

Tanja Poikonen

Frequency-Dependent Selection  
and Environmental Heterogeneity  
as Selective Mechanisms in  
Wild Populations



Tanja Poikonen

Frequency-Dependent Selection  
and Environmental Heterogeneity  
as Selective Mechanisms in Wild Populations

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella  
julkisesti tarkastettavaksi yliopiston Konneveden tutkimusasemalla  
joulukuun 10. päivänä 2010 kello 12.

Academic dissertation to be publicly discussed, by permission of  
the Faculty of Mathematics and Science of the University of Jyväskylä,  
in Konnevesi Research Station, on December 10, 2010 at 12 o'clock noon.



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Frequency-Dependent Selection  
and Environmental Heterogeneity  
as Selective Mechanisms in Wild Populations

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 215

Tanja Poikonen

Frequency-Dependent Selection  
and Environmental Heterogeneity  
as Selective Mechanisms in Wild Populations



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Editor

Jari Haimi

Department of Biological and Environmental Science, University of Jyväskylä

Pekka Olsbo, Sini Rainivaara

Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

Editorial Board

Jari Haimi, Anssi Lensu, Timo Marjomäki, Varpu Marjomäki

Department of Biological and Environmental Science, University of Jyväskylä

URN:ISBN:978-951-39-4138-3

ISBN 978-951-39-4138-3 (PDF)

ISBN 978-951-39-4108-6 (nid.)

ISSN 1456-9701

Copyright © 2010, by University of Jyväskylä

Jyväskylä University Printing House, Jyväskylä 2010

## ABSTRACT

Poikonen, Tanja

Frequency-dependent selection and environmental heterogeneity as selective mechanisms in wild populations

Jyväskylä: University of Jyväskylä, 2010, 44 p.

(Jyväskylä Studies in Biological and Environmental Science

ISSN 1456-9701; 215)

ISBN 978-951-39-4138-3 (PDF), 978-951-39-4108-6 (nid.)

Yhteenveto: Frekvenssistä riippuva valinta ja ympäristön heterogeenisyys luonnonvalintaa ohjaavina tekijöinä luonnonpopulaatioissa

Diss.

A central question in evolutionary biology is the maintenance of genetic variation, the material for natural selection. Several mechanisms have been proposed to contribute to the maintenance of variation. In this thesis, I study the mechanisms maintaining genetic variation in two traits: infanticidal behaviour and immune function. My model species is the bank vole (*Myodes glareolus*), which is the most common small rodent in Finland. I employed quantitative genetic methods to assess the proportion of heritable genetic variation in the studied traits and found that both of these traits show a marked additive genetic basis. Next, I employed experimental field and laboratory studies to determine the fitness effects on survival and reproductive success of manipulating the frequencies of the different genotypes, pathogenic environment or level of food resources, by using individuals that differed genetically in infanticidal behaviour or immune function. These studies provide evidence of two main mechanisms maintaining genetic variation in the studied behavioural and physiological traits: environmental heterogeneity and negative frequency-dependent selection. The results shed new light on the genetic basis of these important traits as well as the factors maintaining variation in natural populations.

Keywords: Frequency-dependent selection; immunocompetence; infanticidal behaviour; *Myodes glareolus*; quantitative genetics; reproductive effort.

*T. Poikonen, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland*

**Author's address** Tanja Poikonen  
Centre of Excellence in Evolutionary Research  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland  
tanja.poikonen@jyu.fi

**Supervisors** Professor Tapio Mappes  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland

Docent Esa Koskela  
Academy Research Fellow  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland

Docent Tuula Oksanen  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland

**Reviewers** Professor Ulrika Candolin  
Dept. of Biosciences  
P.O. Box 65  
FIN-00014  
University of Helsinki  
Finland

Docent Jan Lindström  
College of Medical, Veterinary and Life Sciences  
Graham Kerr building  
University of Glasgow  
Glasgow G12 8QQ  
United Kingdom

**Opponent** Professor Harri Hakkarainen  
Department of Biology  
Section of Ecology  
FI- 20014 University of Turku  
Finland

## CONTENTS

### LIST OF ORIGINAL PUBLICATIONS

1	INTRODUCTION .....	7
1.1	Maintenance of genetic variation .....	7
1.2	Infanticidal behaviour .....	8
1.3	Immunity .....	10
1.4	Aim of the study .....	11
2	METHODS .....	13
2.1	Study species .....	13
2.1.1	Bank vole as a model species in studies of infanticide (I-III) ....	14
2.1.2	Bank vole as a model species in studies of immunocompetence (IV-VI) .....	15
2.2	Laboratory procedures .....	15
2.2.1	Mating success trials (IV) .....	16
2.2.2	Testing individual infanticidal status (I-III) .....	16
2.2.3	Measuring immunity (IV-VI) .....	16
2.2.4	Selection on immune response (IV, V) .....	17
2.3	Estimating genetic parameters of infanticide (II, III) and immunity (IV-VI) .....	17
2.3.1	Parent-offspring regression (IV, V) .....	18
2.3.2	Threshold model with parent-offspring regression (III) .....	18
2.3.3	Restricted maximum likelihood based animal model (II, VI) .....	18
2.4	Field studies .....	19
3	RESULTS AND DISCUSSION .....	21
3.1	Effects of infanticidal behaviour on reproductive success and resource competition .....	21
3.1.1	Infanticidal behaviour and synchronous breeding (I) .....	21
3.1.2	Infanticide's heritability and genetic correlation with reproductive effort (II) .....	22
3.1.3	Competitive abilities of individuals with opposite infanticide tactics (III) .....	22
3.2	Effects of immunocompetence on reproductive success and survival .....	23
3.2.1	Immunocompetence and male fitness (IV) .....	23
3.2.2	Immunocompetence and female fitness (V) .....	23
3.2.3	Genetic basis of immune system in the bank vole (VI) .....	24
4	CONCLUSIONS .....	26
	<i>Acknowledgements</i> .....	30
	YHTEENVETO .....	32
	REFERENCES .....	36



## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-VI. I am the main writer of papers I, II and V, and I carried out a large part of planning, data collection and writing of each paper.

- I Poikonen, T., Koskela, E., Mappes, T. & Mills, S. C 2008. Infanticide in the evolution of reproductive synchrony: effects on reproductive success. *Evolution* 62: 612-621.
- II Poikonen, T., Mappes, T., & Schroderus, E. Is infanticidal behaviour related to reproductive effort? - A quantitative genetic analysis in a small mammal. Manuscript.
- III Mappes, T., Aspi, J., Koskela, E., Mills, S.C., Poikonen, T., & Tuomi, J. Origin and maintenance of infanticide - experimental evidence for negative frequency-dependent selection. Manuscript.
- IV Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes T. & Poikonen, T. 2010. Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution* 64: 166-179.
- V Poikonen, T., Jokinen, I., Koskela, E., Mappes, T., & Mills, S.C. Frequency-dependent selection shaping optimal immune response in female mammals. Manuscript.
- VI 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. *Am. Nat.* 176(4): E90-E97.

# 1 INTRODUCTION

## 1.1 Maintenance of genetic variation

The existence of genetic variation raises a fundamental question in ecology and evolutionary biology: why do populations not consist of only the most fit genotype? Given that natural selection and genetic drift should reduce the amount of genetic variation in a population (Fisher 1930), explaining the actual genotypic variation has been a challenge for evolutionary biologists. There are numerous possible mechanisms that may account for the maintenance of variation, although it is unknown which mechanisms are prominent in nature (Sheenan & Tibbetts 2009). The explanations according to Roff (1992) are mutation–selection balance, heterosis, environmental variability, antagonistic pleiotropy and negative frequency-dependent selection.

In **mutation-selection balance**, mutations are assumed to compensate the erosion of variation caused by selection (Lande 1975). Empirical analyses (Lynch et al. 1998, Houle 1998) support the hypothesis that much of the standing genetic variation in life history traits is due to mutational input (Roff & Fairbairn 2007). **Heterosis** refers to the idea that heterozygotes have a higher fitness than either of the homozygotes, ensuring stable polymorphisms (Ewens & Thompson 1970).

Both spatial and/or temporal **environmental variation** could maintain genetic variation (Levene 1953, Hedrick et al. 1976, Hedrick 1986) (I–VI). Since presently the environment is changing at an accelerating rate due to human activities, there is an increasing need to determine the effects of variable environmental conditions on the adaptations of populations (Candolin & Heuschele 2008). The finding of genotype–environment (G×E) interactions in many, if not most studies in which it has been examined argues in favor of environmental heterogeneity playing a role in the maintenance of additive genetic variation. Different traits respond differentially to environmental variation, so the evolutionary consequences of environmental variation are trait-specific. For environmentally sensitive traits, environmental heterogeneity

increases phenotypic, but not genetic variance, thereby actually reducing the trait's heritability (Mazer & Damuth 2001). It is also evident that the rank of different genotypes varies with the environment (Pigliucci 2001). If there are different optimal phenotypes across environments, this means that selection may favor some genotypes in some environments and other genotypes in other environments. This may explain the maintenance of genetic variation in natural populations experiencing heterogeneous environments (Via & Lande 1985).

In **antagonistic pleiotropy** (IV, VI) a gene has a positive effect on one component of fitness but a negative effect on another; thus, alleles in a particular locus might, for instance, increase reproductive success but decrease survival. Antagonistic pleiotropy is important in life history theory because it is the genetic basis of trade-offs; negative genetic correlations are putative evidence of antagonistic pleiotropy (Roff 1992). In the wild, selection generally acts on suites of traits or whole organisms, not on individual traits in isolation (Dobzhansky 1956, Lewontin 1970).

Frequency dependent selection refers to a situation where the fitness of a given genotype varies as a function of the genotypic composition of the population (Ayala & Campbell 1974, Gromko 1977). Specifically, **negative frequency dependent selection** (III, V) occurs when rare phenotypes are favored by selection. In theory, stable polymorphisms can readily be obtained with this type of selection. Frequency-dependent selection may be important in maintaining phenotypic variation, such as alternative reproductive behaviours (Roff 1992). Although frequency-dependent selection has the potential to be a powerful evolutionary mechanism for the promotion and maintenance of polymorphisms, relatively few studies have documented frequency-dependent selection in natural populations (Mappes et al. 2008, Sheenan & Tibbetts 2009).

The question, still unanswered, is how frequently each of the mechanisms described above operates in the natural world - while we have an abundance of theory, we still have a scarcity of relevant data to test it (Roff 1992). In this thesis, I study variation and its maintenance in two traits: infanticidal behaviour and immunity.

## 1.2 Infanticidal behaviour

Infanticidal behaviour, the killing and harassing of conspecific young, is an intrinsic feature present extensively in mammalian populations (in humans see e.g. Pitt & Bale 1995, in other animals Hrdy 1979, Hausfater & Hrdy 1984, Parmigiani & vom Saal 1994, Ebensperger 1998, van Schaik & Janson 2000). In many mammalian populations, infanticide is considered to be the main or a significant cause of juvenile mortality (Boonstra 1978, Sherman 1981, Packer & Pusey 1983, Caley & Boutin 1985, Hoogland 1985, Borries 1997, Lambin & Yoccoz 1998), thereby providing an excellent model trait to investigate factors that maintain ecologically relevant variation.

Hrdy (1979) originally proposed five hypotheses to explain infanticidal behaviour: parental manipulation, sexual selection, exploitation, resource competition and social pathology. **The parental manipulation hypothesis** proposes that infanticide takes place as a form of parent – offspring conflict, where the parent(s) (actively or passively) reduces the number of dependent young in order to either produce fewer offspring of better quality, or to allocate resources to their own maintenance and future reproduction. **The sexual selection hypothesis** posits that the limited sex (i.e. the sex whose reproductive success is limited by access to the opposite sex) will benefit from killing the dependent young of the limiting sex, thus gaining the access to mate with the caregiver of the killed offspring (Hrdy 1979). While my thesis focuses on female non-parental infanticide, the parental manipulation hypothesis deals with infanticide by the biological parents and the sexual selection hypothesis is usually associated with infanticide by males. The possible costs and benefits of non-parental infanticide by females are presented in Table 1. Benefits 1 and 2 (a, b) underlie Hrdy’s (1979) hypotheses on the adaptive value of female non-parental infanticide, presented below.

TABLE 1 Possible costs and benefits of committing non-parental female infanticide.

Costs	Benefits
1. Time and energy spent on searching for young to kill	1. Direct nutritional benefits
2. Increased risk of own predation during searching	2. Less non-kin pups
3. Increased mortality risk of own offspring (due to predation or infanticide by other adults)	a) Reduced competition for the killer itself (both in the present and in the future)
a) Pups may be left alone when the perpetrator commits infanticide elsewhere	b) Reduced competition for the offspring of the killer (both in the present and in the future)
b) The female victim may stay in the neighborhood and become infanticidal (“revenge”)	c) Greater contribution to next generation (direct fitness benefit)
4. Risk of killing own or kin offspring (inclusive fitness cost)	3. Decreased risk of infanticide towards own pups (the female victim may leave the site)

**The exploitation hypothesis** suggests that infanticidal females benefit from the “use” of the victim itself (Hrdy 1979). This refers to cannibalism where the perpetrator benefits in terms of the nutritional value of the infant as a food item.

**The resource competition hypothesis** (II, III) addresses a factor closely linked to female reproductive success, i.e. access to resources that will increase the survivorship of young (e.g. Darwin 1871, Trivers 1972, Hrdy 1979, Wrangham 1980). The hypothesis predicts that by killing the young of other females, the infanticidal female and her young will have fewer competitors for limited resources, both in the present and future (Hrdy 1979, Sherman 1981,

Hoogland 1995). This hypothesis has not been previously evaluated empirically in rodents with uniparental care.

Considering the amount of the specific knowledge on the conditions for infanticide and the prevailingly accepted view of the adaptability of the behaviour (but see Bartlett et al. 1993, Sussman et al. 1995, Dagg 1998, 1999, 2000), it is surprising that before the current studies (II, III), no evidence on the evolutionary potential or heritability of infanticide has existed. While infanticide as a behavioural event is taking place “in hundreds and probably many thousands of species” (Dagg 2000), its adaptability or evolutionary background has raised a raging debate whether it is adaptive (Hrdy et al. 1995, Silk & Stanford 1999, Packer 2000) or non-adaptive (Bartlett et al. 1993, Sussman et al. 1995, Dagg 1998, 1999, 2000). In her fifth hypothesis, **the social pathology hypothesis**, Hrdy (1979) considered the possibility of infanticide as a non-adaptive behaviour, arising from stressful conditions due to e.g. high population densities. However, infanticide could also be adaptive under conditions of high density where male tenure is short (Poole & Morgan 1973, Huck 1984) or where resources are limited (Ebensperger & Blumstein 2007). In general, the resources that an individual possesses play an important role in fitness (I), and the population density often has a strong influence on life-history traits in plants and animals because it influences per capita resource availability (Mazer & Schich 1991, Mazer & Wolfe 1998, Mazer & Damuth 2001). The optimal allocation to reproduction depends on whether the current environmental state is favourable or unfavourable (reviewed in Lindström 1999), and individuals with limited resources might face trade-offs between different life-history traits, e.g. between reproduction and survival (Noorwijk & de Jong 1986) (IV).

### 1.3 Immunity

While the above concept of resource availability and competition is an obvious example of a situation where fitness is influenced by local intraspecific interactions, the infection rate (IV, V) and host-parasite relationships also deal with interactions between different individuals or genotypes. The relationship between genotype and fitness can be highly sensitive to the identity of the other genotypes with which an individual interacts (Nunney 2001, Wilson 2001), even more so when a pathogenic or infectious environment is concerned. Immunocompetence, an animal’s ability to produce an effective immune response following exposure to an antigen, is a central character affecting the fitness of an individual. The immune system plays a pivotal role in defending an animal against attacks by pathogens (Roitt et al. 1988, Wakelin 1996). It is expensive to activate the immunological defenses to minimize risks of parasitic infections (Martin et al. 2007, Keymer & Read 1991, Sheldon & Verhulst 1996), due to allocation decisions with other nutrient-demanding processes, such as reproduction, growth, and thermoregulation (Lochmiller & Deerenberg 2000).

The complexity of interactions between hosts and their parasites is overwhelming, but there are some shared, general aspects of these interactions that are of particular interest to evolutionary ecologists (Lively 2001). First, parasites may attack in a frequency-dependent way. In other words, the probability of infection for a particular host genotype is expected to be, at least in part, a function of the frequency of that host genotype. This asserts the study of immunity-related traits to assess negative frequency dependent selection as a mechanism maintaining genetic variation.

Second, just as population density and resource competition seem to be related with infanticidal behaviour, they may also be related with the immune response of individuals. Parasites may affect the population density of their hosts, and host density may feedback to affect the numerical dynamics of the parasite (Lively 2001). However, we do not know the effects of infection on the competitive ability of many species (Lively 2001) (IV). In animals, the apparent increase in stress that accompanies high population densities (Goulson & Cory 1995) may promote the release of glucocorticoids, such as the stress hormone corticosterone, which has detrimental effects on an individual's immune response (e.g. Evans et al. 2000).

In vertebrate males, androgens, especially testosterone, can have immunosuppressive effects and are understood to explain the weaker male immune system (Dorner et al. 1980, Greives et al. 2006). On the other hand, androgens affect mating success through the development of sexual signals (Zeller 1971, Fernald 1976, Owen-Ashley et al. 2004, Mank 2007, Mills et al. 2007a). 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 & Karter 1992, Ketterson & Nolan 1999). This is expected to happen in particular in species with a low annual survival rate (Hau 2007), such as our model species, the bank vole *Myodes glareolus*. Yet, data on heritable variation in parasite resistance of wild populations are needed (Read 1988)(VI).

## 1.4 Aim of the study

The aim of this work is to study the mechanisms that maintain genetic variation in different traits. In this thesis, I have concentrated on the fitness effects (I, II) and the genetic basis (II, III) of female infanticidal behaviour, and on fitness effects (IV, V) and the genetic basis (VI) of male and female immunity. So far, measurements of selection have been more abundant on morphological traits than on behavioural, physiological and life history traits (McGlothlin & Ketterson 2008). Infanticidal behaviour is a good example of a behavioural trait that probably has a major effect on individual fitness (Hrady 1979, Hausfater & Hrady 1984, Parmigiani & vom Saal 1994, van Schaik & Janson 2000, Ebensperger & Blumstein 2007). This trait also appears dichotomously, an individual being either infanticidal or non-infanticidal, and the two tactics may

provide different advantages in different environments. Immunity, on the other hand, represents a physiological trait with considerable fitness-related effects (e.g. Oksanen et al. 2003, Møller & Saino 2004). It is also a central trait, containing a lot of variation, that affects life-history evolution. The optimal level of immunity may vary individually, according to different environments and different social contexts (Viney et al. 2005). Furthermore, since immunity is known to trade-off with testosterone level (e.g. Folstad & Karter 1992, Ketterson & Nolan 1999, Reed et al. 2006, Mills et al. 2009), a genetic correlation between immunity and testosterone may contribute to maintaining variation.

Specifically, in study I, I aimed to study fitness effects of infanticidal behaviour and the benefits of asynchronous breeding in lowering resource competition by conducting an enclosure experiment. Paper II then estimated the heritability and potential genetic correlation of infanticide with female reproductive effort. In studies III, IV and V, the frequencies of different types of individuals were manipulated (rare or common) in enclosed field populations to test whether the fitness of individuals depended on their neighbours' genotype (frequency dependent selection). More specifically, study III tested the competitive abilities of the two opposite infanticidal tactics (killing / not killing the conspecific offspring), hypothesizing that the costs and benefits of infanticidal behaviour are related to the competition for food resources. Study IV was conducted to examine the importance of immune function for overall fitness of bank vole **males** by demonstrating possible relationships between immune parameters measured in the laboratory and fitness parameters in field enclosures. Study V assessed the effects of immunity on the most important components of **female** fitness: survival, breeding probability and fecundity. These were monitored in enclosures over nine months. Finally, in study VI we examined the common genetic basis for immunocompetence (acquired immune system) and male testosterone level, as well as the genetic correlation between these traits, which provided data on heritable variation in parasite resistance and its genetic constraints in wild populations.



