traits are termed heterozygosity-fitness-correlations (HFCs hereafter) and require heterozygosity at the neutral marker to correlate with heterozygosity across areas of the genome under selection. This assumption has met wide criticism (Hansson & Westerberg, 2002; Slate et al., 2004; Miller & Coltman, 2014) and the field is receiving renewed interest due to the increased availability of genetic markers for wild populations (Chapman et al., 2009). Two hypotheses are commonly considered to explain HFCs; one possibility is that inbreeding causes associations between neutral markers and genome-wide heterozygosity, 'general effects'. Alternatively, HFCs may arise because particular markers are in linkage disequilibrium with non-neutral genes, 'local effects' (summarized in Hansson & Westerberg (2002)). Where both pedigree ƒ and molecular estimates of heterozygosity are available, their correlation tends to be weak leading to suggestions that heterozygosity does not accurately reflect ƒ (Balloux, Amos & Coulson, 2004; Pemberton, 2004; Slate et al., 2004). Indeed, the rationale in favour of ƒ is strong when concerning large, randomly breeding populations (Pemberton, 2004). In such cases, pedigree ƒ and heterozygosity will not be strongly correlated but not necessarily due to the weakness of the molecular markers. However, many populations deviate from panmixis, creating situations where marker heterozygosity may be a better predictor of inbreeding (Forstmeier et al., 2012; Ruiz-Lopez et al., 2012). The banded mongoose, Mungos mungo, represents an ideal species for which to investigate the relationship between parasite load and genetic diversity. A wild but habituated population has been studied in Queen Elizabeth National park for over 15 years yielding a full genetic pedigree. Faecal analyses show banded mongooses harbour multiple parasite taxa known to have fitness implications in other mammals (Table 1, appendix). Variation in parasite burdens is high across the study population, and there is frequent inbreeding leading to variation in inbreeding coefficients across individuals. This provides an ideal setting to investigate whether genetic diversity explains variation in parasite load, and to detect correlations between genetic diversity and fitness-related traits (Hansson & Westerberg, 2002). We focus on both overall parasite load, and two taxonomically distinct parasite genera, predicting genetically diverse individuals to show reduced parasite burdens compared to their inbred and more homozygous conspecifics.

Materials and methods

Study species

The banded mongoose is a small (<2 kg) diurnal carnivore of sub-Saharan Africa. Packs comprise 5–50 individuals (mean = 29) including a core of 2–5 males and 1–4 females who monopolize reproduction (Cant, 2000; Bell et al., 2012; Cant, Vitikainen & Nichols, 2013). There is little dispersal or immigration meaning many individuals remain in natal groups all their lives (Nichols et al., 2014). Thus, packs are generally composed of relatives and inbreeding is a regular occurrence. A total of 14.3% of pups appear moderately inbred (ƒ = 0.125) and 8% show inbreeding coefficients of ƒ ≥ 0.25, indicating breeding between first-order relatives (Nichols et al., 2014). Both inbreeding avoidance and inbreeding depression also occur; within-group breeding pairs appear less related than would be predicted by chance (Sanderson et al., 2015) and pups sired by extra-group fathers are significantly heavier at birth, more heterozygous and more likely to survive than those of within-group paternity (Nichols, Cant & Sanderson, 2015; Sanderson et al., 2015).

Study site and data collection

Data were gathered from five banded mongoose groups in Queen Elizabeth National Park, Uganda (0°120S; 27°540E) between 21st May and 6th August 2014. This population has been studied continuously for over 15 years under licence from the Uganda National Council for Science and Technology (UNCST) and Uganda Wildlife Authority (UWA). All research conducted on the population has been approved by the University of Exeter's Ethical committee in line with ASAB

