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E-Article

Intra- and Intersexual Trade-Offs between Testosterone and Immune System: Implications for Sexual and Sexually Antagonistic Selection

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ABSTRACT: Parasites indirectly affect life-history evolution of most species. Combating parasites requires costly immune defenses that are assumed to trade off with other life-history traits. In vertebrate males, immune defense is thought to trade off with reproductive success, as androgens enhancing sexual signaling can suppress immunity. The phenotypic relationship between male androgen levels and immune function has been addressed in many experimental studies. However, these do not provide information on either intra- or intersex genetic correlations, necessary for understanding sexual and sexually antagonistic selection theories. We measured male and female humoral antibody responses to a novel antigen (bovine gamma globulin), total immunoglobulin G, and the male testosterone level of a laboratory population of the bank vole (*Myodes glareolus*). Although we studied five traits, factor-analytic modeling of the additive genetic (co)variance matrix within a restricted maximum likelihood–animal model supported genetic variation in three dimensions. Sixty-five percent of the genetic variation contrasted testosterone with both immune measures in both sexes; consequently, selection for the male trait (testosterone) will have correlated effects on the immune system not only in males but also in females. Thus, our study revealed an intra- and intersexual genetic trade-off between immunocompetence and male reproductive effort, of which only indirect evidence has existed so far.

Keywords: animal model, genetic correlation, handicap, immunocompetence, *Myodes glareolus*, testosterone.

Introduction

Parasites influence the life histories of most species by causing hosts to direct resources to immune defense—resources that otherwise would have been available for other functions (Sheldon and Verhulst 1996; French et al. 2007). Every organism is assumed to allocate available resources optimally to maximize total fitness (Stearns 1992), but optimal investment to different fitness components may vary between the sexes. Females may gain higher fitness by investing more in survival, whereas males should increase mating rates at the expense of longevity (Bateman 1948). In vertebrate males, androgens, especially testosterone, affect mating success through the development of sexual signals (Zeller 1971; Fernald 1976; Owen-Ashley et al. 2004; Mank 2007; Mills et al. 2007*b*). On the other hand, androgens can have immunosuppressive effects and are understood to explain the weaker male immune system (Dorner et al. 1980; Greives et al. 2006). Androgen-induced immunosuppression would constrain the development of sexual signals, and in theory, males would then have to trade off between immune defense and sexual signaling (Folstad and Karter 1992; Ketterson and Nolan 1999). This is expected to happen in particular in species with a low annual survival rate (Hau 2007).

In general, there is some support, though not consistent, for testosterone-induced immunosuppression in males (Roberts et al. 2004). In addition, from a different perspective, immune activation has shown a suppressive effect on male plasma testosterone level (Verhulst et al. 1999; Boonekamp et al. 2008). Phenotypic engineering alone does not provide inclusive information on the evolutionary processes connected to hormone-mediated traits (McGlothlin and Ketterson 2008). Despite immunosuppressive effects of exogenous testosterone, individuals with the strongest of immune systems can also possess high levels of testosterone (Peters 2000). Therefore, even a positive genetic correlation between natural testosterone levels

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and measures of immune function could be hypothesized. However, so far, the (very limited) evidence of a genetic relationship between testosterone and immunocompetence in artificial selection lines supports negative covariance (Von Schantz et al. 1995; Verhulst et al. 1999).

Testosterone, although considered a male hormone, is also produced in females, and manipulation of testosterone level has immunosuppressive effects in females (Zysling et al. 2006). Further, the female testosterone level is likely to respond to selection for male testosterone (Ketterson et al. 2005). Thus, through the female testosterone level, a genetic correlation between male testosterone and female immunocompetence would have important implications for sexual selection theories. A weak correlation would leave female immunity intact from selection for higher male testosterone levels and presumably drive a sexual dimorphism in immunity. A negative correlation, on the other hand, would strongly constrain a response in male testosterone levels because of not only the antagonistic effect on male immunity but also the similar intersexual effect on females.