## 2 METHODS

### 2.1 Study species

Our model species, the bank vole *Myodes (Clethrionomys) glareolus* is a common small rodent species in Finland (Kallio et al. 2009) and in Northern Europe (Stenseth 1985). It mainly inhabits forests and fields, and feeds mainly on forbs, shoots, seeds, berries and fungi (Hansson 1985). Some animal material is consumed when encountered (Obrtel 1974, Hansson & Larsson 1978, Gebczynska 1983). The mating system in the bank vole is considered to be promiscuous (Sikoski & Wójcik 1990, Mills et al. 2007b). In our study area, females give birth to a maximum of four litters during the breeding season, which lasts from late April to September. Offspring number per litter ranges from two to ten (Koivula et al. 2003). Pregnancy lasts 19-20 days (in lactating females 21-24 days) and the lactation period on average is 18-20 days, after which the young reach total independence. Females breed in postpartum oestus, i.e. can become pregnant within a few hours after parturition. Thus, reproductive events such as parturition of individual females can occur at regular intervals throughout the breeding season.

For successful breeding, space and food resources are crucial for females (e.g. Koskela et al. 1998). Breeding bank vole females defend an exclusive territory (breeding area) against other females and they do not exhibit communal nursing, but their home ranges (feeding areas) greatly overlap (Koskela et al. 1997, Jonsson 2002). In comparison, males have territories overlapping with several female territories (Mazurkiewicz 1971). In omnivorous microtines, such as the bank vole, scarcity of food may limit reproductive success (Koskela et al. 1998). The energy demands of breeding females are 30%-130% higher than those of non-reproducing females of the same body size (Migula 1969, Grodzinski & Wunder 1975) and thus, simultaneous breeding can increase the energy demands of a population dramatically, particularly so if population density is high.



Reproductive effort in male bank voles is not expressed by elaborate ornaments, but by social dominance (Hoffmeyer 1982, Oksanen et al. 1999) that is increased by exogenous testosterone (Mills et al. 2009). Female bank voles also show preferences for dominant males (Horne & Ylönen 1996, Kruczek 1997) and male sexual attractants are likely to be testosterone-dependent (Radwan et al. 2006). Directional selection acts on testosterone (Mills et al. 2007b) and testosterone levels are heritable (Mills et al. 2009). Experimentally elevated testosterone increases mate searching, mobility, and male bank vole reproductive success, but has costs in terms of decreased immune function and survival (Mills et al. 2009). The bank vole is therefore a good model species in which to demonstrate the reproductive effort-survival trade-off and to test the hypothesized mechanism for its maintenance (IV).

Bank voles breed well in enclosures and in the laboratory, they have a high level of trappability and they are not sensitive to disturbance, e.g. when handled. Consequently, the bank vole is a suitable study species for both laboratory and field experiments. The individuals used in the studies were captured around the study site in Konnevesi (IV,V) or/and represented first to fifth generation laboratory-born descendants of wild-captured individuals (I-VI). In all experiments, during the laboratory breeding, inbreeding was carefully avoided. The experiments were conducted with the permission of the Ethical Committee of Scientific Animal Experiments and the Provincial Administrative Board of Western Finland.

### **2.1.1 Bank vole as a model species in studies of infanticide (I-III)**

Naturally, about  $\frac{2}{3}$  of females and  $\frac{1}{2}$  of male bank voles are infanticidal (III), providing a useful model for studying the evolution and maintenance of genetic variation in infanticidal behaviour (I - III). Before the current studies (II, III), no evidence of the evolutionary potential or heritability of infanticide has existed. Although female territoriality could act as a counterstrategy against infanticide, defense against intruders is never fully complete and this behaviour alone is obviously inadequate to totally prevent infanticide in this species. Female bank voles are solitary breeders and do not receive cooperation during nursing from either male or kin, and thus the litter must occasionally be left without immediate guarding (e.g., during foraging by the mother). Female infanticidal behaviour is blocked on average 0-1 days before parturition and it resumes again on average 7-10 days after parturition (T. Poikonen, E. Koskela & T. Mappes, unpubl. data). In general, infanticidal behaviour is blocked in small rodent females around the time of parturition (McCarthy & vom Saal 1985, Soroker & Terkel 1988, Wolff & Macdonald 2004), enabling synchronous breeding to possibly act as a counterstrategy against infanticide committed by other females (I). Experimental studies in rodents have also found that male infanticidal behaviour is blocked for the period from parturition to the infant stage of the litter that it has sired (vom Saal & Howard 1982, Brooks & Schwarzkopf 1983, vom Saal 1985, McCarthy & vom Saal 1986, Mennella & Moltz 1988, Soroker & Terkel 1988, Cicirello & Wolff 1990). Therefore,

synchronous breeding could minimize the risk of both female and male infanticide (I), which has not been considered previously in the literature.

The maintenance of alternative infanticide tactics is predicted to be determined by the cost-benefit ratio of killing pups in different environments. The bank vole is a suitable study species to assess this by manipulating food resources, which limit reproduction (Koskela et al. 1998). Furthermore, measuring infanticide in the bank vole is straightforward, and by utilizing a known pedigree, it is also possible to understand the genetic background of infanticide.

### **2.1.2 Bank vole as a model species in studies of immunocompetence (IV-VI)**

The survival of bank voles in the wild is compromised by diseases from ecto- and endoparasites as well as pathogens (Soveri et al. 2000, Kallio et al. 2007). Such diseases include intraerythrocytic infection caused by the protozoa *Babesia*, which is transmitted through an *Ixodes* tick bite (Karbowiak & Sinski 1996), and Lyme disease carried by *Borrelia burgdorferi*-infected *Ixodes* nymphs (Tälleklint & Jaenson 1996). Up to nine helminth species that affect host mortality have been found in bank voles from Finland (Haukialmi & Henttonen 2000) as well as coccidiosis, an intestinal infection transmitted by the endoparasite, *Eimeria cernae* (Soveri et al. 2000, Hakkarainen et al. 2007). The genetic disease resistance and immune response therefore have important consequences for survival and thus fitness in this species, making it a suitable model species for studying the fitness effects of immunity. A trade-off between male reproductive effort and testosterone has been found in this species (Mills et al. 2009). Moreover, the methods for measuring immunocompetence are well developed in the bank vole (see below).

## **2.2 Laboratory procedures**

The animals were housed in the Experimental Animal Unit of the University of Jyväskylä in standard mouse cages, and maintained in a 16L:8D photoperiod at  $20 \pm 2$  °C. Wood shavings and hay were provided as bedding, while food (standard laboratory rodent food: Labfor R36; Lantmännen, Sweden) and water were available *ad libitum*. For breeding, a mature female and male were housed together for 1-2 weeks, and pregnant females were checked daily for parturition. For the purposes of study I, the pairings were carried out in two sets with a nine-day interval to create the two groups, the synchronously and asynchronously breeding groups. In laboratory populations, pups were weaned from their mothers at the age of 20 days. The offspring were housed together with same-sex littermates until maturity, after which they were housed individually.

### **2.2.1 Mating success trials (IV)**

Male reproductive effort was determined in the laboratory by a set of mating trials, in which a male competed with another male for a female in estrus. During these trials, two males and one female in estrus were released into an arena (1×1 m), and observations were made until ejaculation occurred (Oksanen et al. 1999, Mills et al. 2007a, Mills et al. 2009). All males tested were unfamiliar with each other, being neither a relative nor a cage partner. Males indicate either aggressive or defensive behaviours within 5 min and, normally, the aggressive male courts the females with successful copulation following female lordosis. A total of 60 males were assessed three times with the three other males from his replicate group (two males per trial, six trials in total per replicate group, total of 190 trials) and random unrelated, unfamiliar females. The male that successfully mated was considered dominant and males were ranked within each replicate group based on their copulation success per trial (only one male copulates per trial). The maximum number of successful copulations per male after three trials equals three and males achieving this score were given the highest reproductive effort rank of 4, whereas males achieving zero copulation were given the lowest score of 1.

### **2.2.2 Testing individual infanticidal status (I-III)**

The infanticidal behaviour of bank vole individuals was tested using the neutral arena procedure (Labov et al. 1985, Ylönen et al. 1997). A newborn pup was placed in the middle of a 43×26×15 cm standard mouse cage with a thin layer of clean sawdust, and an adult test individual was introduced into the cage and placed under continuous observation. If the adult aggressively attacked the pup during the 30 min. observation time, it was considered infanticidal. Usually the infanticidal individuals indicated clear aggression against the pup within 10 minutes, whereas non-infanticidal individuals typically inspected the pup several times during the test but did not harm it. If aggression against the test pup was expressed, the pup was immediately removed from the test arena and euthanized. High repeatability of infanticidal behaviour of the testing procedure (89 %) was confirmed by testing 44 individuals twice ( $\chi^2 = 26.3$ ,  $P < 0.001$ ).

### **2.2.3 Measuring immunity**

To assess immunocompetence in terms of humoral adaptive immunity, we measured the primary antibody response to a novel T-cell dependent protein antigen, bovine gamma globulin (BGG), and total immunoglobulin G level (IgG) in plasma (see below for methods). Anti-BGG reflects the resources allocated into the production of specific antibodies in response to the novel antigen. IgG is a first line of defence against pathogens that aims to neutralize them before a specific immune response is triggered (Greives et al. 2006). These measures display significant estimates of heritability (parent –midson analysis: Mills et al.

2009; animal model: VI). The used measures of immunocompetence also correlate with fitness related characters in our study species. In outdoor enclosures, the anti-BGG antibody response correlates positively with survival and growth (Oksanen et al. 2003; IV), and IgG concentration correlates negatively with ectoparasite number (IV). Consequently, although fully recognising the difficulties in trying to assess and classify immunocompetence of individuals, there is excellent experience and knowledge that these methods provide data that has relevance both in the laboratory as well as in field conditions.

To measure humoral antibody responses to a novel antigen (BGG) and total IgG from all adults, animals were immunized with an intraperitoneal injection (0.1 ml) of bovine gamma globulin (BGG, Sigma Chemical Co, 200 mg) emulsified in complete Freund's adjuvant (Difco laboratories, Detroit, MI). Blood samples, taken before immunization in order to determine the baseline level of anti-BGG, were centrifuged (12000 rpm for 5 min, Heraeus Biofuge) to separate plasma from the blood cells and plasma was stored at -20 °C. On day 28 after immunization, another blood sample (18 µl) was taken to determine anti-BGG antibody and total IgG concentrations with a micro plate enzyme-linked immunosorbent assay (ELISA).

#### **2.2.4 Selection on immune response (IV, V)**

We employed two-way artificial selection on the bank vole to generate divergent groups for immune response (high and low) based on immune tests challenging different components of the immune system (Norris & Evans 2000). A total of 400 males and females were wild captured from Konnevesi, central Finland. Humoral adaptive immunity (bovine gamma globulin [BGG] specific antibody response and total immunoglobulin G [IgG] level) were measured from all adults (see above for methods). A total of 120 parents with the most extreme measures were paired to produce first-generation offspring. A total of 93 litters were produced totaling 460 first-generation offspring. From these 93 first-generation litters, 120 male and 120 female bank voles with the most extreme immune responses were selected for the laboratory and field experiment.

### **2.3 Estimating genetic parameters of infanticide (II, III) and immunity (IV-VI)**

Quantitative genetic experiments aim to partition phenotypic variation to its contributing sources so that the additive genetic contribution ( $G_A$ ) can be accurately extracted from nonadditive genetic effects (epistasis or dominance) and the environment. Although much of the resemblance among relatives is due to additive alleles (Lande 1979, Falconer & Mackay 1996, Lynch & Walsh 1998) nonadditive variance can also contribute. Therefore, experimental designs

in which the additive variance can be cleanly partitioned, such as parent-offspring regression [IV,V], half-sib breeding designs or animal models [II, VI] (Falconer & Mackay 1996, Lynch & Walsh 1998) are preferential statistical methods for assessing genetic parameters in quantitative traits (McGuigan 2006).

### **2.3.1 Parent-offspring regression (IV, V)**

To evaluate the evolutionary significance of intraspecific quantitative trait variation, one must investigate the heritability of the traits. Probably the most familiar way to do so is to use the standard parent-offspring regression techniques of quantitative genetics (Falconer 1960, Falconer & Mackay 1996). The heritability of a character in a population is the slope of the linear regression line of the measurements of the character amongst offspring on the mean of the measurements of the character for their two parents (Jacquard 1983). In IV and V, heritabilities were calculated from the slope of the linear parent-midson/middaughter regression. These parent-midson or -middaughter covariances estimate only half of the additive genetic variance; therefore, they must be doubled to obtain heritability estimates (Falconer & Mackay 1996). Defined in this way, the heritability characterizes the relation between the values ascertained amongst offspring and those ascertained amongst parents. It is a measure based on observation which allows the prediction of the offspring values from those of the parent (Jacquard 1983).

### **2.3.2 Threshold model with the parent-offspring regression (III)**

By definition, quantitative genetics is concerned with the genetic basis of traits that show continuous variation, such as body size, development, and fecundity. However, many traits vary in a discrete fashion even though pedigree analyses indicate they are not inherited in a simple Mendelian manner (e.g., single locus with two alleles). The genetic determination of such traits can be understood using the threshold model of quantitative genetics (Falconer 1989). It posits a continuously distributed underlying trait, called the liability, and a response threshold, where the individuals above the threshold display one morph and individuals below it display the alternate morph (Roff et al. 1997). In study III, we assumed that infanticide is a polygenically and environmentally determined character and applied such a threshold model with the parent-offspring regression (Falconer & Mackay 1996, Roff 1997, Lynch & Walsh 1998) to estimate the detailed genetic parameters of infanticidal behaviour.

### **2.3.3 Restricted maximum likelihood based animal model (II, VI)**

Finally, in studies II and VI, the (co)variance components were estimated with the average information restricted maximum likelihood -procedure using the ASReml 2.0 -program (Gilmour et al. 2002, Gilmour et al. 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).

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, this is not a problem since factor analytic modeling of the  $\mathbf{G}$  -matrix directly estimates only that part of the genetic variation that has statistical support (Blows 2007).

The REML-animal model can underestimate additive genetic variance in the base population, if assumptions of the infinitesimal model do not hold (Goddard, 2001). For example, the heritability of litter size estimated from mother-daughter regression can be four times larger than the REML-animal model estimate from the same data set (Holl & Robison 2003). The differences of estimates may also originate from the smaller sample size used in the parent-offspring regressions together with the methods used.

## 2.4 Field studies

The field experiments of this thesis were conducted at the Konnevesi Research Station, central Finland (62 °37'N, 26 °20 'E) in 20 (I, III) or 15 (IV, V) outdoor enclosures (0.2 ha each) situated in an old field. Enclosure fences were constructed of 1.25 m high galvanized sheet metal that was embedded 0.5 m into the ground. The fences were high enough to enclose the study populations, but did not prevent possible entry of predators (e.g. red fox *Vulpes vulpes* L., least weasel *Mustela nivalis nivalis* L. and avian predators).

In studies I, IV and V, two to three days prior to the expected date of parturition, females were captured and brought to the laboratory in order to record litter sizes and body measurements (weight, head width) of the pups at birth. Trappings were performed in each enclosure with 20 multiple-capture live traps that were distributed in a 5 × 4 grid (10 m apart from each other). Each trap was covered with a galvanized sheet metal chimney that reduced exposure to precipitation and temperature extremes. The newborn pups were individually marked in laboratory by toe-codes to identify them at their weaning age. Females with newborn litters were returned to the enclosures at the site of capture. Based on the day of copulation in the laboratory as well as the visual cues of pregnancy in the field, we could accurately estimate the day of delivery. When the offspring were approximately 30 days old, populations were trapped to record the individual survival of the offspring to independence, and their weaning weight and head width were also measured.

In study III, the populations were founded at the beginning of the breeding season (early June). Two factors in the experiment, the food resources and frequency of infanticidal tactics, were manipulated. In eleven enclosures, extra food (sunflower seeds) was provided *ad libitum* in each trap station (20 per enclosure), while in the nine other enclosures, no supplemental food was



provided. After the breeding season (in September), all individuals were live-trapped in order to monitor the possible change in frequencies of infanticidal and non-infanticidal individuals.

In studies III, IV and V, the frequencies of different types of individuals were manipulated (rare or common) in enclosed populations in the field to test whether the fitness of individuals depended on their neighbours' genotype. This experimental design enabled us to test whether the success of the immune tactic or infanticide tactic depends on its frequency in the population in a negative frequency dependent manner (Ayala & Campbell 1974, Gromko 1977, Mappes et al. 2008).

In studies IV and V, parasite-reduced environments, representing conditions under which immunosuppression would not pose a survival or reproductive handicap, were created using anti-parasite medication.

### **3 RESULTS AND DISCUSSION**

#### **3.1 Effects of infanticidal behaviour on reproductive success and resource competition**

##### **3.1.1 Infanticidal behaviour and synchronous breeding (I)**

The results in the first study provide novel evidence from experimental populations that infanticide is an important factor affecting reproductive success. In general, the data show higher breeding success for females in the non-infanticidal population (mean proportion of offspring weaned in NON-INF vs. INF populations). Infanticide caused a 20% decrease in offspring survival. The absolute numbers of recruits per litter show the same difference in NON-INF vs. INF populations, equating to a 25% loss in recruits due to infanticide. Only a few earlier studies have tried to assess the quantitative impact of infanticide on offspring survival, but previous research indicates an even larger proportion of young killed by conspecifics than found in the current results (Sherman 1981, Hoogland 1985, Borries 1997). Yet, the assessment procedures are not completely comparable with the study design here, and mortality in the absence of infanticidal individuals in the population was not determined in previous studies. Nevertheless, the potential importance of infanticide on experimental results might be considerable when dealing with infanticidal species.

Not only was breeding success higher within the NON-INF population, but offspring weight was also higher compared to INF populations. Therefore, rather than demonstrating a trade-off between the number and quality of offspring, these results suggest actual advantages for reproductive success associated with breeding within the non-infanticidal environment. Although it cannot be ascertained whether the cause of the lower reproductive success in INF populations was due to the act of infanticide per se (actual killing and harassment of the young), or some correlated character (e.g., poorer nursing



ability of infanticidal females), the difference between the infanticidal and non-infanticidal populations was evident.

Furthermore, breeding success in the asynchronously breeding, infanticidal populations (INF/AS) was significantly lower compared to non-infanticidal populations (NON-INF/AS), whereas among synchronously breeding females, infanticide had no negative effects on breeding success (INF/S vs. NON-INF/S). These findings support the idea that synchronous reproduction could have evolved as a counterstrategy against infanticide.

### **3.1.2 Heritability of infanticide and genetic correlation with reproductive effort (II)**

In this study, our aim was to investigate the heritability of infanticide and potential genetic correlations between infanticide and female reproductive effort. According to the resource competition hypothesis, we predicted that reproductive effort of females would have a positive genetic correlation with infanticidal behaviour. An animal model of a large pedigreed data set revealed, for the first time, a significant heritable component of infanticidal behaviour and a positive genetic correlation between the infanticidal tendency and reproductive effort (litter size and litter mass). These findings suggest that the resource competition hypothesis may be an adaptive explanation for infanticide.

Although in some conditions, infanticidal behaviour may be maladaptive (recently Le Boeuf & Campagna 1994, Dolhinow 1999, Knopff et al. 2004, Murray et al. 2007, Feh & Munkhtuya 2008), the genetic correlation with reproductive effort, and the support that this association gives to the resource competition hypothesis, reinforces the argument that infanticide may have adaptive value. Individuals may employ different genetic strategies so that individuals with larger litters may be genetically associated with a higher probability to commit infanticide.

### **3.1.3 Competitive abilities of individuals with opposite infanticide tactics (III)**

In this study, we tested the competitive abilities of the two opposite infanticidal tactics (killing / not killing the conspecific offspring), hypothesizing that the costs and benefits of infanticidal behaviour are related to the competition for food resources. We used an experimental design to test whether the different infanticidal tactics affect each other in environments with different resource levels. The results revealed that it is easier for the infanticidal tactic to invade the population of non-infanticidal individuals if food resources are limited (Tuomi et al. 1997). This study confirms the results of the previous study (II) that differential competitive abilities are linked with different infanticide tactics. As reproductive success is constrained by food resources in bank voles (Koskela et al. 1998), infanticidal females might be able to reduce food competition by killing the pups of non-infanticidal competitors. However, infanticidal behaviour should carry some other reproductive costs as non-infanticidal

individuals could invade the population when food was not a limiting factor. While the exact behavioural and physiological mechanisms are still unclear, we can conclude that the evolutionary stability between the different infanticidal tactics can vary according to the level of food resources in animal populations. Furthermore, we predict that the competition for food can be a sufficient condition for the evolution of infanticidal behaviour.

