JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
333

Eero Schroderus

Evolutionary Trade-Offs in a Small Mammal

A Quantitative Genetics Approach

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa YAA303,
kesäkuun 10. päivänä 2017 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2017

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Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-7113-7

URN:ISBN:978-951-39-7113-7
ISBN 978-951-39-7113-7 (PDF)

ISBN 978-951-39-7112-0 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2017

ABSTRACT

Schroderus, Eero
Evolutionary trade-offs in a small mammal - a quantitative genetics approach
Jyväskylä: University of Jyväskylä, 2017, 48 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 333)
ISBN 978-951-39-7112-0 (nid.)
ISBN 978-951-39-7113-7 (PDF)
Yhteenveto: Pikkunisäkkäään evolutiiviset valinnat tarkasteltuna kvantitatiivisen genetiikan menetelmin
Diss.

Limited resources force individuals to trade-off between life-history traits. A vast diversity of life-history strategies, which optimally combine life history traits, can be found. Knowledge of the genetic basis of this phenotypic variation is key to understanding evolutionary processes. I approached life-history evolution by estimating quantitative genetic parameters for a set of life-history traits in the bank vole (*Myodes glareolus*). The work is based on a laboratory-kept colony subjected to two-way selection for litter size (High- (H) and Low- (L) lines). Costs of the created reproductive strategies were observed in natural conditions. Selection increased the difference in litter size between lines, even though the response was asymmetric. The difference persisted in outdoor enclosures. A life-history trade-off between offspring number and offspring size was explained by environmental correlations as associated genetic correlations were even positive. Lines did not diverge in offspring size. In the enclosures maternal body mass had greater effect on pup survival in the L-line. Litter and maternal environment explained most of the phenotypic variation in pup body mass during the nursing period, while direct genetic variation emerged later, implicating selection on early size in the bank vole. Male and female metabolic rate did not seem to share genetic background; the first lacking additive genetic variation and the latter being bound to fecundity by genetic correlation close to one. Testosterone, a principle determinant of male reproductive success in the bank vole, was contrasted with humoral immune system in the both sexes. In general, with the exception of metabolism, the estimated genetic parameters did not set absolute constraints for life-history evolution. However, dimensionality of the additive genetic co(variance) matrix constrains evolution to act along certain trait combinations. In the future, research should strive to find genetic mechanisms, which, through the endocrine system, produces phenotypic co(variation) in different life-history traits.

Keywords: Bank vole; genetic correlation; heritability; offspring number; quantitative genetics; selection line; trade-off.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–V.

I Schroderus, E., Koivula, M., Koskela, E., Mappes, T. & Oksanen, T.A. Evolutionary decoupling of a fundamental life-history trade-off in a small mammal. Manuscript.

II Schroderus, E., Koivula, M., Koskela, E., Mappes, T., Oksanen, T.A. & Poikonen, T. 2012. Can number and size of offspring increase simultaneously? - a central life-history trade-off reconsidered. BMC Evolutionary Biology 12: 44.

III Boratyński, Z., Koskela, E., Mappes, T. & Schroderus, E. 2013. Quantitative genetics and fitness effects of basal metabolism. Evolutionary Ecology 27: 301–314.

IV Schroderus, E., Koivula, M., Koskela, E., Mappes, T. & Oksanen, T.A. Constrained evolution of growth trajectories in a wild small mammal. Submitted manuscript.

V Schroderus, E., Jokinen, I., Koivula, M., Koskela, E., Mappes, T., Mills, S.C., Oksanen, T.A. & Poikonen, T. 2010. Intra- and intersexual trade-offs between testosterone and immune system: implications for sexual and sexually antagonistic selection. American Naturalist 176: E90–97.

The table shows the contributions to the original papers

	I	**II**	**III**	**IV**	**V**
Original idea	TM, EK, MK, TAO	TM, EK, ES	ZB, TM, EK	ES	TM, EK, ES
Experimental work	TM, EK, TAO, MK, ES, TP	TM, EK, TAO, MK, ES, TP	ZB, EK, TM, TAO	ES, EK	IJ, MK, EK, TM, SCM, TAO, TP
Statistical analysis	ES, TM, EK	ES	ES	ES	ES
Manuscript	ES, MK, EK, TM, TAO	ES, MK, EK, TM, TAO	ZB, EK, TM, ES	ES, MK, EK, TM, TAO	ES, IJ, MK, EK, TM, SCM, TAO

ES = Eero Schroderus, TM = Tapio Mappes, EK = Esa Koskela, MK = Minna Koivula, TAO = Tuula A. Oksanen, TP = Tanja Poikonen, ZB = Zbyszek Boratyński, IJ = Ilmari Jokinen, SCM = Suzie C. Mills

1 INTRODUCTION

Every organism strives to maximize the proportion of its genes in the next generation, i.e. its fitness (Fisher 1930). Individuals in a population, however, differ in their success to transmit genes forward. This phenotypic variation in fitness is result of selection. Evolutionary change results if variation in fitness is heritable, and preferred features are transmitted from parents to offspring. Thus, selection acts on the phenotypes, while responses to selection depend on the underlying genetic architecture (Lande 1982, Falconer and Mackay 1996). Selection acting on fitness produces correlated changes in life-history traits, which have a clear and direct connection with fitness (Stearns 1992, Roff 1992).

Age at sexual maturity, number and size of offspring, and longevity are examples of traits that directly influence fitness (Stearns 1992). The closer a trait is related to fitness, the stronger the selection it is subjected to, and consequently, the less additive genetic variation it should harbor (Fisher 1930). Still, in artificial selection experiments, traits like litter size have responded to selection (e.g. Falconer 1971, de la Fuente and Primitivo 1985, Holt *et al.* 2005). Also in the natural environment, practically every life-history trait studied continues to show additive genetic variation (Roff 1997, Merilä and Sheldon 1999, Roff 2002). Maintenance of additive genetic variation continues to form a major puzzle in the field of life-history evolution. The most popular theories are a mutation-selection balance, negative frequency-dependent selection, environmental heterogeneity and antagonistic pleiotropy (Bulmer 1989, Barton and Turelli 1989, Barton 1990). Antagonistic pleiotropy as a mechanism to preserve genetic variation is based on an idea that alleles that have a positive effect on multiple traits become fixed rapidly, leaving only segregating alleles that create negative correlations (Bell and Burris 1973, Falconer and Mackay 1996, Roff 1997).

In evolutionary ecology, the negative genetic correlation between life-history traits is often associated with the concept of trade-offs. Selection cannot maximize all components of fitness simultaneously: the scarcity of resources force organisms to trade-off between different life functions (Zera and Harshman 2001, Stearns 1989). For example, life-history traits are known to

trade-off both at the intra- and interspecific levels, creating the vast diversity of life-history strategies observed (Roff 2002). Understanding this diversity is the goal of life-history theory (Stearns 1992, Roff 2002). However, this understanding cannot be achieved without knowledge of the genetic background of phenotypic variation in life-history traits.

1.1 Quantitative genetics

1.1.1 Basic concepts

Quantitative genetics is currently the best framework for studying how phenotypic variation is manifested as evolutionary change (Lynch and Walsh 1998). It encompasses the study of variation in continuously distributed traits, in which the effect of a single gene cannot be identified from other genes or from environmental variation (Falconer and Mackay 1996). In the statistical framework, variation in continuous characters is caused by a very large number of unlinked genes, each with only a small effect (infinitesimal model) (Fisher 1918, Bulmer 1980). The phenotype (P) of an individual is the sum of genetic (G) and environmental effects (E) ($P = G + E$). The genetic effect can be further divided into additive (G_A), dominance (G_D) and epistatic effects (G_I) ($P = G_A + G_D + G_I + E$). The additive genetic effect is the sum of average gene effects over several loci. Another definition for the additive genetic effect is breeding value, as it is twice the difference of offspring mean from the population mean. The dominance genetic effect sums within loci interactions, as does the epistatic effect for between loci interactions (Falconer and Mackay 1996).

The gene frequencies of individual loci determine the genetic components of variance. Generally, gene frequencies are not known and the interest lies in estimating variances (Falconer and Mackay 1996). Phenotypic variance (V_P) can be dissected into components reflecting individual effects (G_A, G_D, G_I, E) based on the resemblance between relatives ($V_P = V_G + V_E = V_A + V_D + V_I + V_E$) (Fisher 1918). The level of additive genetic determination is traditionally expressed as a ratio to phenotypic variance (narrow sense heritability, $h^2 = V_A/V_P$), which reflects the correlation between phenotype and genotype. Although heritability is, strictly speaking, considered as a population specific parameter, estimates for the same characters from different species can be strikingly similar (Visscher *et al.* 2008). Dominance genetic variance can increase covariance between close relatives, most importantly between full sibs, while variance due to epistatic gene effects is usually considered low and omitted from analysis (Falconer and Mackay 1996, Mäki-Tanila and Hill 2014).

When the same individual has been measured repeatedly, phenotypic variance can be dissected into within- and between-individual variances (Falconer and Mackay 1996). Within-individual variance (V_{Es}) rises from temporal circumstances, and between-individual variance (V_{Eg}) from permanent differences between individuals including permanent

environmental variance and additive genetic variance. From these can be derived the concept of repeatability ($r = V_G + V_{Eg}/V_P$), which determines the correlation between measurements of the same individual, and sets an upper limit to heritability (Falconer and Mackay 1996). An example of a source of environmental variance is maternal effects, which can further have genetic components of their own (Räsänen and Kruuk 2007).

1.1.2 Inbreeding depression

Mating between related individuals, or inbreeding, usually leads to reduced vigor of the offspring. This inbreeding depression, most evident in life-history traits, is believed to be caused by dominance genetic effects (DeRose and Roff 1999). Directional selection erodes additive genetic variation leaving dominance genetic variation intact (Crnokrak and Roff 1995). Consequently, life-history traits, supposedly under stronger selection than morphological traits, have a higher proportion of dominance genetic variance (Roff and Emerson 2006). Due to continuous directional selection, dominance effects will be mostly in the direction of the selection, since alleles producing unwanted effects will be swept away by selection unless they are recessive. Inbreeding reveals these harmful alleles by increasing homozygosity (Falconer and Mackay 1996). In agreement with additivity of the dominance effects over loci, inbreeding depression is roughly proportional to the level of inbreeding (de Boer and van Arendonk 1992). In addition to a change in the trait mean, inbreeding reduces additive genetic variance (Falconer and Mackay 1996). However, in a trait which initially possessed a high degree of non-additive genetic variation, a low level of inbreeding may also increase additive genetic variation (Van Buskirk and Willi 2006).

1.1.3 Response to selection

Heritability (h^2) and selection differential (S) define a trait's predicted response (R) to selection ($R = h^2S$) (Falconer and Mackay 1996). Traits most closely connected to fitness generally have the lowest heritabilities (Gustafsson 1986, Mousseau and Roff 1987, Merilä and Sheldon 1999, Kruuk *et al.* 2000, Hoffmann *et al.* 2016), while morphological trait heritabilities may even approach one (Schroderus and Ojala 2010). Despite even low heritability allowing evolutionary change, most natural populations seem to be in microevolutionary stasis (Merilä *et al.* 2001). This is in sharp contrast to domestic species in which almost every trait can be changed as long as it has phenotypic variation (Hill and Kirkpatrick 2010). In natural populations, detrimental changes in correlated traits may pose a stronger obstacle for evolutionary change (Hill and Kirkpatrick 2010).

Pleiotropy is a common property of genes. In other words, a gene usually affects more than just one trait. This shared genetic background between traits is reflected in genetic correlation (Falconer and Mackay 1996). A genetic correlation may also be created by linkage disequilibrium, but it will not

influence long-term selection responses (Falconer and Mackay 1996). An additive genetic (co)variance matrix (**G**) conveniently summarizes quantitative genetic architecture for multiple traits. In the multivariate formulation of the breeder's equation ($\Delta \mathbf{z} = \mathbf{G}\mathbf{P}^{-1}\mathbf{s}$), heritability and the selection differential (S), measuring the within-generation change in the mean, are replaced by the **G**-matrix, phenotypic (co)variance matrix (**P**) and vector of selection differentials (**s**) (Lande 1982). Thus, the evolution of any trait does not occur in isolation, but instead the ability to evolve is determined by the pattern of additive genetic and phenotypic covariances (Lande 1982). Empirical work has shown that responses of correlated traits can be predicted when their quantitative genetic architecture is known (Roff and Fairbairn 2012). Although in a multivariate context the accuracy of predictions becomes weaker when the number of traits increases (Kruuk *et al* 2008).

