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ORIGINAL ARTICLE

Heterozygosity and lungworm burden in harbour seals (*Phoca vitulina*)

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In several studies, heterozygosity measured at around 10 microsatellite markers correlates with parasite load. Usually the effect size is small, but while this may reflect reality, it may also be possible that too few markers are used or the measure of fitness contains too much error to reveal what is actually a much stronger underlying effect. Here, we analysed over 200 stranded harbour seals (*Phoca vitulina*) for an association between lungworm burden and heterozygosity, conducting thorough necropsies on the seals and genotyping the samples obtained for 27 microsatellites. We found that homozygosity predicts higher worm burdens, but only in young animals, where the worms have the greatest impact on fitness. Testing each locus separately, we found that a significant majority reveal a weak but similar trend for heterozygosity to be protective against high lungworm burden, suggesting a genome-wide effect, that is, inbreeding. This conclusion is supported by the fact that heterozygosity is correlated among markers in young animals but not in otherwise equivalent older ones. Taken as a whole, our results support the notion that homozygosity increases susceptibility to parasitic infection and suggest that parasites can be effective in removing inbred individuals from the population.
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Introduction

The publication of the measure mean d-squared, an estimator of microsatellite allele similarity, and the demonstration that it predicts aspects of fitness in deer and seals (Coltman *et al*., 1998; Coulson *et al*., 1998) stimulated renewed interest in the link between heterozygosity and fitness (David, 1998). Subsequent studies have developed both new and more effective measures for estimating heterozygosity (Coltman *et al*., 1999; Amos *et al*., 2001; Aparicio *et al*., 2006) and confirmed that panels of as few as 10 presumed neutral microsatellite markers often reveal statistically significant correlations with fitness. Over the last decade, the list of fitness traits found to be associated with heterozygosity has expanded greatly, from the initial analyses based on juvenile survival (Coltman *et al*., 1998), now to cover parasite susceptibility (Coltman *et al*., 1999), reproductive success (Slate *et al*., 2000; Hoffman *et al*., 2004) and even behavioural traits, such as territory size (Seddon *et al*., 2004). Such apparent ubiquity makes these heterozygosity–fitness correlations (HFCs) a potentially important component of natural selection and suggests that understanding their prevalence and basis could help elucidate a number of evolutionary processes.

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Some of the strongest HFCs have been recorded in studies of parasite load (Coltman *et al*., 1999) and infectious disease. In rehabilitating sea lions, all classes of sick animals revealed elevated homozygosity (Acevedo-Whitehouse *et al*., 2003), whereas in studies of infectious disease, heterozygosity has been implicated separately in both viral (Valsecchi *et al*., 2004) and bacterial (Acevedo-Whitehouse *et al*., 2003) diseases, as well as influencing the strength of the innate immune response (Hawley *et al*., 2005). However, the exact mechanism underlying these correlations remains obscure (Hansson and Westerberg, 2002; Pemberton, 2004). On the one hand, many authors have invoked inbreeding depression, arguing that heterozygosity at neutral markers reflects genome-wide heterozygosity, which in turn varies with inbreeding coefficient. On the other hand, both theory and simulations suggest that individuals with detectably non-zero inbreeding coefficients are usually too rare in nature to create HFCs, occurring only in small, isolated populations or species with strong polygyny (Balloux *et al*., 2004; Slate *et al*., 2004).

Here, we examine the relationship between heterozygosity and the macroscopic presence of lungworm infection in harbour seals (*Phoca vitulina*). Lungworm infection is an important cause of morbidity and mortality in harbour seals, in particular in young seals (Measures, 2001; Vercruysse *et al*., 2003; Lehnert *et al*., 2007a). Lungworm infection is caused by two macroparasites (Vercruysse *et al*., 2003), often found concurrently (Lehnert *et al*., 2007a): *Otostrongylus circumlitus*, a large species whose adults are easily visible in the

principal airways and, *Parafilaroides gymnurus*, a smaller species found in the alveoli and smaller bronchioles. For the analysis, we used 27 polymorphic loci genotyped in a sample of 204 harbour seals that stranded along the Dutch coast, taking into account the relationship between lungworm burden and age. In addition, to test whether the relationship between heterozygosity and lungworm burden is dominated by a genome-wide rather than a single-locus effect, we first tested for a correlation in heterozygosity across loci and then compared the number of homozygotes and heterozygotes at every locus among seals in which worms were observed with those in which they were not seen.

