

SIGNAL RELIABILITY COMPROMISED BY GENOTYPE-BY-ENVIRONMENT INTERACTION AND POTENTIAL MECHANISMS FOR ITS PRESERVATION

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Sexual selection based on signaling requires that signals used by females in mate choice are reliable indicators of a male's heritable total fitness. A signal and the preference for it are expected to be heritable, resulting in the maintenance of genetic covariance between these two traits. However, a recent article has proposed that signals may quickly become unreliable in the presence of both environmental variation and genotype-by-environment interaction ($G \times E$) with crossing reaction norms, potentially compromising the mechanisms of sexual selection. Here we examine the heritability and plasticity of a male dominance advertisement in the bank vole, *Clethrionomys glareolus*, in stable and changing rearing environments from father to son. The bank vole is naturally exposed to considerable sources of spatial and temporal environmental variation and male reproductive success is determined by both intra- (male–male competition) and inter- (females prefer to mate with dominant males) sexual selection. Significant $G \times E$ for male dominance was found with crossing reaction norms. Plasma testosterone level (T), rather than condition, determined a male's dominance and T also showed a significant $G \times E$. Dominance showed a considerable plasticity across environments, but was only heritable under stable conditions. We document a negative between-environments correlation of male dominance, suggesting that when the environment changes between father and son, the dominance signal is unreliable to females and sexual selection may be compromised. We discuss how $G \times E$ and environmental variation interacting with other mechanisms may preserve the reliability of signals and thus the mechanism of sexual selection itself.

KEY WORDS: Antagonistic sexual selection, *Clethrionomys glareolus*, condition dependence, dominance, mammal, phenotypic plasticity, reaction norms.

Animal communication theory hypothesizes that signals are reliable (Zahavi 1977a), and the expected outcome for both signaler and receiver is a net benefit (see Hauser 1996; Bradbury and Vehrencamp 1998). Although some signaling systems are cooperative, many kinds of communication involve conflict between signaler and receiver. Sexual selection is no exception; whereas selection acts on males to acquire many mates, it acts on females to acquire superior mates (Trivers 1972; Clutton-Brock 1991; Clutton-Brock and Parker 1992). A female's choice of mate may be based on a signal or other advertisement feature, that is a reliable indicator of a potential mate's phenotype, and ultimately their heritable fitness (Moore 1994; Welch et al. 1998; Møller and Alatalo 1999; Doty and Welch 2001). The simplest mechanism for the maintenance of signal reliability is physical constraint, such as the carotenoid-based plumage coloration in male house finches, *Carpodacus mexicanus*, which accurately indicates nutritional condition, and thus health or foraging ability (Hill and Montgomerie 1994) and females selecting brighter males acquire higher quality mates (Hill 1991). In other signaling systems, Zahavi's handicap principle posits that signals will provide reliable information about the quality of signalers, provided that they are costly to produce (Zahavi 1975, 1977a, b). This sexual selection principal has been proven both theoretically (Enquist 1985; Grafen 1990; Godfray 1991; Maynard-Smith 1991; Johnstone and Grafen 1992) and empirically (e.g., Andersson 1994; Johnstone 1995; Møller 1995; Mappes et al. 1996; Kilpimaa et al. 2004). Most studies that examine the reliability of signals have concentrated solely on the output of sons, and neglected the fitness of daughters, an oversight highlighted by Hunt et al. (2004). Homologous traits, such as reproductive success, that are selected for in different directions between the sexes may result in antagonistic sexual selection (Chippindale et al. 2001; Pai and Yan 2002; Fedorka and Mousseau 2004; B. Sinervo and R. Calsbeek, unpubl. ms.). Therefore, signal reliability should be assessed based on the fitness of both sons and daughters.

The expectation of reliability is inherent in both "viability indicator" and Fisherian mechanisms of sexual selection (that are but a continuum of a single process, Kokko et al. 2002) manifest in sons as either superior survivorship and growth or attractiveness, respectively (Greenfield and Rodriguez 2004). Integral to sexual selection mechanisms is the heritability of both the signal and preference for it (Bakker and Pomiankowski 1995; Jang and Greenfield 2000; Greenfield 2002; Rodriguez and Greenfield 2003) and that linkage disequilibrium established by mate choice maintains a genetic covariance between these two traits (Bakker 1993; Gilburn et al. 1993; Hine et al. 2002). Recently however, Greenfield and Rodriguez (2004) demonstrated graphically how environmental variation coupled with genotype-by-environment interaction could quickly lead to the unreliability of signals, a decrease in signal/preference covari-

ance and a fundamental breakdown in the mechanism of sexual selection.

The bank vole, *Clethrionomys glareolus*, is an example of a small mammal species exposed to considerable environmental sources of variation, both spatially (Morris 2005) and temporally through a seasonal variation in food availability and multiannual population density fluctuations (cyclicality) (Hanski et al. 1993), and therefore a promising model species with which to investigate Greenfield and Rodriguez's predictions. Bank vole populations at our study site in central Finland monitored over a 12 year period from 1995 to 2006, show a threefold variation in density within the breeding season, A maximum tenfold variation in density between years and a three-year cyclicality (T. Mappes, unpubl. data). Rather than an elaborate ornamental signal, bank voles advertise their quality by their dominance. Male bank voles compete intensively with each other for access to females (Hoffmeyer 1982) and selection coefficients reveal strong selection gradients on plasma testosterone (T) level (Mills et al. 2007). Male mating success is also determined by female preferences for dominant males based on cues in their urine (Horne and Ylönen 1996; Kruczek 1997) and the preputial gland, the main source of male sexual attractants, is T-dependent (Radwan et al. 2006). Male bank vole mating success is therefore determined by intra- and intersexual selection and both act in concert imposing a directional selection on the same traits. Not only is the reliability of dominance advertisement enforced through social costs during competition (Enquist 1985), but also via a trade-off between dominance and survival according to the immunocompetence handicap (Grossman 1985; Folstad and Karter 1992; Wedekind and Folstad 1994; S. C. Mills et al., unpubl. ms.), as males implanted with exogenous T have lower immune function, larger parasite loads, and reduced survival (S. C. Mills, unpubl. data). As T has a significant father-son heritability ($h^2 = 0.72$ (SE = 0.28), $F = 6.27$, $df = 1, 52$, $P = 0.015$; S. C. Mills, unpubl. data), females that mate with dominant males are expected to produce dominant sons, and indeed a recent study revealed that mating success in male bank voles is significantly heritable (Oksanen et al. 1999).