1.2 Maternal effects

The parental environment has a major influence on the development of offspring. Due to the disproportionate investment of parents on offspring, research is commonly focuses on maternal effects. By definition, maternal effects occur when a mother's phenotype directly influences her offspring's phenotype (Mousseau and Fox 1998). As an important source of variation in offspring fitness, maternal effects can have marked evolutionary and ecological consequences (Räsänen and Kruuk 2007). Maternal effects can create a time delay in the population's response to environmental conditions (Mousseau and Fox 1998), and even influence population cycles (Inchausti and Ginzburg 1998). Evolutionary momentum due to maternal genetic effects can cause a population to continue evolving after selection has ceased (Kirkpatrick and Lande 1989).

In a classic study, Walton and Hammond (1938) reciprocally crossed Shire horses (the largest horse, *Equus caballus*, breed) and Shetland ponies (one of the smallest horse breeds) and showed that the size of the foal at birth was approximately proportional to the size of the mother. Since then, maternal effects have been shown to influence a variety of traits subject to selection in every organism in which they have been explored (Mousseau *et al.* 2009). Two different approaches in the quantification of maternal effects are trait-based and variance partitioning approaches (McGlothlin and Brodie 2009). In the former method, the aim is to determine how a trait is affected in subsequent generations (e.g. mother's litter size on daughter's litter size) or how the maternal trait (e.g. litter size) influences different offspring traits (e.g. neonatal size) and model their dynamics between generations (Kirkpatrick and Lande 1989). In the variance partitioning or maternal performance model, the goal is to estimate variance due to maternal effects, which then include all unspecified maternal traits (Willham 1963, Willham 1972). The variance partitioning method is similar to indirect genetic effects -model (Griffing 1967, Griffing 1968). It has been criticized for ignoring the biology behind the maternal effects

and for missing the oscillating dynamics between two traits influencing each other in subsequent generations (Kirkpatrick and Lande 1989). However, the variance partitioning method is more flexible, and requires no a priori knowledge of which maternal traits influence offspring traits (McGlothlin and Brodie 2009).

Maternal effects increase resemblance between siblings. Thus, ignoring maternal effects can lead to inflation of direct additive genetic variance and overestimation of the population's potential to respond to selection (Wilson *et al.* 2005a). Furthermore, maternal effects can themselves have a genetic background and thus influence evolutionary trajectories (Wolf *et al.* 1998). A genetic correlation between direct and maternal genetic effects will have dramatic effects on the selection response. A positive correlation would accelerate microevolution, while a negative correlation would impede or even reverse the selection response (Wolf *et al.* 1998). Selection is expected to decrease genetic variation in maternal effects at a slower pace than in direct genetic effects, since they are one generation away from the selection event (Willham 1963, Willham 1972).

1.3 Trade-offs

Fitness components cannot be combined with a strategy that maximizes them all simultaneously. Limited resources force organisms to allocate them optimally between different functions. Improvement in one fitness function is often be accompanied by a decrease in another. Consequently, life-history traits are bound together with trade-offs, which constrain their simultaneous evolution and possibly maintain genetic variation (Stearns 1992). Trade-offs can be roughly classified into physiologically and ecologically mediated, although the two strongly interact (Zera and Harshman 2001). Over the years, a plethora of definitions has accumulated for the terms constraint and trade-off (Antonovics and van Tienderen 1991). In this thesis, the trade-off is used in the sense of a fitness cost in one trait being associated with a fitness gain in another.

The trade-off can be measured with phenotypic correlation, experimental manipulation or genetic analysis (Reznick 1985). Phenotypic correlation can suggest the presence of trade-offs, but trade-offs can be neglected if variation in the acquisition of resources is abundant in relation to variation in allocation (Van Noordwijk and Dejong 1986, Zera and Harshman 2001, Roff 2002). Functional trade-offs can be recognized using phenotypic manipulation (Ketterson *et al.* 1996, Oksanen *et al.* 2002), which helps to achieve a broad range of variation, but typically lacks specificity and can alter trade-off function (Zera and Harshman 2001). The quantitative genetic model has the advantage of predicting not only how traits involved in trade-offs evolve, but also can reveal how the trade-off function itself will change under selection (Roff *et al.* 2002, Roff and Gelinas 2003, Roff and Fairbairn 2012). However, only after distinguishing how phenotypic and genotypic correlations combine with

underlying physiological mechanisms can the evolution of life-histories through trade-offs be understood (Stearns 1989, Zera and Harshman 2001).

In quantitative genetics, the search for life-history trade-offs is traditionally focused on detecting negative genetic correlations (Roff 1992). In general, genetic trade-offs are assumed to exist between life-history traits, since any allele having increasing or decreasing effects on fitness through two or more traits are assumed to be fixed or lost at a faster rate than antagonistically pleiotropic alleles (Falconer and Mackay 1996, Roff 1996). However, negative genetic correlations between life-history traits have been estimated only in a few cases (Roff *et al.* 2002). A positive genetic correlation would also induce a genetic trade-off in a situation where associated traits are selected for in opposite directions (Kruuk *et al.* 2008). Furthermore, while a genetic correlation would slow down evolution in a two trait system, only a perfect genetic correlation of -1 or 1 would set an absolute constraint for evolution (Roff and Fairbairn 2007).

Besides genetic correlations, constraints for evolutionary change exist if the population is missing genetic variation for some trait combinations (Blows 2007). Usually just two or three principal components are enough to explain most of the variation in a **G**-matrix regardless of the number of traits studied (Kirkpatrick and Meyer 2004). A **G**-matrix with one or more zero eigenvalues would constrain evolution to occur along linear combinations of the nonzero eigenvalues (Pease and Bull 1988). Thus, genetic trade-offs may be detected only after multivariate analyses are carried out (Kruuk *et al.* 2008).

1.4 Sexual dimorphism and sexual conflict

Due to different roles in reproduction, sex-specific optima may be different for many traits. Sexual conflict results if females and males cannot reach their optima simultaneously (Chippindale *et al.* 2001, Parker 2006, Brommer *et al.* 2007). Alleles that increase fitness in one sex may be detrimental in the other. In such a situation, selection for some characteristics in one sex may lead to fitness costs in the other sex. For example, in the bank vole (*Myodes glareolus*), multiple-mating behavior governed by testosterone is sexually antagonistic; high testosterone increases male and reduces female mating frequency (Mokkonen *et al.* 2012). Furthermore, in the bank vole, sexually antagonistic genetic variation can be maintained by negative frequency-dependent selection in males only (Mokkonen *et al.* 2011).

The genetic correlation between homologous characteristics in males and females interferes with the evolution of phenotypes between sexes (Lande 1980). A large positive intersexual genetic correlation prevents the evolution of dimorphism, because any response in one sex is accompanied by a concordant response in the other. Thus, sexual dimorphism is predicted to be associated with low intersexual genetic correlation (Fairbairn and Roff 2006). In favor of this prediction, fitness components have, on average, a lower intersexual

correlation than physiological or morphometric characters (Poissant *et al.* 2010). However, the evolution of sexual dimorphism is possible even in the presence of large intersexual genetic correlation if sex-specific variances are different enough (Cheverud *et al.* 1985). Sexual conflict imposed by the intersexual genetic correlation can be relaxed by sex-linked inheritance and sex-limited autosomal gene expression (Fairbairn and Roff 2006) or imprinting (Day and Bonduriansky 2004).

1.5 Traits studied

1.5.1 Offspring number and size

Reproduction imposes numerous costs on animals (Harshman and Zera 2007), and these may reduce survival or possibilities for future reproduction (Williams 1966, Trivers 1974, Stearns 1989). Resources expended for reproduction are drained from a common pool with other life functions. The total reproductive effort is usually thought to be constrained by limited energy availability (Speakman 2008). In addition to energy and essential nutrients, the total reproductive effort may be constrained, for example, by female body volume (Du and Lue 2010). The latter may coincide with the energy constraint, as growing fetuses in late gestation limit mothers' feed intake by displacing the gut (Speakman 2008). The total reproductive effort is proposed to evolve independently from offspring number and size following a two stage decision process, where first, the allocation of the total amount resources used for reproduction is decided, and secondly, the allocation per offspring is decided (Smith and Fretwell 1974). However, other models predict that the total reproductive effort is negatively associated with offspring size (Winkler and Wallin 1987, Caley *et al.* 2001).

One of the fundamental assumptions in the life-history theory is derived from the allocation of limited parental resources during a single reproductive attempt: namely a trade-off between offspring number and quality (Stearns 1992). The trade-off holds under both models, explaining the independent and simultaneous evolution of the total reproductive effort and offspring size (Smith and Fretwell 1974, Winkler and Wallin 1987, Stearns 1992). By investing more per offspring to increase the quality of the offspring (commonly measured as size) and thus its survival, a parent is inevitably forced to produce fewer offspring. Furthermore, the trade-off may not be realized until after hatching or birth of the offspring (Lack 1947, Lack 1948). The amount of convertible energy constrains the number of offspring females can nurse. For example, food-supplemented bank vole females have better weaning success compared to a control group (Koskela *et al.* 1998). Food intake capacity may constrain the reproductive effort when the litter size becomes too high. In addition, lactating female mice (*Mus musculus*) reach a plateau in food intake irrespective of the litter size (Speakman 2008). The optimal offspring number from the mother's

perspective differs from the optima for offspring. Typically, maternal fitness is maximized at higher offspring number than offspring fitness (Wolf and Wade 2001, Oksanen *et al.* 2003, Wilson *et al.* 2005b).

Offspring number and size seem to behave quite differently from the microevolutionary perspective. In oviparous vertebrates, the heritability of egg size is generally higher than the heritability of egg number (e.g. Lessells *et al.* 1989, Brown and Shine 2007, Garant *et al.* 2008). Furthermore, in *Drosophila melanogaster*, egg size responded to selection in both directions, but the phenotypic trade-off was observed only in the large-egged line (Schwarzkopf *et al.* 1999). In snails (*Helix aspersa*), egg number and size showed a phenotypic trade-off, while a genetic correlation was positive (Dupont-Nivet *et al.* 1998). A common phenomenon in oviparous species is a lack of genetic trade-offs in the presence of phenotypic trade-offs between offspring number and size (Lessells *et al.* 1989, Fischer *et al.* 2006, Brown and Shine 2007, Garant *et al.* 2008).

In mammals, the relationship between number and size of offspring is more complicated than in oviparous species. Following ovulation, the number of offspring is further influenced by fertilization, implantation and embryonic mortality (Senger 2003). During pregnancy, not only does the mother influence birth size, but so does the offspring. Maternal effects for birth size in mammals cover numerous factors that influence nutrient supply to the fetus (such as uterine capacity and blood flow) (Gluckman and Hanson 2004). In monotocous species, the size of offspring at birth is usually proportional to the surface area of the placenta (Youngquist and Threlfall 2007), while in polytocous species, the relationship is not so straightforward (Kurz *et al.* 1999, van Rens *et al.* 2005). In swine (*Sus scrofa domesticus*), there is evidence that the mother determines conceptus size, and the offspring controls placental efficiency (Biensen *et al.* 1999). In mice, it has been shown that the smallest placentas transfer amino acids most efficiently to the fetus (Coan *et al.* 2008). The amount of resources used for fetal growth introduces conflict between parent and offspring. The more a mother invests in one offspring or litter, the more it diminishes her residual reproductive value (Trivers 1974). Maternal-fetal conflict is assumed to drive the observed rapid evolution of fetal expressed placental proteins (Chuong *et al.* 2010). In addition, the nutrient transfer capacity of the placenta seems to be under the control of paternally imprinted genes (Fowden *et al.* 2011). Thus, multiple paternity should favor paternal imprinting due to relatedness asymmetries between fetuses in a litter (Haig 1997).

1.5.2 Immune system

Fighting pathogens with the immune system is one of the major pieces of the survival puzzle. The immune system of vertebrates comprises two arms: the innate and adaptive immune systems (Murphy *et al.* 2008). The innate immune system responds rapidly and is based on the recognition of conserved molecular patterns in pathogens (e.g. peptidoglycan in bacterial cell walls or double-stranded viral RNA). The adaptive immune system, on the other hand, takes some time to react but provides a more specific response. Its main

constituents are two populations of lymphocytes (T- and B-cells). Each lymphocyte possesses a unique antigen receptor acquired through random recombination of gene segments used to assemble the receptor-encoding gene combined with targeted somatic mutations. Cytotoxic T-cells fight intracellular parasites by killing infected cells, and T-helper-cells regulate the immune response. After an encounter with an antigen with matching specificity, and a signal from a T-helper-cell, a B-cell transforms into a plasma cell and starts to produce immunoglobulins. An immunoglobulin or antibody is a soluble form of the B-cell receptor and its function is to neutralize extracellular pathogens, promote phagocytosis and activate complement.

