

Saija Piironen

Range Expansion to Novel
Environments

Evolutionary Physiology and Genetics
in *Leptinotarsa decemlineata*



Saija Piironen

Range Expansion to Novel Environments

Evolutionary Physiology and Genetics in
Leptinotarsa decemlineata

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ylistönrinteellä, salissa YAA303
kesäkuun 19. 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 Ylistönrinne, hall YAA303, on June 19, 2010 at 12 o'clock noon.



UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2010

Range Expansion to Novel Environments

Evolutionary Physiology and Genetics in
Leptinotarsa decemlineata

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 209

Saija Piironen

Range Expansion to Novel Environments

Evolutionary Physiology and Genetics in
Leptinotarsa decemlineata



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

Cover picture by Jurian Törnroth

URN:ISBN:978-951-39-3917-5

ISBN 978-951-39-3917-5 (PDF)

ISBN 978-951-39-3905-2 (nid.)

ISSN 1456-9701

Copyright © 2010, by University of Jyväskylä

Jyväskylä University Printing House, Jyväskylä 2010

ABSTRACT

Piiroinen, Saija

Range expansion to novel environments: evolutionary physiology and genetics in *Leptinotarsa decemlineata*

Jyväskylä: University of Jyväskylä, 2010, 51 p.

(Jyväskylä Studies in Biological and Environmental Science

ISSN 1456-9701; 209)

ISBN 978-951-39-3917-5 (PDF), 978-951-39-3905-2 (nid.)

Yhteenvedo: Lajien levinneisyysalueen laajeneminen: koloradonkuoriaisen evolutiivinen fysiologia ja genetiikka

Diss.

Understanding the factors that affect species range expansions is a key issue in ecology and evolutionary biology. Biological invasions provide unique opportunities to study the physiological and evolutionary factors and processes that facilitate and limit species ranges. I investigated some of these attributes affecting the range expansion potential, especially towards higher latitudes, of European Colorado potato beetles (*Leptinotarsa decemlineata*). I found that physiological readiness, i.e. low metabolic rate and high body mass, were associated with high diapause propensity, which in turn determined most of the overwintering survival. Beetles also demonstrated the ability to enter diapause under the long-day conditions that prevail in northern latitudes. Beetles were also able to adjust their metabolic rates and prepare for overwintering in a short time. This enables them to prepare for winter and to overwinter successfully at high latitudes where the time window available for entering diapause may be limited. Although there was phenotypic variation, the evolutionary potential, i.e. heritability, of these traits (metabolic rate, body mass) was limited. Exposure to insecticide stress had subtle, though diverse, effects on life-history and physiological traits. Moreover, the effects were dependent on the temperature conditions experienced by the parents. Furthermore, while European populations have decreased genetic variation in the insecticide resistance gene (*AChE*) due to past intense insecticide selection and a population bottleneck upon arrival in Europe, recombination and pre-adaptation to withstand plant alkaloids have, to some degree, attenuated this loss. Taken together, my results show that the range expansion potential of this species is not limited, though it may be reduced, by the genetic and physiological characteristics examined. The history of invasive populations can be important in understanding the evolutionary processes of invasions and in determining the future of invasive pest populations.

Keywords: adaptation; diapause; energy metabolism; genetic variation; insecticide stress; invasive species.

Saija Piiroinen, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address Saija Piironen
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
saija.p.piironen@jyu.fi

Supervisors Docent Leena Lindström
Professor Johanna Mappes
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Professor Alessandro Grapputo
Department of Biology
University of Padua
Via U. Bassi, 58/B
35121 Padua
Italy

Reviewers Professor Liselotte Sundström
Department of Biosciences
P.O. Box 56
FI-00014 University of Helsinki
Finland

Professor Mats Björklund
Department of Animal Ecology
Uppsala University
SE-752 36 Uppsala
Sweden

Opponent Associate Professor Andrew V. Suarez
Dept. of Entomology & Dept. of Animal Biology
University of Illinois
505 S. Goodwin Ave.
Urbana, IL 61801
USA

CONTENTS

ABSTRACT

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

1	INTRODUCTION	7
1.1	Species range expansion to novel environments	7
1.2	Species range expansion and invasive alien species	8
1.2.1	Ecological and physiological factors of invasions	10
1.2.2	Genetic factors and evolutionary processes of invasions	12
1.2.3	Evolution at the invasion frontier	14
1.2.4	Phenotypic plasticity and invasive populations	15
1.3	Aims of the thesis	16
2	MATERIALS AND METHODS	18
2.1	Study species	18
2.2	Genetic analyses.....	20
2.2.1	Population genetics (I)	20
2.2.2	Quantitative genetics (III).....	21
2.3	Life-history and physiological traits.....	21
2.3.1	Study populations and rearing.....	21
2.3.2	Life-history traits and diapause behavior (III, IV, V)	22
2.3.3	Metabolic rate measurements (II, III).....	22
2.3.4	Insecticide treatments (IV, V).....	23
2.3.5	Lipid and water content (V).....	23
3	RESULTS AND DISCUSSION	26
3.1	The amount of genetic variation.....	26
3.1.1	Genetic variation in the insecticide resistance gene (I)	26
3.1.2	Genetic variation in quantitative traits (III)	27
3.2	Association between energy metabolism, diapause and overwintering.....	28
3.2.1	Energy metabolism during pre-diapause (II).....	28
3.2.2	Relationship of body mass and energy metabolism to diapause behavior and overwintering survival (III)	29
3.3	The effect of light regime on diapause behavior and life history traits (IV).....	30
3.4	The effect of insecticide stress on life-history and physiological traits (IV, V)	31
4	CONCLUSIONS.....	33
	<i>Acknowledgements</i>	36
	YHTEENVETO (RÉSUMÉ IN FINNISH).....	37
	REFERENCES.....	40

LIST OF ORIGINAL PUBLICATIONS

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

- I Piironen, S., Lindström, L., Lyytinen, A., Mappes, J., Grapputo, A. 2010. Genetic variation at the acetylcholinesterase gene in European Colorado potato beetle populations: combined effect of pesticide selection, pre-invasion history and demography. Manuscript.
- II Piironen, S., Lindström, L., Lyytinen, A. 2010. Resting metabolic rate can vary with age independently from body mass changes in the Colorado potato beetle, *Leptinotarsa decemlineata*. Journal of Insect Physiology 56: 277-282.
- III Piironen, S., Ketola, T., Lindström, L., Lyytinen, A. 2010. Energy use, diapause behaviour and range expansion potential. Submitted manuscript.
- IV Lindström, L., Piironen, S., Grapputo, A., Lyytinen, A. 2010. The effect of day length and insecticide treatment on life-history traits and winter survival in *Leptinotarsa decemlineata*. Manuscript.
- V Piironen, S., Lindström, L., Lyytinen, A. 2010. Combined effect of parental temperature stress and progeny insecticide stress on physiological and life-history traits in the invasive *Leptinotarsa decemlineata*. Manuscript.

The table shows the contributions to the original papers. Smaller contributions are stated in the acknowledgements of the original papers

	I	II	III	IV	V
Original idea	AG, JM, LL, AL	SP, LL	SP, LL, AL	SP, LL, AL	SP, LL, AL, JM
Data/ Experimental work	SP, AG	SP, LL, AL	SP, LL, AL	SP, LL, AL, AG	SP, LL, AL, JM
Statistical analyses	SP, AG	SP	SP, TK	LL, SP	SP
Writing	SP, LL, AL, JM, AG	SP, LL, AL	SP, TK, LL, AL	LL, SP, AG, AL	SP, LL, AL

SP = Saija Piironen, LL = Leena Lindström, AL = Anne Lyytinen, AG = Alessandro Grapputo, JM = Johanna Mappes, TK = Tarmo Ketola

1 INTRODUCTION

1.1 Species range expansion to novel environments

Understanding the factors that limit the geographical ranges of species and affect their expansion is a key issue in ecology and evolutionary biology (e.g. Gaston 2003, Holt 2003, Gaston 2009, Sexton et al. 2009). The issue includes several questions that have fascinated biologists for a long time such as why some species or populations occupy constant areas while others extend their ranges more or less rapidly or go through repeated expansions and contractions. What are the relative influences of historical, ecological, physiological and evolutionary factors in shaping species ranges? What are the consequences, in both ecological and evolutionary as well as in economic terms, of species range expansions? The ecological and evolutionary aspects of species ranges have received an explosion of interest in the last few years. Part of the reason stems from the urgency to understand how species will respond to global habitat and climatic change (e.g. Bale & Hayward 2010). The impact of humans on the globe has and will continue to drastically alter species distributions by destroying and deteriorating habitats, and also by creating new suitable habitats for species, as well as creating new selective pressures. The current global climatic change will pose further challenges for species' and populations' existence. Secondly, human-induced species' range expansions, i.e. invasive alien species, have become of particular importance and interest as they pose a major threat to biodiversity and ecosystem function (Wilcove et al. 1998, Parker et al. 1999, Sax et al. 2005). At the same time, species invasions provide unique opportunities to study evolutionary processes and deepen our understanding of the factors that influence species ranges as well as giving insights into historical and biogeographic processes (Sakai et al. 2001, Lee 2002, Kinlan & Hastings 2005, Suarez & Tsutsui 2008, Sexton et al. 2009).

