Negative Frequency-Dependent Selection of Sexually Antagonistic Alleles in *Myodes glareolus*

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Negative Frequency-Dependent Selection of Sexually Antagonistic Alleles in *Myodes glareolus*

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Sexually antagonistic genetic variation, where optimal values of traits are sex-dependent, is known to slow the loss of genetic variance associated with directional selection on fitness-related traits. However, sexual antagonism alone is not sufficient to maintain variation indefinitely. Selection of rare forms within the sexes can help to conserve genotypic diversity. We combined theoretical models and a field experiment with *Myodes glareolus* to show that negative frequency-dependent selection on male dominance maintains variation in sexually antagonistic alleles. In our experiment, high-dominance male bank voles were found to have low-fecundity sisters, and vice versa. These results show that investigations of sexually antagonistic traits should take into account the effects of social interactions on the interplay between ecology and evolution, and that investigations of genetic variation should not be conducted solely under laboratory conditions.

The direction of evolutionary change generally cannot be predicted without taking into account interactions between conspecifics, be they competitors or mates (*1*, *2*). Additionally, the fitness effects of particular genotypes can often be highly sex-specific (*3*). Alleles beneficial to a son's reproduction can be detrimental for a daughter's success, or vice versa, resulting in different optimal trait values (optima) between the sexes (*4*–*8*). The presence of these sexually antagonistic (SA) alleles in a variety of organisms is now widely acknowledged (*9*–*11*), but less is known about how variation in such alleles is maintained. All else being equal, SA selection does not decrease genetic variation as rapidly as selection that favors the same alleles in both males and females (*6*, *12*).

However, SA selection is not as beneficial for genetic diversity as true negative frequency-dependent maintenance of alternative alleles, whereby rare genotypes are favored in reproduction (*13*). The process of variation depletion may end in intralocus sexual conflict that remains unresolved, such that the traits of males, females, or both do not match their optima [leading to a cost of SA alleles, the gender load (*14*)]. It may also end in resolved conflict if gene expression evolves to become more sex-specific (*15*). In the former case sexual antagonism is preserved, in the latter case it disappears, but variation as such is lost in both. Because gender load can exist even if all individuals possess the same genotype—that is, the one that is the best compromise between male and female fitness—variation is not necessarily maintained by gender load per se. Sexual antagonism, however, becomes potentially powerful for variance maintenance once it combines with sex linkage (*16*), maternal effects (*17*), or assortative mating (*18*). Here, we focused on the role of frequency-dependent selection as an alternative for making sexual antagonism successful at maintaining genetic variation.

We tested whether frequency-dependent selection on dominance in males and/or females can maintain SA variation in field populations of a common European mammal, the bank vole (*Myodes glareolus*). The reproductive effort of bank voles is negatively frequency-dependent in the field (*19*), attesting to the direct influence of an individual's neighbors on the population dynamics and life history evolution in this species. Previous experiments with this species have also shown that testosterone is under sexual and SA selection and is the most important determinant of dominance in male-male competition (*20*–*22*). This hormone also affects a variety of evolutionarily important processes such as spermatogenesis (*23*), immune function (*24*, *25*), and secondary sex trait growth (*26*).

A haploid model with intralocus sexual conflict has shown that genetic variation can be maintained if the antagonism is sufficiently "balanced"—that is, if the relative fitness differences within males and females are of similar magnitude (*27*). This can lead to cyclic dynamics of morph frequency (*28*) or a protected polymorphism (*29*) where allele frequencies are roughly invariant over time. The maintenance of polymorphisms can be facilitated if the SA alleles are sex-linked (*16*), or if the fitness costs of SA alleles are nearly neutral when averaged between sexes and over their lifetimes (*30*). If we assume additional negative effects for each competitive type as their frequency becomes stronger, maintaining SA genetic variation becomes considerably easier.