Materials and methods

The sample set

The sample set was an exhaustive selection of tissue samples obtained from seals that stranded and died in the Dutch Wadden Sea in the period 1997–2003, whose death was associated either with lungworm infection ($n=26$), phocine distemper virus infection (PDV; $n=169$) or trauma or drowning in fishing nets ($n=9$) and for which relevant data were available (Figure 1). The necropsies were performed following the procedure detailed in Kuiken and Baker (1991). This included the registration of available stranding data and clinical information, specifically: determination of species and sex, standard measurements, including nose-to-tail body length, and description of gross lesions. During gross necropsy, the bronchi and lung tissue were incised and examined visually for the presence of lungworms and associated lesions. Where necessary, samples for histology, virology, bacteriology, parasitology and toxicology were collected and tested to determine the probable cause of death. Death from lungworm infection or noninfectious causes of death was established based on gross lesions, presence of and numbers of parasites, nutritional state and stomach contents. Death from phocine distemper virus infection was established by gross lesions and confirmed by either reverse-transcriptase PCR or IgM serology (Jensen *et al*., 2002).

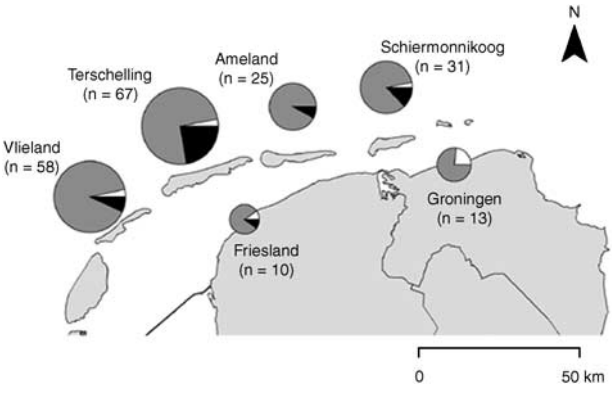

Figure 1 Spatial distribution and probable cause of death of the seals in the sample set. The diameter of the pie chart corresponds to the number of seals stranded at a particular location and the colours in the pie chart indicate the probable cause of death: death associated with lungworm infection (black), phocine distemper infection (grey) or trauma or by-catch (white).

The samples used for DNA extraction were mostly of kidney tissue, initially stored frozen at −20 and −70 °C, later transferred to 96% ethanol and stored at room temperature. In the absence of a kidney sample, lung, spleen or blood was used. A small number of kidney samples ($n=13$) were stored in lysis buffer (6 M Guanidine-HCL, 10 mM Urea, 10 mM Tris–HCl, 20% Triton-X100 (v/v), pH 4.4) at −20 °C and at room temperature. DNA was extracted using an adapted Chelex protocol (Walsh *et al*., 1991).

Genotyping

Microsatellite genotyping, scoring and data entry were conducted as described previously (Hoffman and Amos, 2005). Briefly, PCR reactions were carried out in 10-μl reaction volumes containing 1 μl template DNA, 1 × Thermalase buffer (10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Tween 20, 0.1% gelatine, 0.1% IGEPAL), 60 mM tetramethylammonium chloride (TMAC), 2.5% formamide, 0.1 mM dGTP, 0.1 mM dATP, 0.1 mM dTTP, 0.02 mM dCTP, 4 pmol of each primer, 0.25 units of Taq polymerase and 0.01 μCi [α^{32}P]dCTP. Loci were amplified using the following PCR profile: one cycle of 120 s at 94 °C, 45 s at T1, 50 s at 72 °C; 10 cycles of 30 s at 94 °C, 45 s at T1, 50 s at 72 °C; 25 cycles of 30 s at 89 °C, 45 s at T2, 50 s at 72 °C; and one final cycle of 5 min at 72 °C. For the majority of loci, T1 was 46 °C and T2 was 48 °C. However, for Pv3, Hg6.1, OrrFCB7 and OrrFCB8, T1 and T2 were 50 and 55 °C respectively. PCR products were resolved by electrophoresis on standard 6% polyacrylamide sequencing gels and detected using a phosphoimager (Fujifilm BAS-2500). Genotypes were scored by two independent observers (JMR and JIH) and entered manually into a Microsoft Excel spreadsheet. Genotypes were tested for deviations from Hardy–Weinberg equilibrium and linkage disequilibrium using GENEPOP (http://www.genepop.curtin.edu.au/, Raymond and Rousset, 1995). For each test, we set the dememorization number to 10 000, the number of batches to 1000 and the number of iterations per batch to 10 000.