1.2 Species range expansion and invasive alien species

An invasive alien species can be defined as a species that occurs outside its natural range (therefore defined as alien), whose introduction or presence is often due to human action, intentional or unintentional, and that, after establishment, rapidly expands its range to new environments. Many invasive species have a negative impact on biodiversity, ecosystem function, agriculture and the health of humans and animals (Wilcove et al. 1998, Mack et al. 2000, Kenis et al. 2009). The impact of invasive species on native communities and ecosystems has been recognized for decades (Elton 1985) and the environmental damage and economic costs related to invasions have been estimated to be enormous (Pimentel et al. 2000, 2005, Heikkilä 2006). Consequently, biological invasions have generated a lot of research effort to understand the various processes underlying invasions and to predict, prevent and manage species invasions (e.g. Kolar & Lodge 2001, Sakai et al. 2001, Allendorf & Lundquist 2003, Sax et al. 2005, Suarez & Tsutsui 2008). Although the dynamics of species invasions can and do differ in some ways from natural (or more natural) range expansions (human induced invasions generally involve random introductions of species over large geographical areas characterized by lack of co-adaptation with the local biota in the introduced environment), they offer us real-time, large-scale ecological and evolutionary experiments on species range expansions and their limits that would be difficult or even impossible to artificially simulate.

The invasion of species to new environments is a process which can, in its simplest form, be divided into several successive stages: introduction (or arrival), establishment and spread (range expansion) (Vermeij 1996, Kolar & Lodge 2001, Sakai et al. 2001, Fig. 1). It is important to recognize that the invasion process is, in reality, a continuum and that all the stages can occur continuously and do not cease when one stage in the process has been achieved. For example, the establishment of a population does not preclude the occurrence of new introductions (see Kolbe et al. 2004).

The introduction of species to new areas has much been facilitated by, and has increased in frequency, randomness and number due to, human action (Levine & D'Antonio 2003). Many plants and animals have been intentionally transported for various purposes such as ornament, the pet trade and as biological control agents. In addition, increased international trade and travel have led to the accidental introduction of numerous species, for instance in the ballast water of ships (Ruiz et al. 1997). This increased propagule pressure demonstrably increases the probability of species becoming established (Kolar & Lodge 2001, Suarez et al. 2005, Maron 2006). Establishment means that the population is able to maintain itself by local reproduction and is not dependent on new introductions (naturalized). That is, established individuals must be able to endure the abiotic and biotic conditions of the new environment as well as find and access resources for growth and reproduction. The task can be

challenging. It has been estimated that only a small fraction, approximately 10 %, of species that arrive, survive and successfully establish themselves (Williamson & Fitter 1996a). However, this proportion may vary substantially among taxa (Jeschke & Strayer 2005, Suarez et al. 2005). For instance, it has been estimated that approximately 30 % of immigrant birds and as many as 80 % of immigrant mammals successfully establish themselves (Jeschke & Strayer 2005). After establishment, introduced species may remain confined to one locality, or may continue to expand their range far beyond the original point of introduction (that is becoming invasive). Species' spread across novel environments can be facilitated by human action and/or be influenced by the intrinsic characters of the invasive population such as dispersal ability. However, in the context of species invasions, there is often a lag time between the establishment and spread (Fig. 1). This, as has been suggested, may result from the time needed for evolutionary change to happen (Ellstrand & Schierenbeck 2000, Sakai et al. 2001, Preisser et al. 2008) or it could be that a population becomes invasive only after repeated introductions (Sakai et al. 2001, Kolbe et al. 2004) or after some change in environmental conditions. It has thus far proven very difficult to predict whether a certain species will become invasive and/or engender harmful effects in the introduced environment. This is because the invasion process involves various ecological and evolutionary factors and processes that interact and whose relative significance may differ at different stages of the invasion process.

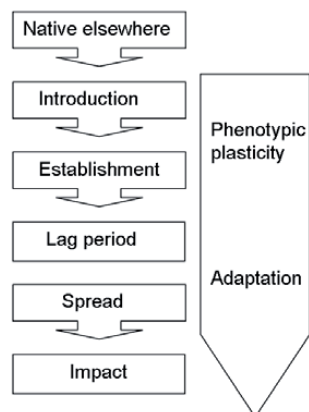


FIGURE 1 Generalized steps of the invasion process and the importance of phenotypic plasticity and adaptation in the process (modified from Sakai et al. 2001 and Sexton et al. 2002). Phenotypic plasticity may enhance initial survival and subsequent establishment in novel environments. Adaptation may have a more crucial role allowing the invasive population to persist and spread in novel environments.

1.2.1 Ecological and physiological factors of invasions

Numerous ecological, physiological and genetic attributes (or biotic and abiotic factors and their interactions) influence species invasion success (arrival, establishment and range expansion) in new environments. While these different attributes can be classified in different ways and discussed separately, it is important to recognize that all are more or less intertwined (Lambrinos 2004).

There are several approaches to studying the ecological aspects of invasion success and range expansion of invasive species. A lot of emphasis has been placed on identifying the life-history characters that contribute to different stages of the invasion process. Indeed, some life-history characteristics have been associated with higher probability of becoming a successful invader (Williamson & Fitter 1996b, Reichard & Hamilton 1997, Kolar & Lodge 2001, van Kleunen et al. 2010). In plants, for instance, characters such as the ability to reproduce vegetatively, rapid growth rate and high tolerance of environmental heterogeneity (or disturbance) promote invasiveness (Baker 1974). Other traits generally associated with invasiveness include high reproductive capacity and dispersal ability. High reproductive capacity on arrival may shorten the period of exposure to the effects of small population size whereas high dispersal may enhance the probability of finding suitable habitats. Invasive populations may also possess pre-adaptations that make them successful in the new environment (Treier et al. 2009). For example, adaptations to stressful conditions in the prior environment may facilitate invasions to novel stressful environments (Reed et al. 2003). However, despite the emphasis, it has been difficult to make any generalizations about the traits that make some species better invaders than others (Kolar & Lodge 2001). It is more likely that different characters are important in different habitats or at different stages of the invasion process (Kolar & Lodge 2001), or that certain combinations of characters explain invasion success (Küster et al. 2008).

Another approach to deciphering the factors affecting invasions and predicting invasion success is the comparison of abiotic and biotic environmental conditions (or the ecological niche) between the native and introduced environments (Peterson & Vieglais 2001). Similar climatic conditions (for instance, in terms of temperature) can obviously facilitate invasion success when the environmental conditions in the newly-invaded area are within the physiological threshold limits of the species or populations. However, as species spread across environmental gradients, they will at some point reach climatic conditions that meet their physiological limits, that is, beyond which they cannot survive, ultimately limiting species ranges. For ectothermic animals, physiological tolerance to various stressors may be especially important in limiting ranges (Lee et al. 2003, Sanford et al. 2006, Lee et al. 2009). For example, for the invasive terrestrial slug species, *Deroceras panormitanum*, the lower and upper elevational range distributions are limited by its tolerance of, respectively, salinity and low temperature (Lee et al. 2009). Furthermore, latitudinal and altitudinal clines in physiological traits such as thermal and desiccation stress tolerance and metabolic rate are considered to reflect

adaptation to local environmental conditions (Addo-Bediako et al. 2000, 2002, Lardies et al. 2004, Gilchrist et al. 2008) illustrating the importance of physiological traits in determining geographical range.