Our integrated theoretical-empirical study included a large-scale field experiment, replicated over 2 years with a total of 31 populations, that exposed bank voles to terrestrial and avian predators as well as naturally occurring weather conditions and food resources (*31*). We first artificially selected groups in the laboratory (lines) to create behaviorally dominant males with sisters of low fecundity, and vice versa (*31*). In a series of male-male competition trials, two males of opposing behavioral dominance competed with each other to mate with a wild female in estrus. The high-dominance males overwhelmingly outcompeted the low-dominance males in mating success trials ($\chi^2 = 59.71$, $N = 168$, $P < 0.001$) (Fig. 1A), and they had a significantly higher plasma testosterone level (generalized linear mixed model; line: $F_{1,161} = 6.18$, $P = 0.014$) (Fig. 1B). We then assigned males and females to large outdoor field enclosures by manipulating the reproductive quality (high or low behavioral dominance for males, high or

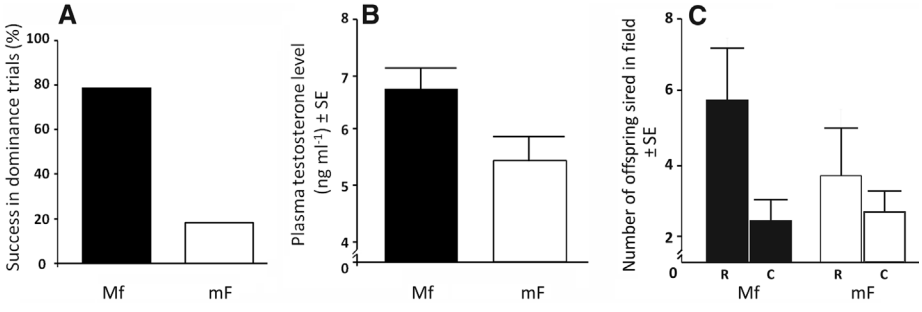

Fig. 1. Experimental data for males artificially selected for high and low dominance. (**A**) Relative mating success in laboratory male-male competition for a female in estrus. (**B**) Mean plasma testosterone level. (**C**) Mean frequency dependence of reproductive success in the field. Post hoc testing showed that the number of offspring sired by rare dominant males was significantly greater than the number sired by common dominant males ($z = 2.97$, $P = 0.015$). No other pairwise groupings differed significantly from each other ($P > 0.1$). Mf (black bars), high-dominance males with low-fecundity sisters; mF (white bars), low-dominance males with high-fecundity sisters; R, rare; C, common. Error bars in (B) and (C) denote SEM.

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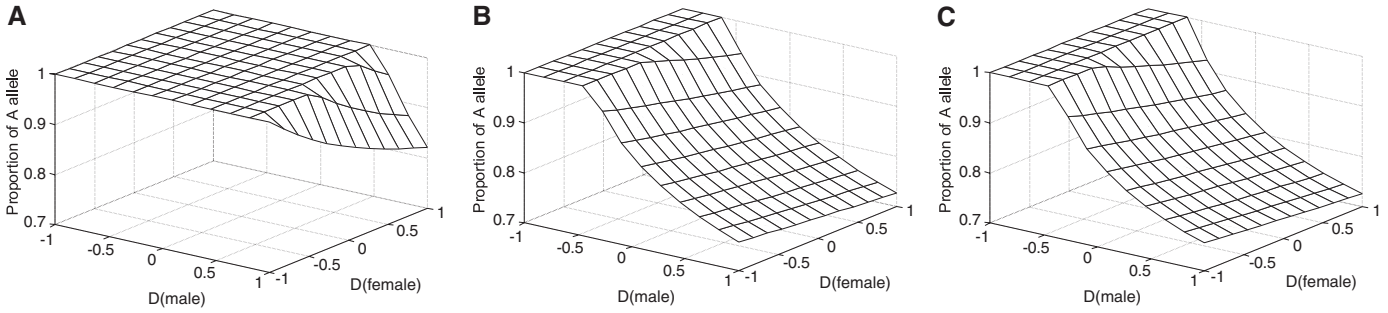