Calculation of heterozygosity

Several alternative ways to calculate heterozygosity have recently been proposed, all of which aim to produce a measure that correlates most strongly with *F*, the inbreeding coefficient. These measures include methods to compensate for missing genotypes (standardized heterozygosity, SH, Coltman *et al*., 1999), to exploit the evolutionary similarity of different alleles (mean d-squared, Coulson *et al*., 1998) and to use allele frequencies to weight scores according to the alleles in the genotype (internal relatedness, IR, Amos *et al*., 2001) or the diversity of the loci being scored (HL, Aparicio *et al*., 2006). Which of these performs best depends on several factors, including the completeness of genotyping, the diversity of the loci being scored and the range of inbreeding coefficients, *F*, represented in the sample. However, mean d-squared is now seen as performing relatively poorly unless two genetically distinct populations have recently mixed (Balloux *et al*., 2004) whereas SH tends to perform marginally less well than IR across a range of scenarios (Amos *et al*., 2001; Balloux *et al*., 2004). Heterozygosity weighted by locus

HL is a recently proposed method that has yet to be used widely, but simulations suggest that it can outperform IR, particularly at loci with higher allelic diversity (Aparicio *et al*., 2006). In view of the above, we chose to use both IR and HL to conduct a comparison.

Data analyses

Seals were categorized as 'infected' or 'uninfected' depending on the presence or absence of lungworms in the respiratory tract at gross necropsy. Using sex and body length to estimate age (McLaren, 1993), males up to 95 cm and females up to 90 cm were considered to be up to 1-year-old, and categorized as 'young' in this paper; the remaining seals referred to as 'older'.

The relationship between heterozygosity and lungworm burden was first analysed by comparing the mean of the measure of heterozygosity (HL, IR) of all infected seals ($n=54$) to that of all uninfected seals ($n=150$; *t*-test). Then, as infection and mortality due to high worm burden is most likely to occur in the first year of life, we performed the same analysis for young seals (total $n=43$; 29 infected, 14 uninfected; *t*-test). To verify that the reduction to this age category was justified, we tested the significance of the interaction between age category (young, older) and lungworm burden (uninfected, infected) by performing a univariate analysis of variance on a model with the measure of heterozygosity (HL, IR) as dependent variable and age category (young, older) and lungworm status (uninfected, infected) as explanatory variables. These analyses were performed in SPSS.

Finally, any relationship between heterozygosity and lungworm burden could be due either to a genome-wide (inbreeding) effect or to a single locus effect caused by chance linkage between one or more of our markers and a gene experiencing balancing selection. To test which is the more likely mechanism in the current data set, we tested whether heterozygosity was correlated across loci using the method of Balloux *et al*. (2004). Here, the loci are divided randomly into two equal groups and then a correlation coefficient is calculated across individuals between the paired heterozygosity estimates, one from each group of markers. By repeating this process 100 times, each time dividing the markers into different groupings, one can assess the robustness of any correlation present. A robust positive correlation suggests that one or more inbred individuals are present in the group, with stronger correlations, suggesting greater numbers of inbred individuals and higher *F*-values. We applied this test to young animals ($n=43$) and older animals ($n=161$) separately. In addition, we used a Fisher's exact test to ask whether, at each locus in turn, there was a difference in heterozygosity between infected and uninfected young individuals. If one or a small number of loci contribute to any effect found, these should yield an equivalent number of significant tests. However, under a genome-wide effect, we expect to find that most loci reveal similar weak trends for heterozygotes to be uninfected.