Understanding and predicting the course of evolution for multiple traits requires multivariate quantitative genetics (Lande 1979), but multivariate additive genetic covariance structure can be difficult to interpret unequivocally. Only a full-rank additive genetic (co)variance matrix (**G**) allows unconstrained evolutionary responses, while **G** with one or more zero eigenvalues would constrain evolution to occur along linear combinations of the nonzero eigenvalues (Pease and Bull 1988). Usually just two or three principal components are enough to explain most of genetic variation (Kirkpatrick and Meyer 2004). However, efforts to evaluate **G** dimensionality by estimating nonzero eigenvalues have only recently been introduced on a larger scale to quantitative genetics (e.g., Kirkpatrick and Meyer 2004; Hine and Blows 2006; Blows 2007; Meyer 2007).

In this study we examined a common genetic basis for immunocompetence and male testosterone level in a polygynous small mammal, the bank vole (*Myodes glareolus*). Reproductive success of bank vole males depends on their testosterone level rather than body size, as shown by both correlative data and testosterone manipulations (Mills et al. 2007*a*, 2007*b*, 2009). Instead of possessing sexual ornaments, males use dominance to advertise their quality through competition with other males for access to females (Hoffmeyer 1982; Oksanen et al. 1999). Testosterone can cause immunosuppression in bank vole males, directly via biochemical pathways but also indirectly through resources expended due to increased mobility and aggressive behavior (Mills et al. 2009). To assess immunocompetence, we measured primary antibody response to a novel T-cell-dependent protein antigen, bovine gamma globulin (BGG), and total immunoglobulin G level (IgG) in plasma. Immunoglobulins are central to the function of the immune system by neutralizing pathogens, promoting phagocytosis, and activating complement, the cascade system of humoral innate immunity. However, measuring T-Helper-2-mediated humoral immune response alone might not suffice for a comprehensive insight into an individual's immunocompetence. Depending on the confronted parasite, for example, T-Helper-1-type responses with cell-mediated immunity can be of substantial importance (Tizard 2008). Moreover, immune response in general might be a trait with an intermediate optimum (Viney et al. 2005). Nonetheless, both of the measures we employed have been found to correlate with fitness-related characters in the bank vole. In outdoor enclosures, the strength of anti-BGG antibody response correlated positively with survival and growth (Oksanen et al. 2003; Mills et al. 2010), whereas IgG concentration correlated negatively with ectoparasite prevalence (Mills et al. 2010). Further, a meta-analytic study in birds showed that individuals with stronger immune responses have dramatically higher survival (Møller and Saino 2004).

Information previously gathered from the bank vole in their natural environment demonstrates the significance of both testosterone and measures of the acquired immune system for life histories and even suggests the presence of a potential genetic trade-off between them (Mills et al. 2009). In this study, we use a pedigreed laboratory population to reliably estimate their common genetic basis. To our knowledge, this is the first time genetic covariances between immunological measures and testosterone level have been estimated.

Material and Methods

Study Species

The bank vole is one of the most common wild mammals in Europe (Stenseth 1985). Population densities are highly variable within and between years, and distinct density cycles are observed in northern Fennoscandia (Kallio et al. 2009). In our study area in central Finland, the breeding period of the bank vole lasts from May to September (Koivula et al. 2003). Breeding females are territorial, while home ranges of males overlap (Bondrup-Nielsen and Karlsson 1985; Koskela et al. 1997). Bank voles have a polygynous mating system, in which males provide no resources to the female or the offspring and compete with other males for possibilities to mate (Oksanen et al. 1999; Mills et al. 2007*a*). Instead of possessing sexual ornaments, bank vole males advertise their quality by dominance, which has been found to depend strongly on testosterone level (Mills et al. 2007*b*). Both male-male competition and

female choice for dominant males cause strong selection for higher levels of testosterone (Mills et al. 2007*a*).