standards for animal ethics. Full details of habitat and climate (Cant, 2000), demography and behaviour (Cant et al., 2013) are described elsewhere. All mongooses are habituated to close (<5 m) human observation and individuals are identifiable by unique haircuts (for full details, see Cant, 2000). Groups are visited by trained observers approximately every 2 days; meaning accurate ages, group compositions and life-history information is available. All animals are individually identified: pups are trapped by trained field staff at around 2–4 weeks of age, anaesthetized (Jordan et al., 2010) and given a unique identifier (a PIT tag or pre 2001, a tattoo), haircut and sexed by examination of the genital region (Jordan et al., 2010). The relationship between parasite load and both inbreeding coefficient (f) and standardized multi-locus heterozygosity (sMLH) was tested for 55 individuals over 6 months old. At this age, banded mongooses are foraging independently and samples can be collected routinely. Faecal samples were taken during morning foraging hours (7–11 am) between 21st May and 6th August 2014, individuals were sampled at least three times during the study period resulting in 185 samples overall. Faeces were collected immediately after deposition and care was taken to avoid other mongooses over-marking or contaminating the sample in any way. Faeces were homogenized and half transferred to a 50 mL Falcon tube containing ~15 mL of 5% formalin. The remaining half was left in the field so as not to disturb natural scent-marking behaviour. Samples were stored at room temperature before transportation to the UK for analysis under import permits from DEFRA.

Parasitology methods

Faecal samples were analysed by a modified McMaster technique in which parasite ova were extracted from faeces via floatation in 15 mL of saline solution (Dunn & Keymer, 1986). A 0.3 mL aliquot was transferred to a McMaster chamber where ova were counted and identified using the veterinary parasitology literature (Urquhart et al., 1996; Bowman, 2014). Ova counts were converted to an eggs per gram (EPG) value using the standard equation (15/0.3)Y/X, where Y represents the sum of all ova counted and X the weight of faecal matter from which the ova were obtained (Dunn & Keymer, 1986). EPG values were averaged across each individual for the duration of the study period. Such counts were calculated for overall parasite load and two specific parasite taxa; a coccidian within the genus Isospora and a cestode (tapeworm) most likely belonging to the genus Dipylidium. These were selected as they occurred in over 20% of individual samples, could be reliably identified at genus level and are known to have negative effects upon host fitness. Infection with Coccidia (coccidiosis) affects growth and survival across various other mammals (Lindsay, Dubey & Blagburn, 1997; Kirkpatrick, 1998; Mundt et al., 2006). Furthermore, as effects are most pronounced in the immunocompromised (Lindsay et al., 1997), coccidia represent ideal targets to consider in relation to immunological costs of inbreeding. Similarly, Dipylidium was selected, as cestode infections are often more severe in individuals with limited immune function (Olson et al., 2003). If inbreeding impacts immunocompetency in the banded mongoose, as it does in other mammals (Charpentier et al., 2008) and birds (Reid et al., 2003; Whiteman et al., 2006), we would therefore expect to see Dipylidium and/or Isospora loads co-vary with inbreeding coefficient. Faecal egg counts (FEC) face criticism regarding their accuracy in quantifying parasite load (Villanua et al., 2006; Gasso et al., 2015). Egg shedding can vary with the life stage of the parasite, environmental and host conditions (Dorchies et al., 1997; Villanua et al., 2006; Raharivololona & Ganzhorn, 2010). Thus, egg numbers in faeces may not represent the in-host parasite community. Nevertheless, FEC remain the best available method for estimating parasite burdens in wild systems. Here, it is unfeasible to kill individuals to gain comprehensive adult parasite counts from the gastrointestinal tract (Poulin & Morand, 2000). We aimed to reduce noise resulting from variation in parasite shedding by averaging FEC per individual. This should provide a comparable estimate parasite loads for the short study period as climatic conditions remained consistent (warm with negligible rainfall), groups retained stable territories and were not subject to large-scale predation events. Thus, average FEC is unlikely to be skewed by stressors such as weather fluctuations, abnormal foraging

patterns or territory shifts. Individuals across the population are also likely to have similar exposure to parasites due to their similar habitats (Cant, 2000) and preference for foraging in faeces of conspecifics and other mammals.

