Supporting Online Material for

Negative Frequency-Dependent Selection of Sexually Antagonistic Alleles in

*Myodes glareolus*

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Material and Methods

Study species

The bank vole (Myodes glareolus) is one of the most common mammalian species in Europe and is found primarily in forest as well as fields (34, 35). They are not sensitive to disturbance and have high trappability, which make them a good study species for both field and laboratory experiments (19, 20). The mating system is polygynandrous (21), such that males frequent territories defended by females to mate with them (36).

Previous work has shown that bank voles experienced sexually antagonistic selection for testosterone, which resulted either in families of low fecundity females with dominant brothers (selected for high T), or families of subordinate males with high fecundity sisters (selected for low T) (22). Based on this male-female association, two selection lines were created for this experiment: wild-caught animals were used as founders and artificially selected according to sexually antagonistic selection on male behavioral dominance and female fertility, so that high-dominance males were mated with females of low fertility and vice versa. This selection resulted in two groups (or tactics): Mf (high-dominance males with low fertility sisters) and mF (low-dominance males with high fertility sisters). We used 168 males in the behavioral (male-male competition) trials, of which we were able to successfully measure the plasma testosterone of 164 males (details below). We were constrained by the number of females available, and hence we used 146 females and 146 males in the field experiment. Study individuals were fourth and fifth generation descendants of wild individuals. Males and females in the laboratory were housed at 22 °C in standard cages measuring 43 x 26 x 15 cm with wood shavings and hay for bedding, ad libitum water and food (Labfor 36; Lactamin AB, Stockholm, Sweden) and kept on a 16:8 L:D photoperiod. The use of these study individuals adhered to ethical guidelines for animal research in Finland.

Laboratory experiment

Prior to releasing the bank voles to the field environment, female fertility was assured by mating them to unrelated males in the laboratory, allowing them to wean their offspring, and then providing them several weeks of recovery. Male dominance was tested in non-repeated male-male competition trials for a female in estrus (20). A high-dominance male was tested against a low-dominance male by placing them simultaneously into an arena with a wild-caught female in estrus. The successful male out-competed the opponent to mate with the female.

Individuals were implanted with electronic identification chips (Trovan, Sweden) using proper antiseptic techniques and isofluorane anesthesia. Next, blood (75μl intra-orbital blood sample, (37)) and DNA samples (1 mm tissue sample from terminal end of ear) were taken. These samples were placed in -20°C storage immediately. Individuals
recovered in the laboratory approximately one week before they were released into enclosures. The plasma obtained from the blood samples was analysed for testosterone using the radioimmunoassay technique (see methods in (21)). A recent study (25) calculated the repeatability of multiple T measures in this species by taking samples twice in a 2 week interval (repeatability = 0.637, N = 56 individuals, F ratio = 4.504), as well as the heritability of this hormone (h² =0.32).

Field experiment

The field experiment was conducted in semi-natural conditions in 11 enclosures near Konnevesi research station in Central Finland (62°37'N, 26°20'E) during the summers of 2008 and 2009. Enclosures were 0.2 ha each in size (40 x 50 m), and were surrounded by sheet metal fencing (1.0 m above ground, 0.5 m below). While high enough to contain the study populations, the fences did not necessarily prevent possible predators (e.g. red fox Vulpes vulpes, least weasel Mustela nivalis or avian predators) from entering the enclosures. Each enclosure contained twenty Ugglan live traps in a grid pattern, approximately 10 metres apart. The traps were covered by galvanized sheet-metal chimneys to reduce exposure of trapped individuals to possible extreme weather conditions. Sunflower seeds, potatoes and pellets were used as bait in the traps, however the study individuals relied on natural food resources during the non-trapping phases in the enclosures.