Results

Microsatellite locus selection

We considered 94 published pinniped microsatellite primers (Allen, 1995; Allen *et al*., 1995; Coltman *et al*., 1996; Gemmell *et al*., 1997; Goodman, 1997, 1998; Buchanan *et al*., 1998; Davis *et al*., 2002; Hernandez-Velaquez *et al*., 2005; Hayes *et al*., 2006; Hoffman *et al*., 2006; Wolf *et al*., 2006). Of these, 38 had previously been tested on four harbour seals from Scotland and were dismissed because they were either monomorphic or failed to amplify. The remaining 56 loci were tested on a panel of 43 Dutch harbour seals, revealing 30 that were polymorphic (Table 1), 19 that were monomorphic and 7 that either gave unscorable results or failed to amplify. Three of the thirty polymorphic loci (Lw18, Pvc74 and ZwcF09) were subsequently excluded because they were not in Hardy–Weinberg equilibrium. As previously reported by Davis *et al*. (2002), Lw18 is likely to be X chromosome-linked because Hardy–Weinberg equilibrium is observed in females (females, test for HWE $P=0.5444$) but not in males (males, test for HWE, $P=0.0000$). This left 27 loci, all but one of which carried six or fewer alleles in the Dutch population, the last locus (Pv3) carrying 23 alleles (four alleles on average). Following sequential Bonferroni correction to compensate for multiple statistical tests, we found no evidence that any locus exhibited significant linkage disequilibrium with any other. Observed heterozygosity varied between 0.023 (M11a) and 0.905 (Pv3) and was on average 0.336 (see Table 1).

Heterozygosity and lungworm burden

In the sample as a whole ($n=204$ individuals), mean measures of heterozygosity did not vary significantly between the uninfected and infected seals, though the difference in means did go in the direction expected if heterozygosity increases resistance to worm infection (mean $HL_{150\ \text{uninfected seals}}=0.512$; mean $HL_{54\ \text{infected seals}}=0.531$; *t*-test: $P=0.314$; mean $IR_{150\ \text{uninfected seals}}=0.014$; mean $IR_{54\ \text{infected seals}}=0.050$; *t*-test: $P=0.313$). However, lungworm burden was more common in young seals (29/43 infected) than in older seals (25/161 infected) and the proportion of deaths due to lungworm was also greater in young seals (Figure 2). When only the group of young seals was considered, the average heterozygosity was significantly greater in uninfected than in infected young seals, suggesting heterozygosity does increase resistance to lungworm infection (mean $HL_{14\ \text{uninfected seals}}=0.431$; mean $HL_{29\ \text{infected seals}}=0.543$; *t*-test: $P=0.006$; mean $IR_{14\ \text{uninfected seals}}=-0.114$; mean $IR_{29\ \text{infected seals}}=0.061$; *t*-test: $P=0.027$). The interaction between age category and lungworm burden was significant for HL but not for IR (HL univariate analysis of variance, $P=0.012$; IR univariate analysis of variance, $P=0.053$), validating the cutoff between age categories for HL but not quite for IR.

Genome-wide or single locus effects

When all adults were used to test for a correlation in heterozygosity among loci, the mean correlation coefficient was 0.018 (± 0.06 s.d.) and did not differ significantly from zero, implying that this group contains few or no appreciably inbred individuals (Figure 3). In contrast, among young animals, the mean correlation coefficient was 0.213 (± 0.087 s.d.), much larger and significantly greater than zero, implying the presence of inbred individuals. In addition, when each locus in turn

Table 1 Number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E) and probability values for derivation from Hardy–Weinberg equilibrium (HWE *P*-value) at 56 microsatellite loci for harbour seals that stranded along the Dutch coast

