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Susceptibility to intestinal parasites and juvenile survival are correlated with multilocus microsatellite heterozygosity in the Capercaillie (*Tetrao urogallus*)

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Inbreeding can have a negative influence on several life-history traits as well as disease resistance in birds and mammals through different genetic mechanisms. Endangered and declining populations may be at particular risk for inbreeding. The level of inbreeding can be estimated by assessing individual heterozygosity at neutral microsatellite markers. We studied the relationships between intestinal helminth infections, age, sex and heterozygosity in Capercaillie (*Tetrao urogallus*). We assessed microsatellite heterozygosity at eight autosomal loci and calculated two different indices (multilocus heterozygosity *MLH* and mean d^2) to quantify individual heterozygosity. Capercaillie were infected by three species of cestodes (*Paroniella urogalli*, *Skrjabinia cesticillus* and *Hymenolepis* sp.) and one nematode species (*Ascaridia compar*). We found that the probability of nematode infection decreased as the level of heterozygosity (measured by mean d^2) increased. Also, the intensity of nematode infection decreased as heterozygosity (measured by *MLH*) increased. However, we did not observe correlation between heterozygosity and the occurrence of cestodes. In addition, heterozygosity (both *MLH* and mean d^2) was dependent on age class: adult Capercaillie had higher heterozygosity than juveniles. Results suggest selection for heterozygosity which can be reinforced by differences in genetic parasite resistance.

1. Introduction

Loss of genetic diversity through inbreeding depression has been reported in a wide variety of wild animal and plant populations (Keller & Waller 2002). Inbreeding depression can result from increased homozygosity of deleterious alleles or decreased number of superior heterozygotes in the case of overdominance (Charlesworth & Charlesworth 1987, Keller & Waller 2002). As inbreeding leads to less heterozygous genotypes, its level has been estimated by evaluating the hete-

rozygosity of the genome using appropriate genetic markers, such as highly variable microsatellites (Coltman *et al*. 1998, Coulson *et al*. 1998). Several indices in addition to simple heterozygosity (H) have been developed to describe genotypes, e.g., mean d^2 (Coulson *et al*. 1998), internal relatedness (IR) (Amos *et al*. 2001) and homozygosity by loci (HL) (Aparicio *et al*. 2006).

Earlier studies have reported that individual heterozygosity at neutral microsatellite markers is correlated positively with components of fitness, such as fecundity (Amos *et al*. 2001) and lifetime reproductive success (Slate *et al*. 2000). In the harbour seal *Phoca vitulina*, juvenile survival has been connected to heterozygosity expressed as mean d^2 (Coltman *et al*. 1998) and similarly recruitment of Great Reed Warbler (*Acrocephalus arundinaceus*) was more successful in fledglings with higher mean d^2 (Hansson *et al*. 2001, 2004). In the Black Grouse (*Tetrao tetrix*), microsatellite heterozygosity was lower in males that never obtained a territory during breeding (lekking) season and consequently failed to breed (Höglund *et al*. 2002). Also, there was a positive relationship between mean d^2 and lifetime copulation success (Höglund *et al*. 2002).

Heterozygosity may also be connected to more specific traits such as resistance against parasites. In the Mountain White-crowned Sparrow (*Zonotrichia leucophrys oriantha*), heterozygosity measured by microsatellite diversity was lower in individuals infected with the blood parasite *Haemoproteus* (MacDougall-Shackleton *et al*. 2005). The heterozygosity level may influence survival in the presence of parasites, as in the case of Soay sheep (Coltman *et al*. 1999). More homozygous, parasitized individuals can suffer from higher mortality while no heterozygosity-related differences in mortality are seen in animals with medically reduced parasite burdens.

Heterozygosity-fitness correlations (HFCs) are generally weak, but they have been consistently found in natural populations (Coltman & Slate 2003, Chapman *et al*. 2009). In order to detect such correlations, sample sizes must be large and the population structure must allow inbreeding (Chapman *et al*. 2009, Szulkin *et al*. 2010). At the population level, HFCs can occur when there are matings between relatives, reduced genetic variation (due to genetic drift or recent bottlenecks) or a flow of genetically different individuals (admixture or immigration) (Szulkin *et al*. 2010). On the other hand, the observed correlations between heterozygosity and fitness should be interpreted with caution when only a small number (up to ten) of microsatellites are available for analysis (Slate & Pemberton 2002). The correlation between microsatellite heterozygosity and true genomic heterozygosity can be poor (DeWoody & DeWoody 2005).