Fig. 2. The outcome of the model, expressed as the frequency of the *A* allele after convergence for three different frequency dependence scenarios. Allele frequencies smaller than 1 indicate that variation is maintained. (**A**) No explicit frequency dependence. (**B**) Explicit negative frequency dependence of SA alleles in all male genotypes. (**C**) Explicit negative frequency dependence only in the behaviorally dominant *AA* males. *AA*, *Aa*, and *aa* males have relative mating success values of $\alpha 1$, $\alpha 2$, and $\alpha 3$, and maximum frequency dependence values of $\varphi 1$, $\varphi 2$, and $\varphi 3$, respectively; *AA*, *Aa*, and *aa* females have fecundity values of $\beta 1$, $\beta 2$, and $\beta 3$. D_{male} and D_{female} represent genetic dominance in males and females; γ and τ determine the shape of the frequency dependence curve [see (*31*) for details on the model and parameter estimates]. Parameter values used: $\alpha_1 = 80$, $\alpha_3 = 20$, $\beta_1 = 4.53$, $\beta_3 = 5.32$, $\gamma = 5$, $\tau = 0.5$, and (A) $\varphi_1 = \varphi_2 = \varphi_3 = 0$, (B) $\varphi_1 = 0.569$, $\varphi_3 = 0.262$, (C) $\varphi_1 = 0.569$, $\varphi_2 = \varphi_3 = 0$. The appropriate values for α_2 and β_2 [and φ_2 for the scenario in (B)], corresponding to different dominance values D_{male} and D_{female}, were calculated as $\alpha_2 = [D_{male}(\alpha_1 - \alpha_3) + \alpha_1 + \alpha_3]/2$, $\beta_2 = [D_{female}(\beta_3 - \beta_1) + \beta_3 + \beta_1]/2$, and $\varphi_2 = [D_{male}(\varphi_1 - \varphi_3) + \varphi_1 + \varphi_3]/2$ according to Falconer's notation (*33*).

low fecundity for females) and frequency (rare or common) of male and female voles in these populations (fig. S1).

The reproductive success of males became negatively frequency-dependent in the field: Dominance was costly for males when it was the common tactic in the population (zero-inflated negative binomial count submodel; frequency, $Z = -2.61$, $P = 0.009$; line, $Z = -1.01$, $P = 0.311$; frequency × line, $Z = 2.00$, $P = 0.046$) (Fig. 1C). In contrast, the fitness of sisters demonstrated a sexually antagonistic effect without evidence for frequency dependence. The high-fecundity sisters of low-dominance males had significantly larger litter sizes than the sisters of high-dominance males (i.e., females selected for low fecundity) [5.32 ± 0.24 versus 4.53 ± 0.27 (SEM)], although, in a clear deviation from the male pattern, the reproductive output of these females did not depend on their frequency in the population (generalized linear model quasi-Poisson; frequency, $t = 0.87$, $P = 0.389$; line, $t = 2.06$, $P = 0.043$).

Combined with previous research on the selection of testosterone (*20*, *22*, *25*), our results suggest that there is potential for the sexes to experience antagonistic selection constraints in adaptation as a result of the physiological and behavioral components of their respective life history strategies. As shown in Fig. 1C, the male mating advantage is negatively frequency-dependent, which helps to explain why fixation of any allele is not predicted to occur.

In the next phase of our study, we extracted parameter estimates from our field data for use in our model (*31*), which expands previous work (*27*) to include diploid genetics and frequency dependence effects within males. Mating propensities of each male type either are constant or change with frequency; the latter choice creates implicit frequency dependence for the mating success of the other male type even if the mating propensity of this type was not set to depend on frequency, because an offspring sired by one male cannot be sired by another (females being a limiting resource). This can lead to the maintenance of genetic variability even if explicit frequency dependence is present in only one male type.

We ran our model with explicit frequency dependence on one or both male types (in the former case, frequency dependence is implicit for subordinate males), as well as with no frequency dependence, using parameters extracted from the experimental results. As we do not know the exact genetic system controlling the antagonistic traits in the study system, we ran the model using the entire range of genetic dominance parameters from full recessiveness to full dominance. In the absence of frequency dependence, genetic variation was maintained only when genetic dominance for both female and male traits was high (in other words, *A* is the dominant allele for males; *a* is dominant for females) (Fig. 2A). Frequency dependence of the magnitude we found for voles makes the maintenance of genetic variation much easier; it is maintained for a large proportion of the parameter range describing genetic dominance (Fig. 2B). It is maintained to a very similar degree even if frequency dependence arises only implicitly for low-dominance males (Fig. 2C). Our proposed mechanism thus appears reasonably robust, and future work could fruitfully combine these findings with other mechanisms known to promote maintenance of variation under SA selection (*16*–*18*).