Locus	*GenBank accession No.*	*Isolated from species (reference)*	*No. of alleles in harbour seals in previous publications*	*No. of alleles in Scottish harbour seals* (n = 4)[a]	*No. of alleles in Dutch harbour seals* (n = 231)	H_O	H_E	*HWE P-value*
The 27 polymorphic loci used in this study								
Aa4	—	South American fur seal *Arctocephalus australis* (Gemmell *et al*., 1997)	2 (Gemmell *et al*., 1997)	2	2	0.028	0.028	1.000
Hg6.1	G02091	Grey seal *Halichoerus grypus* (Allen *et al*., 1995)	7[b], 3[c] (Goodman, 1998)	—	3	0.249	0.271	0.031
Hg6.3	G02092	Grey seal *H. grypus* (Allen *et al*., 1995)	8[b], 4[c] (Goodman, 1998)	3	3	0.383	0.421	0.297
Hg8.9	G02094	Grey seal *H. grypus* (Allen *et al*., 1995)	2 (Gemmell *et al*., 1997)	—	2	0.303	0.347	0.098
Hg8.10	G02093	Grey seal *H. grypus* (Allen *et al*., 1995)	4 (Gemmell *et al*., 1997)	—	2	0.489	0.500	0.789
Hgdii	G02095	Grey seal *H. grypus* (Allen *et al*., 1995)	2 (Gemmell *et al*., 1997)	4	3	0.036	0.035	1.000
Hl2	AF417692	Leopard seal *Hydrurga leptonyx* (Davis *et al*., 2002)	2 (Davis *et al*., 2002)	1	2	0.419	0.437	0.535
Hl15	AF140587	Leopard seal *H. leptonyx* (Davis *et al*., 2002)	2 (Davis *et al*., 2002)	4	4	0.176	0.203	0.180
Hl20	AF140589	Leopard seal *H. leptonyx* (Davis *et al*., 2002)	4 (Davis *et al*., 2002)	4	3	0.224	0.210	0.834
Lw7	AF140591	Weddell seal *Leptonychotes weddellii* (Davis *et al*., 2002)	4 (Davis *et al*., 2002)	2	5	0.714	0.689	0.765
Lw20	AF140595	Weddell seal *L. weddellii* (Davis *et al*., 2002)	4 (Davis *et al*., 2002)	5	3	0.343	0.345	1.000
Lc28	AF140584	Crabeater seal *Lobodon carcinophagus* (Davis *et al*., 2002)	3 (Davis *et al*., 2002)		4	0.096	0.102	0.432
M11a	—	Southern elephant seal *Mirounga leoni* (Hoezel, unpublished data as cited by (Gemmell *et al*., 1997))	2 (Gemmell *et al*., 1997)	2	2	0.023	0.023	1.000
OrrFCB2	G34934	Walrus *Odobenus rosmarus rosmarus* (Buchanan *et al*., 1998)	—	3	5	0.468	0.470	0.950
OrrFCB7	G34928	Walrus *O. rosmarus rosmarus* (Buchanan *et al*., 1998)	—	3	3	0.482	0.504	0.747
OrrFCB8	G34929	Walrus *O. rosmarus rosmarus* (Buchanan *et al*., 1998)	—	4	5	0.582	0.544	0.647
Pv2	U65441	Harbour seal *Phoca vitulina vitulina* (Goodman, 1997)	3 (Goodman, 1997)	—	3	0.309	0.349	0.136
Pv3	U65442	Harbour seal *P. vitulina vitulina* (Goodman, 1997)	33[b], 22[c] (Goodman, 1998)	—	23	0.905	0.928	0.804
Pv9	G02096	Harbour seal *P. vitulina* (Goodman, 1997)	1 (Goodman, 1997)	2	2	0.046	0.063	0.015
Pv10	U65443	Harbour seal *P. vitulina vitulina* (Goodman, 1997)	2[b], 2[c] (Goodman, 1998)		2	0.058	0.056	1.000
Pv11	U65444	Harbour seal *P. vitulina vitulina* (Goodman, 1997)	7[b], 3[c] (Goodman, 1998)	5	3	0.503	0.521	0.871
Pvc30	L40986	Harbour seal *P. vitulina concolor* (Coltman *et al*., 1996)	2 (Coltman *et al*., 1996)	—	4	0.521	0.483	0.483
71HDZ301	—	Steller sea lion *Eumetopias jubatus* (Huebinger *et al*., 2007)	—	3	6	0.629	0.625	0.924
ZcwA12	DQ836320	Galápagos sea lion *Zalophus californianus wollebaeki* (Hoffman *et al*., 2006)	3 (Hoffman *et al*., 2006)	3	5	0.514	0.519	0.717
ZcwD02	AM039816	Galápagos sea lion *Z. californianus wollebaeki* (Wolf *et al*., 2006)	—	3	2	0.126	0.155	0.027
ZcwF07	DQ836326	Galápagos sea lion *Z. californianus wollebaeki* (Hoffman *et al*., 2006)	3 (Hoffman *et al*., 2006)	3	3	0.261	0.240	0.112
ZcCgDh1.8	AY676475	Californian sea lion *Z. californianus* (Hernandez-Velaquez *et al*., 2005)	—	4	3	0.180	0.192	0.127
The three polymorphic loci not in Hardy–Weinberg equilibrium								
Lw18	AF140596	Weddell seal *Leptonychotes weddellii* (Davis *et al*., 2002)	4 (Davis *et al*., 2002)	4	4	0.382	0.642	0.000
Pvc74	L40988	Harbour seal *Phoca vituli concolor* (Coltman *et al*., 1996)	2 (Coltman *et al*., 1996)	—	2	0.000	0.018	0.000
ZcwF09	DQ836327	Galápagos sea lion *Zalophus californianus wollebaeki* (Hoffman *et al*., 2006)	3 (Hoffman *et al*., 2006)	3	3	0.268	0.331	0.000