Species invasions can be strongly influenced by various biotic- and species-community interactions (Lodge 1993, Shea & Chesson 2002). Competition, predation and parasitism can be important attributes in determining invasion success (Lodge 1993). Competition may limit species ranges to smaller areas than would abiotic factors alone (Hersteinsson & Macdonald 1992). On the other hand, escape from natural enemies such as the predators, parasites or pathogens of the earlier environment (the so called enemy release hypothesis), may enhance invasion success in the invaded environment (Porter et al. 1997, Keane & Crawley 2002, Mitchell & Power 2003). Mitchell and Power (2003) found that invasive plant species had fewer fungal and viral infections in their invaded than in their native environment and that the release from enemies was associated with a highly negative impact on invaded ecosystems. One possible mechanism contributing to invasiveness is encapsulated in the hypothesis stating that in absence of enemies, resources released from allocation to defensive processes could be reallocated to growth and reproduction (Blossey & Notzold 1995). So far, there is limited evidence as to whether or not this holds true (Keane & Crawley 2002 and references there in). Other characteristics of the introduced environment or community, such as the level of disturbance and the local species diversity may also affect the success of invading populations (Lodge 1993, Levine 2000). High levels of biodiversity may impede invasions as there are no unoccupied niches for invasive species (Kennedy et al. 2002). It has also been suggested that more disturbed environments are easier to invade by plants as they may offer suitable microhabitats (Lodge 1993, Davis et al. 2000). However, disturbance could impede invasions in some cases by, for example, causing high mortality (Lodge 1993). While landscape structure has rarely been taken into account in the context of species invasions, it can affect their outcome (Zalewski et al. 2009). Landscape structure can have an influence because natural or human-modified environments are rarely homogeneous but provide patches of more and less favorable/suitable habitats. This can, for instance, affect dispersal patterns and gene flow (Zalewski et al. 2009) or hinder finding or reaching suitable food resources or host plants (Shea & Chesson 2002) and, consequently, influence invasions.

Although ecological factors are important in determining invasion success and the further range expansion of invasive species, it has become evident that the ecological factors involved in invasions may interact in complicated ways. So far, there are no specific patterns which would serve as guidelines for making predictions about invasion success.

1.2.2 Genetic factors and evolutionary processes of invasions

It is now well recognized that biological invasions involve evolutionary changes (Sakai et al. 2001, Lee 2002, Suarez & Tsutsui 2008). To enhance our understanding of invasions and species range limits, therefore, it is necessary to examine the evolutionary factors and processes of invasions in tandem with species' ecology.

Genetic attributes may have a considerable effect on the success of invasive species in establishing and expanding their range (Sakai et al. 2001, Lee 2002). As species arrive and spread across new environments they face novel abiotic (e.g. temperature) and biotic (e.g. competition) selection pressures that may reduce or limit further spread (Kellermann et al. 2009, Lee et al. 2009). The ability to adapt to these novel environments, i.e. to respond to natural selection, requires genetic variation (additive genetic variance) in relevant traits (Roff 1997) that facilitate invasions (Lee 2002, Gilchrist & Lee 2007). However, as invasive populations experience population bottlenecks during the invasion process and, consequently, are thought to possess less genetic variability than the source population(s), their ability to evolve in new environments could be limited (Allendorf & Lundquist 2003).

Population bottlenecks can lead to serious reductions in genetic diversity due to the small size of the founder population. Small populations are also subject to genetic drift which may lead to further loss of genetic variation (Nei et al. 1975). Nevertheless, several species have been very successful at expanding to new environments despite having experienced population bottlenecks (Tsutsui et al. 2000, Grapputo et al. 2005, Wares et al. 2005). This discrepancy has spawned considerable research comparing the amount of genetic variation between native and introduced populations as well as deciphering the genetic attributes that contribute to invasions (Wares et al. 2005). Results confirm that many invasive populations have indeed lost genetic variability compared to native populations, but have also revealed that some invasive populations have even higher levels of genetic variation (Lavergne & Molofsky 2007). Moreover, reductions in genetic variability have not, in general, been as large as expected (Novak & Mack 2005, Dlugosch & Parker 2008). One reason for this is that there can be repeated and/or multiple introductions that replenish or increase genetic variability (Lavergne & Molofsky 2007, Fonseca et al. 2010) and contribute to invasion success. It has even been suggested that multiple introductions are a prerequisite for successful invasions, and that multiple introductions may be much more common than was previously thought (Novak & Mack 2005, Dlugosch & Parker 2008).

Although neutral markers are very important in deciphering the source, number and routes of invasions (Wares et al. 2005, Ficetola et al. 2008, Poulet et al. 2009), reductions in genetic variation in neutral genetic markers, the main gauge of population genetic comparisons, do not necessarily translate into a similar reduction in genetic variation in adaptive traits, which are more often quantitative in nature. In fact, there is evidence that quantitative genetic variance may not decrease as readily as neutral genetic variation (Dlugosch &

Parker 2008 and references there in) and, in some cases, population bottlenecks may even increase the amount of additive genetic variance by converting epistatic variance to additive variance (Cheverud & Routman 1996). Currently, however, comparisons of the amount of genetic variation in quantitative traits between native and introduced populations are scarce (Dlugosch & Parker 2008).

While many invasive populations are known to possess sufficient genetic variability, it is the existence of genetic variability in relevant life-history and physiological traits that may determine whether populations are able to expand their range to novel environments (Hoffmann et al. 2003, Kellermann et al. 2009). For example, Hoffmann et al. (2003) found that even though *Drosophila birchii* possessed genetic variation in morphological and neutral genetic markers, the range expansion potential of the species is most likely limited by the lack of genetic variance in desiccation resistance. However, studying the genetic variation of relevant traits may be complicated by the fact that, in many situations, the relevant traits involved in particular species invasions are not known.

There are other genetic attributes that may influence and facilitate the success of invasions. Genomic rearrangements such as chromosomal inversions may have been involved in some invasion events (Prevosti et al. 1988). Particularly, in plants, hybridization between species or between source populations can generate novel genotypes and serve as a stimulus for invasiveness. Hybridization may also influence invasions by increasing genetic variation or heterozygosity (Ellstrand & Schierenbeck 2000). Increased genetic variability as a result of hybridization between two *Ulmus* tree species, *Ulmus pumila* and *U. rubra*, for example, has facilitated the spread of the former species, *U. pumila*, in North America (Zalapa et al. 2010).

In addition, a small number of genes may have a notable influence on invasiveness (Paterson et al. 1995, Krieger & Ross 2002). A mutation in a major gene affecting social behavior may have contributed to the invasion success of the fire ant, *Solenopsis invicta*, in its introduced range in North America (Krieger & Ross 2002, Tsutsui & Suarez 2003). As many invasive species are also pests or weeds, the evolution of pesticide or herbicide resistance, which is often based on one or only a few genes (French-Constant et al. 2004), can greatly affect their invasion success. Furthermore, adaptation based on a few, major genes can happen very rapidly as in the case of pesticide resistance evolution (McKenzie 2001). In addition to resistance evolution, sub-lethal pesticide effects may alter physiological and life-history traits (Hardin et al. 1995, Desneux et al. 2007) and consequently influence selection. In the long term, repeated exposure to pesticides may result in pest populations that are not only adapted to tolerate pesticides but may have evolved traits that contribute to invasiveness.

1.2.3 Evolution at the invasion frontier

While considerable research has focused on studying the history and genetic consequences of invasions, it is also important to be able to assess and predict the potential of invasive species to further expand their range. This is especially relevant in preventing invasions and in the management of invasive species (Genovesi 2005). Thus, invasive populations should be studied at their range limit or the invasion frontier.

Many invasive populations have evolved rapidly after their introduction to novel environments (Huey et al. 2000, Maron et al. 2004, Gilchrist et al. 2008) reflecting the existence of sufficient genetic variation. However, as populations spread across environmental gradients, strong directional selection can reduce additive genetic variation and subsequently limit further adaptation to novel environments (Blows & Hoffmann 1993, Hoffmann & Blows 1994, van Heerwaarden et al. 2010). For instance, directional selection for increased desiccation resistance may limit the southern range expansion of a rainforest *Drosophila burchii* species towards drier environments (van Heerwaarden et al. 2010). Populations at their range limit could also suffer from reduced genetic variation due to small population size (Hoffmann & Blows 1994). In general, range-limit populations are thought to be smaller and more fragmented than more central populations because they are closer to their ecological and physiological limits.