Individuals were assigned randomly to enclosures, but the assignment of siblings to the same enclosure was avoided. The frequency of the two reproductive tactics was manipulated to be either common or rare in each enclosure by releasing one male and one female from one selection group, and three (or four: 1st run in 2008) males and females from the other selection group (e.g. 1 Mf male + 1 Mf female + 3 mF males + 3 mF females, or 1mF male + 1 mF female + 3 Mf males + 3 Mf females). See Figure S1 for a schematic representation of the field enclosure treatments. Two runs were conducted during the first year, and one run during the second year.

The release of females was followed by the release of males four days later, and animals were left to breed in enclosures. Approximately 18 days after males were released, when females were in late pregnancy, all individuals were trapped out of the enclosures and brought to the laboratory for females to give birth. Trapping was done approximately every six hours and stopped when no new individuals were found after three consecutive trappings.

In the laboratory, females were monitored daily for pregnancies and births. Within 24 hours from birth, DNA was sampled from the tip of each pup’s tail. Throughout the experiment, there were no significant differences in mortality or female breeding success between the selection groups (p > 0.05 for all analyses), which validates our use of fecundity as a proxy for fitness.
Genetic analysis

Genomic DNA from the tissue samples was extracted using Qiagen DNeasy Tissue kit and KingFisher magnetic particle processor. Individuals were genotyped for 9 different microsatellite loci: MSCg 04, 09, 15 (38), MSCg 07, 18 and 24 (39), and Cg 5G6, 12E6, 17E9 (40). DNA amplification with polymerase chain reaction (PCR) was done. After amplification, PCR products of three to four microsatellite markers (with different fluorescent labels) from the same sample were mixed together and diluted to 2:3 concentrations. Microsatellite allele length was detected using capillary electrophoresis, the polymorphism was scored, and then paternities were assigned with Cervus 3.0 software (41) using the “most likely candidate with known mother” procedure. Paternity was successfully assigned with strict (95%) statistical confidence for 94.2% of the pups.

Statistical analyses of experimental data

We used a Pearson chi-square test to assess any difference between male mating success in the laboratory competitions trials. A difference in log-transformed male plasma testosterone levels between the selection groups was evaluated using generalized linear mixed models (GLMM), where the ‘line’ was a fixed factor, and ‘mother ID’ and ‘year’ were random factors. For the field data, two random effects (enclosure and run) showed zero variance. So as to reduce superfluous groupings in the analysis and to simplify the model structure, we did not include these random effects in our models. Male (N=146) reproductive success was found to be zero-inflated and overdispersed (ϕ=5.49, z=7.29, p<0.001), so we used a zero-inflated negative binomial model (ZINB) to test if the ‘number of offspring sired’ was affected by the ‘line’ (low/high dominance), ‘frequency’ (rare/common), or their interaction. This model was run by using the `zeroinfl` function from the pscl package in R (R core team 2009). The reported results are from the count component of the zero-inflated models, as all variables were non-significant in the logistic component (all p>0.6). Based on the results of the count model, pairwise post-hoc comparisons based on Tukey contrasts were used for testing the significant interaction. The number of offspring produced by successful females were poisson distributed, however they showed significant underdispersion (ϕ=0.60, z=-3.55, p<0.001), which is common in litter size data. We used a GLM with a quasi-poisson distribution to test if female litter size is affected by ‘line’ (low/high fertility), ‘frequency’ (rare/common) or their interaction. No variables were significant in the model containing the interaction between frequency and line (GLM quasi-poisson: frequency, t=-0.85, p=0.397; line: t=-0.71, p=0.481; line*frequency: t=1.21, p=0.231), so we then sequentially dropped non-significant terms, starting with the interaction (see Main text); the only significant term was line on its own (GLM quasi-poisson: t=2.15, p=0.034).

Model Description

Our aim was to investigate whether the conceptual model of Figure S1, where it is impossible to achieve high success for both males and females with the same genotype, maintains variation when it combines with frequency-dependent male mating success. We assumed diploid inheritance with discrete generations. To simulate sexual antagonism, males of genotype AA are assumed to have the highest reproductive success (via high mating success), whereas females of genotype AA have the lowest reproductive
success (via poor fecundity). Females of genotype $aa$ have the highest fecundity, while males of the same genotype have the lowest mating success.