—: not tested.
The allele frequencies are compared to allele frequencies found in other studies or populations.
[a]Hoffman *et al*., 2006 and Hoffman unpublished data.
[b]In harbour seals of the North Sea (Goodman, 1998).
[c]In harbour seals of the Western Wadden Sea (Goodman, 1998).

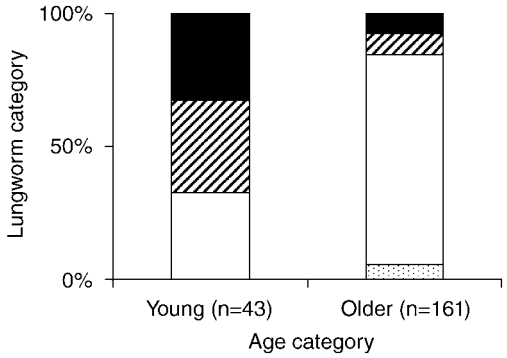

Figure 2 Relative frequency of lungworm infection in the two age groups (total $n=204$). Seals in which lungworms were observed macroscopically (infected) either died of lungworm infection (black areas) or phocine distemper virus infection (slanted hatched areas); seals in which no lungworms were observed macroscopically ('uninfected') died of phocine distemper virus infection (white areas) or by-catch or trauma (dotted area). Seals were classified into age groups based on sex and length. Males up to 95 cm and females up to 90 cm were classified as 'young', all larger seals as 'older'.

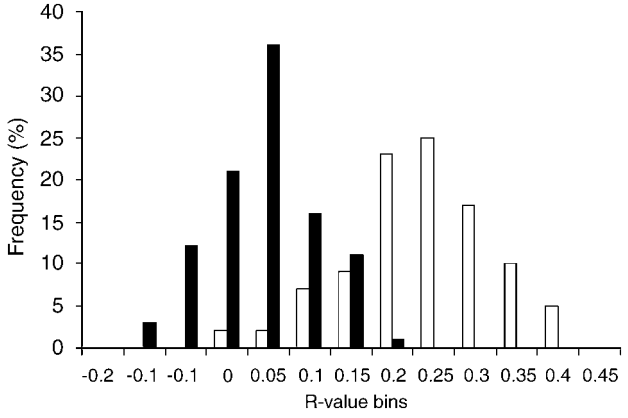

Figure 3 Correlation in heterozygosity among markers in older seals (black bars, $n=161$) compared with young seals (white bars, $n=43$). In each set of animals, the 27 microsatellite markers, for which the animals were typed, were divided randomly into two equal groups and the correlation coefficient, R, between the estimates for each group calculated. The bar chart above presents data for 100 replicates. Values centering on zero suggest that inbred individuals are rare or absent, while increasingly positive values suggest that the presence of inbred individuals (strictly, a higher variance in the underlying inbreeding coefficients of the sample).

was tested for a difference in heterozygosity between infected and uninfected young seals, none of the P-values were significant (Fisher's exact test at each locus). However, 20 of the 27 loci did reveal a tendency for greater homozygosity in the infected animals, a significant imbalance (Sign test, $P=0.019$). Such a pattern is most likely to result from a slight tendency towards heterozygote advantage affecting every locus, that is, a genome-wide or inbreeding effect, rather than one or a few loci showing a big effect.

Discussion

In this paper, we examined the relationship between heterozygosity and lungworm burden in harbour seals that stranded on the Dutch coast and died. Overall, there is no relationship between worm burden and heterozygosity. However, lungworms exert their maximum impact on fitness in young animals soon after weaning. When the analysis was repeated in this light, comparing affected young animals with all others, a significant difference in heterozygosity was found. The pattern appears to be driven by inbreeding depression, with young animals showing evidence of inbreeding not found among adults and no marker standing out as showing a dominant contribution.