In general, individuals with stronger immune responses survive better (Moller and Saino 2004). However, the costs of developing and maintaining an effective immune system may force a trade-off between immune function and other fitness-related traits (Sheldon and Verhulst 1996). In poultry, selection for growth was shown to compromise immune function. However, selection for immune function did not consistently affect growth, which suggests that the costs of growth are large relative to the costs of immune function (van der Most *et al.* 2011). In the soya sheep (*Ovis aries*), responsiveness of the immune system, as measured by the occurrence of self-reactive antibodies, is associated with both reduced reproduction and increased adult female winter survival, reflecting a trade-off between the two (Graham *et al.* 2010). In the rat (*Rattus norvegicus*), mother's litter size correlates negatively with several measures of the offspring immune system (Prager *et al.* 2010). Communication between the hypothalamic-pituitary-gonad axis and the immune system has been proposed as a proximate mechanism to explain reproductive-immune trade-offs between reproduction and immune function across vertebrate taxa (Segner *et al.* 2017).

Females may gain higher fitness by investing more in survival, whereas males should increase mating rates at the expense of longevity (Bateman 1948). Sex-specific optima in immune defense might even lead to a negative intersexual correlation in immune function (Svensson *et al.* 2009). Differences may partly be explained by multiform effects of sex hormones on different arms of the immune system (Oertelt-Prigione 2012). For example, the female sex hormone, estrogen, is found to promote B-cell response in humans (*Homo sapiens*) and mice (Verthelyi 2001), while the male sex hormone, testosterone, inhibits antibody production by human B-cells (Kanda *et al.* 1996). In pipefish (*Syngnathus typhle*) with reversed sex roles, males are the more immunocompetent sex indicating that sexual dimorphism in immunity depends on parental investment and not on sex per se (Roth *et al.* 2011).

1.5.3 Testosterone

Testosterone is the main androgen in vertebrates. It is produced in the testis and adrenal cortex of males and in smaller quantities in ovaries and adrenal cortex of females (Senger 2003). As testosterone is not soluble in water, it is transported in the plasma while bound to the sex hormone binding protein. In tissues, testosterone is further synthesized into dihydrotestosterone (DHT) or

estradiol. Testosterone or its metabolite (DHT) binds to the androgen receptor, and the resulting ligand-receptor complex moves into the nucleus where it influences gene transcription. Testosterone has an influence on the development of male secondary sexual characters and thus it determines male mating success through the development of sexual signals (Zeller 1971, Fernald 1976, Owen-Ashley *et al.* 2004, Mank 2007, Mills *et al.* 2007a).

Androgens can have immunosuppressive effects, which can explain the weaker male immune system (Dorner *et al.* 1980, Greives *et al.* 2006). Especially in species with a low annual survival rate, androgen-induced immunosuppression could constrain the development of sexual signals, and males in these species have to trade-off between immune defense and sexual signaling (Folstad and Karter 1992, Ketterson and Nolan 1999, Hau 2007). Androgen-induced trade-offs between sexual signaling and immunity is evidenced in phenotypic engineering experiments, but its genetic basis is uncertain (Roberts *et al.* 2004).

Testosterone may influence male reproductive fitness in ways beyond mating success. While a high testosterone concentration in the testis is required for sperm synthesis (up to 500 times higher than plasma) (Senger 2003), evidence for the relationship between plasma testosterone levels and the quality of ejaculate are conflicting: males with strong sexual signals have lower quality sperm compared to males that settle for weaker signals (Pizzari *et al.* 2004, Gomendio *et al.* 2007).

1.5.4 Growth and body size

Body size is one of the most important quantitative traits for studying evolutionary biology (Blanckenhorn 2000, Dmitriew 2011). Although strictly considered as morphological trait, body size correlates with several fitness traits. Larger individuals are usually more fecund, perform better in intra-specific competition and are less vulnerable to predation (Blanckenhorn 2000). Selection for weight gain or adult size results in a correlated response in litter size, age at maturity and longevity. Intraspecific covariation usually associates increased body size with increased litter size and reduced life span, while the interspecific pattern is the opposite: larger species have fewer offspring and live longer (Millar and Hickling 1991).

For species living in a cyclic environment, the optimal adult size may change between years (Norrdahl and Korpimäki 2002). In addition, selection between the sexes may be divergent; for example, in the bank vole, smaller males and larger females are likely to survive over winter (Boratyński and Koteja 2009, Boratyński *et al.* 2010). Depending on the selection pressures, the intersexual genetic correlation may pose a constraint on the evolution of sexual dimorphism in adult body size (Badyaev 2002).

1.5.5 Basal metabolic rate

Metabolic rate may well be the most important physiological measure of an animal. Basal metabolic rate (BMR) is the amount of energy consumed at rest. The heat that maintains body temperature during rest comes mainly from the internal organs (Nespolo *et al.* 2011). Endotherms have a high BMR compared to ectotherms, so they require more food than ectotherms of equal size. Thus, the evolution of endothermy is difficult to explain. Different hypotheses try to explain how such a wasteful strategy could, not only once but twice (mammals and birds), have been favored by selection (Bennett and Ruben 1979).

BMR can be considered as a measure of maintenance costs (Jackson *et al.* 2001, Ketola and Kotiaho 2009) or of general efficiency of energy acquisition (Blackmer *et al.* 2005, Boratyński and Koteja 2010). In favor of the latter view, BMR has a positive genetic correlation with maximum metabolic rate (e.g. Wone *et al.* 2009), but the correlation is not high enough to prevent independent evolution of the two. At the species level, BMR corrected for body mass correlates with several physiological and life-history traits such as heart rate, life span and litter size (White and Seymour 2004). Knowing how metabolism is genetically associated with life-history traits is important because it quantifies the costs of various fitness components (e.g. Speakman 2008) and, more broadly, helps understand the evolution of endothermy (Hayes 2010).

1.6 Aims of the study

The objective of this work was to assess life-history evolution through quantitative genetic variation in fitness characters and associated traits in a small mammal. Work was based on a large colony of bank voles (*Myodes glareolus*) kept both in the laboratory and in semi-natural field enclosures. Life-histories of the bank vole are well known due to its extensive use as an experimental model in evolutionary ecology. Vast number of previous studies at the phenotypic level, including observational, physiological and phenotypic manipulation studies, ease interpretation of present results of quantitative genetic studies in the context of life-history theory (e.g. Koskela *et al.* 1998, Oksanen *et al.* 2002, Mappes and Koskela 2004, Koskela *et al.* 2009, Helle *et al.* 2012). Furthermore, bank voles are well-suited for combined laboratory and field studies, as they are relatively easy to handle and breed in these situations. Having a laboratory colony enables the testing of the hypothesis based on physiology separately from ecological factors, which can further be studied in outdoor enclosures. Using a colony descended from a wild population allows deductions on the prevailing genetic variation and selection taking place in nature.

Firstly, the important life-history trade-off between offspring number and size was studied from the perspective of a selection experiment (paper I) and quantitative genetic analysis (paper II). Also the effect of selection for litter size

on adult body size, and the performance of the selected lines in semi-natural field enclosures were studied (paper I). The link between female reproductive fitness, metabolism and body mass was further assessed by including BMR in the analysis (paper III). Moreover, the sources of phenotypic variation in body mass during the growth period were studied (paper IV).The humoral immune system was used as a proxy for survival (paper V). The immune system in males and females were considered as separate traits to take into account possible sexual dimorphism due to different strategies in the two sexes. Further, a trade-off between the immune system and male testosterone, a primary determinant of male reproductive fitness in the bank vole, was also assessed (paper V).

2 METHODS

2.1 Study species

The bank vole (*Myodes glareolus*, formerly *Clethrionomys glareolus*) is a common mammalian species in the Palearctic region (Stenseth 1985). They live in areas of dense vegetation, such as forests and fields, and are omnivorous but mainly feed on leaves, forbs, shoots, seeds and berries (Hansson 1985). Bank vole population densities are highly variable within and between years, and distinct density cycles are observed in northern Fennoscandia (Kallio *et al.* 2009). Life-history characteristics vary along with the density cycles (Innes and Millar 1994). In central Finland, females produce up to four litters during the breeding season (from mid-May to mid-September) (Koivula *et al.* 2003). Pregnancy lasts for 19 to 20 days (Innes and Millar 1994) and phenotypic variation is large in both litter size (2 to 10, mean 5.3 pups) and offspring size (1.0 to 2.5 g, mean 1.76 g) (Koivula *et al.* 2003). Offspring are weaned at 17 days (Innes and Millar 1994). Offspring from the first cohorts start reproducing in the same summer they are born, while immature individuals from later cohorts form the bulk of the overwintering population (Wiger 1979, Prevot-Julliard *et al.* 1999). The maximum life span in nature is 16 months (Innes and Millar 1994).

Breeding bank vole females are territorial, while home ranges of males are large and overlapping (Bondrup-Nielsen and Karlsson 1985, Koskela *et al.* 1997). The bank vole mating system is polygynandrous, that is, both males and females mate multiply, and males show larger variation in their reproductive success compared to females (Mills *et al.* 2007b, Oksanen *et al.* 1999). The bank vole is an induced ovulator (Odberg 1984). Females prefer dominant males as mating partners (Horne and Ylönen 1996), and males use dominance to advertise their quality through competition with other males for access to females (Hoffmeyer 1982, Oksanen *et al.* 1999). The body size of the bank vole is a sexually monomorphic character (Innes and Millar 1994), and reproductive success of bank vole males depends on their testosterone level rather than their body size (Mills *et al.* 2007a, Mills *et al.* 2007b, Mills *et al.* 2009).

2.2 Animal husbandry

Animals were housed in standard mouse cages and were maintained in a 16L:8D photoperiod at 20 ± 2°C. Wood shavings and hay were provided as bedding, while food (labfor 36, Lactamin AB, Stockholm, Sweden) and water were available ad libitum. Pregnant females were checked once a day for parturition. After parturition, the birth size was measured using an electronic scale (± 0.01 g) and head width with a stereomicroscope. Pups were weaned at 20 days and after weaning, sexes were kept in separate cages until the breeding procedure was applied to the new generation.

2.3 Selection procedure

Selection lines were founded from 150 females and 116 males. All males were wild-trapped, but some of the females were laboratory-born offspring of wild-trapped individuals. Thus, the total number of animals with unknown ancestry in the pedigree was 122 females and 134 males. All wild individuals were captured in Konnevesi, central Finland, during summer 2000. The selection was based on between- and within-family selection, and the selection criteria was the average size of the three first litters. The population was divided into two lines based on the phenotype of the females in generation 0 (L low; H high). At first, combined between- and within-family selection was practiced, where one daughter with the highest or lowest mean litter size within the maternal family was selected. In the later generations only within-family selection was practiced.

2.4 Field enclosures

Descendants of the selection lines were released to semi-natural conditions in field enclosures. The field experiment was conducted in 11 enclosures near Konnevesi research station in central Finland (62°37'N, 26°20'E). Enclosures were 0.2 ha each in size (40 x 50 m), and were surrounded by sheet metal fencing (1.0 m above ground, 0.5 m below). The fence was high enough to hold the study populations, but possible predators (e.g. red fox *Vulpes vulpes*, least weasel *Mustela nivalis* or avian predators) were able to enter the enclosures. Each enclosure contained twenty Ugglan live traps organized in a 10 m x 10 m grid pattern. The traps were covered by galvanized sheet-metal chimneys to reduce exposure of trapped individuals to possible extreme weather conditions. Sunflower seeds, potatoes, and pellets were used as bait in the traps; however, in the enclosures, the study individuals relied on natural food resources during the non-trapping phases.

The 11 enclosures were divided into five Low-line and six High-line enclosures, and six females and six males were released to each one. Females were trapped two weeks after they were released to the enclosures and pregnant individuals were taken to the laboratory to give birth. Pups were measured and individually marked, after which the mother and her pups were returned to the same enclosure from where they were trapped for nursing. Enclosures were trapped when the pups were at weaning age (> 20 days). Trapping was continued until no new individuals were caught during two successive trap checks.

2.5 Analytical methods

2.5.1 Humoral immune response (paper V)

To measure the specific immune response, animals were immunized with an intraperitoneal injection (0.1 mL) of bovine gamma globulin (BGG) (200 mg; Sigma) and emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). On day 28 after immunization, a blood sample (18 mL) was taken to determine anti-BGG antibody and total immunoglobulin G (IgG) concentrations with a microplate enzyme-linked immunosorbent assay. These 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).