Our results also suggest that studies of this type should not be restricted to laboratory environments. Seminatural conditions led to a sharp difference in the success of rare versus common behaviorally dominant males (Fig. 1C)—an effect that would have remained undetectable had our study been confined to the mating trials conducted in the laboratory. The difference in optimal food and housing conditions in the lab and the limitations of the field environment can have implications for physiological and behavioral measures (*32*) and fitness consequences alike. Thus, our theoretical and empirical results together show that selection favoring rare male morphs can maintain genetic variation in sexually antagonistic traits, while also indicating that ecological and social environments are important in defining the trait optima for males and females.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/334/6058/972/DC1
Materials and Methods
Figs. S1 to S3
Table S1
References (*34*–*41*)

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X-ray Emission Spectroscopy Evidences a Central Carbon in the Nitrogenase Iron-Molybdenum Cofactor

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Nitrogenase is a complex enzyme that catalyzes the reduction of dinitrogen to ammonia. Despite insight from structural and biochemical studies, its structure and mechanism await full characterization. An iron-molybdenum cofactor (FeMoco) is thought to be the site of dinitrogen reduction, but the identity of a central atom in this cofactor remains unknown. Fe Kβ x-ray emission spectroscopy (XES) of intact nitrogenase MoFe protein, isolated FeMoco, and the FeMoco-deficient Δ*nifB* protein indicates that among the candidate atoms oxygen, nitrogen, and carbon, it is carbon that best fits the XES data. The experimental XES is supported by computational efforts, which show that oxidation and spin states do not affect the assignment of the central atom to C^{4-}. Identification of the central atom will drive further studies on its role in catalysis.

Nitrogenase (N_2ase), found in symbiotic and free-living diazotrophs, catalyzes the reduction of dinitrogen (N_2) to ammonia (NH_3) using eight electrons, eight protons, and 16 MgATPs (ATP, adenosine triphosphate) (*1*). Industrially, the same reaction is performed by the Haber-Bosch process that produces more than 100 million tons of NH_3 each year, thereby accounting for ~1.4% of global energy consumption. Understanding how nature activates the strongest homodinuclear bond in chemistry, the triple bond of N_2, is the key for the future design of molecular catalysts.

The high-resolution crystal structure of N_2ase determined by Einsle *et al*. (*2*) showed that the active site of the molybdenum-iron (MoFe) protein component of N_2ase binds a complex cluster consisting of seven iron ions, one molybdenum ion, and nine sulfides (Fig. 1A); this cluster is referred to as the iron-molybdenum cofactor (FeMoco) and is thought to be the site of dinitrogen activation. For each FeMoco (of which there are two in the $\alpha_2\beta_2$ tetrameric MoFe protein) there is an additional cluster that consists of eight irons and seven sulfides (Fig. 1B); this cluster is referred to as the P cluster. The P clusters serve as electron-transfer sites. Several reaction intermediates in nitrogenase catalysis have recently been observed (*3*, *4*). However, despite the progress in the experimental and theoretical analysis of the FeMoco (*4*–*7*), neither the reaction that occurs at the FeMoco nor the structure of FeMoco has been fully clarified. In 2002, Einsle *et al*. identified a light atom in the center of FeMoco that could be attributed to a single, fully ionized C, N, or O atom (*2*). No consensus has since emerged concerning the nature of this key atom. Study of FeMoco by electron paramagnetic resonance and related techniques is complicated by complex spin-couplings between the open-shell ions, which are not fully understood. Mössbauer spectroscopy suffers from spectral crowding, and neither nuclear resonance vibrational spectroscopy nor extended x-ray absorption fine structure are sufficiently conclusive (*8*).

Herein, we report iron Kβ valence-to-core (V2C) x-ray emission spectroscopy (XES) of N_2ase and demonstrate that these data provide a signature for the presence and identity of the central atom. Kα and Kβ XES monitor the emission of photons after ionization of a metal 1s electron. The $K\beta_{1,3}$ emission line (~7040 to 7070 eV) corresponds to an electric dipole allowed 3p → 1s transition. To higher emission energies,