In this study, we examined the associations between intestinal parasite infections, age class, sex and the level of inbreeding estimated by microsatellite heterozygosity in wild Capercaillie (*Tetrao urogallus*) populations in Finland. The Eurasian Capercaillie lives and breeds in coniferous taiga forests from Scandinavia to Central Siberia and in the Alpine forests of Central Europe (Cramp & Simmons 1980). Finnish Capercaillie appear to form a genetically continuous population (mitochondrial DNA, Liukkonen-Anttila *et al*. 2004) in which dispersing individuals probably cause a homogenising gene flow (Liukkonen *et al*. 2007, Mäki-Petäys *et al*. 2007). Demographic properties that might contribute to inbreeding in a Capercaillie population are male philopatry and a strongly polygynous mating system which prevents random mating. Capercaillie males compete for females in leks in the spring and only a few "top" males get to breed. Intestinal helminth parasites are common in the Capercaillie and previously it has been shown that the occurrence of these parasites depends on host sex and age (Isomursu *et al*. 2006).

We aimed at studying if genomic heterozygosity affects the distribution of parasites and even the overall survival of the Capercaillie. Our first hypothesis was that the more homozygous (inbred) Capercaillie would suffer from lowered parasite resistance and would be more commonly or more intensely infected by parasites. Secondly, we hypothesized that if viability is connected to heterozygosity, the heterozygosity of individuals would increase with age.

2. Material and methods

2.1. Sampling of birds

The Capercaillie is listed in the European Union Birds Directive (2009/147/EC) Article 7 (http://ec.europa.eu/environment/nature/legislation/birdsdirective/index_en.htm). Species referred to in Annex II/Part B may be hunted in the EU Member States under certain regulations; thus, the Capercaillie samples analysed for this study were obtained from the legal annual game bag.

In 1996–2002, hunters from five game management districts in Finland (Lappi, Oulu, Kainuu, Keski-Suomi and Satakunta) collected intestines from shot grouse during the regular hunting season in autumn from September 10th through October 31st. For each individual, one of the wings was also included to determine age (adult or juvenile) and sex (Cramp & Simmons 1980). Altogether 196 Capercaillie were sampled for this study. Samples were frozen in plastic bags as soon as possible and kept frozen until the examination, when the intestines were dissected and macroscopic helminth parasites were extracted and stored in 10% formalin solution for later identification. Nematodes were individually counted and identified microscopically. Cestodes were dyed with carmine red (Sigma Chemicals) and identified microscopically. Cestodes could not be counted due to the fragmentation of most of the worms. Consequently, we analysed the occurrence and intensity of nematode infection while only the occurrence was studied for cestodes.

2.2. DNA extraction

DNA extraction was conducted from the wing primary feather quills. The feather quills were cut into small pieces and put into 100 µl buffer, which contained 0.1 M Tris-HCl (pH 8.5), 0.5 mM EDTA, 0.2% SDS, 0.2 M NaCl and 0.03 mg of Proteinase K. The quills were then incubated in 56ºC for three hours and afterwards centrifuged in 10,000 rpm for 10 min. After this the DNA was precipitated from the supernatant with 200 µl of ice-cold ethanol and 10 µl of 3 M Na-acetate (pH 5.2), washed and diluted into 50 µl of deionized water.

2.3. Microsatellite analyses

We assayed genetic variation at eight microsatellite loci, TUD1-TUD6 and TUT2-TUT3 (Segelbacher et al. 2000, with slight modifications). In the final PCR step we used the temperature 60°C for 30 minutes. To minimize errors in genetic typing (Taberlet *et al*. 1999), we regenotyped 95% of individuals at least once using electrophoresis (ABI Prism 377 system) to ensure consistent allele sizing. We scored altogether 184 individuals for all eight loci, and additionally scored ten individuals for seven and two for six loci.

