Age-dependent genetic effects on a secondary sexual trait in male Alpine ibex, *Capra ibex*

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Abstract

Secondary sexual traits, such as horns in ungulates, may be good indicators of genetic quality because they are costly to develop. Genetic effects on such traits may be revealed by examining correlations between multilocus heterozygosity (MLH) and trait value. Correlations between MLH and fitness traits, termed heterozygosity–fitness correlations (HFC), may reflect inbreeding depression or associative overdominance of neutral microsatellite loci with loci directly affecting fitness traits. We investigated HFCs for horn growth, body mass and faecal counts of nematode eggs in wild Alpine ibex (*Capra ibex*). We also tested if individual inbreeding coefficients ($f''$) estimated from microsatellite data were more strongly correlated with fitness traits than MLH. MLH was more strongly associated with trait variation than $f''$. We found HFC for horn growth but not for body mass or faecal counts of nematode eggs. The effect of MLH on horn growth was age-specific. The slope of the correlation between MLH and yearly horn growth changed from negative to positive as males aged, in accordance with the mutation accumulation theory of the evolution of senescence. Our results suggest that the horns of ibex males are an honest signal of genetic quality.

Keywords: Alpine ibex, heterozygosity–fitness correlations, horns, multilocus heterozygosity, senescence, ungulates

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Introduction

Inbreeding depression is the decrease in fitness of offspring born from matings among related individuals (Charlesworth & Charlesworth 1987). It has been typically measured by regressing fitness-related phenotypic traits on individual inbreeding coefficients. Inbreeding coefficients are generally estimated from complete pedigree data, which are rarely available for wild animals. Alternatively, researchers have used individual multilocus heterozygosity (MLH), the standardized average observed heterozygosity among all microsatellite marker loci in one individual, as a proxy for individual inbreeding. Associations between MLH and fitness-related traits, hereafter termed heterozygosity–fitness correlations (HFC), have been widely reported in wild vertebrate populations in recent years (review in Coltman & Slate 2003).

Three main hypotheses have been proposed to explain HFC: (a) direct overdominance, whereby marker loci directly affect fitness; (b) associative overdominance, whereby marker loci are in linkage disequilibrium with loci directly influencing fitness traits; (c) identity disequilibria, whereby marker heterozygosity reflects genome-wide heterozygosity (Hansson & Westerberg 2002). Hypothesis (a) is possible for allozyme markers, while it can usually be ruled out for nonfunctional and selectively

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neutral microsatellite markers. Hypothesis (b), which is also termed a local effect, assumes that individuals heterozygous at a marker locus, physically linked to an overdominant or dominant locus affecting fitness, tend to be heterozygous also at the latter locus. The identity disequilibria hypothesis (c) assumes that individuals heterozygous at marker loci tend to be heterozygous also at unlinked fitness-influencing loci. Identity disequilibrium is expected to arise only in populations showing interindividual variance in inbreeding, therefore when there is variance in inbreeding a positive HFC may reflect inbreeding depression (Slate et al. 2004). Therefore, hypothesis (c) is also referred to as the inbreeding hypothesis.

Microsatellites are simple DNA sequences repeated in tandem that exhibit variation in repeat unit number. Because most microsatellite loci lie outside of expressed genes, it is assumed that they are selectively neutral (Scribner & Pearce 2000). Mutation rates for microsatellites are extremely high because of slipped-strand mispairing in DNA replication, making them ideal markers for assessing individual genetic variability. Even though microsatellite MLH has been widely used as an indicator of inbreeding, it is not equivalent to the true inbreeding coefficient, which measures the probability that two alleles at a locus are identical by descent. Instead, MLH scores all homozygous loci that are identical by state, regardless of whether they are inherited from a common ancestor. Slate et al. (2004) showed that MLH was only weakly correlated with inbreeding coefficient in a large sample of domestic sheep from a population where inbreeding was common. In the same study, the inbreeding coefficient estimated from pedigree data detected evidence of inbreeding depression in morphological traits, while MLH did not (Slate et al. 2004).

Recently developed statistical methods allow the estimation of individual inbreeding coefficients ($f'$) from microsatellite data in the absence of complete pedigrees (Ritland 1996a; Lynch & Walsh 1998). We will use the notation $f'$ to indicate inbreeding coefficients estimated from microsatellite data to distinguish them from true inbreeding coefficients ($f$) estimated from pedigree data. The estimated inbreeding coefficient $f'$ should, in theory, be superior to MLH for detecting inbreeding depression, as it also includes information on allele frequency (Ritland 1996b).