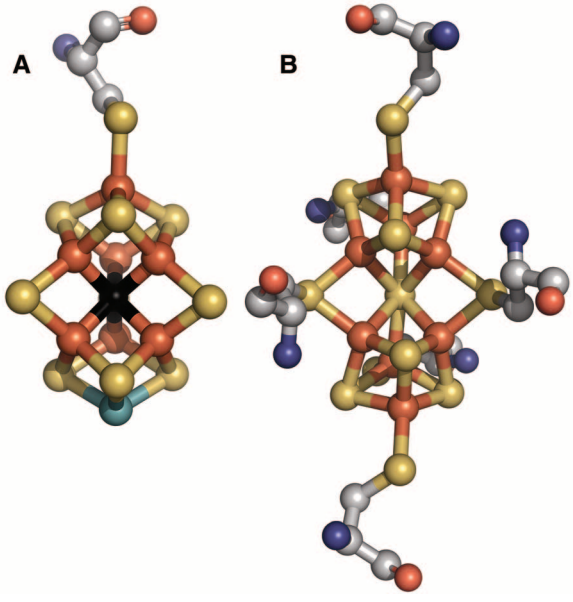

Fig. 1. The FeMoco (**A**) and P cluster (**B**) of nitrogenase (adapted from the Protein Data Bank: identification number 1MIN). Orange, Fe; yellow, S; light blue, Mo; black, C^{4-}, N^{3-}, or O^{2-}; dark blue, nitrogen; gray, carbon. For clarity, the homocitrate and histidine ligands to the Mo have been omitted.

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Supporting Online Material for

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This PDF file includes:

SUPPORTING ONLINE MATERIAL

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 ºC in standard cages measuring 43 x 26 x15 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^{o}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 (h^2 =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 (eg. 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 α_1, α_2 and α_3 (β_1, β_2 and β_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

$$p_{ij} = x_i y_j \tag{1}$$

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

$$y_j = \frac{x_j \alpha_j f_j(x_j)}{\sum_{k=1}^{3} x_k \alpha_k f_k(x_k)} \tag{2}$$

where α_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

$$f_j(x_j) = 1 - \frac{\varphi_j}{1 + e^{\gamma(\tau - x_j)}} \tag{3}$$

Here φ_j determines an upper bound for how much male mating success of genotype *j* can decrease as frequency increases, γ determines how steep this decrease is and τ determines the frequency at which the decline is the steepest. Together with a high α_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 $\varphi_j = 0$, 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 β_1, β_2 and β_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 ½ and ¼ 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 α_1 and α_3, describing relative male mating success, are as in Fig. 1A. Female fecundity parameters β_1 and β_3 are derived directly from the female litter size data in the field, as described in the main text and supplement.

The parameters φ_1 and φ_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: $\varphi_1=(5.75-2.48)/5.75\approx0.569$, $\varphi_3=(3.67-2.71)/3.67\approx0.262$.

For parameters γ and τ, 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. α_2, β_2 and φ_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.