Genetic methods

Inbreeding coefficients (ƒ) were calculated from a nine-generation-deep pedigree of the study population. Pedigree construction used genetic data from 43 microsatellite markers, along with observational data, for full details Sanderson et al. (2015). The final pedigree used both Masterbayes 2.51 (Hadfield, Richardson & Burke, 2006) and COLONY 2.0.5.7 (Jones & Wang, 2010) to infer parentage (1570 maternities and 1476 paternities) at a probability of ≥0.8 across a 14-year period (Sanderson et al., 2015). Although no pedigree collected from the wild can be complete due to the presence of founding members and immigrants, our pedigree has very high coverage of the population (of the 61 individuals sample for parasite analysis, 56 were assigned both parents and grandparents). Previous research has found evidence of inbreeding depression in certain life-history traits, suggesting the pedigree has adequate power to detect relationships between genetic diversity and fitness-related traits. The effect of genome-wide heterozygosity upon parasite load was also considered as pedigree inbreeding may not always accurately reflect very deep inbreeding (Keller, Visscher & Goddard, 2011). This becomes particularly pronounced when the history of founding and immigrant members of a population are unknown (Keller et al., 2011), as is the case for the study population. Thus, standardized multi-locus heterozygosity (sMLH) was calculated from raw allele frequencies of the microsatellite markers (Sanderson et al., 2015). In order to gain a comparable assessment of heterozygosity for individuals with parasite data, we removed the loci with less than 90% coverage. Thus, sMLH was calculated from the 32 best amplified loci. We also calculated the parameter g2, a measure of heterozygosity–heterozygosity correlation between loci. If an effect of heterozygosity is due to a genome-wide effect of inbreeding, we would expect the level of heterozygosity at different loci to correlate and therefore $g2 > 0$. If g2 is exactly zero, then local effects are a more plausible explanation and it would not be possible to detect genome-wide effects of heterozygosity using this marker set (David et al., 2007; Szulkin, Bierne & David, 2010). All analyses involving genetic data used the inbreedR package (Stoffel et al., 2016).

Statistical methods

To investigate whether pedigree ƒ or sMLH affected overall average parasite load in this sample of 55 individuals, a generalized linear mixed effects model was constructed in R version 3.0.2 (R development Core Team, 2013). The MASS package (Venables & Ripley, 2002) fitted the model by a penalized-quasi likelihood (glmmPQL) with a negative binomial error distribution due to the over-dispersion of parasite data. The response variable, average eggs per gram parasite load was multiplied by 1000 to generate positive integer values required for a negative binomial model. Both ƒ and sMLH were included as fixed effects to consider their impact upon egg count. Sex (0 = male, 1 = female) and average age across the study period (in days, continuous numerical value) were included as additional fixed effects because in the closely related meerkat, Suricata suricatta, parasite burdens are recognized to vary with sex and age (Leclaire & Faulkner, 2014; Smythe & Drea, 2015). The possibility of sex-specific interactions with genetic variability merit consideration and thus interactions were included in the model. Pack identity (factor with five levels) was fitted as a random term to account for repeated sampling across social groups. Fitting pack as a fixed effect did not improve model performance nor did it give resolution as to pack-based difference in parasite burden. The model was run in full, then simplified using a backward stepwise process to sequentially remove each non-significant term. To test the effect of sMLH and inbreeding coefficient upon specific parasite taxa (Isospora and Dipylidium), the lme4 package (Bates, Machler & Dai, 2008) ran linear mixed effects models with Gaussian error distributions. Fixed and random effects remained as above.

Results

An average faecal sample harboured 320 _ 40 eggs (mean _ SE). Although four samples were devoid of eggs, all 55 individuals were infected with at least with one type of parasite during the study period. For this subset of the banded mongoose population, variance in ƒ calculated directly from the pedigree was 0.007 _ 0.011 (mean _ SE, range = 0–0.28), suggesting there is variability in inbreeding coefficients which should allow heterozygosity-fitness correlations to be detected. Although g2 was not significantly different from zero (g2 = 0.008, SE 0.008, P = 0.101), values >0 suggest markers should have enough power to detect genome-wide effects of heterozygosity (Szulkin et al., 2010).