Animal Husbandry

The laboratory population used in the study was established from wild individuals captured in Konnevesi, central Finland, during the summer of 2000. Selection lines were founded from 150 females and 116 males. All males used as founders were wild trapped, while some of the females had known parents since they were laboratory-born offspring of wild-trapped individuals. The population was subjected to artificial selection toward small and large litter sizes. The selection procedure was a combination of between- and within-family selections. Animals used in this study for immunological measurements were from the first, third, and fourth generations of the laboratory population and belonged to both of the selection lines in all studied generations. Testosterone was sampled from both lines in all generations. A more detailed description of the selection lines is given in a separate paper (E. Schroderus, M. Koivula, E. Koskela, T. Mappes, and T. A. Oksanen, unpublished manuscript).

The animals were housed in standard mouse cages and maintained on a 16L : 8D photoperiod at 20° ± 2°C. Wood shavings and hay were provided as bedding, and food (standard laboratory rodent food) and water were available ad lib. The animals were housed together with same-sex littermates until maturity, after which they were housed individually.

Analytical Methods

To measure specific immune response, animals were immunized with an intraperitoneal injection (0.1 mL) of BGG (200 mg; Sigma) emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). Before immunization, a 75-μL intraorbital blood sample collected in heparinised capillary tubes was taken from males to measure plasma testosterone level. Blood samples were centrifuged (12,000 rpm for 5 min; Heraeus Biofuge) to separate plasma from the blood cells, and plasma was stored at −20°C.

Plasma testosterone was measured using a radioimmunoassay kit (Testo-CTK, DiaSorin, Byk-Sangtec Diagnostica, Dietzenbach, Germany). Methods are described by Mills et al. (2007*a*). Repeatability was calculated for testosterone values (56 individuals) recorded twice at a 2-week interval using ANOVAs (Lessells and Boag 1987); repeatability = 0.637 (F ratio = 4.504).

On day 28 after immunization, another blood sample (18 μL) was taken to determine anti-BGG antibody and total IgG concentrations with a microplate enzyme-linked immunosorbent assay. Methods are described in detail elsewhere (Oksanen et al. 2003). The period needed for mounting a full antibody response to immunization was determined in a pilot laboratory experiment where anti-BGG antibody levels of adult bank vole males were analyzed 14, 28, and 42 days after injection (E. Koskela, I. Jokinen, T. Mappes, and T. A. Oksanen, unpublished data).

Statistical Analysis

In the preliminary analyses, fixed effects to be used in the animal model were estimated with SPSS 15.0 univariate general linear model (GLM) procedure by excluding random effects other than residuals. The only fixed effect selected was the timing (month) of blood sampling for total IgG.

The (co)variance components were estimated with the average information restricted maximum likelihood (REML) procedure using the ASReml 2.0 program (Gilmour et al. 2002, 2006). The REML-animal model is the default method in quantitative genetic studies as it utilizes all the data and genetic relationships across generations (Kruuk 2004). Appropriate random-effect structure was

Table 1: Natural and transformed trait means for male and female bank vole immune responses and male testosterone level

Trait	n	Natural mean (±SD)	Transformed mean (±SD)
Female:			
Anti-BGG (U/mL)	456	$420 \times 10^3 \pm 134 \times 10^4$	5.14 ± .68
IgG (U/mL)	515	$167 \times 10^4 \pm 106 \times 10^4$	6.16 ± .22
Male:			
Anti-BGG (U/mL)	295	$457 \times 10^3 \pm 906 \times 10^3$	5.32 ± .56
IgG (U/mL)	323	$121 \times 10^4 \pm 679 \times 10^3$	6.02 ± .25
Testosterone (ng/mL)	343	5.23 ± 3.86	2.12 ± .86

Note: Log_{10} transformation was carried out for anti-BGG antibody and IgG levels and square root transformation for testosterone to normalize distributions. BGG = bovine gamma globulin; IgG = immunoglobulin G; n = number of observations.