## **3.2 Effects of immunocompetence on reproductive success and survival**

### **3.2.1 Immunocompetence and male fitness (IV)**

This study showed the importance of immune function for overall fitness of bank vole males by demonstrating strong relationships between immune parameters measured in the laboratory and fitness parameters in field enclosures. Furthermore, we found evidence of a trade-off between male reproductive effort (measured as number of genetic mates and mating success) and survival, which was mediated by parasites and the immune response. As we show in a separate study (Mills et al. 2009), the testosterone level (in addition to immune response, VI) is heritable in bank voles. Thus, females mating with dominant males will benefit in terms of indirect genetic benefits for either reproductive effort or survival for her offspring, but not both. The genetic trade-off between the immune response and testosterone levels implied by this study suggests that the evolution of male reproductive traits is also constrained by a survival handicap. Therefore, the reproductive effort /survival trade-off may represent another mechanism maintaining genetic variation for life-history traits.

### **3.2.2 Immunocompetence and female fitness (V)**

This study, conducted in enclosures over nine months, assessed the effects of immunity on the most important components of female fitness: survival, breeding probability and fecundity. The first component of fitness, female long-term survival, was affected both by the level of immune response (high vs. low) of the female and its frequency in the population. Females with low immunity had significantly higher survival, if they represented the rare morph in the population. Interestingly, among the high immunity females, such a difference between the common and rare morph did not exist. This demonstrates that frequency-dependent survival selection acts differently among high and low immunity females.

With another fitness component, reproductive success, we found a significant interaction for breeding probability between the immune group and parasite medication. Within the high immunity group, the medication had no effect on breeding success, whereas within the low immunity group, parasite

medication improved the breeding probability. This demonstrates that for females with low genetic disease resistance, the costs of mounting an immune response actually reduce their fitness. The significance of frequency-dependent selection was also evident in terms of reproductive success, as rare immunity type females in the population had an advantage in the probability of breeding.

When further analyzing the litter sizes of those females that actually produced a litter (non-reproduced females were excluded from the analysis), we found that the anti-parasite treatment significantly increased the litter size of females compared to controls. This provides evidence for the general costs of pathogens on the fecundity of females.

To summarize the results in the study (V), frequency-dependent selection favored the rare immune type both in terms of survival and breeding probability. According to the Red Queen theory of host-parasite coevolution, such an advantage of the rare type is to be expected if parasites are able to track the common host (Lively 2001). Our data is in accordance with this prediction, since the advantage of the rare morph was visible with ectoparasite number: the tendency for the rare immunity type to carry a lower ectoparasite load suggests this benefit of the rare morph. The high immunity tactic females in particular benefited from being rare as compared to being common in terms of lower ectoparasite load. These findings demonstrate that negative frequency dependent selection may act to maintain genetic variability in populations.

### 3.2.3 Genetic basis of immune system in the bank vole (VI)

In this study, we examined the common genetic basis of immunocompetence (acquired immune system) and male testosterone level. To our knowledge, this is the first time genetic covariances between immunological measures and the testosterone level have been estimated.

In terms of the immunological traits, estimates of additive genetic variance and heritability were higher in females for anti-BGG and in males for IgG. The estimate of heritability for male IgG was quite high (0.48), and moderate (0.20 – 0.32) for the rest of the traits (female and male anti-BGG, female IgG and male testosterone). Genetic correlations for IgG between the sexes was strongly positive (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) ranged from weak to moderate. 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).

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 the 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 of 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. 2007b). Likewise, the heritabilities of dominance related traits, dependent on testosterone level, have been shown to be quite high in the bank vole (Horne & 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. Therefore, despite a high heritability, a response in male testosterone level will be slowed down, because in addition to the antagonistic effect on male survival, there is also an intersexual effect on the survival of females in the population.

To summarize (VI), quantitative genetic analysis of two immunological traits in both sexes and male testosterone level revealed a tight linkage between the traits studied. Selection for higher testosterone level in males will compromise the function of the 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.

## 4 CONCLUSIONS

The maintenance of genetic variation is one of the evolutionary issues of widest concern in conservation biology, however, even in model organisms such as the various *Drosophila* species, the basis of the maintenance process is not clearly understood (Hedrick 2001). The studies of this thesis provide evidence for two main factors maintaining genetic variation in both behavioural and physiological traits: environmental heterogeneity (I-III) and negative frequency dependent selection (III, V).

Based on the results of studies I-III, the fitness of opposite infanticidal tactics differ in relation to their frequency in the population. Different environmental conditions seem to benefit opposite infanticidal tactics, especially the availability of food. Although non-infanticidal females have the highest reproductive success in general (I), low/severe resource conditions allow the infanticidal individuals to invade non-infanticidal populations of bank voles (III). The maintenance of infanticidal behaviour in the population may be sustained by the positive genetic correlation between infanticidal behaviour and reproductive success in females (II). This genetic correlation may have evolved due to the higher competitive ability of infanticidal individuals, indicated by their greater success when the food is scarce (III). For the benefit of non-infanticidal individuals, the possibility that asynchronous breeding staggers resource requirements may enable higher reproductive success (I). This benefit may be particularly important during conditions of low food availability.

While these studies revealed several aspects concerned with infanticidal behaviour and the maintenance of its genetic variation, they have also raised further questions to be answered. One important task in the future is to determine the plasticity of infanticidal behaviour in the wild. Even though infanticidal behaviour in the bank vole is repeatable (I-III) in the laboratory (in the absence of costs associated with searching for, and killing a test pup), there might be more individual variation in the occurrence of the infanticidal act, depending on circumstances. For instance, in the present studies, the degree of relatedness was not a manipulated variable, whereas in wild populations, polymorphism in infanticidal tendency could be expressed conditionally in

relation to the identity of potential recipients (e.g. Sherman 1981, Lambin & Yoccoz 1998, but see Hoogland 1985, Cicirello & Wolff 1990, Wolff & Cicirello 1991). Such plasticity of a trait may influence its fitness value. While a specialist (non-plastic) genotype might perform as well or even better than the plastic one under one condition, it will do significantly worse under the other condition. Depending on the frequencies of the two environments, the plastic genotype may be favoured in the long run. As different traits respond differentially to environmental variation, the evolutionary consequences of environmental variation are trait-specific. For environmentally sensitive traits, environmental heterogeneity inflates phenotypic, but not genetic variance, thereby reducing a trait's heritability (Mazer & Damuth 2001). Since we have shown infanticidal behaviour to have a high heritability (II, III), one might expect it to also have a low level of plasticity in the wild. This prediction remains to be studied.

Secondly, further studies are required to determine whether non-infanticidal females are more prone towards an asynchronized mode of reproduction (actively staggering reproduction to increase the availability of resources) than the infanticidal ones; or whether the infanticidal females actively synchronize reproduction as a counterstrategy against infanticide (I, II). Furthermore, the costs of committing infanticide (such as the increased risk of intra- and interspecific predation of an infanticidal individual itself or its offspring) need to be assessed. These costs may be one factor promoting the maintenance of the non-infanticidal tactic in a population.

The maintenance of variation in immune traits in the wild is due to an array of mechanisms, of which the current thesis shows evidence for: antagonistic pleiotropy, (IV, V), negative frequency dependent selection (V) and environmental variability (IV, V). A large part of the immune status of an individual is genetically determined (VI), and it trades off with testosterone both in females (IV) and males (IV, VI). Thus, immunity trades off with reproductive success at least in males (IV). Female advantages exist through the mate choice they perform, because their (male) offspring inherit preferred male traits. However, the offspring either gain a high testosterone level or high immunocompetence, but not both (IV). Furthermore, immunity is positively correlated with survival (IV). The testosterone level correlates positively with breeding success (mating success in the laboratory trials, number of genetic mates in field, number of offspring in the enclosures as determined using paternity analyses) (IV). Number of ectoparasites (representing one of the varying environmental factors) directly affected male reproductive success (IV). In females, such a direct effect was not found. Different factors may operate on selection in females and males, and the lack of a direct effect of ectoparasites on female fitness components may be partly explained by the multitude of different pathogens that an individual small rodent may be forced to face in the wild. Viruses, bacteria, protozoa, pathogenic fungi as well as prions, however, were beyond the scope of these studies (Playfair & Bancroft 2004).

Testosterone can cause immunosuppression either directly by binding onto immune cells, or indirectly by draining resources from the immune system



(Wedekind & 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). Furthermore, since hormones are generally mediators of evolutionary constraints (McGlothlin & Ketterson 2008) and an efficient immune system should be more important to a female's than a male's fitness (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 & Verhulst 1996) (VI).

In females, study V shows that negative frequency dependent selection can maintain different immune tactics in a population: individuals in the low immunity group benefited from being rare in the population, shown by their higher breeding probability and survival. Furthermore, females with a high immune response benefited in terms of a higher breeding probability and lower ectoparasite count when rare in the population. Also, the fluctuation of parasite numbers (a form of environmental variability) seemed to affect female fitness: removal of parasites (simulating a year/season of low parasite load) caused low immunity females to increase their breeding probability, and females of both high and low immune tactics were able to produce larger litters as compared to the solvent control conditions (a year / season of high parasite pressure).

Negative frequency-dependent selection is one mechanism for low immunity females to survive until the next breeding season. However, during the reproductive season and among individuals of the high immunity tactic, other mechanisms affect female bank vole fitness. As data in study V show, parasites may be able to cause fluctuating changes in host type frequencies, enabling the low immunity strategy to be maintained in the population. Thus, antagonistic pleiotropy (IV), frequency-dependent selection (V) and environmental variability (IV, V) may be important in maintaining variation in physiological traits, such as alternative immunity tactics.

An individual organism in the wild is an entity where different traits are bound up and affect each other in complex ways, forming suites of hormonally determined traits (McGlothlin & Ketterson 2008). For instance, a suite of testosterone-sensitive traits has been identified (reviewed in Ketterson & Nolan 1992, 1999, Reed et al. 2006) that can either accelerate or decelerate individual adaptation. Considering the traits studied in the current thesis, infanticide and immunity, several previous studies have shown that exposure to testosterone can induce or enhance infanticidal behaviour both in female and male rodents (Davis & Gandelman 1972, Gandelman 1972, 1973a,b, Gandelman & vom Saal 1975, 1977, Rosenberg & Sherman 1974, 1975, Svare 1979, Svare & Mann 1981, Mann et al. 1983, Brown 1986, Perrigo et al. 1989). On the other hand, we have shown (in IV and in Mills et al. 2009) that an increased testosterone level is negatively associated with an individual's immunocompetence. These results, taken together, suggest possible alternative tactics to maximize fitness: an

increase in testosterone level, which will increase male (or male offspring) reproductive performance (IV) and infanticidal tendency (which is genetically correlated with larger litter sizes, II), or allocating resources to increase immunity, which leads to a better survival probability in males (IV).



*Acknowledgements*

I would like to thank my supervisors Tapio Mappes, Esa Koskela and Tuula Oksanen for giving me a chance to carry out these studies in this high-grade, ambitious and international research group. Thank you for your help and advice during the whole process and especially for your tremendous patience, support and understanding during my long absences from the scientific work during my maternity leaves.

To all the local and visiting members of this research group during all these years, thank you for your contributions. First, my dearest thanks go to Suzie Mills for so much - for your friendship, advice and support during the three Jyväskylä years at work, at leisure and in private life. Thank you also for checking the language of the review version of this thesis. Zbyszek Boratyński, Anna Dubiec, Heikki Helle, Kikka Kallio, Mikael Mökkönen, Anna-Kaisa Rikalainen, Joanna Rutkowska and Eero Schroderus are all appreciated for companionship and co-operation as part of this research group. I also thank Mikael for the language check of the final version of this thesis. I thank my other co-authors Jouni Aspi, Alessandro Grapputo, Ilmari Jokinen, Minna Koivula and Juha Tuomi for their contributions to the papers included in this thesis. Ulrika Candolin and Jan Lindström kindly reviewed this thesis and provided constructive criticism, and Jari Haimi helped with the editing. Elina Virtanen, Tuija Solismaa, and Maria Tuittu for their invaluable help with the laboratory analyses. During these years, we have also had numerous trainees and assistants whose efforts in vole husbandry and in the field are highly appreciated; especially warmest thanks to the long-term helpers, Tiina Savolainen and Riikka Närä, for help and friendship during (and outside) the work.

I thank professors Rauno Alatalo, Anneli Hoikkala and Janne Kotiaho and for the great atmosphere of the department and especially Johanna Mappes and Mikko Mönkkönen also for the support for my work. There are many wonderful teachers, researchers and fellow students whose presence made the department a pleasant place to work, among others Eira Ihalainen, Katja Kuitunen, Leena Lindström, Anne Lyytinen, Satu Paukku, Mikael Puurtinen, and Heli Siitari. Special thanks to Katjat I. & O. (in alphabetical order), Ines Klemme, Saila Sillanpää, Lenka Trebaticka and Outi Vesakoski for all your friendship, help and discussions, both at work and outside it.

I want to thank the technical and administrative staff of the department and the Experimental Animal Unit in Ylistönrinne. Special thanks to the people in Konnevesi research station: Jussi Viitala and Hannu Ylönen for letting me work in such a wonderful and peaceful place with great facilities, Raija for the all-too-delicious cooking, Tarja, Helinä, Irja as well as Janne, Jyrki & Risto for organizing whatever was asked, and especially during the last year, for the daily "joko nyt" - finally, yes!

My warmest thanks to my parents Tuula and Jouko for always encouraging me to start, to continue and to finish this thesis and my studies all

the way, and for helping and supporting me in all possible ways during these years. Thanks to my brother Jussi, for the humoristic attitude towards the universal significance of my studies. Huge thanks to Ulla, Jukka, Seija and Leena for your flexibility and understanding, without which this work would never have been finished.

Finally and most importantly, thank you Antti - this thesis is not nearly the most important thing I received during my PhD years. Thank you for your love and support and for really making my dreams come true. Thank you Antti, Paavo and Ansa for being *the thing* for me and for never making me think about work at home!

This study was financially supported by the Academy of Finland, the Graduate School of Evolutionary Ecology and University of Jyväskylä.

## YHTEENVETO

### Frekvenssistä riippuva valinta ja ympäristön heterogeisuus luonnonvalintaa ohjaavina tekijöinä luonnonpopulaatioissa

Geneettisen vaihtelun olemassaolo johtaa evoluutiobiologian perimmäiseen kysymykseen: miksi populaatiot eivät koostu vain kaikkein kelpoisimmasta genotyypistä? Miksi heikommilta vaikuttavat geeniyhdistelmät eivät ole karsituneet luonnonvalinnassa? Teoreettisesti mahdollisia geneettistä muuntelua ylläpitäviä mekanismeja tunnetaan useita: mutaatio-valinta -tasapaino, heteroosi, ympäristövaihtelu, antagonistinen pleiotropia ja negatiivinen frekvenssistä riippuva valinta. Kokeellista tietoa näiden mekanismien merkityksestä luonnonpopulaatioissa tarvitaan kuitenkin lisää. Tässä väitöskirjassa keskityn erityisesti ympäristövaihtelun (osatutkimukset I-VI) ja negatiivisen frekvenssistä riippuvan valinnan (III, V) merkitykseen geneettistä vaihtelua ylläpitävinä tekijöinä. Myös antagonistista pleiotropiaa tutkitaan kahdessa osatyössä (IV, VI).

**Ympäristövaihtelu** voi selittää geneettisen muuntelun säilymistä luonnonpopulaatioissa, jos erilaisten ominaisuusyhdistelmien kelpoisuus riippuu ympäristössä vallitsevista olosuhteista. Näin ominaisuusyhdistelmiin kohdistuva valinta voi suosia yhtä genotyyppiä yhdenlaisessa ympäristössä ja toista genotyyppiä toisenlaisessa ympäristössä.

Frekvenssistä riippuva valinta tarkoittaa tilannetta, jossa tietyn genotyypin kelpoisuus riippuu erilaisten genotyyppien esiintymistiheyksistä populaatioissa. **Negatiivinen frekvenssistä riippuva valinta** viittaa tilanteeseen, jossa harvinaisella genotyypillä on valintaetu yleisempiin genotyyppihin verrattuna. Näin ollen negatiivinen frekvenssistä riippuva valinta pystyy teoreettisesti ylläpitämään geneettistä vaihtelua kasvattamalla kulloinkin harvinaisimman genotyypin esiintymistiheyttä populaatioissa.

**Antagonistisessa pleiotropiassa** eli negatiivisessa geneettisessä korrelaatioissa tietyllä geenillä on positiivinen vaikutus yhteen kelpoisuuden osatekijään, mutta samanaikaisesti negatiivinen vaikutus toiseen kelpoisuuden osatekijään. Esimerkiksi lisääntymismenestystä lisäävät alleelit saattavat samanaikaisesti alentaa elinikää. Ilmiö voi ylläpitää geneettistä vaihtelua, koska maksimaalinen kokonaiskelpoisuus saavutetaan usein ominaisuusyhdistelmällä, jossa sekä lisääntymismenestyksellä että eliniällä on suuri merkitys.

Eri ominaisuudet reagoivat eri tavoin ympäristössä esiintyvään vaihteluun, joten myös ympäristövaihtelun evolutiiviset seuraukset ovat ominaisuuskohtaisia. Tässä väitöskirjassa tutkin muuntelun säilymistä kahdessa ominaisuudessa, infantisoivassa käyttäytymisessä (käyttäytymispiirre) ja immunitetissä (fysiologinen piirre). Käyttäytymispiirteisiin ja fysiologisiin piirteisiin kohdistuvaa valintaa on toistaiseksi tutkittu vähemmän kuin morfologisiin piirteisiin kohdistuvaa valintaa.

**Infantisoiva käyttäytyminen** eli samaan lajiin kuuluvien yksilöiden jälkeläisten tappaminen on luonnossa yleinen ilmiö sekä selkärangkaisilla että selkä-

rangattomilla eläimillä. Nisäkkäistä tällaista käyttäytymistä tiedetään esiintyvän yli 50 lajilla. Monissa populaatioissa infantisoivan käyttäytymisen arvioidaan olevan merkittävä tai jopa tärkein poikasten kuolinsyy. Tässä väitöskirjassa esitetyt tutkimukset rajoittuvat naaraiden suorittamaan, ei-parentaaliseen (muiden kuin omien jälkeläisten) infantisointiin. **Immunitaetti** puolestaan tarkoittaa kykyä tuottaa tehokas immuunivaste yksilön altistuessa taudinaiheuttajille. Immunitaetti on merkittävä yksilön kokonaiskelpoisuuteen vaikuttava osatekijä. Immuunipuolustuksen ylläpitämisellä on kuitenkin hintansa. Yksilön on jaettava rajalliset resurssinsa useiden voimavaroja vaativien prosessien kuten lisääntymisen, kasvun ja lämmönsäätelyn välillä, mikä aiheuttaa allokaatiokustannuksia (ns. trade-off -tilanne). Väitöskirjatyössäni olen selvittänyt näiden kahden erilaisen piirteen geneettistä taustaa, geneettisen vaihtelun säilymistä sekä immunitaettiin liittyviä allokaatio-ongelmia metsämyyrällä (*Myodes glareolus*).

Ominaisuuden evolutiivinen potentiaali riippuu siinä esiintyvän periytyvän geneettisen vaihtelun määrästä. Mikäli periytyvää geneettistä vaihtelua ei esiinny, ominaisuus ei voi muuttua luonnonvalinnan ohjaaman evoluution kautta. Tämän vuoksi tutkin infantisoivan käyttäytymisen (II, III) ja immunitaetin (IV-VI) geneettistä taustaa kvantitatiivisen genetiikan menetelmillä. Tulokset osoittavat, että molemmissa ominaisuuksissa esiintyvä geneettinen vaihtelu on suurelta osin periytyvää.

Erilaisia geneettisiä taktiikoita edustavien yksilöiden (infantisoiva vs. ei-infantisoiva taktiikka; korkean immunitaetin vs. alhaisen immunitaetin taktiikka) suhteellista menestymistä tutkittiin sekä laboratorio- että kenttäkokeissa. Tutkimuksia varten koe-eläinten infantisoiva taipumus selvitettiin käyttäytymiskokeilla laboratoriossa ja yksilöiden immunitaettitaso määritettiin mittaamalla immuunivastetta usealla eri menetelmällä. Korkean ja matalan immunitaetin yksilöitä edelleen jalostamalla luotiin eri immuunitasoja edustavat valintalinjat, joiden yksilöitä käytettiin kelpoisuuseroja selvittävässä kenttäkokeissa.