2.5.2 Testosterone (paper V)

A 75-mL intraorbital blood sample collected in a heparinized capillary tube 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 blood cells, and plasma was stored at -20°C. Plasma testosterone was measured using a radioimmunoassay (RIA) technique (Testo-CTK, DiaSorin, Byk-Sangtec Diagnostica, Dietzenbach, Germany). Methods are described in Mills *et al.* (2007b).

2.5.3 Basal metabolic rate (paper III)

BMR measurements trials, including body mass measurements, were conducted on non-reproducing adult individuals belonging to the fourth and fifth generations. BMR was defined as the mean of the (third, fourth and fifth) lowest averages of oxygen consumption calculations based on the measurements conducted in respirometry systems (Boratyński *et al.* 2010, Boratyński and Koteja 2010). Measurements of oxygen consumption (ml h-1)

were conducted in an eight-channel open-flow respirometric system (Sable Systems, Henderson, NV) based on Fc-1B O_2 (Sable Systems) analyzer. Metabolic rate was calculated using O_2 values according to the formula: VO_2 = {Vi (FdO_2)/ [1 - FeO_2 (1 - RQ)]}, (eq. 1b in Koteja (1996)), where VO_2 is the oxygen consumption rate; Vi is the flow rate measured before chamber; FdO_2 is the difference of O_2 fractional concentrations in dry air flow before and after passing through the chamber; FeO_2 is the fractional concentration of O_2 in dry air flow after the chamber, assuming RQ = CO_2 eliminated/O_2 consumed 0.75 for nearly starved animals. Prior to the measurements, seven animals were weighed and placed in chambers (180 mL volume; with the eighth channel remaining empty, as a reference) without access to water or food within the thermal neutral zone (30.0 ± 0.5˚C). The flow of dry air (dried with silica gel) of 260 mL/min was passing through the chambers. Oxygen consumption was recorded sequentially from 8 channels for a period of 7 h 30 min. A total of 29 oxygen consumption measures, sequential recordings for each chamber, were saved throughout one measurement trial.

2.6 Quantitative genetic analysis

2.6.1 REML-animal model

All quantitative genetic analyses in this thesis were performed with the REML-animal model approach (Patterson and Thompson 1971). The animal model is a mixed model which fits the genetic merit of each individual in a pedigree. By incorporating the inverse of the numerator relationship matrix (A^{-1}), it utilizes all the information from the pedigree, takes selection into account and, under the infinitesimal model, gives unbiased estimates of the base population (Lynch and Walsh 1998). The animal model is a flexible method to estimate variance components due to different sources. Depending on the trait in question, we tested and estimated variance in direct additive genetic effects, and the variation in permanent environmental individual effects. In addition, we also estimated environmental and genetic maternal effects as well as litter effects.

Fixed effects used in the models were estimated prior to the estimation of the (co)variance components using the GLM-procedure in SPSS software (versions 15.0-24.0). Linear regression on the inbreeding coefficient as a fixed effect was fitted to estimate inbreeding depression and/or directional dominance. Moreover, it corrected additive genetic estimates from inflation caused by similarity due to directional dominance and/or inbreeding depression (de Boer and van Arendonk 1992).

The (co)variance components were estimated with the ASReml software versions 2.0 and 3.0 which employ an average information restricted maximum likelihood (REML)-algorithm (Gilmour *et al.* 2002, Gilmour *et al.* 2006, Gilmour *et al.* 2009).

For estimation of (co)variance components, different mixed linear models were used. As an example, the most simple (**1**) and most comprehensive models (**2**) are given: (**1**) fitting only direct additive genetic effects; (**2**) fitting maternal genetic, permanent maternal environmental and litter effects besides direct genetic effect:

$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1a} + \boldsymbol{e}$ (**model 1**)
$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1a} + \boldsymbol{Z_2m} + \boldsymbol{Z_3n} + \boldsymbol{Z_4c} + \boldsymbol{e}$ (**model 2**)

where $\boldsymbol{y}$ is a vector of observations, $\boldsymbol{b}$ contains the fixed effects, $\boldsymbol{X}$ is the incidence matrix for fixed effects. The random effects of direct genetic as $\boldsymbol{a}$, maternal genetic as $\boldsymbol{m}$, non-genetic maternal permanent environment as $\boldsymbol{n}$, and common litter as $\boldsymbol{c}$ were related to individual records with the corresponding incidence matrices $\boldsymbol{Z_1}$, $\boldsymbol{Z_2}$, $\boldsymbol{Z_3}$ and $\boldsymbol{Z_4}$, respectively.

The random effects are assumed to be independent. Expectations of the random effect are zero, and their variances are expected to follow a normal distribution. In particular, the variances were assumed to be $V(\boldsymbol{a}) = \boldsymbol{A}\boldsymbol{\sigma}_{\boldsymbol{a}}^{\boldsymbol{2}}$, $V(\boldsymbol{m}) = \boldsymbol{A}\boldsymbol{\sigma}_{\boldsymbol{m}}^{\boldsymbol{2}}$, $\boldsymbol{cov}(\boldsymbol{a},\boldsymbol{m}) = \boldsymbol{A}\boldsymbol{\sigma}_{\boldsymbol{am}}$, $V(\boldsymbol{n}) = \boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{n}}^{\boldsymbol{2}}$, $V(\boldsymbol{c}) = \boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{c}}^{\boldsymbol{2}}$ and $V(\boldsymbol{e}) = \boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}}$ where $\boldsymbol{\sigma}_{\boldsymbol{a}}^{\boldsymbol{2}}$ is direct additive genetic variance, $\boldsymbol{\sigma}_{\boldsymbol{m}}^{\boldsymbol{2}}$ is maternal genetic variance, $\boldsymbol{\sigma}_{\boldsymbol{am}}$ is covariance between direct additive and maternal genetic effects, $\boldsymbol{\sigma}_{\boldsymbol{n}}^{\boldsymbol{2}}$ non-genetic maternal variance, $\boldsymbol{\sigma}_{\boldsymbol{c}}^{\boldsymbol{2}}$ common litter effect variance, and $\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}}$ residual variance. Total phenotypic variance was estimated as the sum of all (co)variance components. Heritability (h^2), litter effect (c^2), maternal permanent environment effect (n^2), and maternal genetic heritability (m^2) for the traits were calculated as the ratio of the relevant variance component to the total phenotypic variance. The genetic correlation between direct and maternal genetic effect was calculated as $\boldsymbol{r_{am}} = \frac{\boldsymbol{\sigma_{am}}}{\sqrt{\boldsymbol{\sigma_a^2}\boldsymbol{\sigma_m^2}}}$.

2.6.2 Reduced rank models

As an alternative to full rank models, the additive genetic (co)variance matrix was modeled in paper V with factor-analytic variance structure:
$\mathbf{G} = \mathbf{\Gamma\Gamma'}$
in which $\mathbf{\Gamma}$ is the matrix of factor loadings. Specific variances were restricted to zero, making analyses similar to principal component analyses.

2.6.3 Model selection

The appropriate random effect structure was selected by comparing the Akaike information criterion (AIC) for a set of models (Burnham and Anderson 2002). Model comparison was based on delta $\mathbf{\Delta}$AIC, i.e. the AIC value of a given model relative to the best model. The selected model was the most parsimonious model within $\mathbf{\Delta}$AIC 2 difference from the best fitted model.

3 RESULTS AND DISCUSSION

3.1 Offspring number and size (I, II)

Estimates of heritability for litter size were low to moderate (0.11–0.21; depending on the model; I, II), but of similar magnitude compared to other polytocous species (Satoh *et al.* 1997, Rastogi *et al.* 2000, Holl and Robison 2003, Menendez-Buxadera *et al.* 2003, Reale *et al.* 2003, Holt *et al.* 2005, Koivula *et al.* 2010), and large enough to enable an observable selection response. Two-way selection for five generations increased the difference between the lines for 0.83 offspring per litter. However, with the exception of the F2 generation, litter size also decreased in the H-line (I). Lines also differed in litter size in the field enclosures, which demonstrates that genetic change acquired in the selection procedure is not restricted to laboratory conditions.

Asymmetric selection responses are commonly observed in fitness traits, with stronger responses towards lower fitness (Frankham 1990, Hill and Caballero 1992). Of the several suggested potential mechanisms causing an asymmetric response to the divergent selection, maternal effects, different selection differentials, inbreeding depression and genetic asymmetry could be relevant in our selection experiment. Maternal effects could particularly explain the antagonistic response in the H-line in the F1 generation. Daughters born in a large litter then give birth to smaller litters as they are exposed to stronger competition on maternal resources (Falconer 1960). However, in the whole dataset, the maternal effect on litter size was found to be non-significant (II). Further, the cumulative selection differential was larger in the H-line, it could thus not explain the stronger response in the L line (I). The most likely explanations for the asymmetric selection response for litter size are inbreeding depression (I) and quantitative genetic architecture with directional dominance and asymmetrical gene frequencies (Frankham 1990, Hill and Caballero 1992, Falconer and Mackay 1996). This result is expected if genes increasing litter size had already been driven close to extreme frequencies by natural selection in the base population (Zhang and Hill 2005). Selection may favor large litter size in

the bank vole, as an offspring number manipulation experiment has shown that number of surviving offspring increase with litter size (Oksanen *et al.* 2001). Thus, results from the selection experiment appear to challenge presumptions of the infinitesimal model (Bulmer 1980). However, when the dataset was analyzed separately for early and late generations, estimates of additive genetic variance did not differ, giving support for an infinitesimal model of the inheritance of bank vole litter size (I). The explanation is likely a compromise: the number of loci is large enough to reasonably follow the infinitesimal model, but the additive genetic variance is caused mainly by deleterious recessive alleles.

Maternal and common litter effects explained most of the phenotypic variation in birth size (II). Together they composed 62% of phenotypic variation in birth mass and 58% in head width at birth. The litter effect was by far the most important source of variation, with 45% and 49% variation explained, while maternal heritabilities were only 0.09 and 0.03, respectively. The estimate of direct heritability was 0.08 for birth mass and 0.07 for birth head width (II). Thus, for structural size, the offspring's own genes were a relatively more important source of variation than for birth mass.

Litter size was an important source of maternal variation in both birth size traits (II). When birth size was adjusted for natal litter size, the variance explained by the litter effect and permanent maternal effect was markedly reduced. This result was expected since the low heritability of litter size indicates that the variation in litter size is mainly due to environmental factors. Thus, removing the effect of litter size on birth size should mainly decrease the amount of environmental variation in birth size. Moreover, when adjusted for natal litter size, more variation was removed from birth mass than from head width at birth. This demonstrates the more substantial trade-off between litter size and birth mass as was observed from the phenotypic correlations between litter size and offspring size (birth mass r = -0.46; birth head width r = -0.35; II).

In agreement with the observed phenotypic trade-off, the decreased litter size in both lines was accompanied with slightly increased offspring birth size in both lines (I). However, lines did not diverge in birth size as for litter size, and in the F5 generation, the difference in both offspring birth mass and head width was statistically non-significant (I). The trade-off between litter size and offspring size was also more apparent in the L-line (I), a result that could reflect evolution of the **G**-matrix during selection experiment. As a correlated response to the selection for litter size in the H-line, female body size also changed and females grew heavier (I). The larger size may have enabled a larger total reproductive capacity allowing females in the H-line to give birth to bigger offspring compared to the L-line despite the larger litter size.

In accordance with the inconsistent correlated selection response in birth size, quantitative genetic analysis revealed both positive and negative genetic correlations between offspring number and size (II). Direct genetic correlation between offspring number and size was positive, suggesting a concordant response in direct genetic effects between the two traits (II). The genetic trade-off was observed as a negative correlation between direct genetic effects for

litter size and maternal genetic effects for offspring size (II). Thus, selection for larger litter size will indirectly increase birth size through genes that are expressed in the offspring, but on the other hand will tend to decrease birth size due to the antagonistic maternal effect. A previously reported negative genetic correlation between litter size and mean offspring birth size in the bank vole (Mappes and Koskela 2004) do not, therefore, contradict the present results. The previous study estimated only the correlation between litter size and the maternal genetic effects for birth size (here shown to be negative) and ignored the positive correlation between litter size and direct genetic effects for birth size. The present results suggest that offspring number and size do not necessarily evolve through antagonistic pleiotropy in the short term. The observed phenotypic trade-off can also be explained with strong negative environmental correlations (II).