Here we investigate the relationship between individual heterozygosity (MLH and $f'$) and three fitness-related traits: faecal counts of nematode eggs (FEC, an index of the combined effects of intensity of gastrointestinal nematode parasitism and host resistance influencing parasite fecundity; Coltman et al. 1999), summer body mass (a determinant of winter survival and reproductive success in mountain ungulates; Festa-Bianchet et al. 1997; Coltman et al. 2002) and horn growth (a sexually selected trait related to reproductive success in mountain ungulates; Coltman et al. 2002) in wild Alpine ibex (Capra ibex) males in the Gran Paradiso National Park (GPNP), Italy. We do so using parameters estimated from a large data set obtained from amplification of 32 microsatellite loci.

We predicted that MLH would be negatively correlated with FEC, and positively correlated with body mass and horn size. As the sign of expected relationships between $f'$ and fitness-related traits is opposite to that expected for MLH, we predicted correlations of the opposite sign for $f'$. According to the handicap principle (Zahavi 1975; Zahavi & Zahavi 1997) exaggerated secondary sexual traits in males that are costly to produce, such as large horns, reliably advertise genetic quality. Secondary sexual traits are usually more phenotypically variable than other traits (Pomiankowski & Møller 1995). A comparison of standardized linear selection gradients on quantitative traits in natural populations suggested that sexual selection is stronger than viability selection (Hoekstra et al. 2001). Moreover, it has been shown that additive genetic variation is higher in sexually selected traits than in other traits (Pomiankowski & Møller 1995). Secondary sexual traits should therefore be more strongly affected by genetic effects (inbreeding depression or local effects) than other morphometric and physiological traits. We therefore expected stronger effects of genome-wide genetic diversity (MLH and $f'$) on horn growth than on mass and FECs.

Alpine ibex males grow a distinct horn segment each year. Horn growth stops over winter, producing a very clear ring (annulus) around each horn. At GPNP the probability of over-winter mortality can be predicted from the size of the yearly horn growth increments (von Hardenberg et al. 2004). Horn growth decreases by up to 19% compared to the age-specific mean in each of the last two years prior to death; therefore yearly horn growth is an indicator of the onset of senescence (von Hardenberg et al. 2004). The two main hypotheses for the evolution of senescence are: (i) the mutation accumulation theory (Medawar 1952), according to which age-specific deleterious mutations accumulate because selection is more efficient at removing those expressed early in life; (ii) the antagonistic pleiotropy hypothesis (Williams 1957), according to which selection favours genes that enhance early performance at the expense of fitness later in life. The mutation accumulation theory predicts that inbreeding effects increase with age, while the antagonistic pleiotropy hypothesis does not predict such an increase (Charlesworth & Hughes 1996). We therefore tested if the effect of individual heterozygosity (MLH and $f'$) on horn growth was age-specific (increasing for MLH and decreasing for $f'$). Because ibex horns show obvious yearly increments, they are ideal to test this hypothesis in a long-lived, iteroparous organism.

Finally, we explored the correlation between MLH and inbreeding in the GPNP Alpine ibex population and estimated the power to detect this correlation.
Methods

Study area and population

The study was conducted in the Gran Paradiso National Park (northwestern Italian Alps; 45°25′N, 07°34′E). Samples for genetic analyses were collected in three of the five main valleys of the Park (Orco, Cogne and Valsavarenche) from 116 live-captured and tagged males and from 37 untagged valleys of the Park (Orco, Cogne and Valsavarenche) from for genetic analyses were collected in three of the five main areas. A. von Hardenberg (unpublished). Horn growth was measured on 109 males captured or found dead. Data on FEC were collected from 53 tagged males and summer mass gain was available for 50 tagged males in the study area of Levionaz, Valsavarenche (GPNP) (von Hardenberg 2005). Most of the Levionaz study area lies above tree line, between 2300 and 3300 m above sea level and is characterized by high-altitude alpine meadows (mainly Festuca varia), moraines, rock cliffs and glaciers. It is used by ibex from the end of May to December. The winter range (at 1700–2300 m above sea level) is characterized by pastures (near villages) and a mixed forest of Larix decidua, Picea abies and Pinus cembra. During this study, about 200 adult ibex were in the study area.

Field methods

Details about capture and marking methods are provided in von Hardenberg (2005). Individual males in Levionaz were repeatedly weighed from June to September each year with an electronic platform scale baited with salt (Bassano et al. 2003). Body mass was adjusted to the 1st of August of each year as described in von Hardenberg (2005).