This article considers how signal reliability (or a mother's fitness payoff from mating with dominant males) is affected by environmental change. We investigate the effects of stable and changing rearing environments between father and son on the heritability and plasticity of male dominance. In addition to the hypothesis that signals should be costly, Zahavi also stated that there should be a necessary link between the formation of a signal and the information that it conveys. Our previous studies in the bank vole have suggested that an individual's dominance conveys plasma T level (Mills et al. 2007) that trades-off against survival (S. C. Mills et al., unpubl. ms.). In recent years many fitness traits are considered to be condition dependent, a form of developmental

plasticity that links phenotypic trait expression to condition (Mappes et al. 1996; Rowe and Houle 1996; David et al. 2000; Kotiaho et al. 2001; Brandt and Greenfield 2004). Therefore, we investigate the link between male dominance, plasma T level, and condition, as well as their plasticity across environments. We also estimate the fitness relationship between sons and daughters across environments.

Materials and Methods

STUDY ANIMALS

Animals were either wild captured during a low density summer in Konnevesi, Finland (62°37'N, 26°20'E), or first generation laboratory-born bank voles, and were housed in standard mouse cages measuring 43 × 27 × 15 cm maintained on a 16L:8D photoperiod. Water and food were continuously available.

MALE DOMINANCE

Two males and a female in oestrus were released into an arena (1 m × 1 m) and observations were made until ejaculation occurred (Oksanen et al. 1999). In addition to urine marking and defecation, males indicated either aggressive or defensive behaviors to each other within 5 min. Normally, the aggressive male then courted the female with successful copulation once the female performed lordosis, whereas the other male retreated to the arena corner. Escalated male aggression only occurred when both males attempted to court the female. On average, copulations took place after 18 min (range 3–67 min). The male that successfully mated was considered dominant. One hundred and forty males were assessed three times with different opponents and random females. Because the success of each male is dependent on the dominance of the three males allotted as his opponents, we corrected the initial dominance score (proportion of contests won) by the success of the opponents. Initial scores were corrected using the equation derived from Alatalo et al. (1991), and also previously used in bank voles (Oksanen et al. 1999),

$$Q = F_{n_F} / [F_{n_F} + (1 - S)n_S],$$

where Q is the corrected dominance estimate, F and S are the mean uncorrected initial dominance scores of the opponents who either failed to copulate (F) or copulated successfully (S); n_F and n_S are the number of unsuccessful and successful opponents, respectively. In this way, the corrected dominance estimate of males that beat successful opponents will increase, whereas the corrected dominance estimate of males that lost to less successful males will be reduced. All males from either the parent or offspring populations were used and males were competed across environments, therefore, a male's dominance value is an absolute rather than a relative value.

No difference in the dominance of fathers was found between those that were wild captured or first generation laboratory born ($t_{1123} = 0.770$, $P > 0.4$). One of three "paternal groups": high, medium, and low dominance was assigned to each father. Males with values of Q ranging from 0 to 0.2, 0.21 to 0.79, and 0.8 to 1 were assigned to the low, medium, and high dominance groups, respectively.

MATING AND CROSS FOSTERING

One hundred and forty fathers were randomly paired for a week to 140 females (which had not been used in the assessment of male dominance). Following the birth of 100 litters (mean ± SE litter size = 5.3 ± 0.1; range 1–8 pups), pups were sexed, individually tagged, weighed (± 0.01g), and head width measured (± 0.1 mm). Within two days of birth, the litters were cross-fostered to exclude any confounding effects of possible variation in postbirth maternal quality—a foster mother's litter was replaced with single pups from different donor mothers (Mappes and Koskela 2004). No differences have been found in this or previous studies between the growth and survival of fostered and nonfostered pups (Mappes et al. 1995).

MANIPULATION OF REARING ENVIRONMENT

We used artificial manipulation of litter size to examine how different environments at nursing affect male bank vole dominance, by moving – 2, ± 0, and + 2 pups to and from litters. The removal of two pups results in a greater amount of resources, such as milk, available per remaining pup, and represents "good" rearing environments in nature. Although mothers increase their milk production after the addition of two pups, the amount received per pup is still lower than that received per pup in unmanipulated litters (E. Koskela, unpubl. data) and enlarging a litter has detrimental effects on pup growth (Oksanen et al. 2003). Thus, enlarged litter treatments represent "poor" rearing environments in nature, such as increased female–female competition at high population density or low food availability, where mothers would receive fewer resources and in turn provide less to their offspring (Koskela et al. 1998).

The manipulation of the rearing environment consisted of a foster mother's original litter being replaced with pups from donor mothers and took place at the same time as cross-fostering. Litter manipulations were carried out in relation to the foster mother's original litter size. Neither the foster nor the donor mother's initial litter size, before manipulation, differed between manipulation groups (one-way ANOVA, donor mother: $F_{2,98} = 1.16$, $P > 0.3$; foster mother: $F_{2,98} = 0.389$, $P > 0.6$). Male and female offspring from a single sire were split between the three manipulated rearing environments.

Sons reared in artificially reduced litters compared to control and enlarged litters were heavier and had larger head widths at weaning (age 20 days) (mean \pm SE; body mass (g): good: 12.3 ± 0.27 , control: 11.2 ± 0.26 , poor: 10.8 ± 0.28 ; $F_{2,82} = 7.99$, $P = 0.001$; head width (mm): good: 12.4 ± 0.06 , control: 12.2 ± 0.06 , poor: 12.1 ± 0.06 ; $F_{2,82} = 4.94$, $P = 0.01$). As there was no difference in the initial body mass or head width at birth (body mass: $F_{2,82} = 0.079$, $P > 0.9$; head width: $F_{2,82} = 0.093$, $P > 0.9$), our rearing manipulations were effective in increasing and decreasing body size, confirming that litter size during nursing is an accurate indicator of conditions for growth (Mappes et al. 1995; Oksanen et al. 2003) and represents variation in environments experienced by nursing mothers in the wild (Koivula et al. 2003).