Our finding of a link between heterozygosity and lungworm burden in young animals but not in the data set analysed as a whole appears to reflect the life history of the parasite. Lungworms appear to infect harbour seals early in life. The mode of infection is unknown, though it is likely that infective larvae develop in fish and infect seals through the oral route (Measures, 2001; Lehnert *et al*., 2007b). Some young seals develop higher burdens than others and, among these, some die as a result of their infections. Generally, in domestic animals, worm burden following primary infection depends on the exposure conditions, for example parasite intake, and on host immunity, first innate and then specific. The specific immunity that develops and helps clear the primary infection also impedes the development of worms in subsequent infections (Tizard, 2004), as has been shown, for example, in calves infected with lungworm (*Dictyocaulus viviparus*) (Eysker *et al*., 1994; Scott *et al*., 1996). The development of protective specific immunity against lungworms in harbour seals has not been proven, but seals do develop antibodies against lungworms (Elson-Riggins *et al*., 2004) and lungworms are uncommon in older seals. Our data reflect this in that the strongest association between genotype and worm burden occurs early in life when the impact of the worms is likely to be largest.

In revealing the lungworm HFCs, we compared two measures of heterozygosity: IR, which weights allele sharing by the frequencies of the alleles in a genotype, and HL, which weights heterozygosity by the variability of each locus at which an individual is homozygous. Our results largely support the claim made by Aparicio *et al*. (2006) that HL is often a 'better' measure in the sense that in most of the significant trends we find, HL generates a lower P-value than IR. We therefore endorse the use of HL as the measure of choice for studying heterozygosity–fitness correlations in natural populations, though it is still of interest to compare the two measures, since they are expected to perform somewhat differently depending on the variability of loci being used (Aparicio *et al*., 2006).

There is currently considerable debate concerning whether heterozygosity–fitness correlations are due mainly to genome-wide changes in heterozygosity brought about by inbreeding or through chance associations between markers and neighbouring genes under balancing selection (Hansson and Westerberg, 2002; Balloux *et al*., 2004; DeWoody and DeWoody, 2005; Ferreira and Amos, 2006). Despite our rather small sample size of affected young animals, our analysis suggests that the relationship between heterozygosity and lungworm burden is dominated by a genome-wide effect, that is, inbreeding. We provide two lines of evidence. First, heterozygosity is essentially uncorrelated across loci among adult animals, but is rather strongly correlated among young animals. Such a pattern is

consistent with the young animals including some individuals with *F*-values that are high enough to detectably impact on the probability that any given marker is heterozygous. Just how many inbred individuals we have sampled cannot be determined without deploying hundreds rather than tens of markers. The second line of evidence is that, when each locus is tested separately for a relationship between heterozygosity and worm burden, no one locus stands out, but instead a significant majority reveal a trend in the direction of homozygosity conferring susceptibility. Such a pattern is consistent with a genome-wide effect.

The presence of a detectable genome-wide effect, and in particular, the large difference between adults and juveniles, is interesting in the context of how selection acts on a population through parasites and disease. It is known that harbour seals exhibit strong population substructure, with significant genetic differences between populations that are easily within the reach of dispersing youngsters (Goodman, 1998). Such structure has the potential to allow or even promote some level of inbreeding. In our sample of individuals from the Dutch population, we do indeed find evidence of inbred individuals, but only among the juveniles, not the much larger sample of adults. By implication, any individuals who are born to related parents are disproportionately likely to suffer lethal worm burdens, which in turn removes them from the population. This therefore agrees with earlier studies that suggested inbred individuals carry more parasites, a greater diversity of parasites and may provide a weak point through which new pathogens can enter the population (Coltman *et al*., 1999; Acevedo-Whitehouse *et al*., 2003; Valsecchi *et al*., 2004).

In conclusion, we reveal a signal in the direction of reduced heterozygosity correlating with lungworm burden in young seals. Our results emphasize the importance of accurately quantifying fitness. Worm burden alone reveals little unless considered alongside measures of age and an appreciation of the age-specific effects of lungworm infection on seal morbidity and mortality.

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