Fecal egg counts

FEC were estimated twice a month from June to September for most tagged males in Levionaz in 2000–2004. Animals were observed from a distance of 10–50 m and at least 20 g of faeces were collected within 1–5 min of defecation. Faecal pellets were kept in plastic bags at 4 °C prior to analysis. FEC followed a modified McMaster technique (Ministry of Agriculture et al. 1971) and were expressed as number of eggs per gram of fresh faeces (EPG). At least two counts per sample were carried out in order to minimize measurement error. EPG was determined to the nearest 20 eggs/gram of faeces. Individuals experimentally treated with antihelmintics (von Hardenberg 2005), were excluded from analyses in the year of treatment. The arithmetic mean of EPG measured for each individual from June to September was used as a yearly index of infection as described in von Hardenberg (2005).

Horn growth measurement

The length of each yearly growth annulus was measured for both horns, along a central line on the front of the horn using a precision calliper to the nearest 1 mm. The average length of the left and right annuli was used for analysis. More details on horn growth measurement can be found in von Hardenberg et al. (2004).

Microsatellite analysis

A sample of cutaneous tissue for microsatellite analysis was collected from each live-captured animal and from carcasses of animals found dead during winter. Using a sterile blade, we cut a tiny piece of tissue (< 2 mm²) from one ear and stored it at room temperature in collection tubes with 1–5 mL of 95% (w/v) ethanol solution. DNA was extracted from about 20 mg of tissue using the DNeasy Kit (QIAGEN GmbH) following the manufacturer’s instructions. Polymerase chain reaction (PCR) were used to amplify 39 polymorphic microsatellite loci: MAF209, MAF36, OarHH35, BMS1350, TGLA441, Texan4, IDVGA30, BM81124, OarHH62, McM73, CSSM47, BM4208, RT1, HUJ1177, McM152, OarJMP29, BM121, BM1225, HEL1, SR6Q, SR26, ETH10, BM1818, SR25, ILST029, INRA185, FCB48, AE54, TGLA122, TGLA126, SR8, HAUT27, ILST030, SR24, SR15, BM4505, OMHC1, OarkP6, TGLA387. Details on methods used for PCR and electrophoresis are described in Maudet et al. (2001, 2002).

Data analysis

Hardy–Weinberg proportions and gametic disequilibrium for all possible pairs of loci were tested using the program GENEPOP version 3.4 (Raymond & Rousset 1995). Individual standardized multilocus heterozygosity was calculated as the ratio of the heterozygosity of an individual to the mean heterozygosity of those loci at which the individual was typed. The standardization avoids confounding because of possible systematic differences in loci used between individuals (Coltman et al. 1999). Individual inbreeding coefficients (f’i) were estimated from microsatellite data using SPAGEDi version 1.1 (Hardy & Vekemans 2002). This coefficient, calculated as intra-individual kinship coefficient (Råtland 1996b), estimates the kinship between alleles within an individual at a locus averaged over all loci according to the following formula:

\[ f' = \sum_i \left( \frac{S_i - P_i}{P_i} \right)^2 / n - 1 \]
where $S_{il}$ is an indicator variable with $S = 1$ if the individual is homozygous for allele $i$ at locus $l$ and $S = 0$ if it is heterozygous; $P_{il}$ is the frequency of allele $i$ at locus $l$ and $n$ is the number of typed loci for the individual. The sign of expected relationships between $f'$ and fitness-related traits is opposite to that expected for MLH (for example, a negative relationship with horn size is expected for $f'$ while a positive one is expected for MLH).

To avoid pseudoreplication because of measurements of the same individuals taken in different years, we fitted linear mixed-effects models (LME) implemented in the nlme package of S-PLUS 2000 (Pinheiro & Bates 2001) to test the relationship between MLH or $f'$ and FEC and body mass. The LME function in S-Plus also allows models to be fitted with heteroscedastic within-group errors. We fitted LME models with the appropriate variance function whenever the within-group errors appeared to have unequal variances. We followed the model-building approach suggested by Pinheiro & Bates (2001) for all fitted LME models. The significance of fixed terms was assessed using conditional $F$-tests. For LME models with different random terms, we chose the model with the lowest Akaike information criterion (AIC). To normalize error terms, we transformed the FEC data to $\ln(\text{EPG} + 1)$ prior to analyses. Treatment contrasts were used to assess differences within factors. Age was centred at 8 years to reduce multicollinearity whenever a quadratic term was included. To minimize the risk of type I error from multiple tests, all probabilities were corrected with the sequential Bonferroni method (Rice 1989). The effect of MLH and $f'$ was tested as a single factor and as an interaction with age for all fitness traits.