Even if populations possess sufficient genetic variation, their range expansion may be limited by opposing selective forces or genetic constraints. If selection favors different traits at the range limit compared to more central areas e.g. in a situation where organisms face a steep environmental gradient, high gene flow from more central to range-limit populations could impede populations at the invasion frontier from adapting to local conditions and prevent further range expansion (Kirkpatrick & Barton 1997, Lenormand 2002). This is because the locally adapted genotypes or alleles would be diluted by the maladapted ones. Recent support for this mechanism comes from Dawson et al. (2010) who found that the primary cause of the northern range limit of the volcano barnacle *Tetraclita rubescens* is the gene flow of maladapted alleles to the peripheral populations. However, the role of gene flow could be dualistic. Whereas high amounts of gene flow can impede adaptation to local conditions, moderate amounts of gene flow may replenish genetic variation and contribute to evolutionary potential (see for a discussion Bridle & Vines 2007, Sexton et al. 2009). As many traits are genetically correlated, selection for one trait can result in correlated responses in other traits and, subsequently, impede successful invasion to novel environments (Blows & Hoffmann 2005). Jenkins and Hoffmann (1999) found that the potential of *Drosophila serrata* populations to expand their range further south could be partly limited by the trade-off between increased cold resistance and decreased fecundity. Range expansion of insect species toward colder environments may also involve a trade-off between development time and body size (Blanckenhorn & Demont 2004). At higher latitudes selection would favor higher body mass as it is associated with,

among other things, higher overwintering survival (Hahn & Denlinger 2007). However, larger body mass is often correlated with longer development time which could be selected against as the length of the favorable growing season at high latitudes may be limited.

1.2.4 Phenotypic plasticity and invasive populations

Genetic adaptation, it has been argued, may be particularly important at the range expansion stage of the invasion process while phenotypic plasticity may play a relatively more important part at the arrival and establishment stages (Sexton et al. 2002, see Fig. 1). Phenotypic plasticity can be defined as the range of phenotypes produced by the genotype in response to environmental variation (Bradshaw 1965). Phenotypic plasticity is thought to allow the introduced species or populations to persist in the novel environment by attaining homeostasis (maintaining fitness) under unfavorable and/or variable conditions until evolutionary change takes place (Williams et al. 1995, Richards et al. 2006, Ghalambor et al. 2007). In other words, phenotypic plasticity can enable physiological tolerance. Furthermore, phenotypic plasticity may allow adaptive responses to unfavorable environmental conditions, thereby increasing fitness and invasion success (Richards et al. 2006). Cano et al. (2008) suggested that the greater plasticity of the introduced plant populations of *Senecio pterophorus* compared to native populations enabled greater ability to increase fitness in response to resource availability. In fact, it has been postulated that successful invaders generally have high phenotypic plasticity (Richards et al. 2006) but, currently, there is no clear evidence that invaders are more plastic. The majority of published studies concern plant species and less is known about the role of plasticity in animal invasions (Chown et al. 2007). It seems likely that differences in plasticity between native and introduced populations vary depending on a trait in question (Brock et al. 2005) and that both phenotypic plasticity and genetic factors play a role in invasions (Sexton et al. 2002, Lee et al. 2003, Cano et al. 2008). Although the phenotypic effects of environmental factors have been most often studied within individual lifetimes, between-generation effects may also be important for invasion success and for the dynamics of invasions (Duckworth 2009, Dyer et al. 2010). This is because the environmental conditions of the parental generation may affect the phenotype of the progeny and their performance (Mousseau & Fox 1998, Räsänen & Kruuk 2007). Plasticity in itself can be adaptive and respond to selection (Via et al. 1995). This is because selection acts on phenotypes and as long as some of the phenotypic variation is genetically based, it can respond to selection. However, it is also possible that plasticity may buffer existing genetic variation from selection and retard adaptive evolution (Ghalambor et al. 2007).

1.3 Aims of the thesis

The objectives of my doctoral thesis were to examine the physiological and evolutionary factors and processes associated with the range expansion of the invasive species Colorado potato beetle (*Leptinotarsa decemlineata*, Say) to novel environments, especially to higher latitudes.

The Colorado potato beetle, a well known invader and pest species, is an excellent model for studying range expansions for several reasons. Its invasion history is well known, it is a currently invading species (EPPO 2006) and the genetics of its insecticide resistances have been well defined (see Alyokhin et al. 2008). A further object of interest is that, despite having experienced a population bottleneck during the invasion of Europe, this species seems to possess a substantial amount of neutral genetic variation (Grapputo et al. 2005) as well as genetic variability in life history traits (Boman et al. 2008, Lyytinen et al. 2008). The first aim of my doctoral thesis was to extend the knowledge of the amount of genetic variation and evolutionary processes associated with the beetles' range expansion in Europe to genes that are or have been targets of selection (I). Insecticide resistance genes offered this possibility. As many invasive species, including the Colorado potato beetle, are pest insects, they face intense selection by insecticides. This selection can reduce genetic variation in the resistance gene locus as well as in the surrounding areas, and may, ultimately, limit adaptive potential (Allendorf & Lundquist 2003). However, the amount of genetic variation can be confounded by other factors, such as recombination and demographic events. The gene acetylcholinesterase (*AChE*) was chosen for this study because organophosphates have been used intensively against the beetle, the gene has been sequenced and the point mutations conferring insecticide (organophosphate) resistance have been defined for the Colorado potato beetle (Zhu et al. 1996, Zhu & Clark 1997).

As insect species expand their range towards northern latitudes, they encounter several selective factors, such as harsh winter conditions, as well as insecticides. The ability to cope with and adapt to harsh winters may depend on physiological tolerance and the amount of genetic variability in relevant physiological and behavioral traits. The Colorado potato beetle's range expansion towards northern latitudes in Europe has been slow in comparison with its initial rapid eastward expansion across Europe. Previous studies have shown that the beetles range expansion to higher latitudes is not limited by summer conditions or by available host plant (potato variety) (Boman 2008, Boman et al. 2008, Lyytinen et al. 2008, 2009) but possibly, by harsh winter conditions (Valosaari et al. 2008). Successful adaptation to northern winters may include traits such as fast development, high metabolic rate and high capacity to acquire energy reserves that enable the species to complete development and prepare for overwintering during the short favorable growing seasons (Chown & Gaston 1999, Hahn & Denlinger 2007). On the other hand, to survive the long, harsh winter conditions, it is important to be able to

enter diapause at the correct time of the year (i.e. during the longer day lengths of higher latitudes) and to have low energy metabolism during overwintering, husbanding limited energy reserves (Kostál 2006, Hahn & Denlinger 2007). That is, insects have to adjust their behavior as well as their physiology to high-latitude environmental conditions.

As the time window for preparation for winter diapause can be narrow for insect species of temperate latitudes, changes in physiological traits take place over very short time periods (Tauber et al. 1986). I addressed this issue in paper II, by studying changes in resting metabolic rate during the beetle's pre-diapause period. Knowledge of age-dependent patterns in energy metabolism was essential for conducting the metabolic measurements reported in paper III. The aims of this experiment (paper III) were to examine the effects of body mass and metabolic rate on the winter diapause behavior and overwintering success of beetles and to assess whether there is adaptive potential in these traits (heritability) to respond to novel selection pressures. I expected high body mass and low metabolic rate to be advantageous in overwintering. In paper IV, my objective was to examine the effect of day length on the diapause behavior and life-history traits of beetle populations collected from high- (northern Russia) and low- (Italy) latitude areas of the species' European distribution. The aim was to determine if beetles from the northern range of the species' distribution have adapted or have the potential to adapt to a long day-length photoperiod and whether there are inter-population differences in response to day length.

Tolerance of various stressors has been regarded as a crucial factor influencing the survival and range expansion of invasive species to new environments (Sakai et al. 2001, Lee et al. 2003). As many invasive species are also pests, they also encounter repeated exposure to insecticides. Exposure to insecticide stress may alter various physiological and life-history traits (Desneux et al. 2007), such as body mass (Bernard & Lagadic 1993), lipid content (Quinlan & Gatehouse 1981) and diapause behavior (Grewal et al. 2001), influencing fitness and thus it may affect the invasion success of insect pest species. Furthermore, the effect of insecticide stress can be complicated by the environmental conditions or stressors experienced by the previous generations (Parsons 1991, Crill et al. 1996, Räsänen & Kruuk 2007). The aim of the paper V was to examine the effect of temperature endured by parents on tolerance to insecticide stress in the offspring. I also addressed the effects of insecticide stress on diapause behavior and life-history traits under two photoperiod regimes in paper IV.