Väitöskirjani tutkimustulokset osoittavat, että negatiivinen frekvenssistä riippuva valinta (III) ja vaihtelevat ympäristöolosuhteet (I, III) edesauttavat geneettisen muuntelun säilymistä metsämyyrien infantisoivassa käyttäytymisessä. Erityisesti vaihtelu ravinnon määrässä vaikuttaa eri taktiikoiden menestykseen populaatiossa. Vaikka naaraat saavuttavat korkeamman lisääntymismenestyksen silloin, kun infantisoivaa taktiikkaa ei esiinny populaatiossa (I), heikot ravinto-olot edesauttavat tämän taktiikan tunkeutumista populaatioon (III). Infantisoivan käyttäytymisen positiivinen geneettinen korrelaatio poikuekoon kanssa (II) ja infantisoivien naaraiden parempi kilpailukyky heikoissa ravinto-oloissa (III) edelleen auttavat infantisoivaa taktiikkaa säilymään populaatiossa. Ei-infantisoivan taktiikan säilymistä taas edistää mahdollisuus eriaikaiseen lisääntymiseen lajitovereiden kanssa (I). Eriaikainen lisääntyminen edesauttaa resurssien riittävyttä, mikä on eduksi erityisesti kilpailukyvyltään heikommille, ei-infantisoiville naaraille (III). Tulokset herättävät kysymyksen, onko ei-infantisoivilla metsämyyränaarilla sitten enemmän taipumusta epäsynkroniseen lisääntymiseen kuin infantisoivilla naarilla? Kysymykseen vastaaminen

vaatisi lisätutkimusta. Lisäksi olisi selvitettävä infantisoivan käyttäytymisen kustannus (kuten lisääntynyt oma ja omien poikasten riski altistua lajinsisäiselle ja lajien väliselle saalistukselle), joka voi olla eräs ei-infantisoivan taktiikan menestymistä edesauttava tekijä. Olisi myös tärkeää selvittää infantisoivan käyttäytymisen tilanneriippuvaisuus (plastisuus) luonnonoloissa.

Erilaisten immuniteettitaktiikoiden säilymiseen luonnossa vaikuttavat tutkimustulosteni mukaan negatiivinen frekvenssistä riippuva valinta (V), vaihtelevat ympäristöolosuhteet (V) ja antagonistinen pleiotropia (IV, VI). Immuniteettitaktiikat ovat vahvasti geneettisesti määräytyneet (VI) ja niiden ja testosteronipitoisuuden välillä esiintyy antagonistista pleiotropiaa sekä naarailla että koirilla (VI). Testosteronipitoisuus korreloi positiivisesti koiraan lisääntymismenestyksen kanssa usealla eri menetelmällä mitattuna (IV). Näin ollen ainakin koirilla on trade-off -tilanne immuniteetin ja lisääntymismenestyksen välillä (IV). Naaraat hyötyvät paritutumiskumppanin valinnasta, koska koiraan ominaisuudet periytyvät niiden omille (koiras)jälkeläisille. Jälkeläiset perivät kuitenkin vain joko korkean testosteronipitoisuuden tai korkean immuniteetin, eivät molempia (IV). Näin antagonistinen pleiotropia voi edesauttaa kahden erilaisen genotyypin menestymistä populaatiossa. Korkea immuniteetti vaikuttaa tulosten mukaan suotuisasti elossasäilyvyyteen (IV). Loisten lukumäärä, joka vaihtelee luonnossa ja näin ollen edustaa yhtä vaihtelevaa ympäristömuuttujaa, vaikutti koirilla suoraan lisääntymismenestykseen (IV). Naarailla tällaista suoraa loisten aiheuttamaa vaikutusta ei löytynyt. Eri ominaisuuksiin kohdistuvat valintapaineet näyttävätkin olevan koirilla ja naarailla ainakin osittain erilaiset.

Havaitsin, että myös negatiivisella frekvenssistä riippuvalla valinnalla on merkitystä yksilön immuniteetissa esiintyvää vaihtelua ylläpitävänä tekijänä. Tutkimukseni (V) osoittaa, että alhaisen immuniteetin omaavilla naarailla sekä elossasäilyvyys seuraavaan lisääntymiskauteen että todennäköisyys lisääntyä olivat korkeammat niiden edustaessa omassa populaatiossaan harvinaisempaa fenotyyppiä. Samoin myös korkean immuniteetin taktiikkaa edustavat naaraat hyötyivät ollessaan harvinainen fenotyyppi populaatiossa: tällöin niiden todennäköisyys lisääntyä oli suurempi ja ulkoloisten määrä alhaisempi. Myös ympäristöstä riippuvat loisten kannanvaihtelut näyttävät vaikuttavan naaraiden kelpoisuuteen. Tutkimuksessani (V) manipuloin loisten määrää joko poistamalla ne myyristä lääkityksen avulla tai jättämällä myyrät ilman loislääkitystä. Havaitsin, että alhaisen immuniteetin omaavien naaraiden todennäköisyys lisääntyä oli suurempi loistiheyden ollessa alhainen. Lisäksi loisten poistaminen kasvatti molempien immuniteettitaktiikoiden naaraiden poikuekokoja. Negatiivinen frekvenssistä riippuva valinta on siis yksi tekijä, joka vaikuttaa alhaisen immuniteetin omaavien naaraiden selviämiseen seuraavaan lisääntymiskauteen, mutta lisääntymiskauden aikana naaraiden kelpoisuuteen voivat vaikuttaa muutkin mekanismit. Tutkimuksen V aineisto osoittaa, että loiset voivat aiheuttaa aaltoilevia muutoksia eri isäntätyyppien frekvensseihin mahdollistaen alhaisen immuunistrategian säilymisen populaatiossa.

Kaiken kaikkiaan väitöstutkimukseni osoittavat, että kaikilla tutkituilla mekanismeilla - antagonistisella pleiotropialla (IV), frekvenssistä riippuvalla valinnalla (III, V) ja ympäristön vaihtelevuudella (I, III, IV, V) - on merkitystä metsämyyrän käyttäytymis- ja fysiologisissa ominaisuuksissa esiintyvää geneettistä muuntelua ylläpitävinä tekijöinä.

## REFERENCES

- Ayala, J. & Campbell, C. A. 1974. Frequency dependent selection. *Annu. Rev. Ecol. Syst.* 5: 115-138.
- Bartlett, T. Q., Sussman, R. W. & Cheverud J. M. 1993. Infant killing in primates: A review of observed cases with specific reference to the sexual selection hypothesis. *Am. Anthropol.* 95: 958-990.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2: 349-368.
- Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *J. Evolution. Biol.* 20: 1-8.
- Boonstra, R. 1978. Effect of adult townsend voles (*Microtus townsendii*) on survival of young. *Ecology* 59: 242-248.
- Borries, C. 1997. Infanticide in seasonally breeding multimale groups of Hanuman langurs (*Presbytis entellus*) in Ramnagar (South Nepal). *Behav. Ecol. Sociobiol.* 41: 139-150.
- Brooks, R. H., & Schwarzkopf, L. 1983. Factors affecting incidence of infanticide and discrimination of related and unrelated neonates in male *Mus musculus*. *Behav. Neural Biol.* 37: 149-161.
- Brown, R.E. 1986. Social and hormonal factors influencing infanticide and its suppression in adult male Long-Evans rats (*Rattus norvegicus*). *J. Comp. Psychol.* 100: 155-161.
- Caley, J., & Boutin, S. 1985. Infanticide in wild populations of *Ondatra zibethicus* and *Microtus pennsylvanicus*. *Anim. Behav.* 33: 1036-1037.
- Candolin U & Heuschele J. 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends Ecol. Evol.* 23: 446-452.
- Cicirello, D. M. & Wolff, J. O. 1990. The effects of mating on infanticide and pup discrimination in white-footed mice. *Behav. Ecol. Sociobiol.* 26: 275-279.
- Dagg, A. I. 1998. Infanticide by male lions hypothesis: A fallacy influencing research in to human behavior. *Am. Anthropol.* 100(4): 940-950.
- Dagg, A. I. 1999. Sexual selection is debatable. *Anthropology News*, December: 20.
- Dagg, A. I. 2000. The infanticide hypothesis: A response to the response. *Am. Anthropol. New Series* 102(4): 831-834.
- Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. Princeton, NJ: Princeton University Press (1981 reprint).
- Davis, P. G. & Gandelman, R. 1972. Pup-killing produced by the administration of testosterone propionate to adult female mice. *Horm. Behav.* 3: 169-173.
- Dobzhansky, T. 1956. What is an adaptive trait? *Am. Nat.* 90: 337-347.
- Dolhinow, P. 1999. A Mystery: Explaining Behavior. In Shirley C. Strum, Donald G. Lindburg, and David Hamburg (eds): *The New Physical Anthropology: Science, Humanism, and Critical Reflection*. Upper Saddle River, NJ: Prentice Hall. Pp. 119-132.
- Dorner, G., Eckert, R. & Hinz, G. 1980. Androgen-dependent sexual dimorphism of the immune-system. *Endokrinologie* 76: 112-114.



- Ebensperger, L. A. 1998. Strategies and counterstrategies to infanticide in mammals. *Biol. Rev. Camb. Philos.* 73: 321–346.
- Ebensperger, L. A. & Blumstein, D. T. 2007. Nonparental infanticide. In Wolff, J. O. & Sherman, P. W. (eds): *Rodent Societies: An Ecological & Evolutionary Perspective*. University of Chicago Press, Chicago. Pp. 267-279.
- Evans, M. R., Goldsmith, A. R. & Norris, S. R. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behav. Ecol. Sociobiol.* 47: 156–163.
- Ewens, W. J & Thomson, G. 1970. Heterozygote selective advantage. *Ann. Hum. Genet.* 33(4): 365–376.
- Falconer, D. S. 1960. *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh. 365 p.
- Falconer, D. S. 1989. *Introduction to quantitative genetics*. Longmans, New York.
- Falconer, D. S., & Mackay, T. F. C. 1996. *Introduction to quantitative genetics*. Longman Sci. and Tech., Harlow, UK.
- Feh, C. H. & Munkhtuya, B. 2008. Male infanticide and paternity analyses Male infanticide and paternity analyses in a socially natural herd of Przewalski's horses: Sexual selection? *Behav. Process.* 78: 335–339.
- Fernald, R. D. 1976. The effect of testosterone on the behavior and coloration of adult male cichlid fish (*Haplochromis burtoni*, Guenther). *Horm. Res.* 7: 172–178.
- Fisher, R. 1930. *The genetical theory of natural selection*. Clarendon, Oxford.
- Folstad, I., & Karter, A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* 139: 603–622.
- Gandelman, R. 1972. Induction of pup killing in female mice by androgenization. *Physiol. Behav.* 9: 101–102.
- Gandelman, R. 1973a. The development of cannibalism in male Rockland–Swiss mice and the influence of olfactory bulb removal. *Dev. Psychobiol.* 6: 159–164.
- Gandelman, R. 1973b. The ontogeny of maternal responsiveness in female Rockland–Swiss albino mice. *Horm. Behav.* 4: 257–268.
- Gandelman, R. & vom Saal, F. S. 1975. Pup-killing in mice: the effects of gonadectomy and testosterone administration. *Physiol. Behav.* 15: 647–651
- Gandelman, R. & vom Saal, F. S. 1977. Exposure to early androgen attenuates androgen-induced pup-killing in male and female mice. *Behav. Biol.* 20: 252–260.
- Gebczynska, Z. 1983. Feeding habits. In Petruszewicz K. (ed.): *Ecology of the bank vole*. *Acta Theriol.* 28: 40–49.
- Gilmour, A. R., Gogel, B. J., Cullis, B. R., Welham, S. J. & Thompson, R. 2002. *ASReml user guide release 1.0*. VSN International, Hemel Hempstead.
- Gilmour, A. R., Cullis, B. R., Harding, S. A. & Thompson, R. 2006. *ASReml update: what's new in release 2.00*. VSN International, Hemel Hempstead.
- Goddard, M. E. 2001. The validity of genetic models underlying quantitative traits. *Livest. Prod. Sci.* 72: 117–127.



- Goulson, D. & Cory, J. S. 1995. Responses of *Mamestra brassicae* (Lepidoptera: Noctuidae) to crowding: Interactions with disease resistance, colour phase and growth. *Oecologia* 104: 416-423.
- Greives, T. J., McGlothlin, J.W., Jawor, J.M., Demas, G. E. & Ketterson E. D. 2006. Testosterone and innate immune function inversely covary in a wild population of breeding Dark-Eyed Juncos (*Junco hyemalis*). *Funct. Ecol.* 20: 812-818.
- Grodzinski, W. & Wunder, B.A. 1975. Ecological energetics of small mammals. In F.P. Golley, K. Petruszewicz, and Ryszkowski, (eds): *Small mammals, their productivity and population dynamics*. Cambridge Univ. Press, Cambridge. Pp. 173-204.
- Gromko, M. H. 1977. What is frequency-dependent selection? *Evolution* 31: 438-442.
- Hakkarainen, H., Koskela, E., Mappes, T., Soveri, T., Huhta, E. & Suorsa, P. 2007. *Eimeria*-parasites are associated with life history traits in island and mainland populations of the bank vole. *Parasitology* 134: 23-31.
- Hansson, L. 1985. *Clethrionomys* food: generic, specific and regional characteristics. *Ann. Zool. Fenn.* 22: 315-318.
- Hansson, L. & Larsson, T.-B. 1978. Vole diet on experimentally managed reforestation areas in northern Sweden. *Ecography* 1(1): 16-26.
- Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* 29: 133-144.
- Haukisalmi, V., & Henttonen, H. 2000. Variability of helminth assemblages and populations in the bank vole *Clethrionomys glareolus*. *Pol. J. Ecol.* 48: 219-231.
- Hausfater, G., & Hrdy, S. B. 1984. *Infanticide: comparative and evolutionary perspectives*. Aldine Press, New York.
- Hedrick, P. H. 2001. Evolutionary conservation biology. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, New York. Pp. 371-383.
- Hedrick, P.W. 1986. Genetic polymorphism in heterogeneous environments. A decade later. *Annu. Rev. Ecol. Syst.* 17: 535-566.
- Hedrick, P. W., Genevan, M.E., & Ewing, E.P. 1976. Genetic polymorphism in heterogeneous environments. *Annu. Rev. Ecol. Syst.* 7: 1-32.
- Hoffmeyer, I. 1982. Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs subordinate conspecific males and to urine odours from dominant vs subordinate males. *Behav. Neural Biol.* 36: 178-188.
- Holl, J.W. & Robison, O.W. 2003. Results from nine generations of selection for increased litter size in swine. *J. Anim. Sci.* 81: 624-629.
- Hoogland, J. L. 1985. Infanticide in prairie dogs: lactating females kill offspring in close kin. *Science* 230: 1037-1040.
- Hoogland, J. L. 1995. *The black-Tailed Prairie Dog: Social Life of a Burrowing Mammal*. University of Chicago Press, Chicago.

- Horne, T. J. & Ylönen, H. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behav. Ecol. Sociobiol.* 38: 401–405.
- Horne, T. J. & Ylönen, H. 1998. Heritabilities of dominance-related traits in male bank voles (*Clethrionomys glareolus*). *Evolution* 52: 894–899.
- Houle, D. 1998. How should we explain variation in the genetic variance of traits? *Genetica* 102–103: 241–253.
- Hrdy, S. B. 1979. Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. *Ethol. Sociobiol.* 1: 13–40.
- Hrdy, S. B. Janson, C. H. & van Schaik, C. P. 1995. Infanticide: let's not throw out the baby with the bath water. *Evol. Anthropol.* 3: 151–154.
- Huck, U. W. 1984. Infanticide and the evolution of pregnancy block in rodents. In Hausfater, G. & Hrdy, S.B. (eds): *Infanticide: Comparative and Evolutionary Perspectives*. Aldine de Gruyter, New York. Pp. 349–365.
- Jacquard, A. 1983. Heritability: One word, three concepts. *Biometrics* 39(2): 465–477.
- Jonsson, P., Hartikainen, T., Koskela, E. & Mappes, T. 2002. Determinants of reproductive success in voles: space use in relation to food and litter size manipulation. *Evol. Ecol.* 16: 455–467.
- Kallio, E. R., Voutilainen, L., Vapalahti, O., Vaheri, A., Henttonen, H., Koskela, E., & Mappes, T. 2007. Endemic hantavirus infection impairs the winter survival of its rodent host. *Ecology* 88: 1911–1916.
- Kallio, E. R., Begon, M., Henttonen, H., Koskela, E., Mappes, T., Vaheri, A. & Vapalahti, O. 2009. Cyclic hantavirus epidemics in humans predicted by rodent host dynamics. *Epidemics*. 1: 101–107.
- Karbowiak, G. & Sinski, E. 1996. The finding of *Babesia microti* in bank vole *Clethrionomys glareolus* in the district of Mazury Lakes (Poland). *Acta Parasitol.* 41: 50–51.
- Ketterson, E. D., & Nolan, V. Jr. 1992. Hormones and life histories: an integrative approach. *Am. Nat.* 140: 533–562.
- Ketterson, E. D., & Nolan, V. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *Am. Nat.* 154(suppl.): S4–S25.
- Keymer, A.E. & Read, A.F. 1991. Behavioural ecology: the impact of parasitism. In Toft, C. A., Aeschliman, A. & Bolis, L. (eds): *Parasite-host associations. Coexistence or conflict?* Oxford University Press, Oxford. Pp. 37–61.
- Knopff, K. H., Knopff, A.R.A. & Pavelka M.S.M. 2004. Observed case of infanticide committed by a resident male central American black howler monkey (*Alouatta pigra*). *Am. J. Primatol.* 63: 239–244.
- Koivula, M., Koskela, E., Mappes, T. & Oksanen, T.A. 2003. Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology* 84: 398–405.
- Koskela, E., Mappes, T. & Ylönen, H. 1997. Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females. *J. Anim. Ecol.* 66: 341–349.

- Koskela, E., Jonsson, P., Hartikainen, T. & Mappes, T. 1998. Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. P. Roy. Soc. Lond. B Bio. 265: 1129-1134.
- Kruczek, M. 1997. Male rank and female choice in the bank vole, *Clethrionomys glareolus*. Behav. Process. 40: 171-176.
- Kruuk, L. E. B. 2004. Estimating genetic parameters in natural populations using the "animal model". Philos. T. Roy. Soc. B 359: 873-890.
- Labov, J. B., Huck, W. U., Elwood, R. W. & Brooks, R. J. 1985. Current problems in the study of infanticidal behavior of rodents. Q. Rev. Biol. 60: 1-20.
- Lambin, X. & Yoccoz, N. G. 1998. The impact of population kin-structure on nestling survival in Townsend's voles, *Microtus townsendii*. J. Anim. Ecol. 67: 1-16.
- Lande, R. 1975. The maintenance of genetic variation by mutation in a polygenic character with linked loci. Genet. Res. 16: 221-235.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33: 402-416.
- Le Boeuf, B. J. & Campagna, C. 1994. Protection and abuse of young in pinnipeds. In Parmigiani, S. and vom Saal, F. S. (eds): Infanticide and Parental Care. Harwood Academic Publishers, Chur, Switzerland. Pp. 257-276.
- Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87: 331.
- Lewontin, R.C. 1970. The units of selection. Annu. Rev. Ecol. Syst 1: 1-18.
- Lindström, J. 1999. Early development and fitness in birds and mammals. Trends Ecol. Evol. 14(9): 343-348.
- Lively, C. M. 2001. Host-Parasite Interactions. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): Evolutionary Ecology. Concepts and Case Studies. Oxford University Press, New York. Pp. 290- 302.
- Lochmiller, R.L. & Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88(1): 87-98.
- Lynch, M., & B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, MA.
- Lynch, M., Latta, L., Hicks, J. & Giorgianni, M. 1998. Mutation, selection, and the maintenance of life-history variation in a natural population. Evolution 52: 727-733.
- Mank, J. E. 2007. The evolution of sexually selected traits and antagonistic androgen expression in actinopterygian fishes. Am. Nat. 169: 142-149.
- Mann, M. A., Kinsley, C., Broida, J. & Svare, B. 1983. Infanticide exhibited by female mice: Genetic, developmental and hormonal influences. Physiol. Behav. 30(5): 697-702.
- Mappes, T., Koivula, M., Koskela, E., Oksanen, T.A., Savolainen, T. & Sinervo, B. 2008. Frequency and density-dependent selection on life-history strategies - a field experiment. PLoS ONE 3: e1687
- Martin, L. B., Navara, K.J., Weil, Z.M. & Nelson, R.J. 2007. Immunological memory is compromised by food restriction in deer mice *Peromyscus maniculatus*. Am. J. Physiol.-Reg. I. 292: R316-R320.