Associations between alleles of specific loci and phenotypic traits were assessed under a dominance and an additive model. For each allele, we scored 1 if the allele was present and 0 if absent for the dominance model. We scored 0 if an allele was absent, 1 if one copy of the allele was present, and 2 if two copies of the allele were present for the additive model. We then fitted the same LME models as for MLH, but with the alleles as genetic terms instead of MLH (once for the dominance and once for the additive model). We fitted $k-1$ alleles because the $k^{th}$ allele is non-independent. The significance of genetic associations was tested comparing the fit of a model with all genetic terms to one with no genetic terms.

The correlation between MLH and true inbreeding was estimated according to Slate et al. (2004) as:

$$r(\text{MLH}, f') = -\frac{\sigma(f')}{\sqrt{[1 - E(f')]\sigma(\text{MLH})}}$$

where $E(f')$ is the mean estimated inbreeding.

The variance in true inbreeding $\sigma^2(f')$ was estimated from the covariance in marker estimates of inbreeding as suggested by Ritland (1996b) using spageti version 1.1 (Hardy & Vekemans 2002). Power analyses followed Zar (1998).

### Results

**Hardy–Weinberg and linkage disequilibrium**

Six of 39 loci (BM81124, OarMP29, BM121, SR25, TGLA126 and SR24) that deviated significantly from Hardy–Weinberg proportions after Bonferroni correction were removed from all further analyses. Two loci (OMHC1 and TGLA387) were in linkage disequilibrium (and thus highly correlated) and we excluded TGLA387 from analyses, leaving 32 loci for subsequent analyses.

**Faecal egg counts**

We found no significant relationship between MLH and FEC or between $f'$ and FEC after accounting for identity, age and year effects (Tables 1 and 2). The interactions MLH $\times$ age and $f' \times$ age did not affect FEC. When we analysed the effect of each single locus on FEC (Fig. 1) we found that heterozygotes had lower mean FEC than homozygotes at 11 of 32 loci. However, the difference was statistically significant at only one locus (ETH110) after sequential Bonferroni correction.

**Body mass**

Body mass was not related to MLH or $f'$ after accounting for identity, age and year effects (Tables 3 and 4). The interactions MLH $\times$ age and $f' \times$ age did not affect mass. When we analysed the effect of each locus separately (Fig. 1) we found that at 14 of 32 loci heterozygotes appeared to be heavier than homozygotes. However, no difference was significant after sequential Bonferroni correction.

### Table 1 Wald test for the significance of terms included in a linear mixed effects (LME) model with faecal output of parasite eggs ($\ln(\text{EPG} + 1)$) of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random slope for age (AIC = 244.87 for the model with a random slope for age vs. AIC = 246.35 for a model with a random intercept only), and age, year and MLH as independent variables. Data were collected at Levionaz (Gran Paradiso National Park, Italy), from 2000 to 2004. Standard errors in parentheses. Model is based on 164 observations from 53 individual ibex.

<table>
<thead>
<tr>
<th>Terms included in the model:</th>
<th>Coefficient</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>+0.21 (0.08)</td>
<td>1.105</td>
<td>20.23</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>Year</td>
<td>+4.106</td>
<td>4.106</td>
<td>20.22</td>
<td>≤ 0.0001</td>
</tr>
<tr>
<td>MLH</td>
<td>+1.54 (0.09)</td>
<td>1.51</td>
<td>0.76</td>
<td>0.39</td>
</tr>
<tr>
<td>Age $\times$ MLH</td>
<td>−0.14 (0.08)</td>
<td>1.105</td>
<td>2.88</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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HETEROZYGOSITY AND HORN GROWTH IN CAPRA IBEX

Table 2 Wald test for the significance of terms included in an LME model with faecal output of parasite eggs [ln(EPG + 1)] of Alpine ibex as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random slope for age (AIC = 242.84 for the model with a random slope for age vs. AIC = 244.97 for a model with a random intercept only), and age, year and inbreeding coefficient f' as independent variables. Data were collected at Levionaz (Gran Paradiso National Park, Italy), from 2000 to 2004. Standard errors in parentheses. Sample size was based on 164 observations from 53 individual ibex individuals.

<table>
<thead>
<tr>
<th>Coefficient d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.105</td>
<td>17.78</td>
</tr>
<tr>
<td>Year</td>
<td>4.105</td>
<td>20.09</td>
</tr>
<tr>
<td>f'</td>
<td>-0.86 (1.19)</td>
<td>1.51</td>
</tr>
<tr>
<td>Age × f'</td>
<td>1.105</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 3 Wald test for the significance of terms included in an LME model with body mass (kg) as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and age, age2, year and MLH as independent variables for male Alpine ibex in Levionaz (Gran Paradiso National Park, Italy), 2000–2004. Standard errors in parentheses. Sample size was based on 122 observations from 50 individuals.

