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

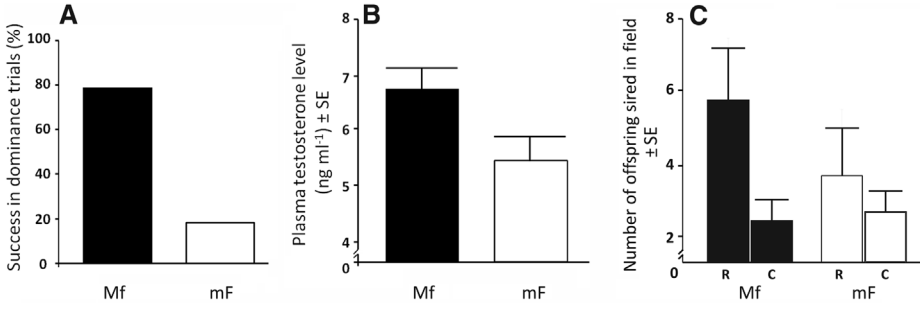

Fig. 1. Experimental data for males artificially selected for high and low dominance. (**A**) Relative mating success in laboratory male-male competition for a female in estrus. (**B**) Mean plasma testosterone level. (**C**) Mean frequency dependence of reproductive success in the field. Post hoc testing 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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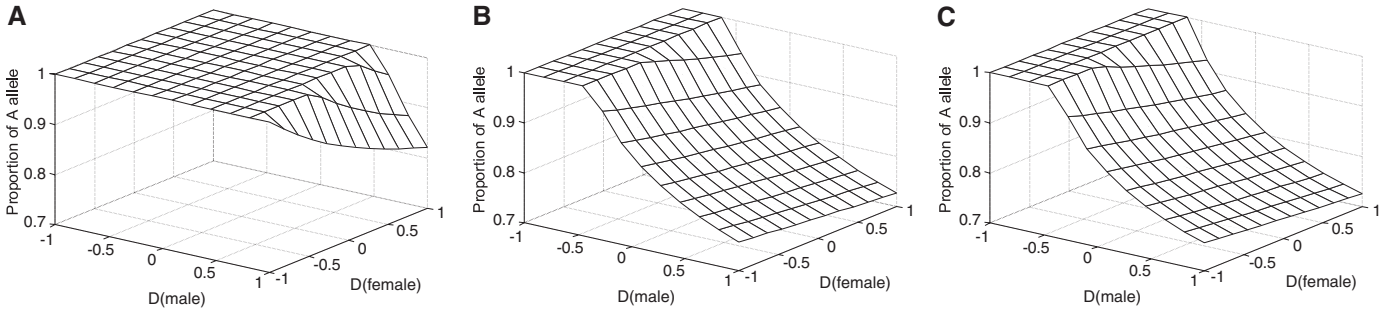

Fig. 2. The outcome of the model, expressed as the frequency of the *A* allele after convergence for three different frequency dependence scenarios. Allele frequencies smaller than 1 indicate that variation is maintained. (**A**) No explicit frequency dependence. (**B**) Explicit negative frequency dependence 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
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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,

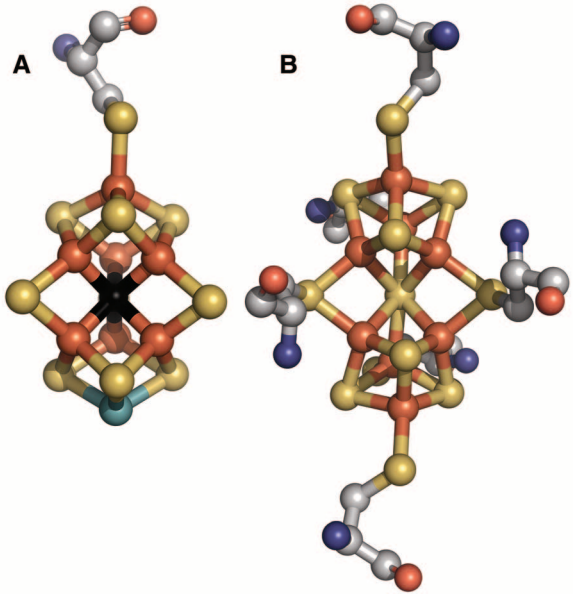

Fig. 1. The FeMoco (**A**) and P cluster (**B**) of nitrogenase (adapted from the Protein Data Bank: identification number 1MIN). Orange, Fe; yellow, S; light blue, Mo; black, C^{4-}, N^{3-}, or O^{2-}; dark blue, nitrogen; gray, carbon. For clarity, the homocitrate and histidine ligands to the Mo have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

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