- Mazer, S. J. & Damuth, J. 2001. Evolutionary significance of variation. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, New York. Pp. 16-28.
- Mazer, S. J. & Schich, C. T. 1991. Constancy of population parameters for life history and floral traits in *Raphanus sativus* L: 1. Norms of reaction and the nature of genotype by environment interactions. *Heredity* 67: 143-156.
- Mazer, S. J. & Wolfe, L. M. 1998. Density-mediated maternal effects on seed in wild radish: Genetic variation and its evolutionary implications. In Mosseau, T.A. & Fox, C. W. (eds): *Maternal effects as adaptations*. Oxford University Press, New York. Pp. 323-343
- Mazurkiewicz, M. 1971. Shape, size and distribution of home ranges of *Clethrionomys glareolus* (Schreber 1780). *Acta Theriol.* 16: 23-60.
- McCarthy, M.M., & vom Saal, F. 1985. The influence of reproductive state on infanticide by wild female house mice (*Mus musculus*). *Physiol. Behav.* 35: 843-849.
- McCarthy, M.M., & vom Saal, F. 1986. Inhibition of infanticide after mating by wild male house mice. *Physiol. Behav.* 36: 203-209.
- McGlothlin, J. W. & Ketterson, E. D. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. *Philos. T. Roy. Soc. B.* 363(1497): 1611-1620.
- McGuigan, K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. *Mol. Ecol.* 15(4): 883-896.
- Mennella, J. A. & Moltz, H. 1988. Infanticide in rats: male strategy and female counter-strategy. *Physiol. Behav.* 42: 19-28.
- Migula, P. 1969. Bioenergetics of pregnancy and lactation in the European common vole. *Acta Theriol.* 14: 167-179.
- Mills, S. C., Alatalo, R. V., Koskela, E., Mappes, J., Mappes, T. & Oksanen T. A. 2007a. Signal reliability compromised by genotype by environment interaction and potential mechanisms for its preservation. *Evolution* 61(7): 1748-1757.
- Mills, S. C., Grapputo, A., Koskela, E. & Mappes, T. 2007b. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *P. Roy. Soc. Lond. B Bio.* 274: 143-150.
- Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., Oksanen, T.A. & Poikonen, T. 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. *Am. Nat.* 173: 475-487.
- Møller, A. P. & Saino, N. 2004. Immune response and survival. *Oikos* 104: 299-304.
- Murray, C. M., Wroblewski, E. & Pusey, A.E. 2007. New case of intragroup infanticide in the chimpanzees of Gombe national park. *Int. J. Primatol.* 28(1): 23-37.
- Noorwijk, A. J. & de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* 128: 137-142.
- Norris, K., & Evans, M. R. 2000. Ecological immunology: life history tradeoffs and immune defense in birds. *Behav. Ecol.* 11: 19-26.

- Nunn, C. L., Lindenfors, P., Pursall, E. R. & Rolff, J. 2009. On sexual dimorphism in immune function. *Philos. T. Roy. Soc. B* 364: 61–69.
- Nunney, L. 2001. Population structure. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, New York. Pp. 70-83.
- Obrtel, R. 1974. Comparison of animal food eaten by *Apodemus flavicollis* and *Clethrionomys glareolus* in a lowland forest. *Zool. Listy* 23: 35-46.
- Oksanen, T. A., Alatalo, R.V., Horne, T. J., Koskela, E., Mappes, J. & Mappes, T. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. *P. Roy. Soc. Lond. B Bio.* 266: 1495–1499.
- Oksanen, T. A., Jokinen, I., Koskela, E., Mappes, T. & Vilpas, H. 2003. Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment. *J. Anim. Ecol.* 72: 321–330.
- Owen-Ashley, N. T., Hasselquist, D. & Wingfield, J. C. 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. *Am. Nat.* 164: 490–505.
- Packer, C. 2000. Infanticide is no fallacy. *Am. Anthropol., New Series* 102(4): 829-831.
- Packer, C. & Pusey, A. E. 1983. Adaptations of female lions to infanticide by incoming males. *Am. Nat.* 121: 716–728.
- Parmigiani, S. & vom Saal, F. S. 1994. *Infanticide and parental care*. Harwood Academic Publishers. Chur, Switzerland.
- Perrigo, G., Bryant, W. C., & vom Saal, F. S. 1989. Fetal, hormonal, and experiential factors influencing the mating-induced regulation of infanticide in male house mice. *Physiol. Behav.* 46: 121–128.
- Pigliucci, M. 2001. Phenotypic plasticity. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, New York. Pp. 58-69.
- Pitt, S. E., & Bale, E. M. 1995. Neonaticide, infanticide, and filicide: a review of the literature. *Bull. Am. Acad. Psychiatry Law* 23: 375– 386.
- Playfair, J. & Bancroft, G. 2005. *Infection and immunity*. Oxford University Press, New York.
- Poole, T. B. & Morgan, D. H. R. 1973. Differences in aggressive behavior between male mice (*Mus musculus* L.) in colonies of different sizes. *Anim. Behav.* 21: 788-795.
- Radwan, J., M. Chadzinska, M. Cichon, S. C. Mills, B. Matula, E. T. Sadowska, K. Baliga, A. Stanis, S. Lopuch, & P. Koteja. 2006. Metabolic costs of sexual advertisement in the bank vole, *Clethrionomys glareolus*. *Evol. Ecol. Res.* 8: 859–869.
- Read, A. F. 1988. Sexual selection and the role of parasites. *Trends Ecol. Evol.* 3(5): 97-102.
- Reed, W. L., Clark, M. E., Parker, P. G., Raouf, S. A., Arguedas, N., Monk, D. S., Snajdr, E., Nolan, V. Jr. & Ketterson, E. D. 2006. Physiological effects on demography: a long-term experimental study of testosterone's effects on fitness. *Am. Nat.* 167: 667–683.



- Roff, D. A. 1992. The evolution of life histories: theory and analysis. 535p. Chapman & Hall, Routledge.
- Roff, D. A. 1997. Evolutionary quantitative genetics. Chapman & Hall, New York.
- Roff, D. A. & Fairbairn, D. J. 2007. The evolution of trade-offs: where are we? *J. Evolution. Biol.* 20(2): 433-447.
- Roff, D. A., Stirling, G. & Fairbairn, D. J. 1997. The evolution of threshold traits: a quantitative genetic analysis of the physiological and life history correlates of wing dimorphism in the sand cricket. *Evolution*, 51(6): 1910-1919.
- Roitt, I.M., Brostoff, J. & Male, D.K. 1988. Immunology. Mosby, London.
- Rolff, J. 2002. Bateman's principle and immunity. *P. Roy. Soc. Lond. B Bio.* 269: 867-872.
- Rosenberg, K.M. & Sherman, G.F. 1974. Testosterone induced pup-killing behavior in the ovariectomized female rat. *Physiol. Behav.* 13: 697-699.
- Rosenberg, K. M. & Sherman, G. F. 1975. The role of testosterone in the organization, maintenance and activation of pup killing in the male rat. *Horm. Behav.* 6: 173-179.
- Sheehan M. J. & Tibbetts, E. A. 2009. Evolution of identity signals: Frequency-dependent benefits of distinctive phenotypes used for individual recognition. *Evolution* 63-12: 3106-3113.
- Sheldon, B. C. & Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* 11: 317-321.
- Sherman, P. W. 1981. Reproductive competition and infanticide in Belding's ground squirrel and other animals. In R. D. Alexander and Tinkle, D.W. (eds): *Natural selection and social behavior: recent research and new theory*. Chiron Press, New York. Pp. 311-331.
- Sikorski, M. S. & Wójcik, A. M. 1990. Mating system and reproductive success in a free-living population of the bank vole, *Clethrionomys glareolus*. In Tamarin, R. H., Ostfeld, R. S., Pugh, S. R. & Bujalska, G. (eds): *Social systems and population cycles in voles*. Springer-Verlag, Basel. Pp. 193-202.
- Silk, J., & Stanford, C. 1999. Infanticide article disputed. *Anthropology News* 9: 27-29.
- Soroker, V., & Terkel, J. 1988. Changes in incidence of infanticidal and parental responses during the reproductive cycle in male and female wild mice *Mus musculus*. *Anim. Behav.* 36: 1275-1281.
- Soveri, T., Henttonen, H., Rudback, E., Schildt, R., Tanskanen, R., Husu-Kallio, J., Haukisalmi, V., Sukura, A. & Laakkonen, J. 2000. Disease patterns in field and bank vole populations during a cyclic decline in central Finland. *Comp. Immunol. Microbiol. Infect. Dis.* 23: 73-89.
- Stenseth, N. C. 1985. Geographic distribution of *Clethrionomys* species. *Ann. Zool. Fenn.* 22: 215-219.
- Sussman, R. W., Cheverud, J. M. & Bartlett T. Q. 1995. Infant killing as an evolutionary strategy: Reality or myth? *Evol. Anthropol.* 3(5): 149-151.
- Svare, B. 1979. Steroidal influences on pup-killing behavior in mice. *Horm. Behav.* 13: 153-164.

- Svare, B. & Mann, M. 1981. Infanticide: Genetic, developmental and hormonal influences in mice. *Phys. Behav.* 27: 921-927.
- Tuomi, J., Agrell, J. and Mappes, T. 1997. On the evolutionary stability of female infanticide. *Behav. Ecol.Sociobiol.* 40: 227-233.
- Tälleklint, L. & Jaenson, T. G. T. 1996. Relationship between *Ixodes ricinus* (Acari: Ixodidae) density and the prevalence of infection with Borrelialike spirochetes and the density of infected ticks. *J. Med. Entomol.* 33: 805– 811.
- Trivers, R. L. 1972. Parental investment and sexual selection. In Campbell, B. (ed.): *Sexual Selection and the Descent of Man 1871-1971*. Chicago: Aldine de Gruyter. Pp. 136-179.
- van Schaik, C. P. & Janson C. H. 2000. *Infanticide by males and its implications*. Cambridge Univ. Press, Cambridge.
- Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39: 505-522.
- Viney, M. E., Riley, E. M. & Buchanan, K. L. 2005. Optimal immune responses: immunocompetence revisited. *Trends Ecol. Evol.* 20: 665–669.
- vom Saal, F. S. 1985. Time-contingent change in infanticide and parental behaviour induced by ejaculation in male mice. *Physiol. Behav.* 34:7–15.
- vom Saal, F.S., & Howard, L. S. 1982. The regulation of infanticide and parental behaviour: implications for reproductive success in male mice. *Science* 215: 1270–1272.
- Wakelin, D. 1996. *Immunity to parasites*. Cambridge University Press, Cambridge.
- Wedekind, C. & Folstad, I. 1994. Adaptive or nonadaptive immunosuppression by sex hormones. *Am. Nat.* 143: 936– 938.
- Wilson, D. S. 2001. Cooperation and altruism. In Fox, C. W., Roff, D. A. & Fairbairn, D. J. (eds): *Evolutionary Ecology. Concepts and Case Studies*. Oxford University Press, New York. Pp. 222-231.
- Wolff, J. O., & Cicirello, D.M. 1991. Comparative paternal and infanticidal behavior of sympatric white-footed mice (*Peromyscus leucopus noveboracensis*) and deermice (*P. maniculatus nubiterrae*). *Behav. Ecol.* 2(1): 38-45.
- Wolff, J. O. & Macdonald, D. W. 2004. Promiscuous females protect their offspring. *Trends Ecol. Evol.* 19: 127–134.
- Wrangham, R. W. 1980. An ecological model of female-bonded primate groups. *Behaviour* 75: 262-299.
- Ylönen, H. Koskela, E. & Mappes, T. 1997. Infanticide in the bank vole (*Clethrionomys glareolus*): occurrence and the effect of familiarity on female infanticide. *Ann. Zool. Fenn.* 34(4): 259-266.
- Zeller, F. J. 1971. Effects of testosterone and dihydrotestosterone on comb, testis, and pituitary gland of male fowl. *J. Reprod. Fertil.* 25: 125–127.
- Zysling, D., Greives, T., Breuner, C., Casto, J., Demas, G. & Ketterson, E. 2006. Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Horm. Behav.* 50: 200–207.

## ORIGINAL PAPERS

### I

#### **INFANTICIDE IN THE EVOLUTION OF REPRODUCTIVE SYNCHRONY: EFFECTS ON REPRODUCTIVE SUCCESS**

by

Tanja Poikonen, Esa Koskela, Tapio Mappes and Suzanne C. Mills 2008

Evolution 62:612-621

Reprinted with kind permission by Wiley-Blackwell publishing ©



## II

# IS INFANTICIDAL BEHAVIOUR RELATED TO REPRODUCTIVE EFFORT? - A QUANTITATIVE GENETIC ANALYSIS IN A SMALL MAMMAL

by

Tanja Poikonen, Tapio Mappes & Eero Schroderus

Manuscript

### **III**

## **ORIGIN AND MAINTENANCE OF INFANTICIDE - EXPERIMENTAL EVIDENCE FOR NEGATIVE FREQUENCY- DEPENDENT SELECTION**

by

Tapio Mappes, Jouni Aspi, Esa Koskela, Suzanne C. Mills, Tanja Poikonen and  
Juha Tuomi

Manuscript

**IV**

**FITNESS TRADE-OFFS MEDIATED BY IMMUNOSUPPRESSION  
COSTS IN A SMALL MAMMAL**

by

Suzanne C. Mills, Alessandro Grapputo, Ilmari Jokinen, Esa Koskela, Tapio  
Mappes and Tanja Poikonen 2010

Evolution 64: 166-179

Reprinted with kind permission by Wiley-Blackwell publishing ©

V

**FREQUENCY-DEPENDENT SELECTION SHAPING OPTIMAL  
IMMUNE RESPONSE IN FEMALE MAMMALS**

by

Tanja Poikonen, Ilmari Jokinen, Esa Koskela, Tapio Mappes and Suzanne C.  
Mills 2010

Manuscript

**VI**

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

by

Eero Schroderus, Ilmari Jokinen, Minna Koivula, Esa Koskela, Tapio Mappes  
Suzanne C. Mills, Tuula A. Oksanen and Tanja Poikonen 2010

American Naturalist 176(4):E90-E97.

Reprinted with kind permission by the American Naturalist ©

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

Eero Schroderus,<sup>1,\*</sup> Ilmari Jokinen,<sup>2</sup> Minna Koivula,<sup>3</sup> Esa Koskela,<sup>2</sup> Tapio Mappes,<sup>1</sup> Suzanne C. Mills,<sup>4</sup> Tuula A. Oksanen,<sup>1</sup> and Tanja Poikonen<sup>1</sup>

1. Centre of Excellence in Evolutionary Research, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland; 2. Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland; 3. MTT, Agrifood Research Finland, Biotechnology and Food Research, Biometrical Genetics, FI-31600 Jokioinen, Finland; 4. Unité de Service et de Recherche 3278 Centre National de Recherche Scientifique–Ecole Pratique des Hautes Etudes, Centre de Biologie et Ecologie Tropicale et Méditerranéenne, Université de Perpignan via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan cedex, France

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. 2007b). 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

\* Corresponding author; e-mail: eero.schroderus@jyu.fi.

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 ( $\mathbf{G}$ ) allows unconstrained evolutionary responses, while  $\mathbf{G}$  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  $\mathbf{G}$  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. 2007a, 2007b, 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 (Koi-vula 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. 2007a). 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. 2007b). Both male-male competition and

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

#### *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  $20^{\circ} \pm 2^{\circ}\text{C}$ . 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- $\mu\text{L}$  intraorbital blood sample collected

in heparinized 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  $-20^{\circ}\text{C}$ .

Plasma testosterone was measured using a radioimmunoassay kit (Testo-CTK, DiaSorin, Byk-Sangtec Diagnostica, Dietzenbach, Germany). Methods are described by Mills et al. (2007a). 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  $\mu\text{L}$ ) 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 ( $\pm$ SD)	Transformed mean ( $\pm$ SD)
Female:			
Anti-BGG (U/mL)	456	$420 \times 10^3 \pm 134 \times 10^4$	$5.14 \pm .68$
IgG (U/mL)	515	$167 \times 10^4 \pm 106 \times 10^4$	$6.16 \pm .22$
Male:			
Anti-BGG (U/mL)	295	$457 \times 10^3 \pm 906 \times 10^3$	$5.32 \pm .56$
IgG (U/mL)	323	$121 \times 10^4 \pm 679 \times 10^3$	$6.02 \pm .25$
Testosterone (ng/mL)	343	$5.23 \pm 3.86$	$2.12 \pm .86$

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

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

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

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

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

in which  $\mathbf{\Gamma}$  is the matrix of factor loadings. Specific variances were restricted to zero, making analyses similar to principal component analyses. Dimensionality of  $\mathbf{G}$  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  $\mathbf{R}$  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  $\mathbf{G}$  matrix directly estimates only that part of the genetic variation that has statistical support (Blows 2007). Eigenanalysis was performed to estimate reduced-rank  $\mathbf{G}$  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 ( $\mathbf{G}$ ) 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 positive.

**Table 3:** Matrix of the factor loadings ( $\mathbf{\Gamma}$ )

	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. 2007a). 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

	$v_1$	$v_2$	$v_3$
%	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

Note: The matrix was based on testosterone and immune measures in the bank vole estimated using a factor-analytic REML-animal model.  $v_1$ ,  $v_2$ , and  $v_3$  = 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.

#### Literature Cited

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2:349–368.

- Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology* 20:1–8.
- Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371–1384.
- Bondrup-Nielsen, S., and F. Karlsson. 1985. Movements and spatial patterns in populations of *Clethrionomys* species: a review. *Annales Zoologici Fennici* 22:385–392.
- Boonekamp, J. J., A. H. F. Ros, and S. Verhulst. 2008. Immune activation suppresses plasma testosterone level: a meta-analysis. *Biology Letters* 4:741–744.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference*. Springer, New York.
- Dorner, G., R. Eckert, and G. Hinz. 1980. Androgen-dependent sexual dimorphism of the immune-system. *Endokrinologie* 76:112–114.
- Evans, M. R., A. R. Goldsmith, and S. R. Norris. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). *Behavioral Ecology and Sociobiology* 47:156–163.
- Fernald, R. D. 1976. The effect of testosterone on the behavior and coloration of adult male cichlid fish (*Haplochromis burtoni*, Guenther). *Hormone Research* 7:172–178.
- Folstad, I., and A. J. Karter. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist* 139:603–622.
- French, S. S., D. F. DeNardo, and M. C. Moore. 2007. Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *American Naturalist* 170:79–89.
- Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. *ASReml user guide release 1.0*. VSN International, Hemel Hempstead.
- Gilmour, A. R., B. R. Cullis, S. A. Harding, and R. Thompson. 2006. *ASReml update: what's new in release 2.00*. VSN International, Hemel Hempstead.
- Greives, T. J., J. W. McGlothlin, J. M. Jawor, G. E. Demas, and E. D. Ketterson. 2006. Testosterone and innate immune function inversely covary in a wild population of breeding dark-eyed juncos (*Junco hyemalis*). *Functional Ecology* 20:812–818.
- Greives, T. J., J. M. Casto, and E. D. Ketterson. 2007. Relative abundance of males to females affects behaviour, condition and immune function in a captive population of dark-eyed juncos *Junco hyemalis*. *Journal of Avian Biology* 38:255–260.
- Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* 29:133–144.
- Hine, E., and M. W. Blows. 2006. Determining the effective dimensionality of the genetic variance-covariance matrix. *Genetics* 173:1135–1144.
- Hoffmeyer, I. 1982. Responses of female bank voles (*Clethrionomys glareolus*) to dominant vs. subordinate conspecific males and to urine odors from dominant vs. subordinate males. *Behavioral and Neural Biology* 36:178–188.
- Horne, T. J., and H. Ylönen. 1998. Heritabilities of dominance-related traits in male bank voles (*Clethrionomys glareolus*). *Evolution* 52:894–899.
- Hosken, D. J. 2001. Sex and death: microevolutionary trade-offs between reproductive and immune investment in dung flies. *Current Biology* 11:R379–R380.
- Huitu, O., I. Jokinen, E. Korpimäki, E. Koskela, and T. Mappes. 2007. Phase dependence in winter physiological condition of cyclic voles. *Oikos* 116:565–577.
- Kallio, E. R. K., M. Begon, H. Henttonen, E. Koskela, T. Mappes, A. Vaehri, and O. Vapalahti. 2009. Cyclic hantavirus epidemics in humans: predicted by rodent host dynamics. *Epidemics* 1:101–107.
- Ketterson, E. D., and V. Nolan. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* 154(suppl.):S4–S25.
- Ketterson, E. D., V. Nolan, and M. Sandell. 2005. Testosterone in females: mediator of adaptive traits, constraint on sexual dimorphism, or both? *American Naturalist* 166(suppl.):S85–S98.
- Kirkpatrick, M., and K. Meyer. 2004. Direct estimation of genetic principal components: simplified analysis of complex phenotypes. *Genetics* 168:2295–2306.
- Koivula, M., E. Koskela, T. Mappes, and T. A. Oksanen. 2003. Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology* 84:398–405.
- Koskela, E., T. Mappes, and H. Ylönen. 1997. Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females. *Journal of Animal Ecology* 66:341–349.
- Kruuk, L. E. B. 2004. Estimating genetic parameters in natural populations using the “animal model.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 359:873–890.
- Lande, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain-body size allometry. *Evolution* 33:402–416.
- Lessells, C. M., and P. T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121.
- Mank, J. E. 2007. The evolution of sexually selected traits and antagonistic androgen expression in actinopterygian fishes. *American Naturalist* 169:142–149.
- McGlothlin, J. W., and E. D. Ketterson. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363:1611–1620.
- McKean, K. A., L. Nunney, and K. Hughes. 2008. Sexual selection and immune function in *Drosophila melanogaster*. *Evolution* 62:386–400.
- Meyer, K. 2007. Multivariate analyses of carcass traits for Angus cattle fitting reduced rank and factor analytic models. *Journal of Animal Breeding and Genetics* 124:50–64.
- Meyer, K., and M. Kirkpatrick. 2008. Perils of parsimony: properties of reduced-rank estimates of genetic covariance matrices. *Genetics* 180:1153–1166.
- Mills, S. C., A. Grapputo, E. Koskela, and T. Mappes. 2007a. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proceedings of the Royal Society B: Biological Sciences* 274:143–150.
- Mills, S. C., R. V. Alatalo, E. Koskela, J. Mappes, T. Mappes, and T. A. Oksanen. 2007b. Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution* 61:1748–1757.
- Mills, S. C., A. Grapputo, I. Jokinen, E. Koskela, T. Mappes, T. A. Oksanen, and T. Poikonen. 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. *American Naturalist* 173:475–487.
- Mills, S. C., I. Jokinen, E. Koskela, T. Mappes, T. A. Oksanen, and T. Poikonen. 2010. Fitness trade-offs mediated by immunosuppression costs in a small mammal. *Evolution* 64:166–179.
- Møller, A. P., and N. Saino. 2004. Immune response and survival. *Oikos* 104:299–304.
- Nunn, C. L., P. Lindenfors, E. R. Pursall, and J. Rolff. 2009. On sexual