<table>
<thead>
<tr>
<th>Coefficient d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.163 (1.86)</td>
<td>1.65</td>
</tr>
<tr>
<td>Age2</td>
<td>-0.70 (0.06)</td>
<td>1.65</td>
</tr>
<tr>
<td>Year</td>
<td>4.65</td>
<td>13.70</td>
</tr>
<tr>
<td>MLH</td>
<td>-3.19 (13.43)</td>
<td>1.48</td>
</tr>
<tr>
<td>Age × MLH</td>
<td>-0.05 (1.19)</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Horn growth

Neither MLH nor f' affected horn annulus length as single factors (Tables 5 and 6), but their interactions with age had significant effects on annulus length (Tables 5 and 6). The slope of the regression between MLH and annulus length increased with age (Fig. 2 and Table 7). The opposite pattern was found for f'. Regressing MLH against annulus length at each age revealed a significant positive relationship at 9 years after sequential Bonferroni correction. The relationship between f' and annulus length at 9 years of age was not significant after sequential Bonferroni correction. The correlations between MLH and the length of annuli grown at 6–8 and at 10–12 years also showed positive trends (Table 7). The correlations between inbreeding coefficient f' and horn annulus size at 7–

Table 4 Wald test for the significance of terms included in an LME model with body mass (kg) as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and age, age2, year and f' as independent variables in Alpine ibex males in Levionaz (Gran Paradiso National Park, Italy), 2000–2004. Standard errors in parentheses. Sample size was 122 observations from 50 individuals.

<table>
<thead>
<tr>
<th>Coefficient d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>+16.23 (1.15)</td>
<td>1.65</td>
</tr>
<tr>
<td>Age2</td>
<td>-0.69 (0.06)</td>
<td>1.65</td>
</tr>
<tr>
<td>Year</td>
<td>4.65</td>
<td>13.76</td>
</tr>
<tr>
<td>MLH</td>
<td>+3.54 (0.92)</td>
<td>1.65</td>
</tr>
</tbody>
</table>

Table 5 Wald test for the significance of terms included in an LME model with horn annulus length (mm) as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and age, year, MLH and the interaction term age × MLH as independent variables in Alpine ibex males from the Gran Paradiso National Park, Italy 2000–2004. Standard errors in parentheses. Sample size was based on 750 observations from 109 individuals.

<table>
<thead>
<tr>
<th>Coefficient d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-5.79 (1.87)</td>
<td>1.623</td>
</tr>
<tr>
<td>Year</td>
<td>16.623</td>
<td>4.59</td>
</tr>
<tr>
<td>MLH</td>
<td>+8.51 (5.00)</td>
<td>1.107</td>
</tr>
<tr>
<td>Age × MLH</td>
<td>+3.54 (0.92)</td>
<td>1.623</td>
</tr>
</tbody>
</table>

Table 6 Wald test for the significance of terms included in an LME model with horn annulus length (mm) as the dependent variable, ibex identity as a grouping factor, a general positive-definite within-group error structure, a random intercept, and age, year, f' and the interaction term age × f' as independent variables in Alpine ibex males from the Gran Paradiso National Park, Italy 2000–2004. Standard errors in parentheses. Sample size was based on 750 observations from 109 individuals.

<table>
<thead>
<tr>
<th>Coefficient d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-5.54 (0.25)</td>
<td>1.623</td>
</tr>
<tr>
<td>Year</td>
<td>16.623</td>
<td>4.51</td>
</tr>
<tr>
<td>f'</td>
<td>+4.22 (6.26)</td>
<td>1.107</td>
</tr>
<tr>
<td>Age × f'</td>
<td>-3.91 (1.35)</td>
<td>1.623</td>
</tr>
</tbody>
</table>

12 years of age showed negative trends which, however, were not significant after sequential Bonferroni correction (Table 7). When we analysed the effect of each single locus on horn annulus length we found that in 17 of 31 loci
Fig. 1 Difference in FEC, body mass, and horn annulus length between heterozygotes and homozygotes at 32 microsatellite loci for adult male Alpine ibex (Capra ibex) in Levionaz (Gran Paradiso National Park, Italy). Values higher than 0 indicate a larger value in heterozygotes compared to homozygotes. Error bars represent Standard Errors. Values and significances have been estimated with Linear mixed effects models with ID as grouping factor, a general positive-definite within-group error structure, and the following fixed terms structures: Body mass $\sim$ Age $+$ Age$^2$ $+$ Year $+$ Locus; FEC $\sim$ Age $+$ Year $+$ Locus; Annulus length $\sim$ Age $+$ Year $+$ Locus. Significance levels were corrected using the Sequential Bonferroni Method. Significant differences are marked with an asterisk. The model could not be computed for HUJ1177 on horn annulus size because of singularity in backsolving.
Fig. 2 Relationship between MLH and horn annuli grown at 2–13 years of age in male Alpine ibex in Gran Paradiso National Park (Italy).
heterozygotes appeared to have longer mean horn annuli than homozygotes even though these differences were not significant after applying the sequential Bonferroni method (Fig. 1).