TESTOSTERONE (T) ASSAY

Plasma T was measured using a radioimmunoassay kit (TESTO-CTK, DiaSorin, Byk-Sangtec Diagnostica GmbH & Co., Dietzenbach, Germany) and the methods are described in detail elsewhere (Mills et al. 2007). The T levels in sons were measured from samples made available from another experiment, therefore a plasma sample was not always available for every son.

CONDITION

Condition is defined as the quantity of metabolic resources an individual can accumulate and the efficiency with which they are translated into reproductive success (Bonduriansky and Rowe 2005). Condition based on resource acquisition and conversion was quantified as growth of body size from birth till weaning (20 days). In addition, as male dominance in bank voles is determined by male–male competition that requires energy (in terms of sugars from fat reserves), we quantified a second condition measure based on the pool of available resources: the residual mass from a linear regression between body mass and head width at weaning (Schulte-Hostedde et al. 2005).

BROTHER–SISTER FITNESS RELATIONSHIP

At the age of 40 days, daughters were given the opportunity to mate with several males until their first parturition was observed. Daughters were placed with a mature male for one week and a new male every week until pregnancy was observed. A daughter's litter size was used as a measure of reproductive success and standardized with respect to the whole population. Previous studies have found that female bank voles do not adjust their litter size or offspring size at birth according to male quality (Oksanen et al. 1999). The relationship between full siblings fitness was measured from Pearson's correlation of a male's standardized

mating success (dominance) with a sister's standardized reproductive success. Although we obtained measures of reproductive success for the majority of daughters and all sons, only 45 brother–sister pairs were reared in the same treatment environment: 10, 16, and 19 from good, control, and poor manipulated rearing environments, respectively. Mean values were used when multiple siblings were reared in the same environment.

STATISTICAL ANALYSES

Genetic, environmental, and genotype-by-environment interaction effects on male dominance and condition were estimated with PROC MIXED model in SAS v. 9.1 (SAS Institute, Cary, NC) using each son as a sample unit. The rearing environment was a fixed factor, paternal dominance or condition the covariate and sire was a random factor (full siblings were compared between treatments). As litter size and composition is highly variable, three brothers were not always available per litter for rearing in each of the three manipulated environments. Therefore, for the G × E analysis of dominance we only used litters that had at least two brothers reared in at least two different manipulated environments, which led to the loss of 49 litters. Of the remaining 51 litters, which were sired by 51 fathers, we used 186 sons.

We used the Gail and Simon (1985) method to identify qualitative or crossover interactions between paternal groups across rearing environments. This method focuses on crossover between paternal groups that are treated as fixed effects and has been used and described in detail by Jia et al. (2000). In our experiment, the mean trait values of the three paternal groups (high, medium, and low paternal dominance) are considered over three environmental conditions. For each environment the difference in means and sample variances of offspring dominance were calculated between two paternal groups and these differences are classified as positive or negative. For two paternal groups at a time, two test statistics Q^+ and Q^- are computed for each group from the sum of squared deviations standardized by their respective sampling variances (for equations see Gail and Simon 1985). To test for the significance of crossover between any two paternal genetic groups, $\min(Q^+, Q^-)$ was compared with critical values from table 1 in Gail and Simon (1985). A large value implies that crossover occurs more frequently than can be expected by chance, given the observed sample variances of the means (Jia et al. 2000).

Heritabilities were calculated from the slope of the linear father–son regression for each environment separately, mean dominance of sons was paired to a father's dominance when full siblings were reared in the same manipulated environment. Father-offspring covariances estimate only half of the additive genetic variance, therefore, they were doubled to obtain heritability estimates (Falconer and Mackay 1996). Additive variance (V_A) is twice the father–son covariance, V_P is the phenotypic variance,

Table 1. PROC MIXED analysis in SAS of covariance on two son traits (A) dominance and (B) condition based on the standardized residual mass from a linear regression of body mass on head width at weaning in the bank vole. Two parameters and their interactions were used in each model: genetic paternal trait—(A) dominance, (B) condition; and rearing environment (manipulation of litter size; + 2, ± 0, - 2 pups). Sire was included in the model as a random factor (estimate ± SE: 0.0 and 0.137 ± 0.11 for the two models [A] and [B], respectively). df, degrees of freedom for numerator (n) and denominator (d); *F*, test statistic; *P*, probability.

Source	(A) Dominance				(B) Condition			
	df (n)	df (d)	<i>F</i>	<i>P</i>	df (n)	df (d)	<i>F</i>	<i>P</i>
Paternal trait (G)	1	108	0.03	0.856	1	68.9	1.78	0.189
Rearing environment (E)	2	108	5.34	0.006	2	45.6	0.45	0.639
Paternal trait × environment (G × E)	2	108	5.38	0.006	2	71.4	0.52	0.599

and \bar{x} the mean mating success of fathers (Houle 1992). These values were used to calculate coefficients of additive genetic variation ($CV_A = 100\bar{x}^{-1} \sqrt{V_A}$), and residual variation ($CV_R = 100\bar{x}^{-1} \sqrt{V_P - V_A}$). In cases when the V_A estimate is negative, CV_A would be zero, so $I_A (= V_A / \bar{x}^2)$ was calculated instead (Houle 1992).

Results

G × E AND HERITABILITY OF SIGNAL

We found a significant interaction between paternal dominance and the rearing environment on offspring dominance (see Table 1A). Manipulated rearing environment had a significant effect on male offspring dominance, however, there was no significant effect of either paternal dominance or sire (see Table 1A).