2 MATERIALS AND METHODS

2.1 Study species

The Colorado potato beetle (*Leptinotarsa decemlineata*, Say) is an invasive pest of the potato (*Solanum tuberosum*) worldwide (Casagrande 1987, Alyokhin et al. 2008). Both larvae and adults feed on potato leaves and can cause serious damage to potato fields (Hare 1990). The beetle is native to Mexico (Tower 1906), where it is not a pest species of potato but uses buffalo bur (*Solanum rostratum*) and other species of Solanaceae as host. A shift from buffalo bur to potato occurred sometime before 1850 in Colorado, US, when potato farming had reached the distribution range of the beetle (Casagrande 1987). On potato, the beetle became numerous and was soon considered a pest species (Walsh 1865). Despite various efforts to manage it, from hand-picking to the use of arsenicals, the beetle spread throughout the US, reaching the east coast before 1880 (Casagrande 1987). The beetle was accidentally introduced to Europe (France) where it established populations in 1920s and spread almost throughout Europe, except for the UK and Scandinavia, in 50 years. The beetle is currently spreading further eastwards and towards higher latitudes (Grapputo et al. 2005, EPPO 2006, Boman et al. 2008). The current northernmost beetle populations are in Russia, approaching the northern latitude of 62°. The beetle has attempted to invade Finland in several years (1998, 2002-2007) but has not yet succeeded in establishing permanent populations.

There are several reasons that have contributed to the beetle's successful and rapid range expansion across continents. First, adaptation to the potato as host provided a new and rich habitat almost totally unoccupied by other herbivores. Second, the beetle has a very high reproductive potential. One female can lay up to 700-1300 eggs in one growing season (Weber & Ferro 1996, personal observation). Even one mated female may be sufficient to successfully establish a population in an invasion event. Furthermore, overwintered post-diapause females can begin reproduction in the spring utilizing sperm from pre-diapause mating the previous autumn (Ferro et al. 1991), although the

viability of such eggs is reduced. A third factor contributing to its high invasion success is the beetle's life-cycle which provides the means to escape exposure to harmful agents and unfavorable periods, such as cold winters. After hatching larvae go through four larval stages and burrow into the soil for pupation. The subterranean pupation period shortens the time window in which the beetle is exposed to insecticides and other unfavorable conditions. The development from egg to adult can take 14-56 days depending on temperature (Ferro et al. 1985, Lyytinen et al. 2008). Adults that emerge from the soil can either start breeding and generate a second generation or begin to prepare for overwintering (e.g. gain energy reserves) and enter the soil for winter diapause. As the growing season is much longer and temperatures higher at lower latitudes, there can be up to three generations in one growing season while in higher latitudes there is typically only one. The decision on whether or not to enter diapause is mainly determined by the day length, but is affected by food availability, temperature and moisture conditions also (de Wilde & Hsiao 1981, Tauber et al. 1988, de Kort 1990, Noronha & Cloutier 2006). The refractory phase of diapause lasts approximately 3 months after which the beetles become sensitive to changes in temperature. They start to emerge from the soil when the soil temperature rises above 10°C (de Kort 1990). Although the majority of beetles stay in diapause only over the winter, some beetles can extend the diapause for 2-9 years (Tauber & Tauber 2002). As the species is freeze-intolerant, winter diapause in the soil provides the means to survive otherwise deadly conditions. The fourth reason is the beetle's aposematic coloration. Having black stripes on a yellow/white background of elytra, beetles' advertise their bad taste or unpalatability to potential predators (Daloze et al. 1986, Hough-Goldstein et al. 1993a). Thus, few birds prey on it and it has relatively few natural enemies (Hough-Goldstein et al. 1993b). Furthermore, natural enemies (and other potential biological control agents) are not capable of suppressing the beetle populations below economically-productive densities in commercial potato fields (Alyokhin et al. 2008, Alyokhin 2009). So far, its natural enemies have not also been able to follow the beetle as it expanded its range to new areas.

In addition to its complex and diverse life-history, a crucial factor that has contributed to its enormous success as an invader is the beetle's remarkable ability to evolve resistance to insecticides (or pesticides) (Forgash 1985, Alyokhin et al. 2008). Insecticide compounds are, so far, the most efficient means to manage beetle populations. However, as a result of intensive prevention practices, the beetle has evolved resistance to at least 52 different compounds covering all the major pesticide classes (see Alyokhin et al. 2008). This high ability to evolve resistance to so many pesticides potentially may stem from its adaptation to digest and neutralize the chemicals of the host plant.

2.2 Genetic analyses

2.2.1 Population genetics (I)

The Colorado potato beetle's resistance to organophosphate insecticides is based on single point mutations (S291G, R30K) at the gene acetylcholinesterase (*AChE*), which is involved in the functioning of the nervous system (Zhu et al. 1996, Zhu & Clark 1997). To examine the amount of genetic variation in the *AChE* gene and the frequency of insecticide resistance mutations among European beetle populations (I), beetles were collected from five regions in Europe: Russia (60°43'N, 33°33'E), Finland (61°41'N, 27°15'E), Estonia (58°23'N, 26°43'E), Poland (53°23'N, 14°32'E) and Italy (45°33'N, 11°33'E) in 2003 and 2005. I also compared the European populations to beetles from Colorado, USA (10 individuals) which were collected in 2003.

Basic genetic methods were adopted. Total genomic DNA was extracted from the legs of the individuals using the DNeasy Tissue Kit (QIAGEN). Primers for PCR reactions were obtained from (Clark et al. 2001) as well as designed from the Colorado potato beetle-*AChE* gene available from Genbank (Accession No L41180.1). A polymerase chain reaction (PCR) mixture with a total volume of 25 µl contained 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of each primer, 1 x buffer of Taq DNA polymerase (Biotools), 1 unit of Taq DNA polymerase (Biotools) and 20-50 ng of genomic DNA. The PCR cycles were as follows: 1 cycle of 94°C for 3min; 10 cycles of 94°C for 30s, 60°C for 30s with a 1°C decrease in temperature per cycle, 72°C for 1min 30s; 30 cycles of 94°C for 30s, 50°C for 30s, 72°C for 1min 30s; 1 cycle of 72°C for 10 min. Sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and were run on an ABI 3100 automated sequencer. SeqScape v2.1.1 (Applied Biosystems) was used to assemble and align the sequences. Haplotypes were inferred from genotypic data using the program PHASE 2.1.1 (Stephens et al. 2001). Haplotypes that could not be reliably inferred by PHASE were, prior to sequencing, cloned to determine the correct phase of the variable sites using the Qiagen PCR Cloning Kit (QIAGEN).

Basic population genetic analyses were performed. These included the construction of haplotype networks with TCS 1.21 (Clement et al. 2000). Genetic diversities, recombination parameters and tests of selective neutrality were calculated using DNASP 4.10.9 (Rozas et al. 2003). The number and position of possible recombination breakpoints in the sequence were estimated with Genetic Algorithm Recombination Detection (GARD) (Kosakovsky Pond et al. 2006). Departures from Hardy-Weinberg equilibrium, the hierarchical analysis of molecular variance (AMOVA) (to estimate the level of differentiation among populations and between years) as well as population pair-wise F_{ST} values were performed with ARLEQUIN 3.1 (Excoffier et al. 2005).

2.2.2 Quantitative genetics (III)

The successful range expansion of a species may require adaptation to novel conditions. The adaptive potential to respond to natural selection, in turn, depends on the amount of heritable genetic variation of a trait (Falconer & Mackay 1996, Roff 1997). I used a full-sib half-sib design to estimate the amount of heritable variation in body mass, metabolic rate, diapause behavior and overwintering survival in Colorado potato beetles (III). Variance components were estimated by restricted maximum-likelihood (REML) method because my experimental design was unbalanced (Lynch & Walsh 1998). Narrow sense heritability (h^2) was presented as the proportion of additive genetic variance (V_A) of the total phenotypic variance (V_P).