2.4. Statistical analyses

We estimated the expected heterozygosities and *F* statistics using FSTAT for Windows version 2.9.3.2 (Goudet 2001). F_{is} relates the individual heterozygosity to the expected heterozygosity in a randomly mating subpopulation. The resulting ratio is positive when there is heterozygote deficiency. At the population level, F_{is} quantifies the heterozygote deficiency caused by non-random mating whereas F_{st} is related to a reduction in heterozygosity due to non-random mating among subpopulations. We estimated the level of inbreeding as the multilocus heterozygosity (*MLH*) and mean d^2 over eight microsatellite loci. Multilocus heterozygosity is the proportion of heterozygous loci within an individual. We calculated mean d^2 according to Coulson *et al.* (1998) as the squared distance in repeat units between the two alleles an individual had at a microsatellite locus, averaged over all loci at which an individual was scored.

We applied generalized linear modelling with a binomial distribution and logit link function in the analysis of both cestode and nematode infection occurrence (presence/absence data). Furthermore, we applied generalized linear modelling with a negative binomial distribution and log link function in the analysis of nematode infection intensity. An earlier study suggests that nematode abundance depends on age and sex (Isomursu *et al*. 2006). Hence we included age and sex as factors and used each of the two measures of heterozygosity as covariates. We estimated model parameters by including only age and sex. Furthermore, we analysed more complex models with

Table 1. Observed and expected heterozygosities, and F_{is} by locus. Statistically significant deviations from Hardy-Weinberg equilibrium are in boldface (*P* values are based on randomizations and Bonferroni adjustment).

Locus	H_o	H_e	F_{is}	N
TUD1	0.54	0.80	**0.33**	195
TUD2	0.57	0.61	0.06	196
TUD3	0.58	0.82	**0.29**	188
TUD4	0.81	0.84	0.04	196
TUD5	0.73	0.89	**0.18**	192
TUD6	0.50	0.86	**0.42**	195
TUT2	0.76	0.79	0.03	196
TUT3	0.63	0.70	0.09	196

both of the heterozygosity indices and their interaction terms with age and sex. We estimated six different models: for the occurrence of each parasite class, two models were calculated, each including one heterozygosity index and for the intensity of nematode infection, two models, one for each heterozygosity index, were calculated. In model selection we used Akaike Information Criteria and Akaike weights (Burnham & Anderson 2002).

We used analysis of variance to assess differences in heterozygosity between age classes and sexes. We performed these statistical analyses using SPSS version 15.0.1.

3. Results

Capercaillie harboured three cestode species (*Paroniella* (*Raillietina*) *urogalli*, *Skrjabinia* (*Raillietina*) *cesticillus* and *Hymenolepis* sp.) and one nematode species (*Ascaridia compar*). All cestodes were pooled together in the statistical analysis because of the similar distribution pattern in regard to host age and sex (data not shown). Prevalence of cestodes and nematodes was 28% and 29%, respectively.

All loci were highly variable with the number of alleles, ranging from 8 to 21. Generally, loci showed heterozygote deficiency (Table 1). These results support an earlier microsatellite study on Finnish Capercaillie (Mäki-Petäys *et al*. 2007). Heterozygote deficiency indicates inbreeding or geographical differences between subpopulations. In theory, both explanations may be valid here due to the relatively short natal dispersal of Capercaillie (Storch & Segelbacher 2000) and the large geographical coverage of samples. We estimated *F* statistics for five different geographic areas (game management districts). Comparison of F_{st} (0.010, 95% CI 0.005–0.014) and mean F_{is} (0.200, 95% CI 0.101–0.299) suggests that non-random mating was the main cause for heterozygote deficiency, not geographical differentiation caused by genetic drift among subpopulations.

Heterozygosity indices did not vary statistically significantly between areas and years (*MLH*, year: $F_{6,165} = 0.48$, $P = 0.82$; *MLH*, area: $F_{4,165} = 0.75$, $P = 0.56$; mean d^2, year: $F_{6,165} = 0.41$, $P = 0.87$; mean d^2, area: $F_{4,165}=0.63$, $P=0.65$) and there was no significant interaction between year and area (*MLH*, $F_{17,165} = 0.46$, $P = 0.97$; mean d^2, $F_{17,165} = 0.67$, $P = 0.83$).