Table 2: Comparison of log likelihood and Akaike Information Criterion (AIC) values for factor-analytic REML-animal models

Factors	Log likelihood	df	AIC	ΔAIC
3	715.706	12	−1,407.412	0
2	711.895	9	−1,405.790	1.62
4	715.734	14	−1,403.468	3.92
1	704.581	5	−1,399.162	8.25

Note: The comparison tests the dimensionality of genetic variation among testosterone and immune measures in the bank vole. REML = restricted maximum likelihood; ΔAIC = AIC difference compared with the best model.

first studied for each of the traits with a univariate model. Common litter or maternal effects were not found to be relevant for any of the traits; the only random effect was direct additive genetic effect. Thus, the following multivariate model was used:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

in which **y** is the vector of phenotypic observations and **b** is the vector of fixed effects (blood sampling month for IgG); **a** and **e** are the vectors of direct additive genetic effects and residuals, respectively. Fixed and random effects are linked to individual records by incidence matrices **X** and **Z**. It is assumed that $E(\mathbf{y}) = \mathbf{Xb}$, and the expectations of random effects are zero; the variances of the random effects are

$$\text{Var}(\mathbf{a}) = \mathbf{G} \otimes \mathbf{A},$$
$$\text{Var}(\mathbf{e}) = \mathbf{R} \otimes \mathbf{I},$$

in which **G** is the additive genetic (co)variance matrix, **A** is the additive genetic numerator relationship matrix, **R** is the residual (co)variance matrix, **I** is the identity matrix, and ⊗ is the Kronecker product. The additive genetic (co)variance matrix was modeled with factor-analytic variance structure:

$$\hat{\mathbf{G}} = \mathbf{\Gamma\Gamma}',$$

in which **Γ** is the matrix of factor loadings. Specific variances were restricted to zero, making analyses similar to principal component analyses. Dimensionality of **G** was evaluated by comparing the Akaike Information Criterion (AIC) of the reduced-rank models with different number of factors fit (Burnham and Anderson 2002). Degrees of freedom associated with each model were $p \times m - p(p-1)/2$, in which p and m are numbers of traits and factors.

Residual (co)variance matrix **R** was unstructured. However, since traits measured from males and females were considered as separate traits, they cannot share residual effects, and consequently, corresponding covariances were fixed at zero.

The program ASReml does not provide standard errors for (co)variance components estimated with the factor-analytic method. Thus, it is not possible to give standard errors for additive genetic variances and covariances or heritabilities and genetic correlations. However, no problem is foreseen because factor-analytic modeling of the **G** matrix directly estimates only that part of the genetic variation that has statistical support (Blows 2007). Eigenanalysis was performed to estimate reduced-rank **G** to extract the underlying genetically independent traits.

Results

Phenotypic Results

In both sexes, the specific antibody response (anti-BGG) was more variable than total IgG level (table 1).

Quantitative Genetic Analysis

In the quantitative genetic analysis, immune traits for both sexes were considered as separate traits; thus, we ran multivariate analyses with all five traits shown in table 1. However, factor-analytic modeling of the additive genetic (co)variance matrix (**G**) gave support for genetic variation in three dimensions. With respect to AIC, a model with three factors better explained the data than a model with one, two, or four factors (table 2). The first factor of the preferred model had positive loadings with all traits except male IgG (table 3). The second factor had negative loadings except that of testosterone, and the third factor had positive loadings with all traits except male anti-BGG (table 3).

For the immunological traits, estimates of additive genetic variance and heritability were higher for females in anti-BGG and for males in IgG (tables 4, 5). Estimates of heritability for male IgG were quite high (0.48) and moderate for rest of the traits (0.20–0.32; table 5). Genetic correlations for IgG between the sexes were strongly pos-

Table 3: Matrix of the factor loadings (**Γ**)

	Factor 1	Factor 2	Factor 3
F anti-BGG	.3304	0	0
F IgG	.0453	−.1066	0
M anti-BGG	.0791	−.1187	−.1969
M IgG	−.0005	−.1650	.0400
M testosterone	.0071	.0071	.4484

Note: Factor-analytic modeling captures covariance between testosterone and immune measures in the bank vole into three underlying factors. F = female; M = male; BGG = bovine gamma globulin; IgG = immunoglobulin G.