The significant genotype-by-environment interaction (G × E) indicates that the environmental effects on dominance differed among sons from different fathers (Roff 1997). Therefore, we calculated the heritability of male dominance for each environmental treatment separately. A large and statistically significant additive genetic component to paternal dominance was observed in good (-2 pups) rearing environments (see Table 2), but not in control environments (±0 pups) and a significant negative heritability was identified in poor environments (+2 pups) (see Table 2). Dominance in control and poor environments has a low

heritability due to high residual variation resulting from high environmental variance (see Table 2). We were unable to carry out a more thorough analysis of heritability using a half-sib/full-sib design, and maternal effects during gestation are not taken into account, therefore our heritability estimates should be treated with caution. Furthermore, heritability may be a poor estimate of variability and evolvability because of high environmental variance, and CV_A is considered a better measure (Houle 1992). In good rearing environments almost all the variation for dominance is additive genetic due to sires ($CV_A = 79.54$ in Table 2) and in control rearing environments, not only is there considerable additive genetic variance ($CV_A = 29.59$) but also high residual variation ($CV_R = 59.74$) (see Table 2). The low additive genetic variance in poor rearing environments shows that there will be little response of the population to selection. However, in good, and to a lesser extent in control rearing environments variation in dominance is correlated with the genetic fitness of the bearer, and high CV_A indicates that dominance and related traits can be true targets of selection.

CONDITION DEPENDENCE

In terms of both our condition measures, growth rate and residual body mass, we found no significant correlations between son's condition and either his dominance or plasma T level (see Table 3).

Table 2. Mean (\bar{x}), estimates of heritability (h^2), and variance components for bank vole dominance in three rearing environments. As V_A is negative in the poor environment, CV_A would be zero, so I_A was calculated (Houle 1992). Number of father-son pairs (either one son or mean of multiple sons paired to a father per treatment) = 36, 42, and 35 for good, control, and poor rearing environments, respectively. V_A , additive genetic variance; V_P , phenotypic variance; V_E , environmental variance; CV_A , coefficient of additive genetic variation; CV_P , coefficient of phenotypic variation; CV_R , coefficient of residual variation.

Rearing environment	$\bar{x} \pm SE$	$h^2 \pm SE$ (95% CI)	V_A	V_P	V_E	CV_A or I_A	CV_P	CV_R
Good	0.352 ± 0.06	0.83** ± 0.31 (0.23–1.43)	0.13	0.15	0.02	79.54	57.73	21.56
Control	0.551 ± 0.06	0.18 ^{NS} ± 0.30 (-0.41–0.77)	0.02	0.18	0.16	29.59	62.97	59.74
Poor	0.438 ± 0.06	-0.69* ± 0.33 (-1.34–0.04)	-0.08	0.13	0.22	-0.41	54.23	69.24

* $P < 0.05$, ** $P < 0.01$, ^{NS} $P \geq 0.05$

Table 3. Pearson's correlations of male bank vole dominance and plasma testosterone level with two estimates of condition: growth of body size from birth till weaning and standardized residual mass from a linear regression between body mass and head width at weaning.

Condition measure:	Treatment	Dominance			Testosterone level		
		<i>n</i>	<i>r</i>	<i>P</i>	<i>n</i>	<i>r</i>	<i>P</i>
Growth	Good	55	0.028	0.838	10	0.116	0.750
	Control	66	-0.054	0.665	15	0.001	0.998
	Poor	65	-0.030	0.813	12	-0.053	0.871
Residual mass	Good	55	0.024	0.861	10	0.261	0.467
	Control	66	0.021	0.868	15	0.027	0.923
	Poor	65	-0.021	0.868	12	0.282	0.375

Using the condition measure based on residual body mass, we also tested whether father condition, rearing environment, or their interaction determined a son's condition, but found no significant effects (see Table 1B). The lack of father effect (in the absence of G × E) based on the measures of condition we used, indicates that there is no genetic variation for condition in bank voles.

PHENOTYPIC PLASTICITY AND ECOLOGICAL CROSSOVER OF SIGNAL

The G × E on dominance indicates that offspring from different fathers responded in different ways to rearing environments (see Table 1A). To understand this G × E, we established three paternal groups: high, medium, and low, based on their absolute value of dominance in the population (see Methods), and examined how each environment influenced offspring dominance advertisement among these paternal groups. A significant crossover interaction of reaction norms was identified (Gail and Simon 1985) (see Fig. 1A), implying that none of the three paternal groups signal the highest dominance in all environments (Schlichting and Pigliucci 1998). In good rearing environments, highly dominant fathers sire offspring of higher dominance than offspring of subordinate fathers. However the situation is reversed in poor rearing environments in which dominant fathers produce subordinate offspring and dominant offspring are sired by subordinate fathers. Offspring of the high and low dominance paternal groups show the greatest plasticity in dominance, that is, the greatest response to environmental change.

DIRECTIONAL SELECTION

We found evidence for directional selection on testosterone (T), as a son's dominance covaried positively with plasma T level when all environments were pooled (simple linear regression: $F_{1,58} = 8.84$, $P = 0.004$, $R^2 = 0.13$, $y = 0.177 + 0.09x$). However, when the environments were analyzed separately significant directional selection on T was only found in good rearing conditions (good: $F_{1,10} = 7.47$, $P = 0.021$, $R^2 = 0.43$, $y = 1.93 + 2.81x$; control: $F_{1,27} = 2.53$, $P = 0.123$, $R^2 = 0.09$, $y = 0.12 + 2.97x$; poor:

$F_{1,17} = 2.21$, $P = 0.156$, $R^2 = 0.12$, $y = 0.145 + 2.66x$). An examination of the effects of rearing environment on offspring T levels from each paternal group revealed a significant interaction between rearing environment and paternal dominance group on offspring plasma T (GLM ANOVA, paternal group (G): $F_{2,45} = 0.217$, $P = 0.814$; rearing environment (E): $F_{2,45} = 0.684$, $P = 0.555$; G * E: $F_{4,43} = 5.23$, $P = 0.002$ in Fig. 1B). However, we did not identify significant crossover interactions of reaction norms on offspring T between the dominance paternal groups (see Fig. 1B). The lack of significance may be due to the low sample size of T values; however, the figure suggests that none of the three paternal groups had high T in all environments.