Both negative (Wilson *et al.* 2005a) and positive (Analla and Serradilla 1998) estimates have been reported for the correlation between the maternal permanent environment and litter size or birth size. A positive environmental correlation could arise if the environment affects the traits through resource acquisition (Van Noordwijk and Dejong 1986). For example, abundant nutrition that causes ovulation of extra eggs allows mothers to support the growth of large fetuses. Conversely, a negative environmental correlation is expected if the environment affects resource allocation. In other words, environmental variation in litter size does not affect the total maternal reproductive resources. From this perspective, negative permanent and temporary environmental correlations between litter size and offspring size in the bank vole can be expected. The bank vole has an extremely variable litter size (Koivula *et al.* 2003) due to large environmental variation (I, II). As a small mammal, the bank vole is an income breeder whose capacity to support the growth of the fetuses during late pregnancy is not likely to be connected with environmental variation affecting offspring number, which is already determined early in pregnancy.

High environmental variation in litter size may also be considered as an evolutionary force maintaining genetic variation in maternal effects for birth size. As a female needs to restrict the growth of the conceptus depending on the litter size, a single offspring has different requirements of maternal effects compared to nine offspring, so that birth size is optimal from both the offspring's and mother's point of view. In domestic species, direct heritability for birth weight is highest in monotocous species and decreases as litter size increases; cattle (*Bos Taurus*) > sheep > swine (Dodenhoff *et al.* 1999, Roehe 1999, Safari *et al.* 2005). Maternal heritability for birth weight increases in the same order. Maternal heritability remains elevated in polytocous species even after correcting birth weight for natal litter size (Roehe 1999).

3.2 Fitness effects of basal metabolism and adult body size (I, III)

The results in this thesis gave inconsistent results on the association between metabolism and fitness (III). Inbreeding increased energy use for maintenance, reflected here by BMR, indicating that the association between BMR and overall fitness in bank voles would be negative (i.e. support compensation hypothesis), while a strong, significant positive genetic correlation between BMR and litter size suggests that traits connected to female fecundity might favor higher metabolism (i.e. support increased intake hypothesis). Adult body mass was reduced by inbreeding depression especially in males (III), which suggests that large body mass is favored by selection. However, earlier findings have shown that overwintering survival favors small males (Boratyński and Koteja 2009, Boratyński *et al.* 2010). Adult body mass had a cross-sex genetic correlation close to one (III), which indicates a strong constraint against the development of sexual dimorphism in this species. The cross-sex correlation for absolute BMR differed from one, suggesting that basal metabolism is not under control of the same set of genes in males and females (III). In the mass corrected, or residual, BMR there even was no support for additive genetic variation for males (III). Association between female reproductive characters and metabolism may have created divergent selection pressures on BMR between sexes. While low BMR is consistently favored in males, females gain a fitness advantage from increased BMR through larger litter size. BMR is largely under the control of thyroid hormones (Wrutniak-Cabello *et al.* 2001). The thyroid gland-epiphysis axis may provide a link between metabolism and reproduction, since thyroxin and thyroid stimulating hormone affect steroid hormone and steroid hormone binding globulin levels, and oocyte development (Dittrich *et al.* 2011).

Litter size had a positive genetic correlation with female body size (I, III). Both female body mass and head width showed a correlative response for selection for litter size (I). Fecundity is generally positively correlated with body size or body mass across strains or breeds in several species (Bunger *et al.* 2005) Females in good condition are known to give birth to larger litters in many species (Risch *et al.* 2007, Evsikov *et al.* 2008). Body mass may well affect ovulation rate and embryo survival, for example, through condition. Individuals in good condition have sufficiently adipose tissue which is besides energy storage also an endocrine organ that produces several hormones (Kershaw and Flier 2004). Hormones produced by adipose tissue include leptin which is known to increase maternal investment (French *et al.* 2009).

3.3 Growth and survival (I, IV)

Body mass during the early growth period possessed no direct additive genetic variation (IV). Maternal and common litter effects explained most of the phenotypic variation in the offspring body mass during the nursing period (IV).

Immediately after weaning, the litter effect was the only source of variation in body mass (IV). Direct additive genetic variation was not detected until two months of age (IV). Thus, there is a short period during ontogeny when there is no maternal or direct additive genetic variation in body mass of the bank vole. Directional selection could have eroded direct additive genetic variation for early growth, as weaning size has been implicated to be an important determinant of long-term survival in the bank vole (Koskela 1998). However, there must be some mechanism to preserve maternal genetic variation for body mass during the nursing period. Maternal genetic variation responds more weakly to selection as it is one step further from the selection event (Willham 1963). Maternal genetic variation for body mass may also be associated with other characters that oppose selection for body mass in the offspring. Lactation and litter size, for example, are characters that are expected to influence offspring growth rate during nursing. The former is linked to metabolic rate (Hammond and Diamond 1992) and the latter is positively genetically correlated with adult size in the bank vole (III). Thus, fecundity selection should favor large adult size in females. In addition, during winter, divergent survival selection operates between the sexes: smaller males and larger females have a higher likelihood of survival over winter (Boratyński and Koteja 2009, Boratyński *et al.* 2010). This, combined with an intersexual genetic correlation close to one for adult body mass (III), could hinder the evolution of body size, but, on the other hand, promote maintenance of additive genetic variation in adult body mass.

To emphasize the importance of female body size for offspring survival, an offspring's probability of surviving until weaning in the field was reduced in the smaller sized L-line (I). Further, an increase in the mother's body size increased offspring survival probability more in the L-line than it did in the H-line (I). Generally, lactation puts mothers under strong metabolic stress. Food intake in lactating mice increases multifold compared to pregnancy (Speakman 2008). Lactating house mice females reduce their litter size if metabolic costs are becoming too high for their own survival (Perrigo 1987). The larger body size in the H-line (I) may help females to support their offspring during the nursing period, as energy intake increases along with body size (Speakman 2008).

During the period after weaning, the litter variance increased (IV), underlining its role in convergent growth. A large part of litter variance is likely explained by variation in the natal litter size. Litter size manipulations in the wild and in enclosures have previously shown an effect of litter size on the weaning size of bank vole offspring (Koivula *et al.* 2003, Oksanen *et al.* 2003). Although, variation in food consumption and milk production of nursing bank vole females influence offspring growth even after controlling for litter size (Oksanen *et al.* 1999). In accordance with the convergent growth due to the maternal environment found in the present study, pups grown in enlarged litters have shown accelerated growth after weaning in the bank vole (Oksanen *et al.* 2002). Factors that reduce growth during nursing within litters (e.g. sibling competition) contribute to faster growth after weaning, when individuals start to show compensation in their growth curves. When convergent growth occurs

in response to environmental effects, it is a plastic process that allows adaptation of the individual to current conditions.

3.4 Testosterone mediated fitness effects (V)

Testosterone was found to have moderate heritability in the bank vole (V). There are only a few estimates of heritability of testosterone for different species in the literature. For example, humans (Bogaert *et al.* 2008) and garter snakes (*Thamnophis sirtalis*) (King *et al.* 2004) have had higher heritability reported than bank voles, but these estimates may be inflated by common environmental effects. The lower estimate in the bank vole may also reflect strong selection for testosterone in this species (Mills *et al.* 2007a, Mills *et al.* 2007b, Mills *et al.* 2009). According to the idea of endocrine-mediated life-history evolution, hormones shape the evolution of several fitness components (Zera *et al.* 2007, McGlothlin & Ketterson 2008). In this thesis, it was found that testosterone can alter the development of immunocompetence in both sexes (V).

Testosterone is commonly viewed as an immunosuppressant (Roberts *et al.* 2004). In accordance with phenotypic engineering experiments in the bank vole (e.g. Mills *et al.* 2009), testosterone had a negative genetic correlation with the humoral immune system (V). This effect was not only found in male, but also in the female humoral immune system, demonstrating that selection for higher testosterone in males would compromise the function of immune system in both sexes. Our results are in agreement with two studies carried out with birds. Selection for the humoral immune response in domestic fowl (*Gallus domesticus*) produced a correlated antagonistic selection response in the male testosterone level (Verhulst *et al.* 1999), while selection for increased comb size, a character dependent on testosterone, led to reduced viability in males (Von Schantz *et al.* 1995). In the dark-eyed junco (*Junco hyemalis*), a male-biased sex ratio led to compromised immunity in both sexes (Greives *et al.* 2007).

It is unclear how male testosterone is adversely connected to female immunocompetence. The easiest explanation would be that testosterone also acts as an immunosuppressant in females, and female and male testosterone levels are probably genetically correlated (Moller *et al.* 2005, Zysling *et al.* 2006). Multivariate analysis of two measures of immune function in both sexes and male testosterone level revealed that this five trait system had genetic variation only for three dimensions (V). Selection will be most efficient if it acts in parallel to the largest eigenvector of the G-matrix (Blows and Hoffmann 2005), which in the bank vole contrasts testosterone with all the immune measures used, but especially with the male antibody response to a novel antigen.

4 CONCLUSIONS AND FUTURE CHALLENGES

In this thesis, I have studied a central hypothesis in life-history theory by estimating the quantitative genetic basis for several fitness components in a wild small mammal, the bank vole. Quantitative genetics provides an effective framework to test the hypothesis proposed by life-history theory (Stearns 1992, Roff 2002). Understanding underlying genetic variation is the premise for understanding evolution, as genetic variances and covariances determine the potential for a set of traits to respond to selection (Lande 1979). Results in this thesis are in rough agreement with the prediction that the less genetic variation a trait possesses, the closer it is to fitness (Fisher 1930). Morphological traits were found to have higher heritabilities than physiological measurements, which in turn had higher heritabilities than focal life-history traits (e.g. litter size). Notable exceptions were mass corrected basal metabolic rate in males and juvenile body mass at four weeks of age. Both are in no doubt important characters in the bank vole life-histories and thus additive genetic variation they possess should be under strong eroding forces of selection. However, we cannot rule out artefacts created by rather small data sets in these studies. Importance of energy metabolism for bank life histories was further shown by strong positive genetic correlations between metabolic rate and female fecundity. Correlations approaching one could set so called absolute constraints for life-history evolution; responses in litter size and metabolic rate in females are bound together. Another trade-off in female life-histories, between offspring number and offspring size, was explained by environmental correlations. Genetic correlations associated with this trade-off were of both signs. A weak negative genetic correlation between litter size and maternal genetic effects for birth size was opposed by a stronger positive genetic correlation between litter size and direct genetic effects for birth size. Slope of offspring number-size trade-off seemed to change during even a short term selection practiced in the study population. Whether this was due to evolution of the **G**-matrix or due to increased maternal resources realized as larger body size in the H-line, or both, remains to be studied in the future. However, present work has demonstrated

that rapid change in even focal life-history trade-off is possible under directional selection.

In general, conclusions based on bivariate correlations should be somewhat cautious (Roff and Fairbairn 2007). The multivariate analysis in the present thesis showed that a system of five traits can evolve only in three dimensions. In the studied system 65% of the total variation contrasted male testosterone level with humoral immune function in both sexes. Furthermore, the validity of the estimates depends on the stability of the **G**-matrix. Changes in gene frequencies will affect genetic variances and covariances; the latter more than the former (Falconer and Mackay 1996). However, there is support for the evolutionary stability of the G-matrix in the short term (10 to 15 generations) (Roff 1997, Roff and Fairbairn 2012) and even for longer periods (Arnold *et al.* 2008). In this thesis, change in the slope of the trade-off between offspring number and size questions the stability if the **G**-matrix, while the consistency of estimates of additive genetic variance in litter size over generations implies that a number of loci affecting litter size is large enough to overcome effects of short-term selection at least on the additive genetic variance. Further, since molecular genetics has provided new tools to assess genetic variation in quantitative traits, evidence has started to accumulate to validate the quantitative genetic model in metric characters (Hill 2012). For example, hundreds of SNPs (single nucleotide polymorphism) that contribute to additive genetic variation in human stature have been found (Yang *et al.* 2010, Makowsky *et al.* 2011). Soon, whole genome sequencing will raise our understanding of genetic variation to a new level (Metzker 2010). Even then, we are likely to be far from understanding how the variation in DNA sequences produces the phenotypic variation (Mackay *et al.* 2009) especially when considering possible epistatic interactions (Mäki-Tanila and Hill 2014). Genes controlling the endocrine system are potent candidates to explain genetic variation in life-history characters (Ketterson and Nolan 1992, Tenghe *et al.* 2016, Cox *et al.* 2016, Mokkonen *et al.* 2016). Understanding how genetic variation in common proximate endocrine mechanisms, through controlling the underlying physiology, produces phenotypic (co)variation in different traits is the key to life-history evolution.