2.3 Life-history and physiological traits

2.3.1 Study populations and rearing

Beetles in papers II and V were descendants of beetles collected in the field in Poland (Bonin, 54°09'N, 16°15'E) in June 2003 and had been maintained in the laboratory for three generations under light conditions of L:D 18:6 (with 2 hrs of crepuscular light imitating sunrise and sunset) and overwintered at 5°C in darkness in controlled environmental chambers (Type B1300, Weiss technic). To examine the influence of thermal stress history on stress tolerance, the field collected beetles were subdivided into two fluctuating temperature regimes, low (mean 17°C) and high (mean 23°C) (paper V). The fourth generation beetles used in the experiments were reared from egg to adult at a constant temperature of 23°C and under same light conditions as above. Beetles were kept in Petri dishes lined with a moisturized filter paper and fed with fresh potato leaves (variety Van Gogh) supplied daily. For the pupation of larvae and overwintering of adults, beetles were transferred to plastic containers filled with soil.

Beetles used in the studies for papers III and IV were third generation descendants of beetles collected in Russia (61°49'N, 34°10'E) in 2006. In paper IV, we also had second generation descendants of beetles collected in Italy (45°48'N, 12°07'E) in 2008. In paper III adult beetles (parent and third generation beetles) were maintained at a constant 23°C (L:D 18:6 h) in conditions as described above. Larvae were reared on whole potato plants (variety Van Gogh) planted in pots and kept in a greenhouse at 20°C ($\pm 1^\circ\text{C}$) under natural light conditions until adult emergence (Agrifood Research Finland, Jokioinen, Finland, 60°48'N, 23°29'E, early August LD 18:6). In paper IV, parent beetles were maintained as above but the experimental beetles (larvae and adults) were reared under two fluctuating light regimes: short (L:D 13:11 h) and long day (L:D 21:3 h).

2.3.2 Life-history traits and diapause behavior (III, IV, V)

I measured several basic life-history parameters in the beetles. These were egg-to-adult development time (papers IV and V), body mass at adult emergence, 10-day body mass (papers III-V) and diapause behavior and overwintering survival (papers III and IV). These traits were chosen because they may have an important role for range expansion across novel environments, especially in northern latitudes where the length of the favorable growing season is relatively shorter and winters longer and harsher than at lower latitudes. In these conditions, it is beneficial to develop quickly and have large body mass before the onset of winter, and to enter diapause at the correct time. I used digging behavior (dug or not dug into the soil for overwintering) as an indicator of the onset of diapause. 10-day-old adult beetles were weighed and placed in plastic containers filled with soil and supplied with food. Digging behavior was observed every day for at least 19 days after placing a beetle in a plastic container. Overwintering survival was recorded until the following June. Survival was recorded for 9-10 months in total.

2.3.3 Metabolic rate measurements (II, III)

Metabolic rate, which describes the energetic cost of living, is an important physiological trait that is closely related to life-history traits (Chown & Gaston 1999). All organisms have to obtain sufficient energy resources, which are then allocated among energy-demanding processes such as maintenance, growth and reproduction. However, there often are trade-offs because all biological processes cannot be maximized at the same time. These energy strategies have been shaped by the environmental conditions under which organisms have evolved. This has resulted in substantial among- and within-species variation in metabolic rate (Chown & Nicolson 2004). Metabolic rate variation has various intrinsic (e.g. body mass) and extrinsic (e.g. temperature) sources, it has been linked to fitness (Crnokrak & Roff 2002, Nilsson 2002, Artacho & Nespolo 2009, Ketola & Kotiaho 2009) and considered to be an adaptive trait (Hoffmann & Parsons 1989, Reinhold 1999, Addo-Bediako et al. 2002).

To examine age-dependent changes (II) and the effect of metabolic rate on beetles' diapause behavior and overwintering survival (III), I used CO₂ production as a measure of metabolic rate. CO₂ production was measured by using flow-through respirometry. Metabolic rates can also be indirectly measured from oxygen consumption. However, small animals such as insects consume such small amounts of oxygen that changes are difficult to measure against a large background level compared to changes in CO₂ production against a zero-CO₂ baseline (which is easily achieved by scrubbing CO₂ from the incurrent air) (Lighton 2008). Metabolic rate was measured using a CO₂ analyzer Li-6252 (LiCor, Lincoln, USA) connected to a flow-through respirometry system. A schematic diagram of the equipment is shown in figure 2. Briefly, ambient air was filtered for CO₂ (soda lime) and water vapor (Drierite), and was pulled through a cylindrical respirometry chamber and the

CO₂ analyzer with a pump (SS-2, Sable Systems, Henderson, USA). The flow rate was controlled to be a constant 150 ml min⁻¹. In paper II the experimental temperature was controlled by setting the entire respirometry system in a temperature controlled room (19 ± 1°C) and in paper III, temperature (23°C) was controlled with a temperature controller (Pelt-5, Sable Systems) that regulated the temperature of a Peltier effect constant-temperature cabinet (PTC-1, Sable Systems) inside which the respirometry chamber was housed.

The general procedure for measuring resting metabolic rates of beetles was as follows: 0-10-day-old (II) or 6-day-old (III) beetles were starved for 4-24 hours before measurements to avoid CO₂ emission resulting from the assimilation of food. Recording of CO₂ production was initiated when the CO₂ level had stabilized and the recording lasted a minimum of 5 minutes, during which the moving activity of the beetle was recorded visually (II) or with an infra-red-light scattering based activity detector (AD-1, Sable Systems) (III). Baselines (empty chamber without the beetle) were recorded before and after each measurement. Beetles were weighed (0.1 ± mg) (AM100, Mettler) after the measurements. Respirometry data was recorded and analyzed, including baseline correction and conversion of the data to ml CO₂ h⁻¹, with the acquisition and analysis software Datacan V (Sable Systems) (paper II) or Expedata (Sable Systems) (paper III).

2.3.4 Insecticide treatments (IV, V)

Exposure to insecticides may alter various physiological and life-history traits (Desneux et al. 2007), influencing fitness which can affect invasion success. To study these effects in the Colorado potato beetle (IV, V), insecticide treatment was performed on 3-day-old larvae by using a filter paper technique. Three-to-five larvae from each family were divided into control and insecticide treatment groups. In the insecticide treatment, 1 ml of insecticide solution was added to filter paper on a Petri dish and allowed to absorb before larvae were placed on a potato leaf on the filter paper. The insecticides and concentrations used were: deltamethrin (a pyrethroid, trademark Decis) in a 3.18 mg/l solution in the insecticide stress experiment (paper V) and in a 0.80 mg/l solution in the light regime experiment (paper IV), and dimethoate (an organophosphate, trademark Danadim, 0.05 % solution) (paper IV). One ml of water was used in the control treatments. After 24 (V) or 48 (IV) hours, the survival of the larvae was observed. Surviving larvae were carefully moved to a new Petri dish with fresh potato leaves and reared to adulthood at 23°C.

2.3.5 Lipid and water content (V)

The amount of lipid reserves can influence overwintering survival (Hahn & Denlinger 2007) and, consequently, invasion success. To investigate whether insecticide stress had an effect on beetles' energy reserves, the lipid and water content of 10-day-old adult beetles were measured (V). Beetles were first weighed and killed by decapitation. The wings and legs were carefully

removed and the rest of the beetle (abdomen and thorax) was weighed before drying at 55°C for 72 hours after which the dry weight was measured. A chloroform-methanol solution (1:1) was used to extract lipids and after drying for 72 hours the lipid-free weight of the beetle was again measured (see Östman 2005). The lipid content was calculated by subtracting the lipid-free weight from the dry weight and is presented as proportional to dry weight. The water content is presented as proportional to the fresh weight.

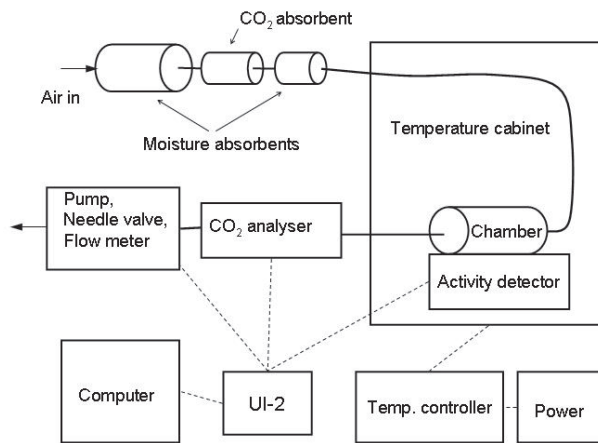


FIGURE 2 Simplified diagram of respirometry system used in the metabolic rate measurements. Solid lines represent the flow of air, dashed lines the electronic connections. UI-2 is the universal interface.