Indices of heterozygosity explained poorly the occurrence of cestodes (Table 2). All *P* values for the heterozygosity indices in the models were greater than 0.05. The occurrence of cestodes was significantly explained by the age of the bird (Table 2). Juveniles were more often infected by cestodes than adults (prevalence 43% in juveniles and 5% in adults).

The occurrence and intensity of nematode infection was explained by the age and sex of the bird as expected (Table 3; see Isomursu *et al*. 2006). Adults were more often and more heavily infected by nematodes than juveniles, mean number of worms in adult males being 7.3, adult females 4.7, juvenile males 0.6 and juvenile females 0.2.

Mean d^2 explained the occurrence of nematode infection (Table 3). Low mean d^2 predicted a high probability to have an infection (Fig. 1). The interaction between sex and mean d^2 was also included in the best model suggesting that the effect of mean d^2 was more pronounced among males (Table 3, Fig. 1). Furthermore, both heterozygosity indices were included into the best models to explain nematode infection intensity (Table 4). The number of nematodes was higher among more homozygous individuals (Fig. 2).

Model including interaction between sex and *MLH* got some support. However, *P* values for these interactions were high. The best model with mean d^2 included its interactions with both age

Table 2. Generalized linear models for the occurrence of cestodes in Capercaillie (presence/absence data). Akaike Information Criteria (AIC_c) and Akaike weight (*w*) are shown for each model. Model 1 fits best to the data having the highest *w*. All analysed variables and interactions are shown, but statistics are shown only for those included in models 1–3.

		Model 1		Model 2		Model 3	
Variable	df	Wald	*P*	Wald	*P*	Wald	*P*
MLH	1	2.20	0.138	–	–	2.20	0.139
Age	1	5.18	0.023	24.09	0.000	5.19	0.023
Sex	1	0.04	0.838	0.05	0.827	0.00	0.981
MLH ×Age	1	3.37	0.066	–	–	3.37	0.069
MLH ×Sex	1	–	–	–	–	0.00	0.970
MLH ×Age×Sex	1	–	–	–	–	–	–
Constant	1	4.53	0.033	33.47	0.000	4.52	0.034
AIC_c	–	72.44	–	73.70	–	74.56	–
w	–	0.426	–	0.226	–	0.147	–
Mean d^2	1	–	–	1.29	0.255	1.72	0.190
Age	1	24.09	0.000	24.77	0.000	9.87	0.002
Sex	1	0.05	0.827	0.08	0.781	0.10	0.753
Mean d^2×Age	1	–	–	–	–	0.41	0.520
Mean d^2×Sex	1	–	–	–	–	–	–
Mean d^2×Age×Sex	1	–	–	–	–	–	–
Constant	1	33.47	0.000	23.77	0.000	15.79	0.000
AIC_c	–	199.56	–	200.36	–	202.08	–
w	–	0.426	–	0.286	–	0.121	–

Table 3. Generalized linear models for the occurrence of nematodes in Capercaillie (presence/absence data). Akaike Information Criteria (AIC_c) and Akaike weight (*w*) are shown for each model. Model 1 fits best to the data, having the highest *w*. All analysed variables and interactions are shown, but statistics are shown only for those included in models 1–3.

		Model 1		Model 2		Model 3	
Variable	df	Wald	*P*	Wald	*P*	Wald	*P*
MLH	1	–	–	0.75	0.387	0.50	0.480
Age	1	30.86	0.000	31.03	0.000	5.76	0.016
Sex	1	3.94	0.047	4.01	0.045	4.14	0.042
MLH ×Age	1	–	–	–	–	0.69	0.406
MLH ×Sex	1	–	–	–	–	–	–
MLH ×Age×Sex	1	–	–	–	–	–	–
Constant	1	31.18	0.000	0.65	0.419	0.76	0.385
AIC_c	–	73.61	–	74.95	–	76.36	–
w	–	0.477	–	0.245	–	0.121	–
Mean d^2	1	4.34	0.037	5.13	0.024	4.72	0.030
Age	1	31.90	0.000	4.91	0.027	3.79	0.051
Sex	1	6.70	0.010	5.67	0.017	3.57	0.059
Mean d^2×Age	1	–	–	1.62	0.204	2.61	0.107
Mean d^2×Sex	1	3.78	0.052	2.64	0.104	–	–
Mean d^2×Age×Sex	1	–	–	–	–	–	–
Constant	1	2.39	0.122	1.43	0.232	1.55	0.213
AIC_c	–	192.81	–	193.09	–	193.70	–
w	–	0.295	–	0.257	–	0.189	–