Acknowledgements

Above all, I would like to thank my supervisors Tapio Mappes, Esa Koskela, Tuula Oksanen and Minna Koivula. You did not give up hope on the completion of this thesis even after surprising change in my upcoming career. Special thanks to Esa for sharing office for three summers and many productive lunch and coffee breaks. Your effort and support during this last year was essential for this work to finish.

I thank my other co-authors Tanja Poikonen, Suzanne Mills, Zbyszek Boratyński and Ilmari Jokinen. Your work in the experiments and with the manuscipts were crucial. Mika Mökkönen checked the language of several of the manuscripts. Mika, your enthusiasm for science is contagious. I thank all the other members of our research group for pleasant company and academic inspiration.

I thank all the people in the Department of Biological and Environmental Science. Professors Anneli Hoikkala, Janne Kotiaho, Johanna Mappes, Mikko Mönkkönen and Jari Ylänne created scientifically productive environment. Jarkko Routtu and Maria Kankare shared office with me during my first years in Jyväskylä. Tarmo Ketola helped with the ASReml software. Experimental Animal Unit and Konnevesi Reasearch Station provided facilities for experiments. I thank all the technical staff in the laboratory and in the vole husbandry who made my research possible.

Matti Ojala my former professor in Helsinki gave useful comments in the beginning of this work. Phill Watts, Ismo Stranden and Joannes van Cann commented manuscripts. Jon Brommer and Erling Strandberg reviewed this thesis with tight schedule. Angela Simms helped with language and Jari Haimi with editing.

I thank my family for support. Mom and dad, I have always been welcome home for weekends and holidays. Home has been my stronghold to plot new schemes. Jukka, I am sorry if you have lost any cows because of my malpractice. Unfortunately this thesis will not make any better a veterinarian. Tiina and Anne, your endless inquiries about my thesis were one piece in a puzzle that pushed me to complete this work.

This PhD work was financially supported by the Academy of Finland, Center of Excellence in Evolutionary Research and University of Jyväskylä.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Pikkunisäkkään evolutiiviset valinnat tarkasteltuna kvantitatiivisen genetiikan menetelmin

Evoluutiota ei voi tapahtua ilman perinnöllistä vaihtelua. Teorian mukaan perinnöllisen vaihtelun pitäisi olla vähäisintä kelpoisuutta lähinnä olevissa ominaisuuksissa. Nämä niin sanotut elinkiertopiirteet liittyvät tyypillisesti lisääntymiseen ja selviytymiseen. Eliöiden on jaettava käytettävissä olevat rajalliset resurssit optimaalisesti eri elinkiertopiirteiden välillä. Esimerkiksi poikasten lukumäärän ja poikasten koon välillä vallitsee negatiivinen suhde samoin kuin lisääntymisen ja hengissä selviytymisen välillä. Elinkiertopiirteiden ilmiasun vaihtelun perinnöllisen taustan tunteminen on välttämätöntä evoluution ymmärtämiseksi. Tutkin tässä väitöskirjassa elinkiertopiirteiden evoluutiota kvantitatiivisen genetiikan keinoin käyttäen tutkimuslajina metsämyyrää (*Myodes glareolus*). Kvantitatiivisessa genetiikassa keskitytään yksittäisten geenien vaikutusten sijaan ilmiasun vaihtelun jakamiseen perinnölliseen ja ympäristöstä johtuviin tekijöihin käyttäen hyväksi sukulaisyksilöiden ilmiasun samankaltaisuutta. Osa ympäristövaikutuksista erityisesti nuoren yksilön ilmiasussa voivat olla peräisin äitivaikutuksista, jotka puolestaan voivat olla perinnöllisiä. Käytin tilastollista menetelmää nimeltä eläinmalli, joka ottaa huomioon kaikki tunnetut sukulaissuhteet tutkitussa populaatiossa.

Metsämyyrä on Suomen yleisin nisäkäs ja siitä on tullut suosittu tutkimuslaji evoluutioekologiassa. Metsämyyrälle on tyypillistä voimakas vaihtelu poikuekoossa. Luonnossa metsämyyrän poikuekoko vaihtelee yhdestä yhdeksään. Naaraat ja urokset parittelevat useiden kumppaneiden kanssa. Urokset eivät osallistu poikasten hoitoon. Naaras vieroittaa poikaset noin kolmen viikon iässä. Tässä väitöskirjassa tutkitut elinkiertopiirteet olivat poikuekoko, poikasen syntymäkoko, poikasen koko kasvun aikana, aikuiskoko, perusaineenvaihdunnan nopeus, humoraalinen immuunivaste, uroksen testosteronitaso ja poikasen selviytyminen vieroitusikäiseksi luonnollisissa tarhaoloissa. Tutkimuksessa käytetty laboratoriopopulaatio perustettiin villeistä yksilöistä ja siihen kohdistettiin kaksisuuntainen valinta poikuekokoon (pieni-linja, suuri-linja). Lopuksi keinotekoisesti luotujen lisääntymisstrategioiden vaikutusta kelpoisuuteen tutkittiin vapauttamalla valintalinjoista periytyviä yksilöitä tarhoihin luonnollisiin oloihin.

Valinta kasvatti eroa poikuekoossa linjojen välillä, mutta valintavaste oli epäsymmetrinen poikuekoon laskiessa myös suuressa linjassa. Ero poikuekoossa linjojen välillä säilyi myös tarhoissa. Yhteisvaihtelu poikuekoossa ja poikasen koossa selittyi pääosin ympäristötekijöillä, koska perinnöllinen vaihtelu näiden välillä oli osittain jopa samansuuntaista. Laboratoriossa valintalinjojen välillä ei ollut eroa poikasen syntymäkoossa. Tarhassa pienen linjan poikaset olivat suurempia, mutta poikuekoon kasvu pienensi poikasen kokoa enemmän pienen poikuekoon linjassa. Suuren linjan poikaset selviytyivät tarhassa paremmin vie-

roitusikään, mutta emon massa vaikutti poikasen selviytymiseen enemmän pienessä linjassa. Poikasen selviytymistodennäköisyys parani emon massan kasvaessa. Emon massa vastasi epäsuorasti valintaan molemmissa linjoissa, kasvaen suuressa linjassa ja vastaavasti pienentyen pienessä linjassa.

Poikueympäristö ja perinnölliset äitivaikutukset selittivät suurimman osan vaihtelusta poikasen massassa ensimmäisten elinviikkojen aikana. Poikasen omat geenit alkoivat vaikuttaa massaan vasta yli kuukauden ikäisenä. Vieroituksen jälkeen metsämyyrän massa jopa vaikutti olevan ilman perinnöllistä vaihtelua, mikä voi kertoa voimakkaasta valintapaineesta aikaiseen kasvunopeuteen. Naaraiden ja urosten aineenvaihdunta on metsämyyrällä perinnöllisesti eriytynyt. Uroksilla massan suhteen korjatussa perusaineenvaihdunnassa ei ollut perinnöllistä vaihtelua. Naaraiden aineenvaihdunta oli puolestaan voimakkaasti kytkeytynyt poikuekokoon perinnöllisen samanlaisuuden asteen ollessa lähellä yhtä. Muutokset poikuekoossa heijastuvat siten suoraan naaraan aineenvaihdunnan tasoon ja siten sen tarvitsemaan energiaan.

Testosteroni on tärkein metsämyyräuroksen lisääntymismenestykseen vaikuttava piirre. Toisaalta testosteronin myös tiedetään heikentävän immuunijärjestelmän toimintaa ja siten huonontavan yksilön selviytymistodennäköisyyttä. Tässä väitöskirjassa immuunijärjestelmän toimintaa kuvattiin kahdella humoraalisen immuunijärjestelmän piirteellä (immuunivaste uuteen antigeeniin ja kokonaisimmunoglobiinin määrä), joiden molempien on aikaisemmissa tutkimuksissa todettu korreloivan selviytymisen kanssa metsämyyrällä. Tutkimuksessa uroksen ja naaraan immuunijärjestelmän piirteet käsiteltiin eri ominaisuuksina. Nämä muodostivat yhdessä uroksen testosteronin kanssa viiden piirteen monimuuttujajärjestelmän. Viiden piirteen järjestelmässä kolme pääkomponenttia riittivät selittämään perinnöllisen vaihtelun. Suurimmassa pääkomponentissa, joka selitti lähes 65 % perinnöllisestä vaihtelusta, testosteronin vaihtelu oli vastakkaismerkkistä molempiin tutkittuihin immuunijärjestelmän piirteisiin molemmilla sukupuolilla.

Tässä väitöskirjassa tutkituista elinkiertopiirteiden välisistä ilmiasussa havaittavista valinnoista osalla oli geneettinen tausta ja osa johtui ympäristötekijöistä. Poikuekoon ja poikasen koon välinen valinta johtui ympäristötekijöistä ja periaatteessa voisi mahdollistaa poikuekoon ja poikasen koon riippumattoman evoluution. Kuitenkin poikuekoon evoluutiota voi rajoittaa sen voimakas kytkeytyminen aikuiskokoon ja naaraiden aineenvaihdunnan nopeuteen. Naaraan pieni aikuiskoko heikentää poikasten selviytymistä ja toisaalta suuri koko nostaa aineenvaihdunnan kautta elinkustannuksia. Vain kahta piirrettä kerralla tarkastellessa ei välttämättä havaita kaikkia perinnöllisen vaihtelun evoluutiolle asettamia rajoitteita. Tämä paljastui urosten testosteronipitoisuuden ja molempien sukupuolten humoraalisen immuunivasteen välisestä yhteydestä.

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ORIGINAL PAPERS

I

EVOLUTIONARY DECOUPLING OF A FUNDAMENTAL LIFE-HISTORY TRADE-OFF IN A SMALL MAMMAL

by

Eero Schroderus, Minna Koivula, Esa Koskela, Tapio Mappes and Tuula A. Oksanen 2017

Manuscript

II

CAN NUMBER AND SIZE OF OFFSPRING INCREASE SIMULTANEOUSLY? - A CENTRAL LIFE-HISTORY TRADE-OFF RECONSIDERED

by

Eero Schroderus, Minna Koivula, Esa Koskela, Tapio Mappes, Tuula A. Oksanen and Tanja Poikonen 2012

BMC Evolutionary Biology 12: 44.

Schroderus et al. BMC Evolutionary Biology 2012, 12:44
http://www.biomedcentral.com/1471-2148/12/44

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

Can number and size of offspring increase simultaneously? - a central life-history trade-off reconsidered

Eero Schroderus[1*], Minna Koivula[2], Esa Koskela[3], Tapio Mappes[1], Tuula A Oksanen[1] and Tanja Poikonen[1]

Abstract

Background: To maximize their fitness, parents are assumed to allocate their resources optimally between number and size of offspring. Although this fundamental life-history trade-off has been subject to long standing interest, its genetic basis, especially in wild mammals, still remains unresolved. One important reason for this problem is that a large multigenerational pedigree is required to conduct a reliable analysis of this trade-off.

Results: We used the REML-animal model to estimate genetic parameters for litter size and individual birth size for a common Palearctic small mammal, the bank vole (*Myodes glareolus*). Even though a phenotypic trade-off between offspring number and size was evident, it was not explained by a genetic trade-off, but rather by negative correlations in permanent and temporary environmental effects. In fact, even positive genetic correlations were estimated between direct genetic effects for offspring number and size indicating that genetic variation in these two traits is not necessarily antagonistic in mammals.

Conclusions: Our results have notable implications for the study of the life-history trade-off between offspring number and size in mammals. The estimated genetic correlations suggest that evolution of offspring number and size in polytocous mammals is not constrained by the trade-off caused by antagonistic selection responses *per se*, but rather by the opposing correlative selection responses in direct and maternal genetic effects for birth size.

Keywords: *Myodes glareolus*, Litter size, Birth size, Genetic correlation, Heritability

Background

Fitness is determined by the number of offspring that reproduce successfully. The probability of offspring to reproduce in time can be increased with a larger investment per offspring, which inevitably decreases offspring number [1]. This fundamental life-history trade-off between offspring number and quality (which is most commonly measured as body size) is derived from the allocation of limited parental resources during a single reproductive attempt, such as energy and abdominal space [2]. Moreover, offspring size can be constrained by pelvic size and shape [3,4]. It is crucial to recognize whether this phenotypic trade-off between offspring number and size is due to a negative genetic correlation, since that would constrain the short term evolution of these central life-history traits. In theory, genetic correlations between life-history traits are expected to be negative [5], however, they have frequently been estimated as positive [6].