Effect of genetic diversity on overall parasite load

Standardized multi-locus heterozygosity (sMLH) was the only factor to have a significant effect upon parasite load. More heterozygous individuals had lower average parasite loads (Table 1 and Fig. 1), while there was no effect of sex, age or inbreeding coefficient (ƒ). To confirm the effects of pedigree ƒ were not masked by collinearity with sMLH (correlation between sMLH and ƒ = 0.437 in the full model), the original model was re-run excluding sMLH. Following model simplification, neither ƒ nor any other fixed effects were significant (effect of ƒ from GLMM: Effect size = 1.822, SE = 1.544, P = 0.244). The relationship between parasite load and sMLH, but not ƒ suggests local genetic effects may influence parasite burdens. We thus ran negative binomial GLMMs considering the effect of heterozygosity at each locus on average parasite load (Table 2). Separate models were run for each locus with locus heterozygosity (coded 0 or 1) as their fixed effect and pack as a random factor. As before, models were fitted by glmmPQL. P-values were collated for the effect of heterozygosity at each loci and corrected by the Bonferroni multiple test correction (Abdi, 2007). Initially, four loci showed a significant correlation ($P < 0.05$) with parasite load, however, three were only marginally significant ($P > 0.03$) and none remained significant following the correction step (Mon35 P = 0.004, Bonferroni corrected level of acceptance P = 0.0016). Bonferroni corrections are conservative (Moran, 2003; Narum, 2006) meaning loci showing significant relationship with parasite load may be worthy of further empirical attention. Locus Mon35 showed the strongest effect (Table 2), and thus, we excluded it from sMLH calculations and re-ran our original, minimal model. Removing Mon35 from sMLH calculations rendered the effect of heterozygosity on parasite load non-significant (Table 3), suggesting this locus may impact parasite burden.

Effect of genetic diversity upon specified parasite taxa

sMLH had no effect on Isospora (LMM: Effects size -1.617, SD = 0.932, t-value -1.734, P-value 0.089) or tapeworm loads (LMM: Effect size = -13.51, SD = 14.91, t-value = -0.906, P-values = 0.369). All other fixed effects (age, sex and ƒ) remained non-significant. For full model output, see Tables S3 and S4.

Discussion

In the banded mongoose, heterozygous individuals showed significantly lower overall parasite loads than more homozygous conspecifics. Although only marginally significant, this implies genetic diversity may explain some variation in overall parasite burdens across this population. It also suggests an heterozygosity-fitness correlation (HFC) and supports studies in other animals where high pathogen loads correlate with reduced genetic diversity (Coltman et al., 1999; Luong, Heath & Polak, 2007; Ilmonen et al., 2008). There was no effect of heterozygosity upon the average load of Isospora or tapeworm ova, suggesting genetic diversity does not underpin variation patterns for these taxa. However, measures of individual parasites may be subject to error due to the limitations of FEC discussed previously. In all cases, pedigree inbreeding coefficient (ƒ) failed to explain parasite burdens. Why heterozygosity but not ƒ correlates with parasite load in the banded mongoose is puzzling. Considering the current pedigree's depth and detail, we would predict ƒ to accurately reflect genome-wide heterozygosity. One caveat may be assumptions made during initial pedigree construction. Generally, founding members of a population are assumed unrelated and outbred, yet