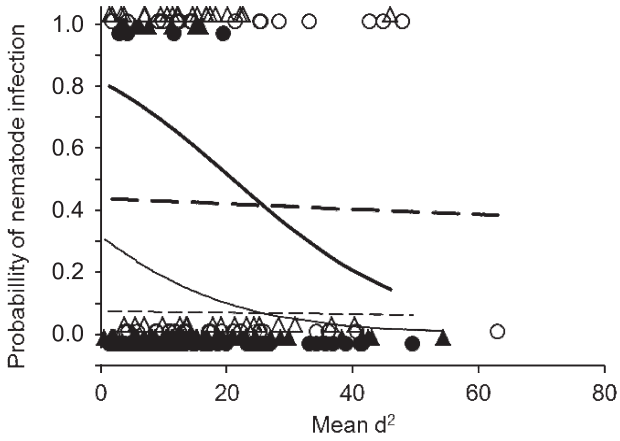

Fig. 1. Predicted nematode infection probability (lines) by sex and age class in relation to mean d^2 based on the best generalized linear model model (see Table 3). Observed values: Open triangle = adult male, open circle = adult female, solid triangle = juvenile male, solid circle = juvenile female.

and sex. The impact of heterozygosity on nematode intensity was stronger among males and adults (Fig. 2). Adults were also more commonly infected with nematodes than juveniles, prevalences being 53% and 13%, respectively.

Also, we conducted complementary analyses where we included nematode-infected birds only. The power of the analyses was reduced due to reduction of sample size (53 infected birds). Especially, the number of infected juveniles was low due to low infection prevalence among them. In the analysis of *MLH*, the best model remained the same as for the whole dataset including variables *MLH* (coefficient = –1.257±0.830), age and sex. However, in the analysis of mean d^2 the best model included only variables age and sex.

Table 4. Generalized linear models for the nematode infection intensity. Akaike Information Criteria (AIC_c) Akaike weight (*w*) and coefficient for heterozygosity measure are shown for each model. Model 1 fits best to the data, having the highest *w*. All analysed variables and interactions are shown, but statistics are shown only for those included in models 1–3.

		Model 1		Model 2		Model 3	
Variable	df	Wald	*P*	Wald	*P*	Wald	*P*
MLH	1	10.57	0.001	10.37	0.001	8.86	0.003
Age	1	159.36	0.000	155.56	0.000	15.23	0.000
Sex	1	11.73	0.001	0.10	0.749	11.77	0.001
MLH ×Age	1	–	–	–	–	0.05	0.820
MLH ×Sex	1	–	–	0.47	0.494	–	–
MLH ×Age×Sex	1	–	–	–	–	–	–
Constant	1	18.28	0.000	17.88	0.000	16.34	0.000
AIC_c	–	644.61	–	646.27	–	646.66	–
w	–	0.490	–	0.216	–	0.175	–
Coefficient for *MLH*±SE	–	–1.789±0.550		–1.408±0.777		–1.876±0.675	
Mean d^2	1	14.16	0.000	13.75	0.000	14.28	0.000
Age	1	29.56	0.000	156.75	0.000	27.97	0.000
Sex	1	8.99	0.003	11.68	0.001	9.23	0.002
Mean d^2×Age	1	2.72	0.099	–	–	2.96	0.085
Mean d^2×Sex	1	4.20	0.041	6.71	0010	3.90	0.048
Mean d^2×Age×Sex	1	–	–	–	–	0.23	0.630
Constant	1	27.76	0.00	25.89	0.000	27.97	0.000
AIC_c	–	639.97	–	640.84	–	641.88	–
w	–	0.408	–	0.263	–	0.156	–
Coefficient for mean d^2±SE		–0.054±0.022		–0.071±0.019		–0.053±0.022	

Table 5. Mean ± SE of *MLH* and mean d^2 for both sexes and age classes, and results of ANOVAs showing separate models for *MLH* and mean d^2. Full factorial models, statistics for interaction and intercept are not shown. Statistically significant factors are in boldface. * = mean d^2 was ln-transformed prior to statistical testing.