**Testing specific allele associations using dominance and additive models**

In light of the significant effect of heterozygosity at ETH10 on FEC, we tested for association between specific ETH10 alleles and FEC and other traits using both dominance and additive models of genetic effects. We also tested allele-specific associations for OMHC1, a locus located in the MHC. Significant associations were found between ETH10 alleles and FEC and between ETH10 alleles and horn growth, but not body mass (Table 8). We found no significant associations of specific OMHC1 alleles with FEC or mass. However, the interaction between age and specific OMHC1 alleles on the length of horn annuli was significant (additive model only; Table 8).

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**Table 7** Relationships between MLH, f and annulus size (Ln transformed) grown at different ages in Alpine ibex in the Gran Paradiso National Park. P (Bonf.) is the P value after Bonferroni sequential correction (Rice 1989). Significant results are underlined.

<table>
<thead>
<tr>
<th>Annulus Value</th>
<th>Standard error</th>
<th>F</th>
<th>d.f.</th>
<th>P</th>
<th>P (Bonf.)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH 2</td>
<td>−0.2765</td>
<td>0.1445</td>
<td>3.662</td>
<td>1107</td>
<td>0.0583</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 3</td>
<td>−0.1087</td>
<td>0.1079</td>
<td>0.2141</td>
<td>1105</td>
<td>0.3158</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 4</td>
<td>−0.0825</td>
<td>0.1141</td>
<td>0.2237</td>
<td>1100</td>
<td>0.4716</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 5</td>
<td>−0.0604</td>
<td>0.1219</td>
<td>0.2453</td>
<td>1.81</td>
<td>0.6217</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 6</td>
<td>+0.0573</td>
<td>0.1164</td>
<td>0.4921</td>
<td>1.64</td>
<td>0.6243</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 7</td>
<td>+0.1194</td>
<td>0.1243</td>
<td>0.9233</td>
<td>1.56</td>
<td>0.3407</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 8</td>
<td>+0.1724</td>
<td>0.1288</td>
<td>1.339</td>
<td>1.52</td>
<td>0.1864</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 9</td>
<td>+0.4113</td>
<td>0.129</td>
<td>10.16</td>
<td>1.47</td>
<td>0.0025</td>
<td>0.05</td>
</tr>
<tr>
<td>MLH 10</td>
<td>+0.3738</td>
<td>0.2063</td>
<td>1.8114</td>
<td>1.43</td>
<td>0.0771</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 11</td>
<td>+0.5119</td>
<td>0.4097</td>
<td>1.2495</td>
<td>1.30</td>
<td>0.2211</td>
<td>NS</td>
</tr>
<tr>
<td>MLH 12</td>
<td>+0.0167</td>
<td>0.4994</td>
<td>0.0334</td>
<td>1.23</td>
<td>0.9736</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 8** Genetic associations between alleles of ETH10 and OMHC1 loci and FEC, horn annulus size and body mass in male Alpine ibex in the Gran Paradiso National Park. Significant values are underlined.

<table>
<thead>
<tr>
<th>Term</th>
<th>Dependent</th>
<th>Dominance model</th>
<th>Additive model</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETH10</td>
<td>FEC</td>
<td>6.91</td>
<td>12.09</td>
</tr>
<tr>
<td>ETH10</td>
<td>Body Mass</td>
<td>0.39</td>
<td>0.67</td>
</tr>
<tr>
<td>ETH10 × age</td>
<td>Annu</td>
<td>14.24</td>
<td>38.28</td>
</tr>
<tr>
<td>OMHC1</td>
<td>FEC</td>
<td>5.22</td>
<td>7.03</td>
</tr>
<tr>
<td>OMHC1</td>
<td>Body Mass</td>
<td>2.63</td>
<td>9.17</td>
</tr>
<tr>
<td>OMHC1 × age</td>
<td>Annu</td>
<td>10.28</td>
<td>25.91</td>
</tr>
</tbody>
</table>
MLH-inbreeding correlation and variance in inbreeding