considering their demography, this is unlikely the case for banded mongooses. Groups form via budding dispersal where same-sex coalitions leave (or are forcibly evicted from) natal groups and seek out opposite-sex individuals (Nichols et al., 2012). Such coalitions will often be relatives from the same pack where inbreeding will have been a common occurrence (Nichols et al., 2012, 2014). Thus, founding members were likely inbred and related which may have led to biased assessments of ancestral inbreeding. However, we only selected individuals for which all four grandparents were present within the pedigree. This should have successfully removed bias in ƒ estimates resulting from assumptions during pedigree construction. Additionally, Keller et al. (2011) showed theoretically that only a small proportion of the variation in ƒ is missed due to ignorance of ancestral inbreeding >five generations ago. It has been suggested that heterozygosity better reflects inbreeding than pedigree ƒ in certain systems (Ruiz-Lopez et al., 2012). In zebra finches, Taeniopygia guttata, just 11 microsatellites generated stronger correlations with phenotypes than did ƒ. Authors attribute this to the high allelic diversity of their microsatellites and that much of the zebra finch genome is inherited in blocks which rarely experience meiotic cross-over (Forstmeier et al., 2012). Data are not currently available regarding segregation and cross-overs in the banded mongoose genome, yet this may explain why heterozygosity provides a better estimate of inbreeding depression. A sequenced genome could also consider the location of microsatellites, as laying within gene-rich regions would substantially increase their chances of linkage disequilibrium with fitness loci (Hansson & Westerberg, 2002). High rates of linkage disequilibrium can make heterozygosity a better predictor of inbreeding depression than ƒ values (Ruiz-Lopez et al., 2012) and inbreeding is one mechanism recognized to increase levels of linkage disequilibrium (Hansson & Westerberg, 2002; Slate et al., 2004; Miller & Coltman, 2014). Banded mongoose appear to tolerate substantial levels of inbreeding (Nichols et al., 2014), suggesting linkage disequilibrium remains high across the population and heterozygosity may indeed better reflect inbreeding than pedigree ƒ. However, the current pedigree has successfully identified inbreeding depression within juvenile life-history traits (Sanderson et al., 2015) supporting its power to uncover inbreeding and inbreeding depression. Alternatively, heterozygosity may reflect local genetic effects rather than a genome-wide association related to inbreeding. Although several loci showed significant correlations with heterozygosity, Bonferroni corrections rendered all nonsignificant (although one locus only marginally so). This may have occurred because local effects are small and difficult to detect (Hansson & Westerberg, 2002). Although other tests can detect the presence of local effects (Szulkin et al., 2010), these lose power once the number of individuals approaches the number of loci in the dataset. This would have been the case for our subset of the banded mongoose population which includes 55 individuals and 32 microsatellite loci. Nevertheless, once parasitic data are available for a larger sample, Szulkin's test could be employed for future consideration of local genetic effects. Secondly, Bonferroni corrections are conservative (Moran, 2003; Narum, 2006) and may dismiss local effects because of their small impact. Indeed, removing the most significant loci (Mon35) from sMLH calculations removed the effect of overall heterozygosity. Mon35 may thus be linked to an important immune-related gene that impacts parasite burden and future research should consider its position within the genome. Alternatively, prior to Bonferroni correction, heterozygosity at loci Mon9 and 41 appears to correlate with increased parasite loads. This opposite effect requires further empirical attention, but competition between multiple parasitic infections can protect hosts from exploitation by single, costly parasites. Thus, heterozygotes with multiple parasites may have a fitness advantage over more homozygous hosts with fewer pathogens.

To summarize, genetic diversity appears to impact overall parasite variation across this banded mongoose population. Heterozygosity correlated with lower overall parasite loads; however, the effect was contingent on one microsatellite marker and ƒ did not show a similar relationship. This implies local effects are at play. Yet, it is possible that frequent inbreeding within this population means linkage disequilibrium is high, leading sMLH to better predict genome-wide heterozygosity

than ƒ. To fully understand the relationship between inbreeding and parasite burdens will require further research on a larger subset of the population, ideally using genomic techniques.

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Figures and tables

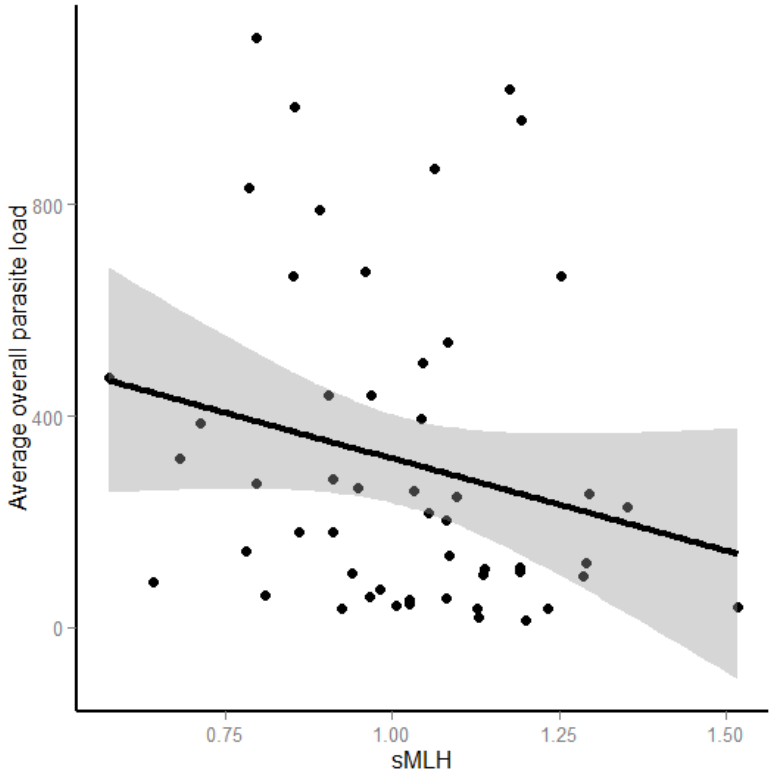

Figure 1 The correlation between standardized multi-locus heterozygosity (where 1 = population average) and average parasite load for adult banded mongooses. Points represent mean parasite burdens of each individual banded mongoose over the duration of this study period. Shading represents confidence intervals of 95%.