TABLE 1 An overview of the experimental designs.

	Experiment			
	II	III	IV	V
Population	Poland	Russia	Russia, Italy	Poland
Experimental temperature	23°C, 19°C	23°C	23°C	23°C
Light Conditions	18:6	18:6	13:11, 21:3	18:6
Treatment			Insecticide	Insecticide, parents maintained under mean 17°C or 23°C for 3 generations
Traits measured	Body mass of 0-10-day-old beetles	Emergence and 10-day body mass	Emergence and 10-day body mass	Emergence body mass
	Resting metabolic rate of 0-10-day-old beetles	Resting metabolic rate at age 6 days	Development time	Development time
		h ²	Larval survival	Larval survival, larvae-to-adult survival
		Diapause behavior Winter survival	Diapause behavior Winter survival	Lipid content Water content

3 RESULTS AND DISCUSSION

3.1 The amount of genetic variation

3.1.1 Genetic variation in the insecticide resistance gene (I)

Intense selection by insecticides generally leads to an increase in the resistance allele frequency (e.g. Labbé et al. 2007) and reduction in genetic variation in the locus targeted by selection as well as in the surrounding areas through linkage and genetic hitch-hiking (Maynard-Smith & Haigh 1974, Newcomb et al. 2005). Consequently, this may limit the adaptive potential to respond to further selection (Allendorf & Lundquist 2003). However, the amount of genetic variation can be confounded by other factors, such as recombination and demographic events including population bottlenecks. My objective was to examine the relative impact of selective and demographic forces on genetic variation in the organophosphate resistance conferring gene (*AChE*) and the prevalence of organophosphate resistance (resistance mutation S291G) among European Colorado potato beetle populations. I also analyzed beetles from Colorado, USA to compare the level of genetic diversity between North American and European populations.

Analysis of the 110 Colorado potato beetles (5 European populations in two different years, 2003 and 2005, and one North American population in 2003) yielded a total of 23 haplotypes containing 39 polymorphic sites. The level of genetic variation in the *AChE* gene was higher than the mtDNA variability (Grapputo et al. 2005) with about three times as many haplotypes as in the mtDNA. There was genetic variation in the *AChE* gene among European beetles despite the strong past insecticide selection. Interestingly, all genetic variability was associated with the resistance mutation S291G. The haplotype network showed no population-specific patterns but the European and the North American haplotypes intermingled in the network. Haplotypes were substantially differentiated with long branches connecting them. Such genetic variation and the substantial differentiation among these haplotypes suggests the role of recombination and/or an old origin of variation originating before

the onset of insecticide selection and the invasion of the beetle to Europe. Recombination analysis indicated several recombination events in both the US and European samples and GARD indicated the presence of a recombination breakpoint in the middle of the fragment. The portion of the *AChE* gene after the recombination breakpoint, which was associated with the resistance mutation S291G, showed less variability than the portion before the recombination breakpoint. This indicates that selection for resistance could have reduced the genetic variability but this was subsequently enhanced by recombination creating the high variability among the haplotypes. The high level of observed variation, especially in the North American population, could also be related to the adaptation to Solanaceous plants, including the beetle's original host plant, buffalo burr (*Solanum rostratum*), which have high concentrations of steroidal alkaloids that inhibit the AChE enzyme (Wierenga & Hollingworth 1992, Zhu & Clark 1995). Colorado potato beetle's AChE has been found to be more resistant to these alkaloids than that of other insects (Wierenga & Hollingworth 1992). Thus, most of the observed genetic variation could be related to the beetle's historic association with alkaloids which caused a pre-adaptation to withstand organophosphate insecticides.

The frequency of resistant genotypes (carrying the resistance mutation S291G) was very high among European beetles (89 %) as well as in the North American population where 90% were resistant homozygotes and 10% were heterozygous for the S291G mutation. This high frequency suggests the role of selection for organophosphate resistance, though no clear signs of positive insecticide selection were detected by the tests of neutrality.

The level of genetic diversity was lower in the European than in the North American population (both in the form of lost of rare variants and reduced nucleotide diversity) indicating a population bottleneck. This was also reflected in the opposite signs of Tajima's neutrality values and could also explain the lack of genetic structure and differentiation among European populations. Overall, the amount of genetic variation among European populations has been affected by recombination, pre-adaptation to withstand plant alkaloids, population bottleneck and selection for insecticides.

3.1.2 Genetic variation in quantitative traits (III)

Successful range expansion may require that a species is able to adapt to novel environments. This, in turn, requires genetic variation (additive genetic variance) in relevant traits (see e.g. Kellermann et al. 2009). I performed a half-sib/full-sib rearing experiment to estimate the amount of heritable variation in metabolic rate as well as in body mass and diapause behavior, all of which may be essential for the beetle's successful overwintering at high latitudes.

Although there was phenotypic variation in the measured traits, I found that all traits had low and insignificant heritability estimates, ranging from 0 to 0.19. The low heritability estimate of metabolic rate (0.01-0.10) was not unexpected as it accords with other studies which have also found low and insignificant heritability estimates of insect resting metabolic rate (Rantala &

Roff 2006, Nespolo et al. 2007, Ketola & Kotiaho 2009, Klemme & Hanski 2009), characteristic of fitness-related traits (Mousseau & Roff 1987, DeRose & Roff 1999). I estimated the evolutionary potential of a marginal population at the species' range limit. It is possible that founder effects could explain the lack of additive genetic variance (Hoffmann & Blows 1994) or that selection has eroded the amount of additive genetic variance (Blows & Hoffmann 1993, Hoffmann & Blows 1994, van Heerwaarden et al. 2010).

Overall, the low evolutionary potential in these traits may suggest that, if the beetle's range expansion further north requires adaptive genetic changes in these traits, its potential to expand its range beyond its current distribution via evolutionary adaptation could be limited (see Roff 1997). Evolutionary change is not, however, the only prerequisite for species to successfully spread to new areas. The beetle is able to overwinter successfully at latitudes of its most northern range and the beetle has high overwintering survival under optimal conditions (see below). This suggests that, as for overwintering survival, benign winters may facilitate the range expansion further north. In which case, other factors, such as population size and propagule pressure, will likely regulate the beetle's northward range expansion.

3.2 Association between energy metabolism, diapause and overwintering

3.2.1 Energy metabolism during pre-diapause (II)

Harsh winter conditions are the limiting factor for the successful range expansion of insect species towards high latitudes. Many insects, however, have several behavioral and physiological mechanisms to survive winter periods at high latitudes, including diapause (Tauber et al. 1986, de Kort 1990). However, in high latitudinal environments the time window for preparation for winter diapause can be very narrow and, thus, physiological changes must take place in very short time periods (Tauber et al. 1986). I set out to investigate changes in the energy metabolism of pre-diapause Colorado potato beetles by examining resting metabolic rate for eleven days, from adult emergence until the age of 10 days. I found that metabolic rate changed independently of changes in body mass and formed a bell shaped pattern with adult age. First, metabolic rate increased almost two fold from the emergence day to the first day after emergence and peaked in 2-day-old beetles. It then decreased gradually until 10-day-old beetles had metabolic rates that were at the same level as those of newly emerged beetles. This pattern in energy metabolism reflects the physiological changes that take place in the adult pre-diapause beetles that have adapted to temperate latitudes. Beetles must first accumulate sufficient energy reserves for overwintering and also develop flight muscles (May 1989) (increasing energy metabolism) after which it is essential to shut down the energetically expensive processes. This lowered metabolic rate saves the limited

energy reserves during the overwintering period (Hahn & Denlinger 2007). As for variation in metabolic rate, adult age evidently matters and should be taken into account in studies of energy metabolism. It is also worth noting that variation in the age-related patterns of resting metabolic rate and/or diapause propensity among populations (e.g. comparing this study to de Wilde & Stegwee 1958, de Wilde & Hsiao 1981, Yocum et al. 2009) may reflect different physiological adaptations to specific environmental conditions, especially to photoperiod (day length is the main cue initiating diapause development in the beetle (de Kort 1990).

3.2.2 Relationship of body mass and energy metabolism to diapause behavior and overwintering survival (III)

Diapause is an important physiological adaptation of insects to survive prolonged and harsh winter periods (Hahn & Denlinger 2007). As shown above (II), the pre-diapause period may involve fast changes in physiology as energy reserves must be first gained, after which metabolism is lowered. Behavior may also play a part in successful overwintering. Therefore, I examined the influence of metabolic rate and body mass on diapause behavior and overwintering success in beetles from the species' northern range limit.