	Female		Male		Age		Sex	
	Juvenile	Adult	Juvenile	Adult	$F_{1,192}$	*P*	$F_{1,192}$	*P*
N	56	38	62	40				
MLH	0.621±0.025	0.672±0.031	0.613±0.024	0.690±0.030	5.28	**0.023**	0.03	0.854
Mean d^2	15.65±1.60	20.66±2.28	14.94±1.44	16.48±1.62	4.67*	**0.032***	1.00*	0.319*

Analysis of variance revealed that age significantly explained the variance of both heterozygosity indices (Table 5). Adults had higher values of *MLH* and mean d^2 than juveniles (Table 5, Fig. 3).

4. Discussion

We obtained partial support for our first hypothesis: susceptibility to nematodes in Capercaillie was dependent on the level of heterozygosity. Both heterozygosity indices were included in the best models to explain the intensity of nematode infection i.e., the number of worms. The number of nematodes was higher among more homozygous individuals. The occurrence of nematodes was correlated with only mean d^2: individuals with lower mean d^2 were more likely to be infected with nematodes. This pattern suggests that a low level of heterozygosity both impairs the individual ability to control the infection and, to some extent, the ability to prevent the infection altogether. We did not observe a correlation between heterozygosity and the occurrence of cestodes. However, we

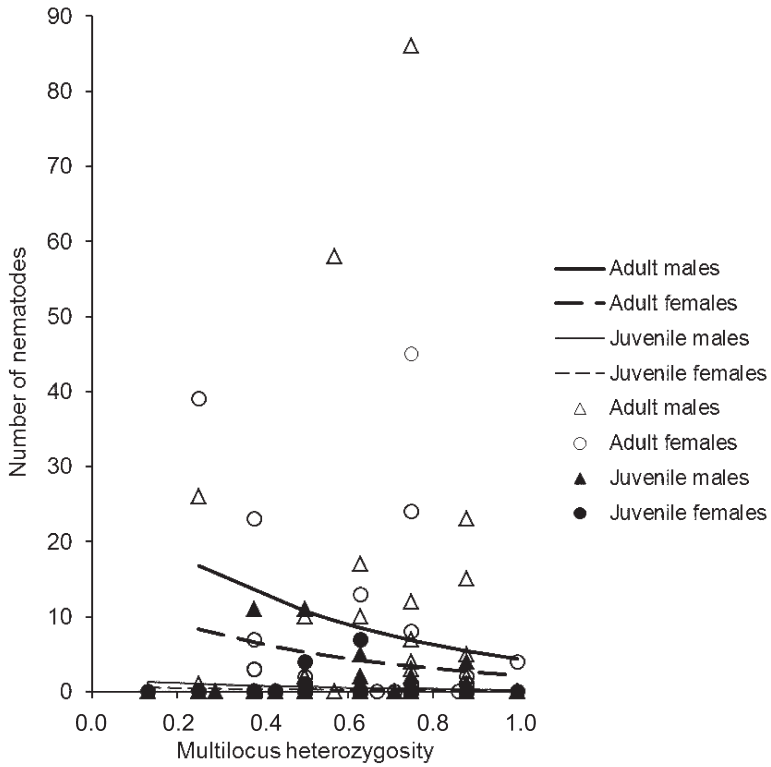

Fig. 2. Predicted nematode infection intensity (lines) by sex and age class in relation to multilocus heterozygosity (*MLH*) based on the best generalized linear model (see Table 4). Observed values: Open triangle = adult male, open circle = adult female, solid triangle = juvenile male, solid circle = juvenile female.