The correlation observed between MLH and individual estimated inbreeding coefficients ($f'f$) was $-0.65 (P < 0.001)$. Mean $f'$ of the population was $E(f') = 0.026$. The covariance among marker loci in the estimated individual inbreeding coefficients was very low [$\sigma^2(f) = 0.00032$] and not different from 0 ($SE = 0.00023$) suggesting low variance in true inbreeding. The estimated correlation between MLH and true inbreeding was low [$r(\text{MLH}, f) = -0.095$; power($\alpha = 0.05, n = 159) = 0.22$]. It would require a minimum sample of $n = 870$ to demonstrate that this correlation coefficient is significantly different from 0 with a power of 0.8.

Discussion

We found no relationship between MLH or estimated individual inbreeding coefficients ($f'f'$, estimated from microsatellite data) and faecal egg counts or body mass in Alpine ibex males. Moreover, trends were typically opposite to expectations, except for the relationship between $f'$ and body mass. These results are not surprising given a recent meta-analysis of HFC in wild populations which revealed a weak effect size for MLH (mean $r = 0.0274$) (Coltman & Slate 2003) and which estimated that a minimum sample size of 600 was required for a nominal power of 80% for detecting HFC. The same study revealed an even lower mean effect size of MLH on physiological traits (including parasite resistance) only (mean $r = 0.0075$) or on morphometric traits (including body mass) only (mean $r = 0.0052$) (Coltman & Slate 2003). Alternatively, the choice of microsatellite loci used in this study might also have influenced the results. Acevedo-Whitehouse et al. (2005) found that heterozygosity at microsatellites linked to immunogenetic regions was an important predictor of bovine tuberculosis in wild boars Sus scrofa. Perhaps if we used more microsatellites linked to other immunogenetic regions local effects would have contributed more to the relationship between MLH and FEC.

Nevertheless, we suspect that we have detected local effects at two loci in our data set. Despite the lack of an overall effect of heterozygosity on FEC and body mass, heterozygotes at locus ETH10 had significantly lower FEC than homozygotes. Significant effects were not found for any other locus, and the sign of the difference between heterozygotes and homozygotes appeared to be randomly distributed among loci. We also found associations between specific ETH10 alleles and both FEC and the length of horn annuli (but not body mass). ETH10 is in a region of chromosome 5 known to be linked to growth rate and carcass traits in cattle (Li et al. 2004). Recent investigations have shown that ETH10 seems to be under selection in reintroduced Alpine ibex populations in Switzerland (I. Biebach and L. Keller personal communication). The positional candidate gene of ETH10 is insulin-like growth factor 1 (igf1). While an extensive literature suggests that igf1 plays an important role in immunity, other findings suggest that it is not an obligate immunoregulator (review in Dorshkind & Horseman 2000). Further studies should analyse the possibility that ETH10 is in linkage disequilibrium with this or other genes affecting parasite resistance in Alpine ibex. Associative overdominance is at least partially responsible for an association between birth weight and MLH in red deer Cervus elaphus (Slate & Pemberton 2002). We did not attempt to analyse directly the relationship between igf1 and FEC in Alpine ibex because, even though surely interesting, such a study would be beyond the scopes of the present research.

The loci we analysed included OMHC1 which is linked to the MHC and has been shown to be associated with FEC in Soay sheep (Paterson et al. 1998). In Alpine ibex, we did not find significant association between specific OMHC1 alleles and neither FEC nor body mass. However, we found a significant interaction between age and specific OMHC1 alleles on the length of horn annuli. This result agrees with Ditchkoff et al. (2001) who showed that antler development of white-tailed deer was associated with variation of loci at the major histocompatibility complex (MHC) level.

Unlike FEC or body mass, MLH was positively correlated with the length of horn annuli grown at 6–13 years of age and the slope of the relationship between annulus length and MLH increased with age. The relationship between individual inbreeding coefficient ($f'$) and the length of annuli grown at 7–13 years of age was negative as predicted. While it has been argued that HFC should decline with age, because growth and survival differences are maximal early in life (David 1998), the positive age-specific relationship between MLH and horn growth is as predicted by the mutation accumulation theory for the evolution of senescence (Charlesworth & Hughes 1996). The relative roles of mutation accumulation vs. antagonistic pleiotropy in the evolution of senescence remains controversial, and data mostly come from laboratory studies on semelparous invertebrates (for example see Hughes et al. 2002 and Rose et al. 2002).