BROTHER-SISTER FITNESS RELATIONSHIP

We standardized the mating success (dominance) of sons and reproductive success of daughters (litter size), then compared the fitness of full siblings that had been reared in the same environment. No significant relationship was found in either good or control rearing environments (see Fig. 2), but the reproductive successes of full siblings that had been reared in poor environments showed a significantly negative relationship (see Fig. 2).

Discussion

We tested the reliability of dominance advertisement in the bank vole, *C. glareolus*, in stable and changing rearing environmental conditions from father to son. Greenfield and Rodriguez (2004) recently hypothesized that a signal is likely to become unreliable in species that show both environmental variation and genotype-by-environment interaction (G × E) with crossing reaction norms. We found that male dominance in bank voles shows considerable plasticity across different environments and a significant G × E with crossing reaction norms. Furthermore, we confirm Greenfield and Rodriguez's (2004) predictions of signal unreliability, as we found a negative between-environments correlation of male dominance when environmental rearing conditions are different

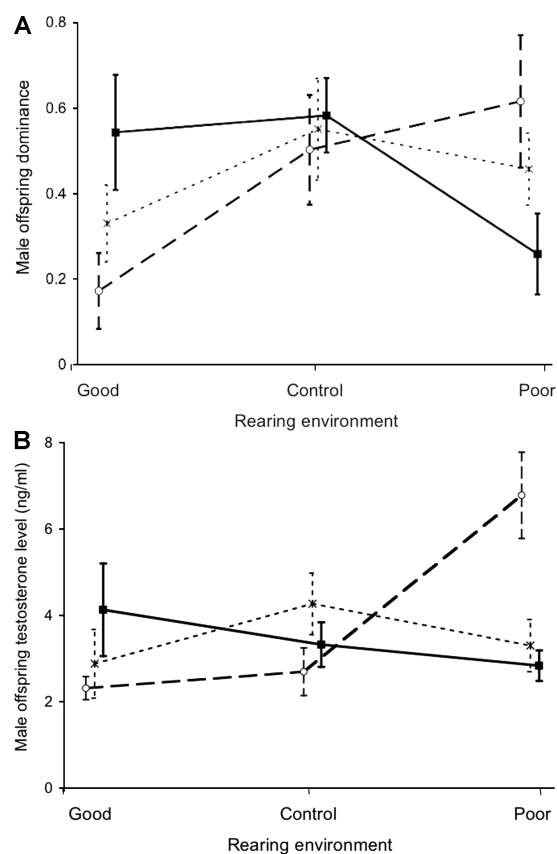


Figure 1. Offspring (A) dominance (mean $Q \pm 1$ SE) and (B) plasma testosterone level (mean ± 1 SE) produced by the three paternal groups (based on dominance) across the different rearing environments. (A) Dominance: \circ = low paternal dominance ($Q: 0 - 0.2$): $y = 0.227x - 0.014$, $n = 31$, $P = 0.024$; \times = medium paternal dominance ($Q: 0.21 - 0.79$): $y = 0.06x + 0.31$, $n = 42$, $P = 0.343$; \blacksquare = high paternal dominance ($Q: 0.8 - 1$): $y = -0.138x + 0.77$, $n = 41$, $P = 0.115$. Using the Gail and Simon (1985) method we identified a significant level of crossover between the high and middle paternal groups ($\min(Q^+, Q^-) = 4.61$, $I = 3$, $P < 0.05$). No crossover was found between the high and low paternal groups ($\min(Q^+, Q^-) = 1.20$, $I = 3$, $P > 0.2$) or between the low and middle paternal groups ($\min(Q^+, Q^-) = 0.32$, $I = 3$, $P > 0.2$). (B) Testosterone: \circ = low paternal dominance ($Q: 0 - 0.2$): $y = 2.199x - 1.08$, $n = 10$, $P = 0.079$; \times = medium paternal dominance ($Q: 0.21 - 0.79$): $y = 0.465x + 2.675$, $n = 11$, $P = 0.542$; \blacksquare = high paternal dominance ($Q: 0.8 - 1$): $y = -0.923x + 5.281$, $n = 12$, $P = 0.083$. We identified no significant crossovers between any groups: ($\min(Q^+, Q^-) = 0.88, 0.73$ and 0.25 , $I = 3$, $P > 0.2$ for the high and middle, high and low, and low and middle paternal groups, respectively).

between fathers and sons. Although we did not find any evidence that dominance is condition dependent, we found a directional selection on plasma T level in stable conditions, as well as plasticity in male T levels across environments.

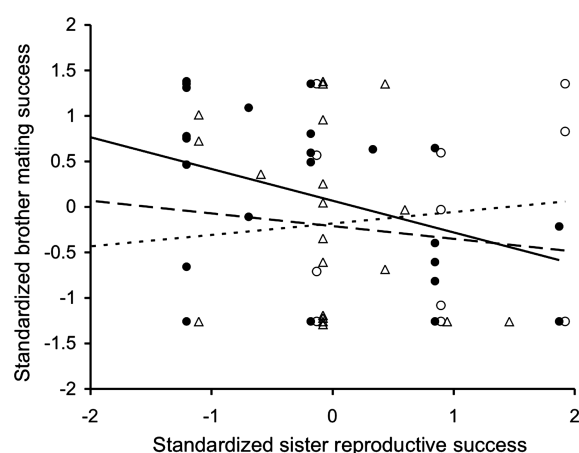


Figure 2. Fitness relationship between a brother's dominance and his sister's reproductive success (litter size) when reared in the same treatment. Mating and reproductive successes were standardized for the whole population. \circ = good (reduced litter): $y = 0.125x - 1.82$, $n = 11$, $P = 0.769$, $R^2 = 0.01$; \triangle = control: $y = -0.14x - 0.223$, $n = 21$, $P = 0.645$, $R^2 = 0.011$; \bullet = poor (enlarged litter): $y = -0.35x + 0.087$, $n = 24$, $P = 0.045$, $R^2 = 0.17$.