To shorten the notation, from now on we denote genotypes $AA$, $Aa$ and $aa$ by the numbers 1, 2 and 3, and the RS of $AA$, $Aa$ and $aa$ males (females) by $\alpha_1$, $\alpha_2$ and $\alpha_3$ ($\beta_1$, $\beta_2$ and $\beta_3$) respectively.

We note that the model can accommodate any combination of parameters and could be used to perform a much more general analysis, but in this study our aim was to explore the parameters for our study system.

Since we considered discrete generations with a non-biased primary sex ratio, there will always be an equal number of males and females of the same genotype within each generation. Therefore it was sufficient to keep track of the genotype frequencies without sex specificity. We denote the frequencies of genotypes 1, 2 and 3 as $x_1$, $x_2$ and $x_3$ respectively.

We assumed that all females mate, as the majority of females found at the end of the experiment were pregnant, thus female mating success was not affected by genotype or frequency (while her fecundity is dependent on genotype). Thus, the probability that, in a given mating, the female is of genotype $i$ and the male of genotype $j$ is

$$p_{ij} = x_i y_j$$

where $y_j$ is the probability that the sire is of genotype $j$. If mating success was independent of genotype, this probability would simply be equal to the frequency of the genotype in question. However, this probability is elevated for males of high dominance, and their relative advantage may also decline with the frequency of highly dominant males. To take such effects into account, $y_j$ is calculated as

$$y_j = \frac{x_j \alpha_j f_j(x_j)}{\sum_{k=1}^{3} x_k \alpha_k f_k(x_k)}$$

where $\alpha_j$ is the intrinsic mating propensity of male type $j$, and $f_j(x_j)$ incorporates any frequency-dependent changes in this propensity. Specifically, we investigate the functions

$$f_j(x_j) = 1 - \frac{\varphi_j}{1 + e^{\gamma(\tau-x_j)}}$$

Here $\varphi_j$ determines an upper bound for how much male mating success of genotype $j$ can decrease as frequency increases, $\gamma$ determines how steep this decrease is and $\tau$ determines the frequency at which the decline is the steepest. Together with a high $\alpha_j$ this captures the assumptions that high-dominance males achieve a disproportionate share of matings.
but only when they are not very common in the population. By setting $\phi_j = 0$, we can make explicit frequency dependence disappear for any genotype independently. However, implicit frequency dependence is still possible if the other genotype changes its mating propensity with frequency, since we assume that all females mate and mating propensities of males are evaluated relative to each other.

Given that sires and mothers are known, the model can now specify the number of offspring of a given genotype. Denoting the total population size of females as $N$ and their genotype-specific fecundities as $\beta_1$, $\beta_2$ and $\beta_3$, the number of offspring of each genotype becomes

$$n_1 = N \left( p_{11} \beta_1 + \frac{1}{2} p_{12} \beta_1 + \frac{1}{2} p_{21} \beta_2 + \frac{1}{4} p_{22} \beta_2 \right) \quad (4a)$$

$$n_2 = N \left( \frac{1}{2} p_{12} \beta_1 + p_{13} \beta_1 + \frac{1}{2} p_{21} \beta_2 + \frac{1}{2} p_{22} \beta_2 + \frac{1}{2} p_{23} \beta_2 + p_{31} \beta_3 + \frac{1}{2} p_{32} \beta_3 \right) \quad (4b)$$

$$n_3 = N \left( \frac{1}{4} p_{22} \beta_2 + \frac{1}{2} p_{23} \beta_2 + \frac{1}{2} p_{32} \beta_3 + p_{33} \beta_3 \right) \quad (4c)$$

The coefficients $\frac{1}{2}$ and $\frac{1}{4}$ represent probabilities that a given combination of parental genotypes leads to the expected offspring genotype. Finally, genotype frequencies in the next generation are given by

$$\hat{x}_i = \frac{n_i}{\sum n_i} \quad (5)$$

Note that $N$ from equations (4a-4c) will cancel out in equation (5), and the total number of individuals in the population does not affect the predicted frequencies. By replacing $x_i$ with $\hat{x}_i$ and repeating equations (1-5) any given number of times, we can simulate genotype frequency changes over several generations.