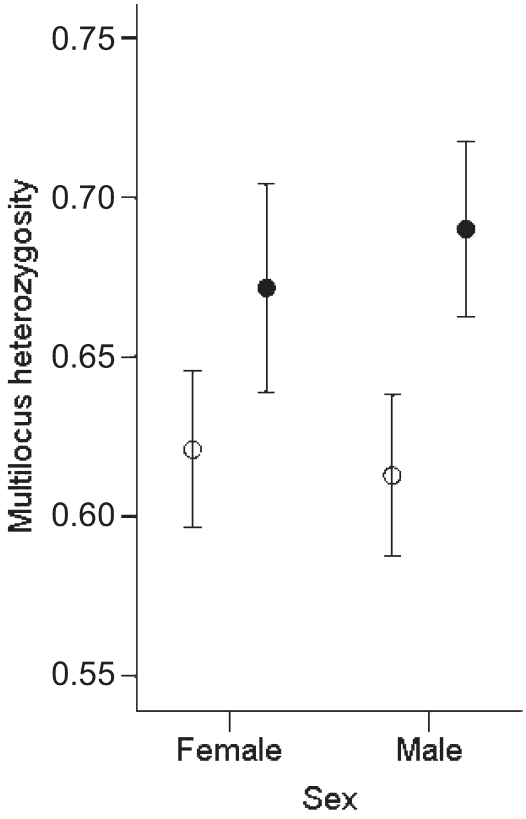

Fig. 3. Multilocus heterozygosity (*MLH*) (±SE) of different age classes and sexes. Open circles = juveniles, solid circles = adults.

could not study the intensity of cestode infection.

The age class of the host explained the occurrence of both cestodes and nematodes. Juvenile Capercaillie were more often infected by cestodes, whereas nematode infections were more common and more intense in adults. In the case of nematodes, host sex was also a significant factor, as males were more heavily infected. These findings are in accordance with Isomursu *et al*. (2006).

The best model for mean d^2 and the second best model for *MLH* also included the interaction term between heterozygosity index and sex. The negative correlation between nematode intensity and heterozygosity tended to be stronger among males. Sex-dependent differences among Capercaillie are not surprising as sexual size dimorphism of the species is extreme: the male weighs twice as much as the female (Cramp & Simmons 1980). This size difference influences the prevalence of nematode infections, males being more often parasitized (Isomursu *et al*. 2006). The present results suggest that there might also be a genetic difference between the female and male immune systems or their allocation of resources.

The best model for mean d^2 included the interaction term between the heterozygosity index and age class. In adults, the effect of mean d^2 on nematode intensity tended to be stronger than among juveniles. Mean d^2 is expected to reflect genetic distance between the two parental gametes (Coltman & Slate 2003). Consequently, our result could be interpreted as a selective advantage for individuals having dissimilar parents. Mean d^2 may reveal influence of events deeper in individual's ancestry, such as historical population division and admixture (Coulson *et al*. 1998). On the other hand, the use of mean d^2 may be less useful than mean heterozygosity (Tsitrone *et al*. 2001, Slate & Pemberton 2002, Coltman & Slate 2003).

There are few known examples in natural populations where heterozygosity is directly correlated with some aspects of parasitism. Mountain White-crowned Sparrows infected by the protozoan blood parasite *Haemoproteus* had lower microsatellite heterozygosity than uninfected individuals (MacDougall-Shackleton *et al*. 2005). In stranded California sea lions (*Zalophus californianus*) the level of inbreeding measured by microsatellite heterozygosity was high in animals that suffered from helminth infection (Acevedo-Whitehouse *et al*. 2003). In these two cases, the parasites had a known negative effect on the host: an infection by *Haemoproteus* negatively affected the fitness of Mountain White-crowned Sparrows, and the helminth infection in stranded California sea lions was considered their primary health problem.

But why did we not detect a correlation between cestode infections and heterozygosity? The sampling procedure may play a role in the results concerning parasite infections: the method applied here gives a snapshot over a short time period. If homozygous individuals had weaker resistance, we would expect negative correlation between the probability of infection and the heterozygosity level. However, this negative correlation could disappear (or even turn positive) if parasite infection was lethal for the least heterozygous individuals. They would therefore not be included in the

hunted samples. The impact of intestinal helminths on the health of wild Capercaillie has not been investigated in detail. The specimens of this study were apparently healthy, hunted birds, but subtle differences in general condition could not be measured. However, indirect evidence from an earlier study suggests that especially cestodes may be deleterious for juvenile grouse by increasing their vulnerability to canine predators (Isomursu *et al*. 2008).