HERITABILITY AND SIGNAL RELIABILITY

Bank voles show additive genetic variation in good rearing environments, but little or no genetic variation in control and poor environments, as all the variance is environmental due to the change in rearing conditions from father to son. Therefore, although sexual selection may be operating when environmental conditions change, there will be no response in T levels nor dominance of males in the population and females cannot ensure the phenotype of her sons.

Our study shows that signal reliability, or the genetic benefit to female bank voles from mating with dominant males, depends on the stability of environments and the environment experienced by their offspring, rather than mate quality per se, as hypothesized by Hunt et al. (2004). As predicted by Greenfield and Rodriguez (2004), $G \times E$ with crossing reaction norms has the potential to render signals unreliable and reduce the genetic benefits to females from mating with dominant males. Indeed, if the linkage disequilibrium between the signal and the preference for it is also disrupted, $G \times E$ also has the potential to interfere with the operation of both the viability indicator and Fisherian mechanisms of sexual selection (Greenfield and Rodriguez 2004). Therefore, why do females continue to show mate choice for dominant males? The females used in our male dominance trials were reared in only control environments, therefore, it would be interesting to measure whether female choice differs as a function of their rearing environment, such as the three environments used in this study, as well as across stable and unpredictable environments. Female bank voles are known to be polyandrous and $G \times E$ coupled with the prospect of an unpredictable environment may provide one

explanation for the presence of multi male mating in this species (Mills et al. 2007), as females may gain genetic benefits through bet hedging. The degree of female choice in mating is another factor considered important for the consequences of G × E on sexual selection and warrants further investigation in the bank vole (Greenfield and Rodriguez 2004).

CONDITION DEPENDENCE

We estimated condition using two measures: growth rate and residual body mass. Our study revealed that neither plasma T nor dominance itself is condition dependent (see Table 3). Furthermore, we found no evidence for genetic variation in condition (see Table 1B), suggesting that male dominance in bank voles, measured as mating success, may not be condition dependent.

PHENOTYPIC PLASTICITY AND ECOLOGICAL CROSSOVER OF SIGNAL

The presence of crossover interactions of reaction norms between paternal groups for male dominance (see Fig. 1A) suggests that bank voles may display alternative male strategies in different environments, such that each strategy is able to bear the costs of dominance in their own specialist environment. Increased mortality due to lowered immune function and increased parasite load (S. C. Mills et al., unpubl. ms.) are likely candidates for the costs of dominance (Grossman 1985; Folstad and Karter 1992; Wedekind and Folstad 1994). Bank voles may exhibit different life-history trajectories in interaction with the rearing environment that determine their T level and social status in adulthood and such strategies may explain our lack of evidence for condition dependence. One strategy, high dominance, is successful in stable favorable environments, but may not be able to afford the costs of dominance when rearing environments deteriorate from father to son, such as following an increase in population density or decrease in available food. A second strategy, low dominance, is unable to compete with dominant males in stable favorable environments, yet may be able to bear the costs of dominance when rearing environments deteriorate. The different bank vole genotypes may be able to persist in the population due to temporal or spatial variation in the environment (Hanski et al. 1993; Morris 2005). Natural bank vole populations are exposed to three-year density cycles (T. Mappes unpubl. data), seasonal (Hanski et al. 1993) and spatial variation (Morris 2005), therefore generation and/or population overlap may maintain the different genotypes and thus additive genetic variance for male dominance.

ANTAGONISTIC SEXUAL SELECTION

A conflict of interest between the sexes may cause certain traits to be selected for in opposite directions, resulting in a negative intersexual correlation for fitness, such as reproductive success. Antagonistic selection has previously been demonstrated in adult

Drosophila melanogaster (Chippindale et al. 2001), the red flour beetle *Tribolium castaneum* (Pai and Yan 2002), the cricket *Ailonemobius socius* (Fedorka and Mousseau 2004) and the side-blotched lizard *Uta stansburiana* (B. Sinervo and R. Calsbeek, unpubl. ms.). Here we only found weak evidence for sexually antagonistic effects in the bank vole, *C. glareolus*, (see Fig. 2) and only in response to environmental stress, our enlarged litter treatment. Antagonistic selection may not have been observed in our good and control environments due to foster-maternal effects (such as milk production) compensating for female and male detrimental alleles. Furthermore, litter size may not accurately represent female fitness, and mating competition among females would have been a better measure (S. C. Mills unpubl. data), as such the finding in this paper should be treated with caution.

SIGNAL RELIABILITY AND MAINTENANCE OF ITS GENETIC VARIANCE

Although directional selection is predicted to drive genes that confer a fitness advantage to fixation depleting the additive genetic variance for fitness-related traits (Charlesworth 1987; Falconer and Mackay 1996), considerable genetic variance persists (Pomiankowski and Møller 1995; Rowe and Houle 1996). Theoretical and empirical evidence for the maintenance of this genetic variance have been found from condition dependence (Rowe and Houle 1996; David et al. 2000; Kotiaho et al. 2001; Brandt and Greenfield 2004) antagonistic pleiotropy (Williams 1957; Rose 1982; Forsman 1995; Chippindale et al. 2001; Rice and Chippindale 2001; Sinervo and Calsbeek 2003; Charmantier et al. 2006), and from fluctuating selection including G × E (Falconer 1952; Via and Lande 1985; Gillespie and Turelli 1989; West-Eberhard 1989; Jia and Greenfield 1997; Schlichting and Pigliucci 1998; Qvarnström 1999; Jia et al. 2000; Welch 2003; Hunt et al. 2004; Turelli and Barton 2004).