A negative genetic correlation between offspring number and size has been reported in oviparous vertebrates (fish [7,8], reptiles [9,10] and birds [11]). In contrast to these taxa in which offspring (egg) size is purely a maternal character, in mammals, the determination of the offspring size is more complicated. Offspring birth size in mammals is influenced by both the phenotype of the mother (maternal effects) and genes of the offspring. Maternal effects for birth size cover numerous factors that influence nutrient supply to the foetus (such as uterine capacity and blood flow) and are expected to have a substantial effect on offspring birth size [12,13]. Maternal effects themselves can be heritable or

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environmentally induced; the latter can either influence a single reproductive event or span over several reproductive bouts [14]. Furthermore, maternal genetic effects can increase or decrease the potential of populations to respond to selection on offspring size depending on the correlation between direct and maternal genetic effects [15,16]. A large direct genetic effect (genes in the offspring) on birth size would decrease the maternal flexibility in resource allocation between the number and size of the offspring in mammals. Thus, when estimating genetic parameters it is important to use individual records for birth size traits, together with the modelling of direct and maternal genetic effects [17]. However, this causes the trade-off at the genetic level to be divided into correlations between offspring number and two separate genetic effects (direct and maternal) for birth size, which makes the estimation and interpretation of the results challenging. Given the complexity of this offspring number-offspring size trade-off in mammals, it is not surprising that only a few studies have attempted to determine the genetic basis of the number-size trade-off in mammals [14,18,19].

As a litter bearing small mammal, the bank vole (*Myodes glareolus*) is ideally suited for the study of the offspring size-number trade-off. Bank voles can be bred intensively in the laboratory which allows for the collection of a deep and large pedigree, which is necessary to efficiently study maternal genetic effects [20]. A common garden experiment also avoids complications in the estimation of the genetic parameters created by environmental heterogeneity [21,22]. The use of the animal model ensures that estimated genetic parameters refer to a wild caught base population. The animal model is a flexible method to estimate variance components due to different sources without the need for complicated breeding designs. It utilizes all the information from the pedigree, takes selection into account and, under the infinitesimal model, gives unbiased estimates of the base population [20].

Here we report, to the best of our knowledge, the first estimation of the genetic correlation between offspring number and individual offspring size in a polytocous wild species. Our analysis is based on a large (over 10 000 animals) pedigreed laboratory colony founded by wild-caught bank voles. It shows how direct, maternal genetic and environmental effects contribute to the phenotypic trade-off between offspring number and size at birth in small mammals.

Methods

Study species and data recording

The bank vole is a common mammalian species in the Palearctic region [23]. In central Finland, females produce up to four litters of 1-9 pups during the breeding season, and there is substantial variation in both litter size and offspring size both among females and between litters of the same female [24]. Both the number and the size of offspring at birth are important fitness components. The size of offspring indicates quality, as it correlates positively with survival and breeding success [19,25,26], while litter size is adjusted to environmental conditions and is subject to balancing selection [25,27,28].

A laboratory population was established from wild individuals captured in Konnevesi, central Finland, during the summer of 2000 and subjected to artificial selection toward small and large litter sizes. Selection lines were founded from 150 females and 116 males. Both lines were pooled together in the analysis. All founder males were wild-caught, while some of the "founder" females were laboratory-born offspring of wild-trapped individuals (and thus had known parents). The selection procedure was a combination of between- and within-family selections. Litter size records were collected from generations 1-5 and birth size records were taken from generations 2-6. 1025, 874, 863 and 83 females had 1, 2, 3 and 4 litters respectively.

Animals were housed in standard mouse cages and maintained in a 16 L:8D photoperiod at 20 ± 2°C. Wood shavings and hay were provided as bedding, while food (labfor 36, Lactamin AB, Stockholm, Sweden) and water were available *ad libitum*. Pregnant females were checked once a day for parturition. After parturition, the birth size was measured using an electronic scale (± 0.01 g) and head width with a stereomicroscope.

The use of the animals adhered to ethical guidelines for animal research in Finland (The Finnish Act on Animal Experimentation, 62/2006) as well as the institutional guidelines. The study was conducted under permissions from the National Animal Experiment Board.

Statistical analysis

Statistical significance of fixed factors was initially studied with univariate models using SPSS statistical software (version 18.0). All random effects were excluded except for the residual and 'individual' for the litter size. Fixed effects fitted for the birth mass were the number of parity (four classes) and sex (two classes); for head width, the factors were sex and measurer (ten classes); for litter size, the factors were parity, age (in days) nested as a linear covariate within the parity. Alternatively in the univariate analysis, the size of the birth litter was used as a covariate for birth mass and head width to estimate variance components after the effect of litter size was removed. The effect of inbreeding on the studied traits was found to be statistically non-significant.

The (co)variance components were estimated with the average information Restricted Maximum Likelihood (REML) -procedure using ASReml version 2.0 [29,30].

The following linear models were used:

Birth mass and head width:

$$\mathbf{y_1} = \mathbf{X_1 b_1} + \mathbf{Z_1 a_1} + \mathbf{Mm} + \mathbf{Nn} + \mathbf{Lc} + \mathbf{e_1}$$

Litter size:

$$\mathbf{y_2} = \mathbf{X_2 b_2} + \mathbf{Z_2 a_2} + \mathbf{Qq} + \mathbf{e_2}$$

In which $\mathbf{y_1}$ and $\mathbf{y_2}$ are the vectors of phenotypic observations for birth mass/head width and litter size; $\mathbf{b_1}$ and $\mathbf{b_2}$ are the vectors of fixed effects for birth size and litter size; $\mathbf{a_1}$, $\mathbf{a_2}$ and $\mathbf{m}$ are the vectors of direct additive genetic effects for birth size, litter size and maternal additive genetic effects for birth size; $\mathbf{n}$ and $\mathbf{q}$ are the vectors of maternal permanent effects for birth size (non-genetically determined effects that the mother has on all her offspring in all litters) and permanent individual effects for litter size (non-genetic effect on sizes of all litters of one female); $\mathbf{c}$ and $\mathbf{k}$ are the vectors of litter effects for birth size (environmental effect common for all the offspring in one litter) and temporary environmental effect for litter size (described later); finally, $\mathbf{e_1}$ and $\mathbf{e_2}$ are the vectors of residuals for birth size and litter size respectively. Fixed and random effects are fitted to individual records by incidence matrices $\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, $\mathbf{M}$, $\mathbf{N}$, $\mathbf{Q}$, $\mathbf{L}$ and $\mathbf{K}$.

$\mathrm{E}\begin{bmatrix}\mathbf{y_1}\\ \mathbf{y_2}\end{bmatrix} = \begin{bmatrix}\mathbf{X_1\,b_1}\\ \mathbf{X_2\,b_2}\end{bmatrix}$ and the expectations of random effects are zero.

Variances and covariances:

$$\mathrm{Var}\begin{bmatrix}\mathbf{a_1}\\ \mathbf{a_2}\\ \mathbf{m}\\ \mathbf{n}\\ \mathbf{q}\\ \mathbf{c}\\ \mathbf{k}\\ \mathbf{e_1}\\ \mathbf{e_2}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma^2_{a1} & \mathbf{A}\sigma_{a2} & \mathbf{A}\sigma_{a1m} & 0 & 0 & 0 & 0 & 0 & 0\\ \mathbf{A}\sigma_{a1a2} & \mathbf{A}\sigma^2_{a2} & \mathbf{A}\sigma_{a2m} & 0 & 0 & 0 & 0 & 0 & 0\\ \mathbf{A}\sigma_{a1m} & \mathbf{A}\sigma_{a2m} & \mathbf{A}\sigma^2_{m} & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & \mathbf{I}\sigma^2_{n} & \mathbf{I}\sigma_{nq} & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & \mathbf{I}\sigma_{nq} & \mathbf{I}\sigma^2_{q} & 0 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{c} & \mathbf{I}\sigma_{ck} & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma_{ck} & \mathbf{I}\sigma^2_{k} & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e1} & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \mathbf{I}\sigma^2_{e2}\end{bmatrix}$$

In which $\mathbf{A}$ and $\mathbf{I}$ are the numerator relationship matrix and identity matrix respectively. σ^2_{a1}, σ^2_{a2} and σ^2_{m} are the direct additive genetic variance for birth size and litter size and maternal additive genetic variance for birth size; σ^2_{n} and σ^2_{q} are the permanent environmental maternal variance for birth size and permanent individual variance for litter size; σ^2_{c} and σ^2_{k} are the common litter variance for birth size and temporary environmental variance for litter size; σ^2_{e1} and σ^2_{e2} are the residual variances for the birth size trait and litter size. σ_{a1a2}, σ_{a1m} and σ_{a2m} are the additive genetic covariances between corresponding genetic effects; σ_{nq} is the covariance between permanent maternal effects for birth size and permanent individual effects for litter size; σ_{ck} is the covariance between common litter effects for birth size and the temporary environmental effect for litter size. In the univariate models, direct-maternal genetic covariance for birth size traits was set to zero because models including covariance did not converge. In the bivariate analysis, the residual covariance was set to zero because the dataset was composed of two separate parts: one containing records for litter size and the other for birth mass and head width. However, due to the functional relationship between litter size and offspring size, residual variation in the former is presumably correlated with the common litter environment of the latter. For that reason, actual residual variance of litter size was fixed to 0.01, and a dummy 'temporary environmental effect' was fitted for litter size in the bivariate models which was then allowed to correlate with the fitted litter effect for offspring. Without this temporary environmental effect for litter size, the correlation between permanent environmental effects tended to converge outside the parameter space.

Estimates of the ratios of variance components to the total phenotypic variance were calculated as: (heritability) h^2 = Va/Vp; (maternal heritability) m^2 = Vm/Vp; (maternal permanent environmental effect) n^2 = Vn/Vp; (common litter effect) c^2 = Vc/Vp; (permanent individual effect) q^2 = Vq/Vp, in which the total phenotypic variance (Vp) was determined as a sum of the appropriate (co)variance components.

Statistical significance of the genetic and environmental covariances was assessed with Log Likelihood ratio tests by comparing a full model with a model in which tested covariance was constrained to zero.

Results

There was large variation in both offspring number and offspring size (body mass and head width) at birth. The coefficient of variation was largest in the litter size and lowest in the head width at birth (Table 1). The average litter size and offspring birth mass of the study population were close to values we have earlier observed in nature (5.3 pups, 1.76 g) [24]. Phenotypic correlations between litter size and mean offspring birth size were

Table 1 Number of observations (n), trait means (± SD), coefficients of variation (CV) and range

Trait	n	Mean	CV	Range
Birth mass (g)	10986	1.89 ± 0.22	0.11	0.84-3.28
Head width (mm)	10971	8.22 ± 0.38	0.05	5.78-10.56
Litter size	2665	4.48 ± 1.53	0.34	1-9

moderately negative (birth mass r = -0.46, $P < 0.001$; birth head width r = -0.35, $P < 0.001$) and similar to estimates from a natural population (-0.41 and -0.38 respectively) [19].

Estimates of direct heritability (h^2) from the univariate models were low: 0.10 ± 0.03 for litter size, 0.08 ± 0.03 for birth mass and 0.07 ± 0.03 for birth head width (Tables 2, 3 and 4 respectively). The permanent individual effect (q^2) for litter size was 0.14 ± 0.03. The litter effect (c^2) explained approximately half of the phenotypic variance in the birth size. Maternal heritabilities (m^2) were 0.09 ± 0.03 for birth mass and 0.03 ± 0.02 for birth head width. Permanent maternal effects (n^2) were 0.08 ± 0.03 for birth mass and 0.06 ± 0.02 for birth head width, while litter effects were 0.45 ± 0.02 and 0.49 ± 0.02, respectively. Adjusting the birth mass and head width for size of the birth litter decreased total phenotypic variance by 19% in the birth mass and 13% in the birth head width. The variance due to a common litter and maternal permanent environment decreased with the adjustment, whereas direct genetic variance increased. When birth mass and head width were analyzed in bivariate models with litter size, there were only minor differences in variance components compared to those derived from univariate models.

Direct genetic correlations between litter size and birth size traits were positive (birth mass 0.54 ± 0.23; birth head width 0.47 ± 0.26), whereas the correlations between direct genetic effects for litter size and maternal genetic effects for birth size were negative (birth mass -0.30 ± 0.23; birth head width -0.47 ± 0.38) (Table 5). The latter were statistically non-significant. Correlations between direct and maternal genetic effects in both the birth mass and head width were weakly positive (0.04 ± 0.32 and 0.34 ± 0.76) but statistically non-significant.