Table 1: The effect of standardised multi-locus heterozygosity (sMLH) upon average parasite load.

Fixed effect	Effect size	Standard error	t-value	p-value
Intercept	11.712	0.671		
sMLH	**-1.378**	**0.660**	**-2.090**	**0.042**
Sex (female)	0.080	0.299	0.272	0.787
Inbreeding coefficient	1.258	1.643	0.766	0.448
Age (in days)	<0.001	<0.001	0.666	0.509

GLMM fit by PQL, testing the effect of age, sex, inbreeding coefficient (f) and sMLH upon average ova load for the study period. sMLH had a significant effect, with more heterozygous individuals showing lower egg counts. The initial model included all fixed effects and second order interactions, and following a backward simplification method where non-significant (NS) terms were sequentially dropped from the model, only sMLH had a significant effect. NS fixed effects are presented in the table with p-values upon which they were dropped from the model.

Table 2: Relationship between single-locus heterozygosity and parasite load prior to Bonferroni corrections.

Loci ID	Effect size	Standard error	t-value	p-value
Mon16	0.171	0.255	0.668	0.508
Mon17	-0.144	0.254	-0.567	0.573
Mon25	-0.178	0.260	-0.684	0.497
Mon41	**0.606**	**0.259**	**2.342**	**0.023**
Mon69	***-0.642***	***0.292***	***-2.197***	***0.033***
Mon19	-0.353	0.273	-1.293	0.202
Mon32	-0.449	0.267	-1.685	0.098
Mon38	**-0.603**	**0.262**	**-2.300**	**0.026**
Mon65	-0.038	0.260	-0.146	0.885
Mon66	-0.284	0.254	-1.119	0.269
Mon67	0.487	0.347	1.403	0.167
Mon68	-0.144	0.265	-0.542	0.590
Mon70	0.007	0.292	0.024	0.980
Mon29	0.224	0.291	0.770	0.445
Mon31	-0.198	0.266	-0.744	0.460
Mon35	***-0.831***	***0.278***	***-2.99***	***0.004***
Mon36	***-0.535***	***0.257***	***-2.08***	***0.042***
Mon42	0.002	0.255	0.010	0.992
Mon49	0.150	0.301	0.498	0.621
Mon9	0.519	0.259	2.003	0.051
A226	0.237	0.338	0.702	0.486
A248	0.181	0.266	0.681	0.499
Ag6	0.025	0.292	0.087	0.931
Hj35	***-0.658***	***0.319***	***-2.063***	***0.046***
M53	-0.009	0.267	-0.032	0.975
Mm10-7	0.013	0.255	0.051	0.959
Mm5-1	0.008	0.276	0.030	0.976
ss10-4	-0.041	0.261	-0.158	0.875
ss13-8	-0.184	0.275	-0.667	0.508
TGN	0.188	0.259	0.726	0.471
FS15	-0.272	0.266	-1.020	0.313
FS44	0.242	0.313	0.774	0.443

Output of multiple GLMMs testing the effect of heterozygosity at each loci upon parasite load. Six loci initially showed significant effects ($p<0.05$), and are displayed in bold italics. However, Bonferroni corrections adjusted the critical p-value to 0.0016 (0.05/32) rendering all single-locus effects non-significant.

Table 3: Output of GLMM testing the effect of sMLH (minus Mon35) upon parasite load.

Fixed effect	**Effect size**	**Standard error**	**t-value**	**p value**
Intercept	11.402	0.680		
sMLH (minus locus Mon35)	-1.045	0.668	-1.564	0.124

Heterozygosity no longer has a significant effect upon parasite load when Mon35 is removed from sMLH calculations. This suggested there may be a local genetic effect of heterozygosity at this locus influencing parasite burdens.