Genetic variance persists for male plasma T level and dominance advertisement in bank voles and our results suggest that G × E, rather than condition dependence, maintains variation in these traits. Greenfield and Rodriguez (2004) suggested that signal advertisement may become unreliable in the presence of both changing environmental conditions and G × E with crossing reaction norms, potentially compromising sexual selection. However, our study suggests that the additional presence of antagonistic sexual selection may provide one mechanism that maintains signal reliability. Antagonistic sexual selection may cause the reproductive strategies of bank vole sons and daughters to be diametrically opposed which, facilitated by population density cycles in bank voles (Hanski et al. 1993), has the potential to result in signal reliability and adaptive female choice for dominant males in both peak and low density years (good and poor environments, respectively).

This and a previous study (Mills et al. 2007) provide evidence for directional selection and yet also considerable plasticity and

genetic variance for dominance advertisement in the bank vole. Environmental variation combined with fluctuating selection on $G \times E$ provides one mechanism not only for the maintenance of genetic variation, but also for signal unreliability and thus for the potential breakdown of sexual selection (Greenfield and Rodriguez 2004). Our results suggest that fluctuating selection and antagonistic selection may be complimentary mechanisms that can act in concert to maintain additive genetic variance for fitness traits and signal reliability and essentially preserve the mechanisms of sexual selection.

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LITERATURE CITED

- Alatalo, R. V., J. Höglund, and A. Lundberg. 1991. Lekking in the black grouse—a test of male viability. *Nature* 352:155–156.
- Andersson, M. 1994. *Sexual selection*. Princeton Univ. Press, Princeton, NJ.
- Bakker, T. C. M. 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 363:255–257.
- Bakker, T. C. M., and A. Pomiankowski. 1995. The genetic basis of female mate preferences. *J. Evol. Biol.* 8:129–171.
- Bonduriansky, R., and L. Rowe. 2005. Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostoma* (Piophilidae). *Evolution* 59:138–151.
- Bradbury, J. W., and S. L. Vehrencamp. 1998. *Principles of animal communication*. Sinauer, Sunderland, MA.
- Brandt, L. S. E., and M. D. Greenfield. 2004. Condition-dependent traits and the capture of genetic variance in male advertisement song. *J. Evol. Biol.* 17:821–828.
- Charlesworth, B. 1987. The heritability of fitness. Pp. 21–40 in J. W. Bradbury and M. B. Andersson, eds. *Sexual selection: testing the alternatives*. Wiley, New York.
- Charmantier, A., C. Perrins, R. H. McCleery, and B. C. Sheldon. 2006. Quantitative genetics of age at reproduction in wild swans: support for antagonistic pleiotropy models of senescence. *Proc. Nat. Acad. Sci.* 103:6587–6592.
- Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Nat. Acad. Sci.* 98:1671–1675.
- Clutton-Brock, T. H. 1991. Lifetime data and the measurement of selection. *Evolution* 45:454.
- Clutton-Brock, T. H., and G. A. Parker. 1992. Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* 67:437–456.
- David, P., T. Bjorksten, K. Fowler, and A. Pomiankowski. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* 406:186–188.
- Doty, G. V., and A. M. Welch. 2001. Advertisement call duration indicates good genes for offspring feeding rate in gray tree frogs (*Hyla versicolor*). *Behav. Ecol. Sociobiol.* 49:150–156.
- Enquist, M. 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. *Anim. Behav.* 33:1152–1161.
- Falconer, D. S. 1952. The problem of environment and selection. *Am. Nat.* 86:293–298.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to quantitative genetics*. Longman, Harlow, U.K.
- Fedorka, K. M., and T. A. Mousseau. 2004. Female mating bias results in conflicting sex-specific offspring fitness. *Nature* 429:65–67.
- Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139:603–622.
- Forsman, A. 1995. Opposing fitness consequences of colour pattern in male and female snakes. *J. Evol. Biol.* 8:53–70.
- Fry, J. D. 1992. The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution* 46:540–550.
- Gail, M., and R. Simon. 1985. Testing the qualitative interactions between treatment effects and patient subsets. *Biometrics* 41:361–372.
- Gilburn, A. S., S. P. Foster, and T. H. Day. 1993. Genetic correlation between a female mating preference and the preferred male character in seaweed flies (*Coelopa frigida*). *Evolution* 47:1788–1795.
- Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics* 121:129–138.
- Godfray, H. C. J. 1991. Signalling of need between parents and offspring. *Nature* 352:328–330.
- Grafen, A. 1990. Biological signals as handicaps. *J. Theor. Biol.* 144:517–546.
- Greenfield, M. D. 2002. *Signalers and receivers: mechanisms and evolution of arthropod communications*. Oxford Univ. Press, Oxford, U.K.
- Greenfield, M. D., and R. Rodriguez. 2004. Genotype-environment interaction and the reliability of mating signals. *Anim. Behav.* 68:1461–1468.
- Grossman, C. J. 1985. Interactions between the gonadal steroids and the immune system. *Science* 227:257–260.
- Hanski, I., P. Turchin, E. Korpimäki, and H. Henttonen. 1993. Population oscillations of boreal rodents: regulation by mustelid predators leads to chaos. *Nature* 364:232–235.
- Hauser, M. D. 1996. *The evolution of communication*. MIT Press, Cambridge, MA.
- Hill, G. E. 1991. Plumage colouration is a sexually selected indicator of male quality. *Nature* 350:337–339.
- Hill, G. E., and R. Montgomerie. 1994. Plumage colour signals nutritional condition in the house finch. *Proc. R. Soc. Lond. B* 258:47–52.
- Hine, E., S. Lachish, M. Higgle, and M. W. Blows. 2002. Positive genetic correlation between female preference and offspring fitness. *Proc. R. Soc. Lond. B* 269:2215–2219.
- Hoffmeyer, I. 1982. Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs subordinant conspecific males and to urine odours from dominant vs subordinant males. *Behav. Neur. Biol.* 36:178–188.
- Horne, T. J., and H. Ylönen. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behav. Ecol. Sociobiol.* 38:401–405.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- Hunt, J., L. F. Bussière, M. D. Jennions, and R. Brooks. 2004. What is genetic quality? *Trends Ecol. Evol.* 19:330–333.
- Jang, Y., and M. D. Greenfield. 2000. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*: variation and evolvability of preference along multiple dimensions of the male advertisement signal. *Heredity* 84:73–80.