Table 4: Additive genetic (co)variance matrix for testosterone and immune measures in the bank vole estimated with the three-dimensional factor-analytic REML-animal model

	F anti-BGG	F IgG	M anti-BGG	M IgG	M testosterone
F anti-BGG	.1090				
F IgG	.0150	.0134			
M anti-BGG	.0264	.0162	.0591		
M IgG	−.0002	.0176	.0117	.0288	
M testosterone	.0026	−.020	−.1102	.0199	.2378

Note: F = female; M = male; BGG = bovine gamma globulin; IgG = immunoglobulin G.

itive (0.89) and for anti-BGG moderately positive (0.33). Genetic correlations between anti-BGG and IgG within (female = 0.39, male = 0.28) and between the sexes (0.58 and 0.00) were from weak to moderate (table 5). Genetic correlations were negative between male testosterone and male anti-BGG (−0.93) and between male testosterone and female IgG (−0.35), while the correlation between male testosterone and male IgG was weakly positive (0.24; table 5).

Eigenanalysis performed for the estimated reduced-rank **G** summarized genetic variation into three principal components (table 6). Sixty-five percent of the additive genetic variation (first principal component) contrasted testosterone with immunological measures in both sexes. The second principal component summarized 26% of the genetic variation that was parallel in all traits, with the lowest weight given to male IgG. The third principal component (9%) contrasted female anti-BGG with the rest of the traits.

Discussion

In this study we estimated the common genetic basis of male testosterone level and acquired immune system in the bank vole, a polygynous vertebrate with strong selection on male testosterone. The additive genetic (co)variance matrix of the whole five-trait system had the strongest statistical support in three dimensions. The largest genetic principal component, explaining 65% of the additive genetic variation, contrasted testosterone with plasma antibody response (anti-BGG) and total immunoglobulin G level (IgG) in both sexes, while the second principal component, explaining 26% of the additive genetic variation, summarized genetic variation in the same direction for all studied traits. The remaining 9% of the additive genetic variation contrasted female anti-BGG with all the other traits. Our results revealed an intra- and intersexual genetic trade-off between immunocompetence and male reproductive effort.

A moderate heritability of male testosterone level would suggest a rapid evolutionary response, as it is favored by both strong intra- and intersexual selection in the bank vole (Mills et al. 2007*a*). Likewise, heritabilities of dominance-related traits, dependent on testosterone level, have been shown to be quite high in the bank vole (Horne and Ylönen 1998). However, the first genetic principal component, explaining 65% of the total variation, contrasted testosterone with both measures of immune function, especially with male anti-BGG level (table 6). Therefore, despite high heritability, a response in male testosterone level will be slowed, because in addition to the antagonistic effect on male survival, there is also an intersexual effect on the survival of females in the population. Our results are in agreement with two studies carried out on the domestic fowl (*Gallus domesticus*). Selection for humoral immune response produced a correlated antagonistic selection response in male testosterone level (Verhulst et al. 1999). Furthermore, selection for increased comb size, a character dependent on testosterone, led to reduced viability in males (Von Schantz et al. 1995). Further, in the dark-eyed junco (*Junco hyemalis*), a male-biased sex ratio led to compromised immunity in both sexes (Greives et al. 2007), as did selection experiments with a male-biased

Table 5: Heritabilities (diagonal) and genetic correlations for testosterone and immune measures in the bank vole estimated with the three-dimensional factor-analytic REML-animal model

	F anti-BGG	F IgG	M anti-BGG	M IgG	M testosterone
F anti-BGG	.26	.39	.33	−.00	.02
F IgG		.28	.58	.89	−.35
M anti-BGG			.20	.28	−.93
M IgG				.48	.24
M testosterone					.32

Note: F = female; M = male; BGG = bovine gamma globulin; IgG = immunoglobulin G.