Our results also supported our second hypothesis on survival and heterozygosity. Heterozygosity was higher in adult than in juvenile Capercaillie, suggesting that survival is highest among the most heterozygous juvenile individuals. This is most probably due to a range of heterozygosity-related fitness factors, one of which is resistance to parasites. Juvenile mortality of Capercaillie is high: only 12% of hatchlings survive the first winter (Lindén 1981), which results in strong selection pressure among young Capercaillie.

Heterozygosity has earlier been correlated to some aspects of juvenile vitality. The more heterozygous proportion of the population can have higher neonatal survival (Coltman *et al*. 1998), higher juvenile survival up to one year of age (Da Silva *et al*. 2006) or more successful recruitment (Hansson *et al*. 2001). In the frog *Rana perezi*, the oldest age class out of three had the highest heterozygosity (Schmeller *et al*. 2007).

The problems in correlating heterozygosity, inbreeding and fitness are increasingly often acknowledged. Heterozygosity at molecular markers is expected to correlate with true genomic heterozygosity poorly if only a small number of markers are assayed (DeWoody & DeWoody 2005). Also, associations between multilocus heterozygosity and fitness traits seem to be weak when only up to ten markers are typed (Slate & Pemberton 2002). In general, inbreeding coefficient and heterozygosity are poorly correlated (Slate *et al*. 2004). According to Balloux *et al*. (2004), polygamic mating system, which is the case in Capercaillie, seems to strengthen the correlation between inbreeding coefficient and heterozygosity. These authors also found that the correlation is strongest when the population under study is divided in several subpopulations.

The Finnish Capercaillie population has experienced a marked decline in the whole country during the last 40 years. One of the most important reasons for the decline has been intensified forestry, resulting in loss and fragmentation of forest habitat (Ranta *et al*. 2004). Population decline due to habitat fragmentation may in turn lead to loss of heterozygosity (Keyghobadi 2007). Our results suggest that increased homozygosity may decrease fitness, e.g., the ability to cope with parasites, and consequently the observed population decline may accelerate. Further studies focusing on, e.g., the pathogenic effects of parasites and causal effects of genetic properties of Capercaillie on parasite infections are needed to elucidate the present findings.

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Metson suolistoloistartunta ja nuoruusiän selviytyvyys ovat yhteydessä usean mikrosatelliittilokuksen heterotsygotiaan

Sisäsiittoisuudella voi olla negatiivisia vaikutuksia useisiin lintujen ja nisäkkäiden elämänkiertoominaisuuksiin sekä tautien vastustuskykyyn erilaisten geneettisten mekanismien välityksellä. Uhanalaisilla ja pienenevillä populaatioilla saattaa olla erityinen riski sisäsiittoisuuteen. Sisäsiittoisuuden tasoa voidaan arvioida selvittämällä yksilön heterotsygoottisuutta neutraalien mikrosatelliittimarkkerien avulla.

Tutkimme riippuvuussuhteita suolistoloismatotartuntojen, ikäluokan, sukupuolen ja heterotsygotian välillä metsolla (*Tetrao urogallus*). Selvitimme mikrosatelliittiheterotsygotian kahdeksan autosomaalisen lokuksen kohdalla, ja laskimme kaksi eri indeksiä (monilokusheterotsygotia *MLH* ja keskimääräinen d^2), jotka määrittävät yksilön heterotsygoottisuutta. Metsoilla todettiin kolmea heisimatolajia (*Paroniella urogalli*, *Skrjabinia cesticillus* ja *Hymenolepis* sp.) sekä yksi sukkulamatolaji (kanalintusuolinkainen, *Ascaridia compar*).

Totesimme, että suolinkaistartunnan voimakkuus väheni heterotsygotian (*MLH*) tason noustessa. Suolinkaistartunnan todennäköisyys väheni heterotsygotian (keskimääräinen d^2) kasvaessa. Heterotsygotia (*MLH* ja keskimääräinen d^2) oli myös riippuvainen ikäluokasta: aikuisilla metsoilla oli korkeampi heterotsygotia kuin nuorilla. Tulokset viittaavat heterotsygotiaa suosivaan valintaan, jota voivat vahvistaa erot perinnöllisessä loisten vastustuskyvyssä.

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