SUPPORTING FIGURES

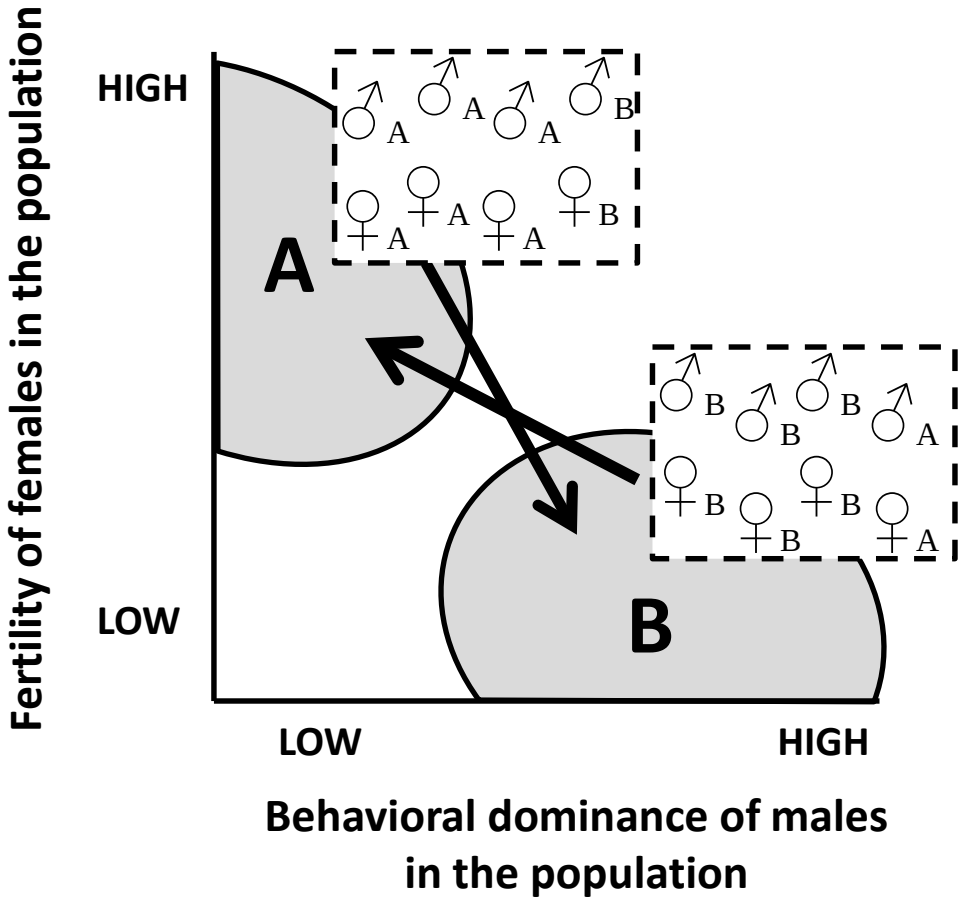

Figure S1. The two treatment groups (broken rectangles) in the enclosure experiment and the prediction (solid arrows) for how the frequency treatment could affect the populations. Population A has males with low behavioral dominance and females of high fertility. Population B has males of high behavioral 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.

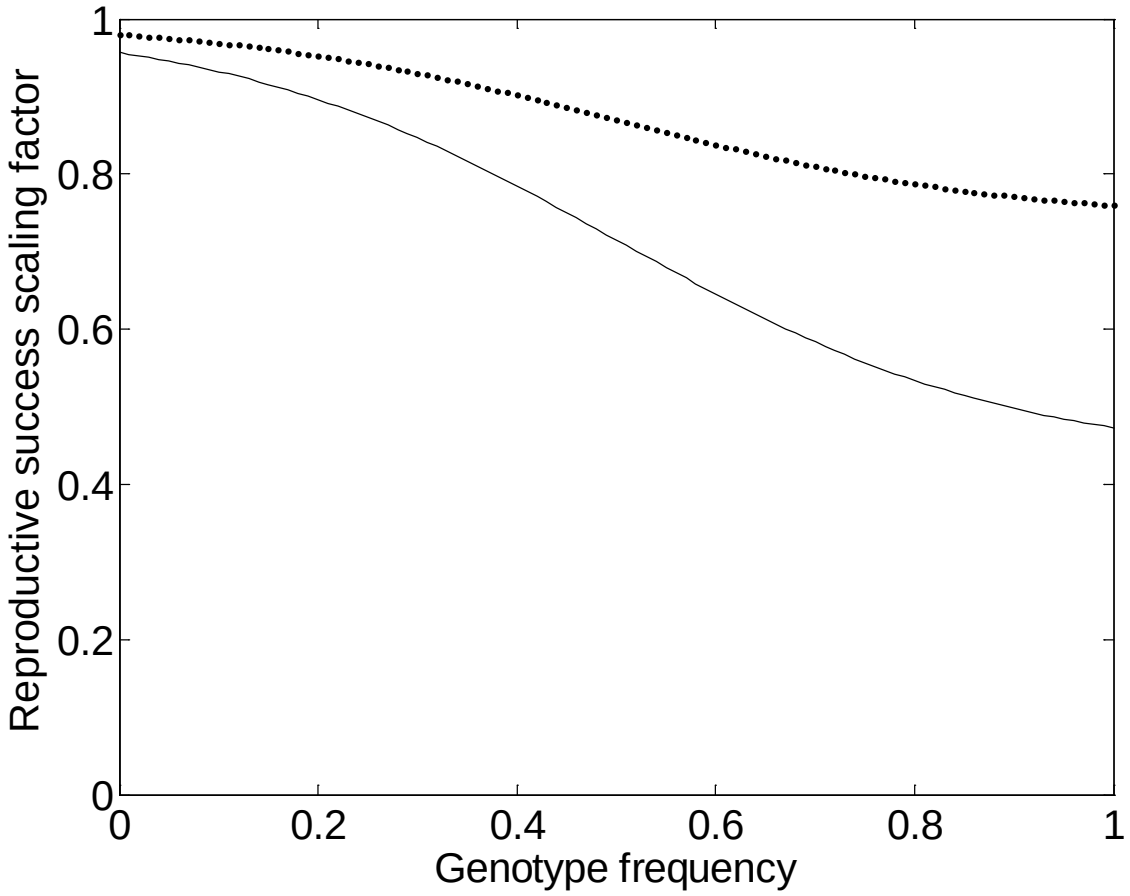

Figure S2. Frequency dependence functions for low-dominance males (dotted line) and high-dominance males (solid line). Parameter values used: φ_1=0.57, φ_3=0.26, γ=5, τ=0.5 (see Eq. 3).