- dimorphism in immune function. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:61–69.
- Oksanen, T. A., R. V. Alatalo, T. J. Horne, E. Koskela, J. Mappes, and T. Mappes. 1999. Maternal effort and male quality in the bank vole, *Clethrionomys glareolus*. *Proceedings of the Royal Society B: Biological Sciences* 266:1495–1499.
- Oksanen, T. A., I. Jokinen, E. Koskela, T. Mappes, and H. Vilpas. 2003. Manipulation of offspring number and size: benefits of large body size at birth depend upon the rearing environment. *Journal of Animal Ecology* 72:321–330.
- Owen-Ashley, N. T., D. Hasselquist, and J. C. Wingfield. 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. *American Naturalist* 164:490–505.
- Pease, C. M., and J. J. Bull. 1988. A critique of methods for measuring life-history trade-offs. *Journal of Evolutionary Biology* 1:293–303.
- Peters, A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proceedings of the Royal Society B: Biological Sciences* 267:883–889.
- Roberts, M. L., K. L. Buchanan, and M. R. Evans. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. *Animal Behaviour* 68:227–239.
- Rolff, J. 2002. Bateman's principle and immunity. *Proceedings of the Royal Society B: Biological Sciences* 269:867–872.
- Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 11:317–321.
- Soveri, T., H. Henttonen, E. Rudback, R. Schildt, R. Tanskanen, J. Husu-Kallio, V. Haukisalmi, A. Sukura, and J. Laakkonen. 2000. Disease patterns in field and bank vole populations during a cyclic decline in central Finland. *Comparative Immunology Microbiology and Infectious Diseases* 23:73–89.
- Stearns, S. C. 1992. *The evolution of life histories*. Oxford University Press, Oxford.
- Stenseth, N. C. 1985. Geographic distribution of *Clethrionomys* species. *Annales Zoologici Fennici* 22:215–219.
- Tizard, I. R. 2008. *Veterinary immunology*. Saunders, St. Louis.
- Verhulst, S., S. J. Dieleman, and H. K. Parmentier. 1999. A trade-off between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences of the USA* 96:4478–4481.
- Viney, M. E., E. M. Riley, and K. L. Buchanan. 2005. Optimal immune responses: immunocompetence revisited. *Trends in Ecology & Evolution* 20:665–669.
- Von Schantz, T., M. Tufvesson, G. Goeransson, M. Grahm, M. Wilhelmson, and H. Wittzell. 1995. Artificial selection for increased comb size and its effects on other sexual characters and viability in *Gallus domesticus* (the domestic chicken). *Heredity* 75:518–529.
- Wedekind, C., and I. Folstad. 1994. Adaptive or nonadaptive immunosuppression by sex hormones. *American Naturalist* 143:936–938.
- Zeller, F. J. 1971. Effects of testosterone and dihydrotestosterone on comb, testis, and pituitary gland of male fowl. *Journal of Reproduction and Fertility* 25:125–127.
- Zysling, D., T. Greives, C. Breuner, J. Casto, G. Demas, and E. Ketterson. 2006. Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*). *Hormones and Behavior* 50:200–207.

Associate Editor: Ellen D. Ketterson  
Editor: Mark A. McPeck

BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

- 1 RAATIKAINEN, M. & VASARAINEN, A., Damage caused by timothy flies (*Amaurosoma* spp.) in Finland, pp. 3-8.  
SÄRKKÄ, J., The numbers of *Tubifex tubifex* and its cocoons in relation to the mesh size, pp. 9-13.  
ELORANTA, P. & ELORANTA, A., Keuruselän kalastosta ja sen rakenteesta. - On the fish fauna of Lake Keuruselkä, Finnish Lake District, pp. 14-29.  
ELORANTA, P. & ELORANTA, A., Kuusveden veden laadusta, kasviplanktonista ja kalastosta. - On the properties of water, phytoplankton and fish fauna of Lake Kuusvesi, Central Finland, pp. 30-47. 47 p. 1975.
- 2 ELORANTA, V., Effects of different process wastes and main sewer effluents from pulp mills on the growth and production of *Ankistrodesmus falcatus* var. *acicularis* (Chlorophyta), pp. 3-33.  
ELORANTA, P. & KUNNAS, S., A comparison of littoral periphyton in some lakes of Central Finland, pp. 34-50.  
ELORANTA, P., Phytoplankton and primary production in situ in the lakes Jyväsjärvi and North Päijänne in summer 1974, pp. 51-66. 66 p. 1976.
- 3 RAATIKAINEN, M., HALKKA, O., VASARAINEN, A. & HALKKA, L., Abundance of *Philaenus spumarius* in relation to types of plant community in the Tvärminne archipelago, southern Finland. 38 p. 1977
- 4 HAKKARI, L., On the productivity and ecology of zooplankton and its role as food for fish in some lakes in Central Finland. 87 p. 1978.
- 5 KÄPYLÄ, M., Bionomics of five woodnesting solitary species of bees (Hym., Megachilidae), with emphasis on flower relationships. 89 p. 1978.
- 6 KANKAALA, P. & SAARI, V., The vascular flora of the Vaarunvuoret hills and its conservation, pp. 3-62.  
TÖRMÄLÄ, T. & KOVANEN, J., Growth and ageing of magpie (*Pica pica* L.) nestlings, pp. 63-77. 77 p. 1979.
- 7 VIITALA, J., Hair growth patterns in the vole *Clethrionomys rufocanus* (Sund.), pp. 3-17.  
NIEMI, R. & HUHTA, V., Oribatid communities in artificial soil made of sewage sludge and crushed bark, pp. 18-30. 30 p. 1981.
- 8 TÖRMÄLÄ, T., Structure and dynamics of reserved field ecosystem in central Finland. 58 p. 1981.
- 9 ELORANTA, V. & KUIVASNIEMI, K., Acute toxicity of two herbicides, glyphosate and 2,4-D, to *Selenastrum capricornutum* Printz (Chlorophyta), pp. 3-18.  
ELORANTA, P. & KUNNAS, S., Periphyton accumulation and diatom communities on artificial substrates in recipients of pulp mill effluents, pp. 19-33.  
ELORANTA, P. & MARJA-AHO, J., Transect studies on the aquatic macrophyte vegetation of Lake Saimaa in 1980, pp. 35-65. 65 p. 1982.
- 10 LAKE PÄIJÄNNE SYMPOSIUM. 199 p. 1987.
- 11 SAARI, V. & OHENOJA, E., A check-list of the larger fungi of Central Finland. 74 p. 1988.
- 12 KOJOLA, I., Maternal investment in semi-domesticated reindeer (*Rangifer t. tarandus* L.). 26 p. *Yhteenveto* 2 p. 1989.
- 13 MERILÄINEN, J. J., Impact of an acid, polyhumic river on estuarine zoobenthos and vegetation in the Baltic Sea, Finland. 48 p. *Yhteenveto* 2 p. 1989.
- 14 LUMME, I., On the clone selection, ectomy-corrhizal inoculation of short-rotation willows (*Salix* spp.) and on the effects of some nutrients sources on soil properties and plant nutrition. 55 p. *Yhteenveto* 3 p. 1989.
- 15 KUITUNEN, M., Food, space and time constraints on reproduction in the common treecreeper (*Certhia familiaris* L.) 22 p. *Yhteenveto* 2 p. 1989.
- 16 YLÖNEN, H., Temporal variation of behavioural and demographical processes in cyclic *Clethrionomys* populations. 35 p. *Yhteenveto* 2 p. 1989.
- 17 MIKKONEN, A., Occurrence and properties of proteolytic enzymes in germinating legume seeds. 61 p. *Yhteenveto* 1 p. 1990.
- 18 KAINULAINEN, H., Effects of chronic exercise and ageing on regional energy metabolism in heart muscle. 76 p. *Yhteenveto* 1 p. 1990.
- 19 LAKSO, MERJA, Sex-specific mouse testosterone 16 $\alpha$ -hydroxylase (cytochrome P450) genes: characterization and genetic and hormonal regulations. 70 p. *Yhteenveto* 1 p. 1990.
- 20 SETÄLÄ, HEIKKI, Effects of soil fauna on decomposition and nutrient dynamics in coniferous forest soil. 56 p. *Yhteenveto* 2 p. 1990.
- 21 NÄRVÄNEN, ALE, Synthetic peptides as probes for protein interactions and as antigenic epitopes. 90 p. *Yhteenveto* 2 p. 1990.
- 22 ECOTOXICOLOGY SEMINAR, 115 p. 1991.
- 23 ROSSI, ESKO, An index method for environmental risk assessment in wood processing industry. 117 p. *Yhteenveto* 2 p. 1991.
- 24 SUHONEN, JUUKA, Predation risk and competition in mixed species tit flocks. 29 p. *Yhteenveto* 2 p. 1991.
- 25 SUOMEN MUUTTUVUUS LUONTO. Mikko Raatikaiselle omistettu juhla-kirja. 185 p. 1992.
- 26 KOSKIVAARA, MARI, Monogeneans and other parasites on the gills of roach (*Rutilus rutilus*) in Central Finland. Differences between four lakes and the nature of dactylogyrid communities. 30 p. *Yhteenveto* 2 p. 1992.
- 27 TASKINEN, JOUNI, On the ecology of two Rhipidocotyle species (Digenea: Bucephalidae) from two Finnish lakes. 31 p. *Yhteenveto* 2 p. 1992.
- 28 HUOVILA, ARI, Assembly of hepatitis B surface antigen. 73 p. *Yhteenveto* 1 p. 1992.
- 29 SALONEN, VEIKKO, Plant colonization of harvested peat surfaces. 29 p. *Yhteenveto* 2 p. 1992.



BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

- 30 JOKINEN, ILMARI, Immunoglobulin production by cultured lymphocytes of patients with rheumatoid arthritis: association with disease severity. 78 p. Yhteenveto 2 p. 1992.
- 31 PUNNONEN, Eeva-Liisa, Ultrastructural studies on cellular autophagy. Structure of limiting membranes and route of enzyme delivery. 77 p. Yhteenveto 2 p. 1993.
- 32 HAIMI, JARI, Effects of earthworms on soil processes in coniferous forest soil. 35 p. Yhteenveto 2 p. 1993.
- 33 ZHAO, GUOCHANG, Ultraviolet radiation induced oxidative stress in cultured human skin fibroblasts and antioxidant protection. 86 p. Yhteenveto 1 p. 1993.
- 34 RÄTTI, OSMO, Polyterritorial polygyny in the pied flycatcher. 31 p. Yhteenveto 2 p. 1993.
- 35 MARJOMÄKI, VARPU, Endosomes and lysosomes in cardiomyocytes. A study on morphology and function. 64 p. Yhteenveto 1 p. 1993.
- 36 KIHILSTRÖM, MARKKU, Myocardial antioxidant enzyme systems in physical exercise and tissue damage. 99 p. Yhteenveto 2 p. 1994.
- 37 MUOTKA, TIMO, Patterns in northern stream guilds and communities. 24 p. Yhteenveto 2 p. 1994.
- 38 EFFECT OF FERTILIZATION ON FOREST ECOSYSTEM 218 p. 1994.
- 39 KERVINEN, JUKKA, Occurrence, catalytic properties, intracellular localization and structure of barley aspartic proteinase. 65 p. Yhteenveto 1 p. 1994.
- 40 MAPPES, JOHANNA, Maternal care and reproductive tactics in shield bugs. 30 p. Yhteenveto 3 p. 1994.
- 41 SIKAMÄKI, PIIRKKO, Determinants of clutch-size and reproductive success in the pied flycatcher. 35 p. Yhteenveto 2 p. 1995.
- 42 MAPPES, TAPIO, Breeding tactics and reproductive success in the bank vole. 28 p. Yhteenveto 3 p. 1995.
- 43 LAITINEN, MARKKU, Biomonitoring of the responses of fish to environmental stress. 39 p. Yhteenveto 2 p. 1995.
- 44 LAPPALAINEN, PEKKA, The dinuclear Cu centre of cytochrome oxidase. 52 p. Yhteenveto 1 p. 1995.
- 45 RINTAMÄKI, PEKKA, Male mating success and female choice in the lekking black grouse. 23 p. Yhteenveto 2 p. 1995.
- 46 SUURONEN, TIINA, The relationship of oxidative and glycolytic capacity of longissimus dorsi muscle to meat quality when different pig breeds and crossbreeds are compared. 112 p. Yhteenveto 2 p. 1995.
- 47 KOSKENNIEMI, ESA, The ecological succession and characteristics in small Finnish polyhumic reservoirs. 36 p. Yhteenveto 1 p. 1995.
- 48 HOVI, MATTI, The lek mating system in the black grouse: the role of sexual selection. 30 p. Yhteenveto 1 p. 1995.
- 49 MARTTILA, SALLA, Differential expression of aspartic and cyteine proteinases, glutamine synthetase, and a stress protein, HVA1, in germinating barley. 54 p. Yhteenveto 1 p. 1996.
- 50 HUHTA, ESA, Effects of forest fragmentation on reproductive success of birds in boreal forests. 26 p. Yhteenveto 2 p. 1996.
- 51 OJALA, JOHANNA, Muscle cell differentiation in vitro and effects of antisense oligodeoxyribonucleotides on gene expression of contractile proteins. 157 p. Yhteenveto 2 p. 1996.
- 52 PALOMÄKI, RISTO, Biomass and diversity of macrozoobenthos in the lake littoral in relation to environmental characteristics. 27 p. Yhteenveto 2 p. 1996.
- 53 PUSENIUS, JYRKI, Intraspecific interactions, space use and reproductive success in the field vole. 28 p. Yhteenveto 2 p. 1996.
- 54 SALMINEN, JANNE, Effects of harmful chemicals on soil animal communities and decomposition. 28 p. Yhteenveto 2 p. 1996.
- 55 KOTIAHO, JANNE, Sexual selection and costs of sexual signalling in a wolf spider. 25 p. (96 p.). Yhteenveto 2 p. 1997.
- 56 KOSKELA, JUHA, Feed intake and growth variability in Salmonids. 27p. (108 p.). Yhteenveto 2 p. 1997.
- 57 NAARALA, JONNE, Studies in the mechanisms of lead neurotoxicity and oxidative stress in human neuroblastoma cells. 68 p. (126 p.). Yhteenveto 1 p. 1997.
- 58 AHO, TEIJA, Determinants of breeding performance of the Eurasian treecreeper. 27 p. (130 p.). Yhteenveto 2 p. 1997.
- 59 HAAPARANTA, AHTI, Cell and tissue changes in perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in relation to water quality. 43 p. (112 p.). Yhteenveto 3 p. 1997.
- 60 SOIMASUO, MARKUS, The effects of pulp and paper mill effluents on fish: a biomarker approach. 59 p. (158 p.). Yhteenveto 2 p. 1997.
- 61 MIKOLA, JUHA, Trophic-level dynamics in microbial-based soil food webs. 31 p. (110 p.). Yhteenveto 1 p. 1997.
- 62 RAHKONEN, RIITTA, Interactions between a gull tapeworm *Diphyllobothrium dendriticum* (Cestoda) and trout (*Salmo trutta* L.). 43 p. (69 p.). Yhteenveto 3 p. 1998.
- 63 KOSKELA, ESA, Reproductive trade-offs in the bank vole. 29 p. (94 p.). Yhteenveto 2 p. 1998.
- 64 HORNE, TAINA, Evolution of female choice in the bank vole. 22 p. (78 p.). Yhteenveto 2 p. 1998.
- 65 PIRHONEN, JUHANI, Some effects of cultivation on the smolting of two forms of brown trout (*Salmo trutta*). 37 p. (97 p.). Yhteenveto 2 p. 1998.
- 66 LAAKSO, JOUNI, Sensitivity of ecosystem functioning to changes in the structure of soil food webs. 28 p. (151 p.). Yhteenveto 1 p. 1998.
- 67 NIKULA, TUOMO, Development of radiolabeled monoclonal antibody constructs: capable of transporting high radiation dose into cancer cells. 45 p. (109 p.). Yhteenveto 1 p. 1998.

## BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

- 68 AIRENNE, KARI, Production of recombinant avidins in *Escherichia coli* and insect cells. 96 p. (136 p.). Yhteenveto 2 p. 1998.
- 69 LYYTIKÄINEN, TAPANI, Thermal biology of underyearling Lake Inari Arctic Charr *Salvelinus alpinus*. 34 p. (92 p.). Yhteenveto 1 p. 1998.
- 70 VIHINEN-RANTA, MAIJA, Canine parvovirus. Endocytic entry and nuclear import. 74 p. (96 p.). Yhteenveto 1 p. 1998.
- 71 MARTIKAINEN, ESKO, Environmental factors influencing effects of chemicals on soil animals. Studies at population and community levels. 44 p. (137 p.). Yhteenveto 1 p. 1998.
- 72 AHLROTH, PETRI, Dispersal and life-history differences between waterstrider (*Aquarius najas*) populations. 36 p. (98 p.). Yhteenveto 1 p. 1999.
- 73 SIPPONEN, MATTI, The Finnish inland fisheries system. The outcomes of private ownership of fishing rights and of changes in administrative practices. 81 p. (188 p.). Yhteenveto 2 p. 1999.
- 74 LAMMI, ANTTI, Reproductive success, local adaptation and genetic diversity in small plant populations. 36 p. (107 p.). Yhteenveto 4 p. 1999.
- 75 NIVA, TEUVO, Ecology of stocked brown trout in boreal lakes. 26 p. (102 p.). Yhteenveto 1 p. 1999.
- 76 PULKKINEN, KATIJA, Transmission of *Triaenophorus crassus* from copepod first to coregonid second intermediate hosts and effects on intermediate hosts. 45 p. (123 p.). Yhteenveto 3 p. 1999.
- 77 PARRI, SILJA, Female choice for male drumming characteristics in the wolf spider *Hygrolycosa rubrofasciata*. 34 p. (108 p.). Yhteenveto 2 p. 1999.

## JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 78 VIROLAINEN, KAIJA, Selection of nature reserve networks. - Luonnonsuojelualueiden valinta. 28 p. (87 p.). Yhteenveto 1 p. 1999.
- 79 SELIN, PIRKKO, Turvevarojen teollinen käyttö ja suopohjan hyödyntäminen Suomessa. - Industrial use of peatlands and the re-use of cut-away areas in Finland. 262 p. Foreword 3 p. Executive summary 9 p. 1999.
- 80 LEPPÄNEN, HARRI, The fate of resin acids and resin acid-derived compounds in aquatic environment contaminated by chemical wood industry. - Hartsihappojen ja hartsihappoppe- räisten yhdisteiden ympäristökohtalo kemiallisen puunjalostusteollisuuden likaamissa vesistöissä. 45 p. (149 p.). Yhteenveto 2 p. 1999.
- 81 LINDSTRÖM, LEENA, Evolution of conspicuous warning signals. - Näkyvien varoitussignaalien evoluutio. 44 p. (96 p.). Yhteenveto 3 p. 2000.
- 82 MATTILA, ELISA, Factors limiting reproductive success in terrestrial orchids. - Kämmeköiden lisääntymismenestystä rajoittavat tekijät. 29 p. (95 p.). Yhteenveto 2 p. 2000.
- 83 KARELS, AARNO, Ecotoxicity of pulp and paper mill effluents in fish. Responses at biochemical, individual, population and community levels. - Sellu- ja paperiteollisuuden jätevesien ekotoksisuus kaloille. Tutkimus kalojen biokemiallisista, fysiologisista sekä populaatio- ja yhteisövasteista. 68 p. (177 p.). Yhteenveto 1 p. Samenvatting 1 p. 2000.
- 84 AALTONEN, TUULA, Effects of pulp and paper mill effluents on fish immune defence. - Met- säteollisuuden jätevesien aiheuttamat immunologiset muutokset kaloissa. 62 p. (125 p.). 2000.
- 85 HELENIUS, MERJA, Aging-associated changes in NF-kappa B signaling. - Ikääntymisen vaikutus NF-kappa B:n signalointiin. 75 p. (143 p.). Yhteenveto 2 p. 2000.
- 86 HUOVINEN, PIRJO, Ultraviolet radiation in aquatic environments. Underwater UV penetration and responses in algae and zooplankton. - Ultraviolettisaiteilyn vedenalain- nen tunkeutuminen ja sen vaikutukset leviin ja eläinplanktoniin. 52 p. (145 p.). Yhteenveto 2 p. 2000.
- 87 PÄÄKKÖNEN, JARI-PEKKA, Feeding biology of burbot, *Lota lota* (L.): Adaptation to profundal lifestyle? - Mateen, *Lota lota* (L), ravinnon- käytön erityispiirteet: sopeumia pohja- elämään? 33 p. (79 p.). Yhteenveto 2 p. 2000.
- 88 LAASONEN, PEKKA, The effects of stream habit restoration on benthic communities in boreal headwater streams. - Koskikunnostuksen vaikutus jokien pohjaeläimistöön. 32 p. (101 p.). Yhteenveto 2 p. 2000.
- 89 PASONEN, HANNA-LEENA, Pollen competition in silver birch (*Betula pendula* Roth). An evolutionary perspective and implications for commercial seed production. - Siitepölykilpailu koivulla. 41 p. (115 p.). Yhteenveto 2 p. 2000.
- 90 SALMINEN, ESA, Anaerobic digestion of solid poultry slaughterhouse by-products and wastes. - Siipikarjateurastuksen sivutuotteiden ja jätteiden anaerobinen käsittely. 60 p. (166 p.). Yhteenveto 2 p. 2000.
- 91 SALO, HARRI, Effects of ultraviolet radiation on the immune system of fish. - Ultravioletti- säiteilyn vaikutus kalan immunologiseen puolustusjärjestelmään. 61 p. (109 p.). Yhteenveto 2 p. 2000.
- 92 MUSTAJÄRVI, KAISA, Genetic and ecological consequences of small population size in *Lychnis viscaria*. - Geneettisten ja ekologisten tekijöiden vaikutus pienten mäkitervakko- populaatioiden elinkykyyn. 33 p. (124 p.). Yhteenveto 3 p. 2000.



JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 93 TIKKA, PÄIVI, Threatened flora of semi-natural grasslands: preservation and restoration. - Niittykasvillisuuden säilyttäminen ja ennallistaminen. 35 p. (105 p.). Yhteenveto 2 p. 2001.
- 94 SIITARI, HELI, Ultraviolet sensitivity in birds: consequences on foraging and mate choice. - Lintujen ultravioletin ekologinen merkitys ravinnon- ja puolisonvalinnassa. 31 p. (90 p.). Yhteenveto 2 p. 2001.
- 95 VERTAINEN, LAURA, Variation in life-history traits and behaviour among wolf spider (*Hygrolycosa rubrofasciata*) populations. - Populaatioiden väliset erot rummuttavan hämähäkin *Hygrolycosa rubrofasciata* kasvussa ja käyttäytymisessä. 37 p. (117 p.) Yhteenveto 2 p. 2001.
- 96 HAAPALA, ANTTI, The importance of particulate organic matter to invertebrate communities of boreal woodland streams. Implications for stream restoration. - Hiukkasmaisen orgaanisen aineksen merkitys pohjoisten metsäjokien pohja-eläinyhteisöille - huomioita virtavesien kunnostushankkeisiin. 35 p. (127 p.) Yhteenveto 2 p. 2001.
- 97 NISSINEN, LIISA, The collagen receptor integrins - differential regulation of their expression and signaling functions. - Kollageeniin sitoutuvat integriinit - niiden toisistaan eroava säätely ja signalointi. 67 p. (125 p.) Yhteenveto 1 p. 2001.
- 98 AHLROTH, MERVI, The chicken avidin gene family. Organization, evolution and frequent recombination. - Kanan avidiini-geeniperhe. Organisaatio, evoluutio ja tiheä rekombinaatio. 73 p. (120 p.) Yhteenveto 2 p. 2001.
- 99 HYÖTYLÄINEN, TARJA, Assessment of ecotoxicological effects of creosote-contaminated lake sediment and its remediation. - Kreosotilla saastuneen järvisedimentin ekotoksikologisen riskin ja kunnostuksen arviointi. 59 p. (132 p.) Yhteenveto 2 p. 2001.
- 100 SULKAVA, PEKKA, Interactions between faunal community and decomposition processes in relation to microclimate and heterogeneity in boreal forest soil. - Maaperän eliöyhteisön ja hajotusprosessien väliset vuorovaikutukset suhteessa mikroilmastoon ja laikut-taisuuteen. 36 p. (94 p.) Yhteenveto 2 p. 2001.
- 101 LAITINEN, OLLI, Engineering of physicochemical properties and quaternary structure assemblies of avidin and streptavidin, and characterization of avidin related proteins. - Avidiinin ja streptaviidiinin kvaternäärirakenteen ja fysiokemiallisten ominaisuuksien muokkaus sekä avidiinin kaltaisten proteiinien karakterisointi. 81 p. (126 p.) Yhteenveto 2 p. 2001.
- 102 LYYTINEN, ANNE, Insect coloration as a defence mechanism against visually hunting predators. - Hyönteisten väritys puolustuksessa vihollisia vastaan. 44 p. (92 p.) Yhteenveto 3 p. 2001.
- 103 NIKKILÄ, ANNA, Effects of organic material on the bioavailability, toxicokinetics and toxicity of xenobiotics in freshwater organisms. - Orgaanisen aineksen vaikutus vierasaineiden biosaatavuuteen, toksikokinetiikkaan ja toksisuuteen vesieliöillä. 49 p. (102 p.) Yhteenveto 3 p. 2001.
- 104 LIIRI, MIRA, Complexity of soil faunal communities in relation to ecosystem functioning in coniferous forest soil. A disturbance oriented study. - Maaperän hajottajaeliöstön monimuotoisuuden merkitys metsäekosysteemin toiminnassa ja häiriön-siedossa. 36 p. (121 p.) Yhteenveto 2 p. 2001.
- 105 KOSKELA, TANJA, Potential for coevolution in a host plant - holoparasitic plant interaction. - Isäntäkasvin ja täysloiskasvin välinen vuorovaikutus: edellytyksiä koevoluutiolle? 44 p. (122 p.) Yhteenveto 3 p. 2001.
- 106 LAPPIVAARA, JARMO, Modifications of acute physiological stress response in whitefish after prolonged exposures to water of anthropogenically impaired quality. - Ihmistoiminnan aiheuttaman veden laadun heikentymisen vaikutukset planktonsiian fysiologisessa stressivasteessa. 46 p. (108 p.) Yhteenveto 3 p. 2001.
- 107 ECCARD, JANA, Effects of competition and seasonality on life history traits of bank voles. - Kilpailun ja vuodenaikaisvaihtelun vaikutus metsämyyrän elinkiertopiirteisiin. 29 p. (115 p.) Yhteenveto 2 p. 2002.
- 108 NIEMINEN, JOUNI, Modelling the functioning of experimental soil food webs. - Kokeellisten maaperäravintoverkkojen toiminnan mallintaminen. 31 p. (111 p.) Yhteenveto 2 p. 2002.
- 109 NYKÄNEN, MARKO, Protein secretion in *Trichoderma reesei*. Expression, secretion and maturation of cellobiohydrolase I, barley cysteine proteinase and calf chymosin in Rut-C30. - Proteiinien erittyminen *Trichoderma reesei*ssä. Sellobiohydraasi I:n, ohran kysteiini-proteiinaasin sekä vasikan kymosiinin ilmeneminen, erittyminen ja kypsyminen Rut-C30-mutanttikannassa. 107 p. (173 p.) Yhteenveto 2 p. 2002.
- 110 TIIROLA, MARJA, Phylogenetic analysis of bacterial diversity using ribosomal RNA gene sequences. - Ribosomaalisen RNA-geenin sekvenssien käyttö bakteeridiversiteetin fylogeneettisessä analyysissä. 75 p. (139 p.) Yhteenveto 2 p. 2002.
- 111 HONKAVAARA, JOHANNA, Ultraviolet cues in fruit-frugivore interactions. - Ultravioletin ekologinen merkitys hedelmiä syövien eläinten ja hedelmäkasvien välisissä vuorovaikutussuhteissa. 27 p. (95 p.) Yhteenveto 2 p. 2002.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 112 MARTTILA, ARI, Engineering of charge, biotin-binding and oligomerization of avidin: new tools for avidin-biotin technology. - Avidiinin varauksen, biotiininsitomisen sekä oligomerisaation muokkaus: uusia työkaluja avidiini-biotiiniteknologiaan. 68 p. (130 p.) Yhteenveto 2 p. 2002.
- 113 JOKELA, JARI, Landfill operation and waste management procedures in the reduction of methane and leachate pollutant emissions from municipal solid waste landfills. - Kaatopaikan operoinnin ja jätteen esikäsittelyn vaikutus yhdyskuntajätteen biohajoamiseen ja tyyppipäästöjen hallintaan. 62 p. (173 p.) Yhteenveto 3 p. 2002.
- 114 RANTALA, MARKUS J., Immunocompetence and sexual selection in insects. - Immunokompetenssi ja seksuaalivalinta hyönteisillä. 23 p. (108 p.) Yhteenveto 1 p. 2002.
- 115 OKSANEN, TUULA, Cost of reproduction and offspring quality in the evolution of reproductive effort. - Lisääntymisen kustannukset ja poikasten laatu lisääntymispanostuksen evoluutiossa. 33 p. (95 p.) Yhteenveto 2 p. 2002.
- 116 HEINO, JANI, Spatial variation of benthic macroinvertebrate biodiversity in boreal streams. Biogeographic context and conservation implications. - Pohjaeläinhyteisöjen monimuotoisuuden spatiaalinen vaihtelu pohjoisissa virtavesissä - eliömaantieteellinen yhteys sekä merkitys jokivesien suojelulle. 43 p. (169 p.) Yhteenveto 3 p. 2002.
- 117 SIIRA-PIETIKÄINEN, ANNE, Decomposer community in boreal coniferous forest soil after forest harvesting: mechanisms behind responses. - Pohjoisen havumetsämaan hajottajayhteisö hakkuiden jälkeen: muutoksiin johtavat mekanismit. 46 p. (142 p.) Yhteenveto 3 p. 2002.
- 118 KORTET, RAINE, Parasitism, reproduction and sexual selection of roach, *Rutilus rutilus* L. - Loisten ja taudinaiheuttajien merkitys kalan lisääntymisessä ja seksuaalivalinnassa. 37 p. (111 p.) Yhteenveto 2 p. 2003.
- 119 SUVILAMPI, JUHANI, Aerobic wastewater treatment under high and varying temperatures - thermophilic process performance and effluent quality. - Jätevesien käsittely korkeissa ja vaihtelevissa lämpötiloissa. 59 p. (156 p.) Yhteenveto 2 p. 2003.
- 120 PÄIVINEN, JUSSI, Distribution, abundance and species richness of butterflies and myrmecophilous beetles. - Perhosten ja muurahaispesissä elävien kovakuoriaisten levinneisyys, runsaus ja lajistollinen monimuotoisuus 44 p. (155 p.) Yhteenveto 2 p. 2003.
- 121 PAAVOLA, RIKU, Community structure of macroinvertebrates, bryophytes and fish in boreal streams. Patterns from local to regional scales, with conservation implications. - Selkärangattomien, vesisammalten ja kalojen yhteisö rakenne pohjoisissa virtavesissä - säännönmukaisuudet paikallisesta mittakaavasta alueelliseen ja luonnonsuojelullinen merkitys. 36 p. (121 p.) Yhteenveto 3 p. 2003.
- 122 SUIKKANEN, SANNA, Cell biology of canine parvovirus entry. - Koiran parvovirusinfektion alkuvaiheiden solubiologia. 88 p. (135 p.) Yhteenveto 3 p. 2003.
- 123 AHTIAINEN, JARI JUHANI, Condition-dependence of male sexual signalling in the drumming wolf spider *Hygrolycosa rubrofasciata*. - Koiraan seksuaalisen signaalin kunto riippuvuus rummuttavalla susihämähäkillä *Hygrolycosa rubrofasciata*. 31 p. (121 p.) Yhteenveto 2 p. 2003.
- 124 KAPARAJU, PRASAD, Enhancing methane production in a farm-scale biogas production system. - Metaanintuoton tehostaminen tilakohtaisessa biokaasuntuotantojärjestelmässä. 84 p. (224 p.) Yhteenveto 2 p. 2003.
- 125 HÄKKINEN, JANI, Comparative sensitivity of boreal fishes to UV-B and UV-induced phototoxicity of retene. - Kalojen varhaisvaiheiden herkkyys UV-B säteilylle ja reteenin UV-valoindusoituvalle toksisuudelle. 58 p. (134 p.) Yhteenveto 2 p. 2003.
- 126 NORDLUND, HENRI, Avidin engineering; modification of function, oligomerization, stability and structure topology. - Avidiinin toiminnan, oligomerisaation, kestävyden ja rakennetopologian muokkaaminen. 64 p. (104 p.) Yhteenveto 2 p. 2003.
- 127 MARJOMÄKI, TIMO J., Recruitment variability in vendace, *Coregonus albula* (L.), and its consequences for vendace harvesting. - Muikun, *Coregonus albula* (L.), vuosiluokkien runsauden vaihtelu ja sen vaikutukset kalastukseen. 66 p. (155 p.) Yhteenveto 2 p. 2003.
- 128 KILPIMAA, JANNE, Male ornamentation and immune function in two species of passerines. - Koiraan ornamentit ja immuunipuolustus varpuslinnuilla. 34 p. (104 p.) Yhteenveto 1 p. 2004.
- 129 PÖNNIÖ, TIIA, Analyzing the function of nuclear receptor Nor-1 in mice. - Hiiren tumareseptori Nor-1:n toiminnan tutkiminen. 65 p. (119 p.) Yhteenveto 2 p. 2004.
- 130 WANG, HONG, Function and structure, subcellular localization and evolution of the encoding gene of pentachlorophenol 4-monooxygenase in sphingomonads. 56 p. (90 p.) 2004.
- 131 YLÖNEN, OLLI, Effects of enhancing UV-B irradiance on the behaviour, survival and metabolism of coregonid larvae. - Lisääntyvän UV-B säteilyn vaikutukset siikakalojen poikasten käyttäytymiseen, kuolleisuuteen ja metaboliaan. 42 p. (95 p.) Yhteenveto 2 p. 2004.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 132 KUMPULAINEN, TOMI, The evolution and maintenance of reproductive strategies in bag worm moths (Lepidoptera: Psychidae). - Lisääntymisstrategioiden evoluutio ja säilyminen pussikehrääjillä (Lepidoptera: Psychidae). 42 p. (161 p.) Yhteenveto 3 p. 2004.
- 133 OJALA, KIRSI, Development and applications of baculoviral display techniques. - Bakulovirus display -tekniikoiden kehittäminen ja sovellukset. 90 p. (141 p.) Yhteenveto 3 p. 2004.
- 134 RANTALAINEN, MINNA-LIISA, Sensitivity of soil decomposer communities to habitat fragmentation - an experimental approach. - Metsämaaperän hajottajayhteisön vasteet elinympäristön pirstaloitumiseen. 38 p. (130 p.) Yhteenveto 2 p. 2004.
- 135 SAARINEN, MARI, Factors contributing to the abundance of the ergasilid copepod, *Paraergasilus rylovi*, in its freshwater molluscan host, *Anodonta piscinalis*. - *Paraergasilus rylovi* -loisäyriäisen esiintymiseen ja runsauteen vaikuttavat tekijät *Anodonta piscinalis* -pikkujärvisimpukassa. 47 p. (133 p.) Yhteenveto 4 p. 2004.
- 136 LILJA, JUHA, Assessment of fish migration in rivers by horizontal echo sounding: Problems concerning side-aspect target strength. - Jokeen vaeltavien kalojen laskeminen sivuttaisuuntaisella kaikuluotauksella: sivuaspektikohdevoimakkuuteen liittyviä ongelmia. 40 p. (82 p.) Yhteenveto 2 p. 2004.
- 137 NYKVIST, PETRI, Integrins as cellular receptors for fibril-forming and transmembrane collagens. - Integriinit reseptoreina fibrillaarisille ja transmembraanisille kollageeneille. 127 p. (161 p.) Yhteenveto 3 p. 2004.
- 138 KOIVULA, NIINA, Temporal perspective of humification of organic matter. - Orgaanisen aineen humuistuminen tarkasteltuna ajan funktiona. 62 p. (164 p.) Yhteenveto 2 p. 2004.
- 139 KARVONEN, ANSSI, Transmission of *Diplostomum spathaceum* between intermediate hosts. - *Diplostomum spathaceum* -loisen siirtyminen kotilo- ja kalaisännän välillä. 40 p. (90 p.) Yhteenveto 2 p. 2004.
- 140 NYKÄNEN, MARI, Habitat selection by riverine grayling, *Thymallus thymallus* L. - Harjuksen (*Thymallus thymallus* L.) habitatiinvalinta virtavesissä. 40 p. (102 p.) Yhteenveto 3 p. 2004.
- 141 HYNYNEN, JUHANI, Anthropogenic changes in Finnish lakes during the past 150 years inferred from benthic invertebrates and their sedimentary remains. - Ihmistoiminnan aiheuttamat kuormitusmuutokset suomalaisissa järvisä viimeksi kuluneiden 150 vuoden aikana tarkasteltuina pohjaeläinyhteisöjen avulla. 45 p. (221 p.) Yhteenveto 3 p. 2004.
- 142 PYLKKÖ, PÄIVI, Atypical *Aeromonas salmonicida* -infection as a threat to farming of arctic charr (*Salvelinus alpinus* L.) and european grayling (*Thymallus thymallus* L.) and putative means to prevent the infection. - Epätypinen *Aeromonas salmonicida* -bakteeritartunta uhkana harjukselle (*Thymallus thymallus* L.) ja nierialle (*Salvelinus alpinus* L.) laitoskasvatuksessa ja mahdollisia keinoja tartunnan ennaltaehkäisyyn. 46 p. (107 p.) Yhteenveto 2 p. 2004.
- 143 PUURTINEN, MIKAEL, Evolution of hermaphroditic mating systems in animals. - Kaksineuvoisten lisääntymisstrategioiden evoluutio eläimillä. 28 p. (110 p.) Yhteenveto 3 p. 2004.
- 144 TOLVANEN, OUTI, Effects of waste treatment technique and quality of waste on bioaerosols in Finnish waste treatment plants. - Jätteenkäsittelytekniikan ja jätelaadun vaikutus bioaerosolipitoisuuksiin suomalaisilla jätteenkäsittelylaitoksilla. 78 p. (174 p.) Yhteenveto 4 p. 2004.
- 145 BOADI, KWASI OWUSU, Environment and health in the Accra metropolitan area, Ghana. - Accran (Ghana) suurkaupunkialueen ympäristö ja terveys. 33 p. (123 p.) Yhteenveto 2 p. 2004.
- 146 LUKKARI, TUOMAS, Earthworm responses to metal contamination: Tools for soil quality assessment. - Lierojen vasteet metallialtistukseen: käyttömahdollisuudet maaperän tilan arvioinnissa. 64 p. (150 p.) Yhteenveto 3 p. 2004.
- 147 MARTTINEN, SANNA, Potential of municipal sewage treatment plants to remove bis(2-ethylhexyl) phthalate. - Bis-(2-etyyliheksyyli)ftalaatin poistaminen jätevesistä yhdyskuntajätevedenpuhdistamoilla. 51 p. (100 p.) Yhteenveto 2 p. 2004.
- 148 KARISOLA, PIILA, Immunological characterization and engineering of the major latex allergen, hevein (Hev b 6.02). - Luonnonkumiallergian pääallergeenin, heveinin (Hev b 6.02), immunologisten ominaisuuksien karakterisointi ja muokkaus. 91 p. (113 p.) Yhteenveto 2 p. 2004.
- 149 BAGGE, ANNA MARIA, Factors affecting the development and structure of monogenean communities on cyprinid fish. - Kidusloisyhteisöjen rakenteeseen ja kehitykseen vaikuttavat tekijät sisävesikaloilla. 25 p. (76 p.) Yhteenveto 1 p. 2005.
- 150 JÄNTTI, ARI, Effects of interspecific relationships in forested landscapes on breeding success in Eurasian treecreeper. - Lajienvälisen suhteen vaikutus puukiipijän pesintämenestykseen metsäympäristössä. 39 p. (104 p.) Yhteenveto 2 p. 2005.
- 151 TYNKKYNNEN, KATJA, Interspecific interactions and selection on secondary sexual characters in damselflies. - Lajienväliset vuorovaikutukset ja seksuaaliominaisuuksiin kohdistuva valinta sudenkorennoilla. 26 p. (86 p.) Yhteenveto 2 p. 2005.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 152 HAKALAHTI, TEIJA, Studies of the life history of a parasite: a basis for effective population management. - Loisen elinkiertopiirteet: perusta tehokkaalle torjunnalle. 41 p. (90 p.) Yhteenveto 3 p. 2005.
- 153 HYTÖNEN, VESA, The avidin protein family: properties of family members and engineering of novel biotin-binding protein tools. - Avidiini-proteiiniperhe: perheen jäsenten ominaisuuksia ja uusia biotiiniä sitovia proteiiniyökaluja. 94 p. (124 p.) Yhteenveto 2 p. 2005.
- 154 GILBERT, LEONA, Development of biotechnological tools for studying infectious pathways of canine and human parvoviruses. 104 p. (156 p.) 2005.
- 155 SUOMALAINEN, LOTTA-RIINA, *Flavobacterium columnare* in Finnish fish farming: characterisation and putative disease management strategies. - *Flavobacterium columnare* Suomen kalanviljelyssä: karakterisointi ja mahdolliset torjuntamenetelmät. 52 p. (110 p.) Yhteenveto 1 p. 2005.
- 156 VEHNIAINEN, EEVA-RIIKKA, Boreal fishes and ultraviolet radiation: actions of UVR at molecular and individual levels. - Pohjoisen kalat ja ultraviolettisäteily: UV-säteilyn vaikutukset molekyyli- ja yksilötasolla. 52 p. (131 p.) 2005.
- 157 VAINIKKA, ANSSI, Mechanisms of honest sexual signalling and life history trade-offs in three cyprinid fishes. - Rehellisen seksuaalisen signaaloinnin ja elinkiertojen evoluution mekanismit kolmella särkikalalla. 53 p. (123 p.) Yhteenveto 2 p. 2005.
- 158 LUOSTARINEN, SARI, Anaerobic on-site wastewater treatment at low temperatures. Jätevesien kiinteistö- ja kyläkohtainen anaerobinen käsittely alhaisissa lämpötiloissa. 83 p. (168 p.) Yhteenveto 3 p. 2005.
- 159 SEPPÄLÄ, OTTO, Host manipulation by parasites: adaptation to enhance transmission? Loisten kyky manipuloida isäntiään: sopeuma transmission tehostamiseen? 27 p. (67 p.) Yhteenveto 2 p. 2005.
- 160 SUURINIEMI, MIIA, Genetics of children's bone growth. - Lasten luuston kasvun genetiikka. 74 p. (135 p.) Yhteenveto 3 p. 2006.
- 161 TOIVOLA, JOUNI, Characterization of viral nanoparticles and virus-like structures by using fluorescence correlation spectroscopy (FCS). - Virus-nanopartikkelien sekä virusten kaltaisten rakenteiden tarkastelu fluoresenssi korrelaatio spektroskopiolla. 74 p. (132 p.) Yhteenveto 2 p. 2006.
- 162 KLEMME, INES, Polyandry and its effect on male and female fitness. - Polyandria ja sen vaikutukset koiraan ja naaraan kelpoisuuteen 28 p. (92 p.) Yhteenveto 2 p. 2006.
- 163 LEHTOMÄKI, ANNIMARI, Biogas production from energy crops and crop residues. - Energia-kasvien ja kasvijätteiden hyödyntäminen biokaasun tuotannossa. 91 p. (186 p.) Yhteenveto 3 p. 2006.
- 164 ILMARINEN, KATJA, Defoliation and plant-soil interactions in grasslands. - Defoliaatio ja kasvien ja maaperän väliset vuorovaikutukset niittyekosysteemeissä. 32 p. (111 p.) Yhteenveto 2 p. 2006.
- 165 LOEHR, JOHN, Thinhorn sheep evolution and behaviour. - Ohutsarvilampaiden evoluutio ja käyttäytyminen. 27 p. (89 p.) Yhteenveto 2 p. 2006.
- 166 PAUKKU, SATU, Cost of reproduction in a seed beetle: a quantitative genetic perspective. - Lisääntymisen kustannukset jyväkuoriaisella: kvantitatiivisen genetiikan näkökulma. 27 p. (84 p.) Yhteenveto 1 p. 2006.
- 167 OJALA, KATJA, Variation in defence and its fitness consequences in aposematic animals: interactions among diet, parasites and predators. - Puolustuskyvyn vaihtelu ja sen merkitys aposemaattisten eläinten kelpoisuuteen: ravinnon, loisten ja saalistajien vuorovaikutus. 39 p. (121 p.) Yhteenveto 2 p. 2006.
- 168 MATILAINEN, HELI, Development of baculovirus display strategies towards targeting to tumor vasculature. - Syövän suonitukseen kohdentuvien bakulovirus display-vektorien kehittäminen. 115 p. (167 p.) Yhteenveto 2 p. 2006.
- 169 KALLIO, EVA R., Experimental ecology on the interaction between the Puumala hantavirus and its host, the bank vole. - Kokeellista ekologiaa Puumala-viruksen ja metsämyyrän välisestä vuorovaikutussuhteesta. 30 p. (75 p.) Yhteenveto 2 p. 2006.
- 170 PIHLAJA, MARJO, Maternal effects in the magpie. - Harakan äitivaikutukset. 39 p. (126p.) Yhteenveto 1 p. 2006.
- 171 IHALAINEN, EIRA, Experiments on defensive mimicry: linkages between predator behaviour and qualities of the prey. - Varoitussignaalin jäljittely puolustusstrategiana: kokeita petosaalis-suhteista. 37 p. (111 p.) Yhteenveto 2 p. 2006.
- 172 LÓPEZ-SEPULCRE, ANDRÉS, The evolutionary ecology of space use and its conservation consequences. - Elintilan käytön ja reviiirikäyttäytymisen evoluutioekologia luonnonsuojellullisine seuraamuksineen. 32 p. (119 p.) Yhteenveto 2 p. 2007.
- 173 TULLA, MIRA, Collagen receptor integrins: evolution, ligand binding selectivity and the effect of activation. - Kollageenireseptori-integriinien evoluutio, ligandin sitomisvalikoivuus ja aktivaation vaikutus. 67 p. (129 p.) Yhteenveto 2 p. 2007.
- 174 SINISALO, TUULA, Diet and foraging of ringed seals in relation to helminth parasite assemblages. - Perämeren ja Saimaan norpan suolistolohyönteisöt ja niiden hyödyntäminen hylkeen yksilöllisen ravintoekologian selvittämisessä. 38 p. (84 p.) Yhteenveto 2 p. 2007.



JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 175 TOIVANEN, TERO, Short-term effects of forest restoration on beetle diversity. - Metsien ennallistamisen merkitys kovakuoriaislajiston monimuotoisuudelle. 33 p. (112 p.) Yhteenveto 2 p. 2007.
- 176 LUDWIG, GILBERT, Mechanisms of population declines in boreal forest grouse. - Kanalintukantojen laskuun vaikuttavat tekijät. 48 p. (138 p.) Yhteenveto 2 p. 2007.
- 177 KETOLA, TARMO, Genetics of condition and sexual selection. - Kunnon ja seksuaalivalinnan genetiikka. 29 p. (121 p.) Yhteenveto 2 p. 2007.
- 178 SEPPÄNEN, JANNE-TUOMAS, Interspecific social information in habitat choice. - Lajienvälinen sosiaalinen informaatio habitaatinvalinnassa. 33 p. (89 p.) Yhteenveto 2 p. 2007.
- 179 BANDILLA, MATTHIAS, Transmission and host and mate location in the fish louse *Argulus coregoni* and its link with bacterial disease in fish. - *Argulus coregoni* -kalatäin siirtyminen kalaisäntään, isännän ja parittelukumppanin paikallistaminen sekä loisinnan yhteys kalan bakteeritautiin. 40 p. (100 p.) Yhteenveto 3 p. Zusammenfassung 4 p. 2007.
- 180 MERILÄINEN, PÄIVI, Exposure assessment of animals to sediments contaminated by pulp and paper mills. - Sellu- ja paperiteollisuuden saastuttamat sedimentit altistavana tekijänä vesieläimille. 79 p. (169 p.) Yhteenveto 2 p. 2007.
- 181 ROUTTU, JARKKO, Genetic and phenotypic divergence in *Drosophila virilis* and *D. montana*. - Geneettinen ja fenotyyppinen erilaistuminen *Drosophila virilis* ja *D. montana* lajien mahlakärpäisillä. 34 p. (106 p.) Yhteenveto 1 p. 2007.
- 182 BENESH, DANIEL P., Larval life history, transmission strategies, and the evolution of intermediate host exploitation by complex life-cycle parasites. - Väkäkärsämatotoukkien elinkierto- ja transmissiostrategiat sekä välisännän hyväksikäytön evoluutio. 33 p. (88 p.) Yhteenveto 1 p. 2007.
- 183 TAIPALE, SAMI, Bacterial-mediated terrestrial carbon in the foodweb of humic lakes. - Bakteerivälitteisen terrestrisen hiilen merkitys humusjärvien ravintoketjussa. 61 p. (131 p.) Yhteenveto 5 p. 2007.
- 184 KILJUNEN, MIKKO, Accumulation of organochlorines in Baltic Sea fishes. - Organoklooriyhdisteiden kertyminen Itämeren kaloihin. 45 p. (97 p.) Yhteenveto 3 p. 2007.
- 185 SORMUNEN, KAI MARKUS, Characterisation of landfills for recovery of methane and control of emissions. - Kaatopaikkojen karakterisointi metaanipotentiaalin hyödyntämiseksi ja päästöjen vähentämiseksi. 83 p. (157 p.) Yhteenveto 2 p. 2008.
- 186 HILTUNEN, TEPPO, Environmental fluctuations and predation modulate community dynamics and diversity. - Ympäristön vaihtelut ja saalistus muokkaavat yhteisön dynamiikkaa ja diversiteettiä. 33 p. (100 p.) Yhteenveto 2 p. 2008.
- 187 SYVÄRANTA, JARI, Impacts of biomanipulation on lake ecosystem structure revealed by stable isotope analysis. - Biomanipulaation vaikutukset järviökosysteemin rakenteeseen vakaiden isotooppien avulla tarkasteltuna. 46 p. (105 p.) Yhteenveto 4 p. 2008.
- 188 MATTILA, NIINA, Ecological traits as determinants of extinction risk and distribution change in Lepidoptera. - Perhosten uhanalaisuuteen vaikuttavat ekologiset piirteet. 21 p. (67 p.) Yhteenveto 1 p. 2008.
- 189 UPLA, PAULA, Integrin-mediated entry of echovirus 1. - Echovirus 1:n integriinivälitteinen sisäänmeno soluun. 86 p. (145 p.) Yhteenveto 2 p. 2008.
- 190 KESKINEN, TAPIO, Feeding ecology and behaviour of pikeperch, Sander lucioperca (L.) in boreal lakes. - Kuhan (Sander lucioperca (L.)) ravinnonkäyttö ja käyttäytyminen boreaalisissa järvissä. 54 p. (136 p.) Yhteenveto 3 p. 2008.
- 191 LAAKKONEN, JOHANNA, Intracellular delivery of baculovirus and streptavidin-based vectors *in vitro* - towards novel therapeutic applications. - Bakulovirus ja streptavidiini geeninsiirtovektoreina ihmisen soluissa. 81 p. (142 p.) Yhteenveto 2 p. 2008.
- 192 MICHEL, PATRIK, Production, purification and evaluation of insect cell-expressed proteins with diagnostic potential. - Diagnostisesti tärkeiden proteiinien tuotto hyönteissolussa sekä niiden puhdistus ja karakterisointi. 100 p. (119 p.) Yhteenveto 2 p. 2008.
- 193 LINDSTEDT, CARITA, Maintenance of variation in warning signals under opposing selection pressures. - Vastakkaiset evolutiiviset valintapaineet ylläpitävät vaihtelua varoitussignaalinnissa. 56 p. (152 p.) Yhteenveto 2 p. 2008.
- 194 BOMAN, SANNA, Ecological and genetic factors contributing to invasion success: The northern spread of the Colorado potato beetle (*Leptinotarsa decemlineata*). - Ekologisten ja geneettisten tekijöiden vaikutus koloradonkuoriaisen (*Leptinotarsa decemlineata*) leviämismenestykseen. 50 p. (113 p.) Yhteenveto 3 p. 2008.
- 195 MÄKELÄ, ANNA, Towards therapeutic gene delivery to human cancer cells. Targeting and entry of baculovirus. - Kohti terapeuttista geeninsiirtoa: bakuloviruksen kohdennus ja sisäänmeno ihmisen syöpäsoluihin. 103 p. (185 p.) Yhteenveto 2 p. 2008.
- 196 LEBIGRE, CHRISTOPHE, Mating behaviour of the black grouse. Genetic characteristics and physiological consequences. - Teeren pariutumiskäyttäytyminen. Geneettiset tekijät ja fysiologiset seuraukset. 32 p. (111 p.) Yhteenveto 2 p. 2008.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

- 197 KAKKONEN, ELINA, Regulation of raft-derived endocytic pathways – studies on echovirus 1 and baculovirus. - Echovirus 1:n ja bakuloviruksen soluun sisäänmenon reitit ja säätely. 96 p. (159 p.) Yhteenveto 2 p. 2009.
- 198 TENHOLA-ROININEN, TEIJA, Rye doubled haploids – production and use in mapping studies. - Rukiin kaksoishaploidit – tuotto ja käyttö kartoituksessa. 93 p. (164 p.) Yhteenveto 3 p. 2009.
- 199 TREBATICÁ, LENKA, Predation risk shaping individual behaviour, life histories and species interactions in small mammals. - Petoriskin vaikutus yksilön käyttäytymiseen, elinkiertojärjestelmään ja yksilöiden välisiin suhteisiin. 29 p. (91 p.) Yhteenveto 3 p. 2009.
- 200 PIETIKÄINEN, ANNE, Arbuscular mycorrhiza, resource availability and belowground interactions between plants and soil microbes. - Arbuskelimykorrhiza, resurssien saatavuus ja maanalaiset kasvien ja mikrobien väliset vuorovaikutukset. 38 p. (119 p.) Yhteenveto 2 p. 2009.
- 201 AROVIITA, JUUKA, Predictive models in assessment of macroinvertebrates in boreal rivers. - Ennustavat mallit jokien pohjaeläimistön tilan arvioinnissa. 45 p. (109 p.) Yhteenveto 3 p. 2009.
- 202 RASI, SAIJA, Biogas composition and upgrading to biomethane. - Biokaasun koostumus ja puhdistaminen biometaaniksi. 76 p. (135 p.) Yhteenveto 3 p. 2009.
- 203 PAKKANEN, KIRSI, From endosomes onwards. Membranes, lysosomes and viral capsid interactions. - Endosomeista eteenpäin. Lipidikalvoja, lysosomeja ja viruskapsidin vuorovaikutuksia. 119 p. (204 p.) Yhteenveto 2 p. 2009.
- 204 MARKKULA, EVELIINA, Ultraviolet B radiation induced alterations in immune function of fish, in relation to habitat preference and disease resistance. - Ultraviolet B -säteilyn vaikutus kalan taudinvastustuskykyyn ja immunologisen puolustusjärjestelmän toimintaan. 50 p. (99 p.) Yhteenveto 2 p. 2009.
- 205 IHALAINEN, TEEMU, Intranuclear dynamics in parvovirus infection. - Tumansisäinen dynamiikka parvovirus infektiossa. 86 p. (152 p.) Yhteenveto 3 p. 2009.
- 206 KUNTTU, HEIDI, Characterizing the bacterial fish pathogen *Flavobacterium columnare*, and some factors affecting its pathogenicity. - Kalapatogeeni *Flavobacterium columnare* -bakteerin ominaisuuksia ja patogeenisuuteen vaikuttavia tekijöitä. 69 p. (120 p.) Yhteenveto 3 p. 2010.
- 207 KOTILAINEN, TITTA, Solar UV radiation and plant responses: Assessing the methodological problems in research concerning stratospheric ozone depletion. - Auringon UV-säteily ja kasvien vasteet: otsonikatoon liittyvien tutkimusten menetelmien arviointia. 45 p. (126 p.) Yhteenveto 2 p. 2010.
- 208 EINOLA, JUHA, Biotic oxidation of methane in landfills in boreal climatic conditions. - Metaanin biotekninen hapettaminen kaatopaikoilla viileässä ilmastossa. 101 p. (156 p.) Yhteenveto 3 p. 2010.
- 209 PIIRAINEN, SAIJA, Range expansion to novel environments: evolutionary physiology and genetics in *Leptinotarsa decemlineata*. - Lajien levinneisyysalueen laajeneminen: koloradonkuoriaisen evolutiivinen fysiologia ja genetiikka. 51 p. (155 p.) Yhteenveto 3 p. 2010.
- 210 NISKANEN, EINARI, On dynamics of parvoviral replication protein NS1. - Parvovirusten replikaationproteiini NS1:n dynamiikka. 81 p. (154 p.) Yhteenveto 3 p. 2010.
- 211 PEKKALA, SATU, Functional characterization of carbomoyl phosphate synthetase I deficiency and identification of the binding site for enzyme activator. - Karbamyylifosfaatti syntetaasi I:n puutteen patologian toiminnallinen karakterisaatio ja entsyymien aktivaattorin sitoutumiskohdan identifikaatio. 89 p. (127 p.) Yhteenveto 2 p. 2010.
- 212 HALME, PANU, Developing tools for biodiversity surveys - studies with wood-inhabiting fungi. - Työkaluja monimuotoisuustutkimuksiin - tutkimuskohteina puulla elävät sienet. 51 p. (125 p.) Yhteenveto 2 p. 2010.
- 213 JALASVUORI, MATTI, Viruses are ancient parasites that have influenced the evolution of contemporary and archaic forms of life. - Virukset ovat muinaisia loisia, jotka ovat vaikuttaneet nykyisten ja varhaisten elämänmuotojen kehitykseen. 94 p. (192 p.) Yhteenveto 2 p. 2010.
- 214 POSTILA, PEKKA, Dynamics of the ligand-binding domains of ionotropic glutamate receptors. - Ionotrooppisten glutamaattireseptoreiden ligandin-sitomisdomeenien dynamiikka. 54 p. (130 p.) Yhteenveto 3 p. 2010.
- 215 POIKONEN, TANJA, Frequency-dependent selection and environmental heterogeneity as selective mechanisms in wild populations. - Frekvenssistä riippuva valinta ja ympäristön heterogeenisuus luonnonvalintaa ohjaavina tekijöinä luonnonpopulaatioissa. 44 p. (115 p.) Yhteenveto 4 p. 2010.