Ibex males in GPNP do not reach an asymptote in body mass until 10–12 years (von Hardenberg 2005). Male reproductive success, which we assume to be dependent on horn and body size, should peak at about the same age. It is possible that genetic effects on horn growth start to be evident only when male ibex participate actively in the rut. As rut-related energetic expenditure peaks, only high-quality males can afford the costs of reproduction while investing in horn growth (Pelletier et al. in press). There is much evidence that horns and antlers reliably advertise...
male quality in ungulates. Coltman et al. (2002) found a
correlation between horn length and reproductive success
in mature bighorn rams Ovis canadensis. Positive effects of
horn length on lifetime and seasonal reproductive success
have also been shown in feral sheep (Preston et al. 2001),
and Kruuk et al. (2002) found a positive relationship
between antler size and mating success in red deer. Antler
size in red deer also correlates positively with sperm
quantity and quality (Malo et al. 2005). In Alpine ibex, males
aged 5–11 years that grew annuli shorter than the popula-
tion age-specific average had reduced survival chances over
the following two years (von Hardenberg et al. 2004).
Because the survival of male Alpine ibex aged 2–8 years is
extremely high (typically over 98% a year, Toïgo et al. 1997),
our results are unlikely to be due to selective mortality of
young males. The larger sample size for younger males is
mostly due to the fact that we targeted young adults for
capture and marking.

Our results suggest that horns are honest signals of male
genetic quality in Alpine ibex (von Hardenberg et al. 2004).
Indirect evidence for a relationship between secondary
sexual traits and genetic variability in ungulates was also
provided by Ditchkoff et al. (2001) and by Fitzsimmons
et al. (1995) who showed that large-horned bighorn rams
were more heterozygous than small-horned rams aged 6–
8 years. Body weight and FEC are less accurately measured
than horn growth because both may vary with season and
time of day. Measurement error may therefore have limited our power to detect a similar relationship for
these traits.

Correlations between MLH, based on microsatellite
markers, and fitness traits have usually been interpreted as
evidence of inbreeding depression (Pemberton 2004). Two
recent papers, however, show that individual heterozy-
gosity is only weakly correlated with individual inbreeding
coefficients (Balloux et al. 2004; Slate et al. 2004). We
found that the correlation between MLH and inbreeding
was extremely low. In addition, the variance in inbreeding,
estimated as the covariance among marker loci inbreeding
estimates, was very low and not significantly different from
0, suggesting that HFC for horn growth may be largely due
to the local effects of specific microsatellites in linkage
disequilibrium with loci directly affecting fitness.

Theoretically f′ should be a better measure of inbreeding
than MLH because it includes information on allele fre-
frequency. Acevedo-Whitehouse et al. (2003) showed that an
index of ‘internal relatedness’, estimated from microsatel-
rite markers similarly as is f′, influenced the susceptibility
to pathogens in wild Californian sea lions (Zalophus califor-
nianus). Inbreeding coefficients (estimated from pedigree
data) but not MLH detected inbreeding depression for
morphological traits in domestic sheep (Slate et al. 2004).
Contrary to predictions, however, we found that estimated
individual inbreeding coefficients performed worse than
MLH as an index of individual genetic variability. Indeed,
while we found a significant relationship between MLH
and horn annulus length grown at 9 years of age, such
significant relationship could not be detected using f′. The
inherent, possibly inevitable, inaccuracy of the indirect
regression approach used to estimate f′ in the absence of
degree information (Ritland 1996a; Lynch & Walsh 1998)
may have caused too much noise around the inferred
parameters, limiting their usefulness. Thomas et al. (2002)
showed that several indirect marker-based methods were
poor estimators of heritability of body weight in free-
ranging Soay sheep, Ovis aries. Coltman (2005) showed that
marker-based estimates of heritability were imprecise and
downwardly biased.

In conclusion, we found evidence for a heterozygosity–
fitness correlation in a secondary sexual trait (horn growth)
but not in body mass or in FEC in Alpine ibex. The effect of
heterozygosity on horn growth was strongly age depen-
dent in accordance with the predictions of the mutation
accumulation theory for the evolution of senescence (Char-
lesworth & Hughes 1996). To the best of our knowledge,
this is the first study to find support for the mutation
accumulation theory in a free-ranging vertebrate.

We also found lower FECs for heterozygotes compared
to homozygotes at a single microsatellite locus. The
possibility that this result may be due to associative over-
dominance with loci directly involved in parasite resistance
merits further exploration. Inbreeding coefficients,
estimated from microsatellite data, did not appear to be a
better indicator of individual genetic variation than MLH.
This is consistent with Pemberton (2004) who suggested that
inbreeding coefficients in HFC studies should be used
only if they can be calculated from pedigree data.

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References


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