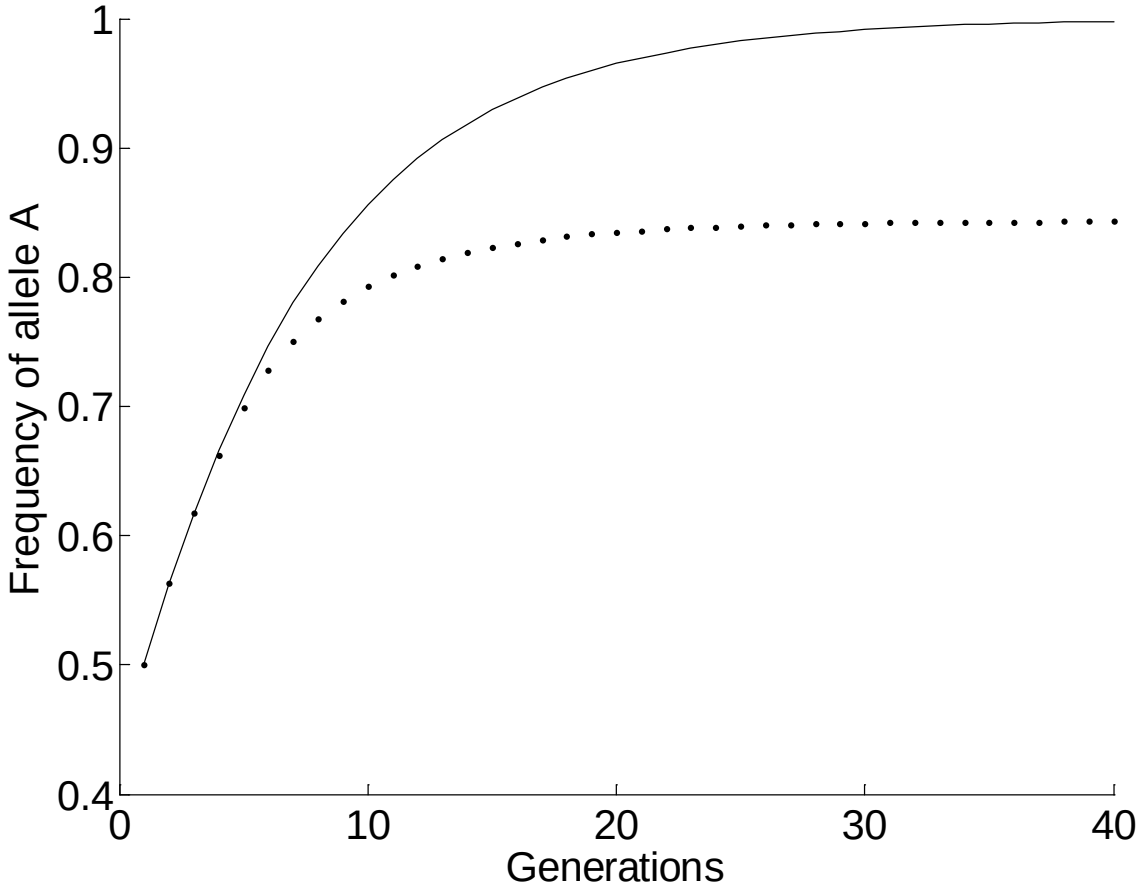

Figure S3. Frequency of *A* allele in a population over time when dominance equals 0 for both sexes (codominance). Dotted line = explicit frequency dependence, solid line = no explicit frequency dependence. Parameter values as in Fig. 2, with $D_\alpha=D_\beta=0$. The first 40 out of 1000 simulated generations 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.

Parameter	Description	Estimate
α_1, α_3	Relative male dominance	80, 20
β_1, β_3	Female fecundity	4.53, 5.32
φ_1, φ_3	Male frequency dependence	0.569, 0.262
γ and τ	Shape of frequency dependence curve	5, 0.5

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