- Jia, F.-Y., and M. D. Greenfield. 1997. When are good genes good? Variable outcomes of female choice in wax moths. *Proc. R. Soc. Lond. B* 264:1057–1063.
- Jia, F.-Y., M. D. Greenfield, and R. D. Collins. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype × environment interaction. *Evolution* 54:953–967.
- Johnstone, R. A. 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biol. Rev.* 70:1–65.
- Johnstone, R. A., and A. Grafen. 1992. The continuous Sir Philip Sidney game: a simple model of biological signalling. *J. Theor. Biol.* 156:215–234.
- Kilpimaa, J., R. V. Alatalo, and H. Siitari. 2004. Trade-offs between sexual advertisement and immune function in the pied flycatcher (*Ficedula hypoleuca*). *Proc. R. Soc. Lond. B* 271:245–250.
- Koivula, M., E. Koskela, T. Mappes, and T. A. Oksanen. 2003. Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology* 84:398–405.
- Kokko, H., R. Brooks, J. M. McNamara, and A. I. Houston. 2002. The sexual selection continuum. *Proc. R. Soc. Lond. B* 269:1331–1340.
- Koskela, E., P. Jonsson, T. Hartikainen, and T. Mappes. 1998. Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. Lond. B* 265:1129–1134.
- Kotiaho, J. S., L. W. Simmons, and J. L. Tomkins. 2001. Towards a resolution of the lek paradox. *Nature* 410:684–686.
- Kruczek, M. 1997. Male rank and female choice in the bank vole, *Clethrionomys glareolus*. *Behav. Proc.* 40:171–176.
- Mappes, J., R. V. Alatalo, J. Kotiaho, and S. Parri. 1996. Viability costs of condition-dependent sexual male display in a drumming wolf spider. *Proc. R. Soc. Lond. B* 263:785–789.
- Mappes, T., and E. Koskela. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* 58:645–650.
- Mappes, T., E. Koskela, and H. Ylönen. 1995. Reproductive costs and litter size in the bank vole. *Proc. R. Soc. Lond. B* 261:19–24.
- Maynard-Smith, J. 1991. Honest signalling: the Philip Sidney game. *Anim. Behav.* 42:1034–1035.
- Mills, S. C., A. Grapputo, E. Koskela, and T. Mappes. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proc. R. Soc. Lond. B* 274:143–150.
- Moore, A. J. 1994. Genetic evidence for the “good genes” process of sexual selection. *Behav. Ecol. Sociobiol.* 35:235–241.
- Morris, D. L. 2005. On the roles of time, space and habitat in a boreal small mammal assemblage: predictably stochastic assembly. *Oikos* 109:223–238.
- Møller, A. P. 1995. Sexual selection and the Barn Swallow. Oxford Univ. Press, Oxford, U.K.
- Møller, A. P., and R. V. Alatalo. 1999. Good-genes effects in sexual selection. *Proc. R. Soc. Lond. B* 266:1495–1499.
- Oksanen, T. A., R. V. Alatalo, T. J. Horne, E. Koskela, J. Mappes, and T. Mappes. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. *Proc. R. Soc. Lond. B* 266:1495–1499.
- Oksanen, T. A., I. Jokinen, E. Koskela, T. Mappes, and H. Vilpas. 2003. Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment. *J. Anim. Ecol.* 72:321–330.
- Pai, A., and G. Yan. 2002. Polyandry produces sexy sons at the cost of daughters in red flour beetles. *Proc. R. Soc. Lond. B* 269:361–368.
- Pomiankowski, A., and A. P. Møller. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* 260:21–29.
- Qvarnström, A. 1999. Genotype-by-environment interactions in the determination of the size of a secondary sexual character in the collared flycatcher (*Ficedula albicollis*). *Evolution* 53:1564–1572.
- Radwan, J., M. Chadzinska, M. Cichon, S. C. Mills, B. Matula, E. T. Sadowska, K. Baliga, A. Stanis, S. Lopuch, and P. Koteja. 2006. Metabolic costs of sexual advertisement in the bank vole, *Clethrionomys glareolus*. *Evol. Ecol. Res.* 8:859–869.
- Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. *J. Evol. Biol.* 14:685–693.
- Rodriguez, R. L., and M. D. Greenfield. 2003. Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution* 57:1304–1313.
- Roff, D. A. 1997. Evolutionary quantitative genetics. International Thomson Publishing, New York.
- Rose, M. R. 1982. Antagonistic pleiotropy, dominance and genetic variation. *Heredity* 48:63–78.
- Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variation by condition dependence. *Proc. R. Soc. Lond. B* 263:1415–1421.
- Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer Associates, Inc., Sunderland, MA.
- Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. Restitution of mass-size residuals: validating body condition indices. *Behav. Ecol. Sociobiol.* 57:155–163.
- Sinervo, B., and R. Calsbeek. 2003. Physiological epistasis, ontogenetic conflict and natural selection on physiology and life history. *Integr. Comp. Biol.* 43:419–430.
- Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 in B. Campbell, ed. *Sexual selection and the descent of man*. Aldine Press, Chicago, IL.
- Turelli, M., and N. H. Barton. 2004. Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G×E interactions. *Genetics* 166:1053–1079.
- Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–522.
- Wedekind, C., and I. Folstad. 1994. Adaptive or nonadaptive immunosuppression by sex hormones. *Am. Nat.* 143:936–938.
- Welch, A. M. 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. *Evolution* 57:883–893.
- Welch, A. M., R. D. Semlitsch, and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science* 280:1928–1930.
- West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* 20:249–278.
- Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11:389–411.
- Zahavi, A. 1975. Mate selection—a selection for a handicap. *J. Theor. Biol.* 67:603–606.
- . 1977a. Reliability in communication systems and the evolution of altruism. Pp. 253–259 in B. Stonehouse and C. M. Perrins, eds. *Evolutionary ecology*. Macmillan, London.
- . 1977b. The cost of honesty (further remarks on the handicap principle). *J. Theor. Biol.* 67:603–605.

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