**Parameter Estimation and Convergence**

All parameter estimates are shown in Table S1. Parameter estimates for $\alpha_1$ and $\alpha_3$, describing relative male mating success, are as in Fig. 1A. Female fecundity parameters $\beta_1$ and $\beta_3$ are derived directly from the female litter size data in the field, as described in the main text and supplement.

The parameters $\phi_1$ and $\phi_3$, describing frequency dependence for high and low behavioral dominance males respectively, are calculated from the relative drop in the number of offspring sired in the field in high and low frequency trials, as shown in Fig. 1C: $\phi_1=(5.75-2.48)/5.75\approx0.569$, $\phi_3=(3.67-2.71)/3.67\approx0.262$.

For parameters $\gamma$ and $\tau$, which determine the shape of the frequency dependence curve, we used the values 5 and 0.5 respectively. These parameterize a sigmoidal curve.
describing a relatively gradual and conservative frequency dependence function (see Fig. S2). These two parameters were not derived from the data.

Since the dominance for alleles $A$ and $a$ is unknown, we tested all combinations of male and female genetic dominance from full recessiveness to full dominance. $\alpha_2$, $\beta_2$ and $\phi_2$ were calculated using these dominance values, as shown in Fig. 2 and described in the figure legend.

To ensure convergence, for each genetic dominance scenario the simulation was run until there was no change in gene frequencies in the last 100 generations, with a tolerance level of $10^{-7}$.

**Additional Model Notes**

Figure S3 shows a specific case of genetic dominance ($D = 0$ for both sexes, i.e. co-dominance). Again, with parameters extracted from the experimental data, without explicit frequency dependence the $A$ allele evolves to fixation. With explicit frequency dependence included, both alleles ($A$ and $a$) are maintained.
Figure S1. The two treatment groups (broken rectangles) in the enclosure experiment and the prediction (solid arrows) for how the frequency treatment could affect the populations. Population A has males with low behavioral dominance and females of high fertility. Population B has males of high behavioral dominance and females of low fertility. In the field experiment, one unrelated male and female from each population were included as rare individuals in a treatment group containing unrelated members of the other population (3-4 common individuals per sex). We tested whether there was frequency dependent selection for these rare (high/low dominance) male and/or (high/low fertility) female individuals in a population.
Figure S2. Frequency dependence functions for low-dominance males (dotted line) and high-dominance males (solid line). Parameter values used: $\phi_1=0.57$, $\phi_3=0.26$, $\gamma=5$, $\tau=0.5$ (see Eq. 3).
Figure S3. Frequency of A allele in a population over time when dominance equals 0 for both sexes (codominance). Dotted line = explicit frequency dependence, solid line = no explicit frequency dependence. Parameter values as in Fig. 2, with $D_\alpha=D_\beta=0$. The first 40 out of 1000 simulated generations are shown. Gene frequencies were converged by 250 generations (with a tolerance of $10^{-7}$), but there was very little change after the first 40 generations.
SUPPORTING TABLES

**TABLE S1. List of parameter estimates used in mathematical model.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Estimate</th>
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</thead>
<tbody>
<tr>
<td>$\alpha_1, \alpha_3$</td>
<td>Relative male dominance</td>
<td>80, 20</td>
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<tr>
<td>$\beta_1, \beta_3$</td>
<td>Female fecundity</td>
<td>4.53, 5.32</td>
</tr>
<tr>
<td>$\phi_1, \phi_3$</td>
<td>Male frequency dependence</td>
<td>0.569, 0.262</td>
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<tr>
<td>$\gamma$ and $\tau$</td>
<td>Shape of frequency dependence curve</td>
<td>5, 0.5</td>
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Additional References