In both birth size traits, the temporary environmental correlations between litter size and birth size traits were significantly negative (Table 5). The correlation between permanent individual and maternal effects was -0.35 ± 0.17 between litter size and birth mass and -0.44 ± 0.17 between litter size and birth head width. The correlations between the temporary environmental effect for litter size and litter effects for birth size were -0.67 ± 0.02 and -0.52 ± 0.02 respectively.

Table 2 Estimated variance components, heritabilities (h^2) and permanent animal effects (q^2) for litter size (Standard error)

	Univariate	Bivariate	
		With birth mass	With birth head width
Vp	2.011 (0.059)	2.013 (0.059)	2.011 (0.059)
Va	0.193 (0.055)	0.194 (0.054)	0.194 (0.054)
Vq	0.279 (0.062)	0.274 (0.061)	0.270 (0.061)
h^2	0.10 (0.03)	0.10 (0.03)	0.10 (0.03)
q^2	0.14 (0.03)	0.14 (0.03)	0.13 (0.03)

Vp = phenotypic variance; Va = additive genetic variance; Vq = permanent individual effect variance

Table 3 Estimated variance components, heritabilities (h^2), common litter effects (c^2), maternal heritabilities (m^2) and permanent maternal effects (n^2) for birth mass (Standard error)

	Univariate		Bivariate
	Not adjusted for litter size	Adjusted for litter size	(with litter size)
Vp	0.054 (0.001)	0.044 (0.001)	0.055 (0.001)
Va	0.004 (0.002)	0.005 (0.002)	0.005 (0.002)
Vm	0.005 (0.002)	0.005 (0.001)	0.004 (0.002)
Vc	0.024 (0.001)	0.014 (0.001)	0.025 (0.001)
Vn	0.004 (0.001)	0.004 (0.001)	0.004 (0.001)
h^2	0.08 (0.03)	0.11 (0.03)	0.09 (0.03)
c^2	0.45 (0.02)	0.32 (0.02)	0.44 (0.02)
m^2	0.09 (0.03)	0.11 (0.03)	0.08 (0.03)
n^2	0.08 (0.03)	0.08 (0.03)	0.08 (0.03)

Vp = phenotypic variance; Va = additive genetic variance; Vc = common litter variance; Vm = maternal genetic variance; Vn = maternal permanent environmental variance

Discussion

In this study, we quantified the genetic basis of a fundamental life-history trade-off between offspring number and size. This is essential, as understanding the relative influence of genetic versus environmental causes behind this important phenotypic trade-off is necessary for predicting the strength and direction of evolution. By using a sufficiently deep pedigree, we found that the

Table 4 Estimated variance components, heritabilities (h^2), common litter effects (c^2), maternal heritabilities (m^2) and permanent maternal effects (n^2) for head width at birth (Standard error).

	Univariate		Bivariate
	Not adjusted for litter size	Adjusted for litter size	(with litter size)
Vp	0.134 (0.003)	0.117 (0.003)	0.137 (0.003)
Va	0.010 (0.004)	0.013 (0.004)	0.010 (0.005)
Vm	0.005 (0.003)	0.005 (0.003)	0.003 (0.004)
Vc	0.066 (0.003)	0.050 (0.002)	0.068 (0.003)
Vn	0.008 (0.003)	0.005 (0.003)	0.009 (0.003)
h^2	0.07 (0.03)	0.11 (0.03)	0.07 (0.03)
c^2	0.49 (0.02)	0.43 (0.02)	0.49 (0.02)
m^2	0.03 (0.02)	0.04 (0.02)	0.02 (0.03)
n^2	0.06 (0.02)	0.04 (0.02)	0.06 (0.02)

Vp = phenotypic variance; Va = additive genetic variance; Vc = common litter variance; Vm = maternal genetic variance; Vn = maternal permanent environmental variance

Table 5 Genetic and environmental correlations between litter size and birth size traits (standard error)

	Birth mass	χ^2	Birth head width	χ^2
Genetic correlations				
LS direct - BS direct	0.54 (0.23)*	5.00	0.47 (0.26)*	3.34
LS direct - BS maternal	-0.30 (0.23)	1.20	-0.47 (0.38)	0.30
BS direct - BS maternal	0.04 (0.32)	0.00	0.34 (0.76)	1.26
Environmental correlations				
Permanent environmental	-0.35 (0.17)*	3.00	-0.44 (0.17)*	4.32
LS residual - BS litter	-0.67 (0.02)***	635.40	-0.52 (0.02)***	345.22

LS litter size; BS birth size (mass or head width)

Statistical significance: * $P < 0.1$; ** $P < 0.01$;*** $P < 0.001$

χ^2 Chi square test statistic for loglikelihood-ratio test of the covariance

phenotypic trade-off between offspring number and size in the bank vole was due to environmental effects rather than additive genetic effects. Our results emphasize that despite being functionally bound to a phenotypic trade-off, the common genetic basis of litter size and birth size is comprised of antagonistic as well as parallel genetic variation.

Heritability of litter size

The heritability of litter size was low, but similar to estimates of h^2 in other polytocous mammals [31-35]. This is expected since litter size is a composite trait, with ovulation rate setting the maximum value, while the number of offspring is further influenced by fertilization, implantation and embryonic mortality. Irrespective of the underlying genetic component of litter size, these effects add environmental variation to the total phenotypic variation in litter size. Indeed, the heritability of the ovulation rate is greater than the heritability of litter size in several vertebrates (swine [36] and mice [37]). Previous estimates of litter size heritability in bank voles were substantially overestimated [19], most likely because of the smaller sample size and methods used (i. e. mother-daughter regression). Heritability estimates based on mother-daughter regression can be four times larger than animal model estimates of h^2 [33].

Sources of variation in offspring size at birth

Direct genetic effects that describe the genetic potential for a foetus to grow and absorb nutrients through the placenta explained 7-8% of the phenotypic variation in both birth size traits. These values are comparable to estimates of h^2 reported for birth size in other mammals [14,17,38]. Birth size is expected to be largely determined by maternal effects, and, including a litter effect, the overall combination of maternal effects accounted for 58-62% of phenotypic variation. However, only a small proportion of maternal effects was explained by additive genetic effects, since the maternal heritability was only 9% in birth mass and 3% in birth head width. This is a bit surprising as selection is less efficient on maternal genetic effects compared to direct genetic effects [39]. Adjusting birth size for natal litter size reduced the variance explained by the litter effect and permanent maternal effect. This result was expected since the low heritability of litter size indicates that the variation in litter size was mainly due to environmental factors; removing the effect of litter size on birth size should therefore decrease the amount of environmental variation in birth size. Moreover, when adjusted for natal litter size, more variation was removed from birth mass than from birth head width. This demonstrates the more substantial trade-off between litter size and birth mass as was observed already from the phenotypic correlations.

Genetic basis of resource allocation between offspring number and size

Our estimation of co-variation between different genetic and environmental effects showed only weak support for a genetic trade-off between litter size and offspring size. The genetic correlation between litter size and direct genetic effects for birth size was positive, which indicates that the genes that increase female litter size tend to also enhance that individual's size at birth. Previous reports of a negative genetic correlation between litter size and mean offspring birth size in a bank vole [19] do not disagree with the present results (see Table 5). The previous study estimated the correlation only between the litter size and maternal genetic effects for birth size (here proven to be negative) and ignored the positive correlation between litter size and direct genetic effects for birth size.

Other studies have reported both negative [14] and positive [38] estimates for the maternal environmental correlation between litter size and birth size. A positive environmental correlation could arise if the environment affects the traits through resource acquisition [40]. For example, in the case of offspring number and size, abundant nutrition that causes ovulation of extra eggs allows mothers to support the growth of large foetuses. Conversely, a negative environmental correlation is expected if the environmental source of variation in litter size does not affect total maternal reproductive resources. The latter case is likely to happen in the bank vole, in which permanent and temporary environmental correlations between litter size and offspring size were strongly negative. The bank vole has an extremely variable litter size [19,24], and large environmental variation in the litter size demonstrated by a low heritability (this study).

As a small mammal, the bank vole is an income breeder whose capacity to support growth of the foetuses during late pregnancy is not likely to be connected with environmental variation affecting offspring number, which is already determined early in pregnancy.

Selection for offspring number and size

In general, selection on offspring size at birth and litter size acts antagonistically on the mother and offspring [19,25,41-43]. This combined with a presumed negative genetic correlation between offspring number and size is thought to constrain the evolution of these traits [44]. Our results indicate that genetic variation in offspring viability and offspring number are not necessarily antagonistic in mammals. A positive genetic correlation between direct genetic effects for litter size and offspring birth size can reduce parent-offspring conflict in offspring size as the same genes increase fitness at both levels. Also, an effectively null correlation between direct and maternal genetic effects for birth size implies a low level of parent-offspring conflict in the bank vole. Genetic correlations in natural populations of bank voles should be stronger than those estimated here, since genetic correlations are typically weaker in good environments such as laboratory conditions [22,45].

These data were obtained using a population that has been subject to short-term, two-way selection for litter size. Under an infinitesimal model and with complete pedigree information, the animal model takes selection into account when the selective events are included in the data set [20]. The infinitesimal model, whereby quantitative genetic variation is explained with a large number of unlinked genes of small effect, is not a realistic assumption but it does work reasonably well for short-term selection experiments [46]. In our selection experiment, litter size in the two lines (towards small and large) has diverged. However, divergence in offspring size has not been so straightforward, thus demonstrating the complex nature of this important life-history trade-off. (Schroderus, Koivula, Koskela, Mappes, Oksanen and Poikonen; Unpublished data).

Conclusions

We have shown that the phenotypic trade-off observed between offspring number and size in a polytocous small mammal was due to environmental effects rather than additive genetic effects. Our results indicate that genetic variation in offspring number and size are not necessarily antagonistic in mammals. This finding is in line with the many positive estimates of genetic correlations reported between life-history traits [6]. Our results emphasize the complex nature of offspring number - size trade-off in mammals in terms of both environmental and genetic variation. The structure of the additive genetic covariance matrix suggests that evolution of offspring number and size in polytocous mammals may not be constrained by the trade-off *per se* caused by antagonistic selection responses, but rather by the opposing correlative selection responses in direct and maternal genetic effects for birth size.

Acknowledgements

We thank Matti Ojala for comments on the statistical models and the results and two anonymous reviewers for comments on the earlier version of the manuscript. Phill Watts and Mikael Mokkonen commented and checked the English of this manuscript. We thank the Academy of Finland for financial support (grant no. 132190 to T.M.; 218107, 119200, 115961 to E.K.) and the Centre of Excellence in Evolutionary Research of the Academy of Finland.

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Authors' contributions

ES participated in the collection of the data, performed statistical analysis and wrote the manuscript. All other authors participated in the collection of the data, and contributed and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 23 March 2011 Accepted: 31 March 2012
Published: 31 March 2012

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doi:10.1186/1471-2148-12-44
Cite this article as: Schroderus *et al.*: Can number and size of offspring increase simultaneously? - a central life-history trade-off reconsidered. *BMC Evolutionary Biology* 2012 **12**:44.

III

QUANTITATIVE GENETICS AND FITNESS EFFECTS OF BASAL METABOLISM

by

Zbyszek Boratyński, Esa Koskela, Tapio Mappes and Eero Schroderus 2013

Evolutionary Ecology 27: 301–314.

IV

CONSTRAINED EVOLUTION OF GROWTH TRAJECTORIES IN A WILD SMALL MAMMAL

by

Eero Schroderus, Minna Koivula, Esa Koskela, Tapio Mappes and Tuula A. Oksanen 2017

Submitted manuscript

V

INTRA- AND INTERSEXUAL TRADE-OFFS BETWEEN TESTOSTERONE AND IMMUNE SYSTEM: IMPLICATIONS FOR SEXUAL AND SEXUALLY ANTAGONISTIC SELECTION

by

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Suzanne C. Mills, Tuula A. Oksanen and Tanja Poikonen 2010

American Naturalist 176: E90–97.

vol. 176, no. 4 the american naturalist october 2010

E-Article

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

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Submitted June 12, 2009; Accepted May 3, 2010; Electronically published August 16, 2010

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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Am. Nat. 2010. Vol. 176, pp. E90–E97.
DOI: 10.1086/656264

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

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

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.

Acknowledgments

We thank three anonymous reviewers for comments, I. Stranden for comments on the earlier version of this manuscript, and the Experimental Animal Unit of University of Jyväskylä for providing the facilities. The Academy of Finland (grants 132190 to T.M.; 100143, 78777, and 103148 to E.K.; 104568 and 108955 to T.A.O.; and 103508 and 108566 to S.C.M.) and the Centre of Excellence in Evolutionary Research of the Academy of Finland financially supported this study.

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