Table 6: Principal components (proportion of the total genetic variance [%] and respective eigenvectors) for a three-dimensional additive genetic (co)variance matrix

	$\mathbf{v}_1$	$\mathbf{v}_2$	$\mathbf{v}_3$
%	64.94	26.38	8.68
F anti-BGG	.072322	.978584	−.103776
F IgG	.098942	.140393	.485821
M anti-BGG	.318688	.086777	.022788
M IgG	.072688	.019872	.859555
M testosterone	−.937089	.121400	.117704

Notee: The matrix was based on testosterone and immune measures in the bank vole estimated using a factor-analytic REML-animal model. $\mathbf{v}_1$, $\mathbf{v}_2$, and $\mathbf{v}_3$ = the first, second, and third eigenvectors. F = female; M = male; BGG = bovine gamma globulin; IgG = immunoglobulin G; REML = restricted maximum likelihood.

sex ratio in the yellow dung fly *Scathophaga stercoraria* (Hosken 2001) and *Drosophila melanogaster* (McKean et al. 2008).

A strong contrast in the leading genetic principal component between male testosterone and male anti-BGG response is consistent with a previously described testosterone-induced immunosuppression in this species using phenotypic manipulation (Mills et al. 2009). Testosterone can cause immunosuppression either directly by binding onto immune cells or indirectly by draining resources from the immune system (Wedekind and Folstad 1994) or via glucocorticoids (e.g., Evans et al. 2000). It is unclear how male testosterone is adversely connected to female immunocompetence. One plausible explanation is that female testosterone acts as an immunosuppressant in females, as male and female testosterone levels are presumably genetically correlated (Zysling et al. 2006). Further, since hormones are generally mediators of evolutionary constraints (McGlothlin and Ketterson 2008) and an efficient immune system should be more important to a female's fitness than a male's (Bateman 1948; Rolff 2002; Nunn et al. 2009), these results indicate that the genetic trade-off between female reproductive fitness and immunocompetence would make an interesting prospect for future research (Sheldon and Verhulst 1996).

The AIC difference between the two- and three-factor models was not very large (table 2). However, we favor the three-factor model, since possible bias decreases with an increasing number of fitted principal components (Meyer and Kirkpatrick 2008). This is a five-trait system, yet genetic variation occurs in only three dimensions; therefore, there are directions in the multivariate trait space in which no genetic variation exists and response to selection would be constrained to occur along linear combinations of the nonzero eigenvalues (Pease and Bull 1988). Thus, possible evolutionary responses are limited, compared to the situation with a full-rank **G** matrix (Blows 2007). In general, response will be fastest when selection acts in the direction of the major axis of **G** (the first principal component; Blows and Hoffmann 2005), which in this case contrasted male reproductive fitness with immunity of both sexes. The second principal component, however, extracted genetic variation in the same direction in all traits, with most weight being given to male testosterone and female immune traits. Thus, selection acting in the direction of the second principal component would increase both male and female fitness. Whether selection in the bank vole acts more in the direction of the first or the second genetic principal component probably depends on current environmental conditions. In general, survival selection favors individuals with the strongest immune response (Møller and Saino 2004; Mills et al. 2009). However, vole populations in northern Fennoscandia show distinctive density cycles (Kallio et al. 2009) where pathogen pressure (Soveri et al. 2000) and immunological parameters of voles (Huitu et al. 2007) differ between peak and crash years, indicating variation in selection for immune-related traits.

To summarize, quantitative genetic analysis of two immunological traits in both sexes and male testosterone level revealed tight linkage between the traits studied. Selection for higher testosterone level in males will compromise the function of immune system in both sexes. Our study demonstrates the importance of both intra- and intersexual connections for the genetic trade-off between immunocompetence and male reproductive effort in mammals, of which only indirect evidence has existed so far. Keeping in mind the context-specific nature of optimal immune responses and that the relative importance of the different arms of the vertebrate immune system still remain uncharacterized, the significance of our findings on the evolution of the whole immune system invites further investigation.

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