I found that high body mass and low metabolic rate were associated with high diapause propensity which was linked to high overwintering survival (survival in the soil 69.2%, on the surface 10.8%). Results here and elsewhere suggest that there is and has been selection on body size and metabolic rate at the onset of diapause (Munyiri et al. 2004, Hiiesaar et al. 2006, Noronha & Cloutier 2006, Hahn & Denlinger 2007). That is, the insects must achieve certain thresholds for body mass (energy reserves) and metabolic rate (lowered energy use) to be able to successfully enter diapause. Not all beetles dug into the soil, some stayed on the surface. These beetles were possibly in weak physiological condition or, alternatively, they may have not been in the pre-diapause stage. Whatever the case, survival on the surface was reduced dramatically.

Furthermore, winter diapause behavior seems to be of adaptive value to beetles as the earlier a beetle entered diapause, the higher the overwintering survival. However, it was unclear what caused this differential survival. The physiological traits (body mass and metabolic rate) measured in my study did not explain it. It is well known that diapause propensity in response to photoperiod can be highly variable among and within insect populations (Tauber et al. 1988, Tanaka 1992, Hard et al. 1993, Schmidt et al. 2005). So it could be that the variation in diapause propensity might stem from the photoperiod used in the experiment.

Another important result of the experiment was that the effects of metabolic rate and body mass on overwintering survival were mostly indirect, mediated by diapause behavior. Once beetles had entered diapause, large body mass was associated with high overwintering survival only in males. In general, large body size contributes to high overwintering survival because larger individuals usually have proportionally larger energy (lipid) reserves (Hahn &

Denlinger 2007). My finding that male, but not female, body mass increased overwintering survival could be related to the fact that in this species, males are smaller than females and thus, even a small increase in size is beneficial to males but less significant to females. It must be noted that while my experiment was conducted under stable, optimal conditions in the laboratory, in the wild the environmental conditions during the winter can be much more variable and harsher, in which conditions the body mass and the level of energy metabolism may have more crucial influence on survival (Irwing & Lee 2000, 2003, Hiiesaar et al. 2006).

3.3 The effect of light regime on diapause behavior and life history traits (IV)

Range expansion towards northern latitudes may require changes in behavior as well as physiology. Insects inhabiting temperate zones rely on diapause for their overwintering survival (Denlinger 2002). As winters arrive earlier in the northern latitudes than in lower latitudes, species expanding their range northwards have to enter diapause when the day length is relatively long. My objective was to examine whether beetles have adapted or have the potential to adapt to the photoperiod of northern latitudes and whether there are interpopulational differences in response to photoperiod. I examined the effect of short (L:D 13:11) and long (L:D 21:3) day-lengths on life-history traits, diapause behavior and overwintering survival in beetle populations collected from high (northern Russia) and low (Italy) latitudinal areas of the species' European distribution.

While the long photoperiod delayed development time, there was no difference in the body mass on emergence between beetles developing under the long- and short-photoperiod conditions. This suggests that beetles responded to the short day-length conditions by accelerating their feeding or food assimilation (see also Dolezal et al. 2007). The Russian beetles seemed to have a shorter development time than the Italian beetles. This could reflect an adaptation to the shorter growing season of northern latitudes. Italian beetles had larger overwintering body mass (mass at 10 days) when reared under short than under long-day conditions, whereas Russian beetles gained similar overwintering body mass irrespective of the photoperiod. Russian beetles were more prone to enter diapause under long-day conditions than Italian beetles. However, even though beetles were prone to enter diapause under the long-day conditions, overwintering survival was lower in the beetles that were reared under the long-day than those reared under the short-day conditions. These results suggest that beetles at northern latitudes have adapted to enter winter diapause even when the photoperiod is relatively long, suggesting that invasion towards higher latitudes is possible. However, the lower overwintering survival of the beetles reared under the long-day conditions suggests that the

physiological machinery to enter diapause at long-day lengths is not fine tuned, which might be the explanation for the slower invasion towards northern latitudes.

3.4 The effect of insecticide stress on life-history and physiological traits (IV, V)

Tolerance to various stressors is a crucial factor influencing the survival and range expansion of invasive species to new environments (Sakai et al. 2001, Lee et al. 2003, Gilchrist et al. 2008, Lee et al. 2009). Exposure to insecticide stress may have diverse effects on physiological and life-history traits (Michaelides et al. 1997, Wei & Du 2004, Desneux et al. 2007) which can influence fitness in general and affect the invasion success of insect pest species. Furthermore, the effect of insecticide stress can be complicated by the environmental conditions or stressors experienced by previous generations (Parsons 1991, Crill et al. 1996, Räsänen & Kruuk 2007). I investigated the effect of insecticide stress on life-history and physiological traits (IV, V) as well as diapause behavior and overwintering survival (IV).

Exposure to pyrethroid insecticide did affect the physiological and life-history traits of Colorado potato beetles (paper V), but these effects were quite subtle. Moreover, temperature stress experienced by parental generations influenced the tolerance of progeny to insecticide stress. Beetles descendant of parents that experienced a low, stressful temperature regime (mean 17°C) had, in general, lower tolerance of insecticide stress than beetles descendant of parents that experienced a high, more favorable, temperature regime (mean 23°C). In beetles whose ancestral generations had been reared on a low temperature regime, insecticide stress prolonged male development time by about 2 days and decreased adult lipid content while no negative effects on these traits were observed in beetles descendant of parents that experienced a higher temperature regime. A likely explanation is that, the low temperature regime was stressful for the beetles (Ferro et al. 1985, Lyytinen et al. 2008, Lyytinen et al. 2009) and thus reduced the fitness of the parents. This then had negative cross-generational effects (Crill et al. 1996, Steigenga & Fischer 2007) decreasing tolerance to insecticide stress in the next generation.

Beetles descendant from parents in high temperature regime tolerated insecticide stress well and there was some indication that insecticide stress resulted in an increase in adult weight. This suggests that larvae were able to compensate for the harmful effects of insecticide stress by increasing feeding or the assimilation of food (Campero et al. 2007).

The fact that the effect of temperature history manifested itself only when larvae were exposed to insecticide supports the view that the organism's ability to endure additional stress is diminished when it has already experienced other stressors (Sih et al. 2004), which, in our case, was be the low temperature

experienced by the parents. Furthermore, as for fitness consequences, the effect of insecticide stress may be positive in some traits (increased weight) but negative in others (decreased lipid content). The complexity of the factors that influence tolerance to stress and the diverse effects that stress can incur, make it difficult to predict the outcome of the invasion of pest insect species to new environments and of procedures to manage them.

Organophosphate insecticide treatment of larvae had no significant effects on diapause behavior or overwintering survival (paper IV). This could be related to the fact that although organophosphates affect the nervous system of the insects and diapause induction is also controlled by neural pathways, these two processes probably have different modes of action.

It is notable that larval survival of insecticide stress varied between populations in different experiments and with the insecticides used. Polish larvae (V) were very tolerant of pyrethroid insecticide (80 % survived) while Russian larvae (IV) were more susceptible (20 % survived) despite the much lower concentration used on Russian larvae. In contrast, Russian larvae were very resistant to organophosphate insecticides (90 % survived the treatment). These differences reflect the differential insecticide selection pressures in the original populations. The Polish populations have been heavily controlled by pyrethroids and thus the prevalence of pyrethroid resistance in this population is high (~ 100% Grapputo et al. ms). In contrast, in Russia, pyrethroids have not been used as long as in Poland for managing beetle populations. The prevalence of organophosphate resistance is still very high among European populations (I), including Russia, reflecting the intense past use of this insecticide. Although the use of organophosphates against beetles has dramatically decreased since the 1990s, the possibility that organophosphates or other pesticides targeting the AChE are still used against the beetle in some regions cannot be ruled out.

4 CONCLUSIONS

Biological invasions provide unique opportunities to study the physiological and evolutionary factors and processes that facilitate and limit species ranges (Sakai et al. 2001, Lee 2002). I investigated some of these attributes affecting the range expansion potential of European Colorado potato beetles (*Leptinotarsa decemlineata*), especially towards higher latitudes. More specifically, I focused on studying the factors involved in winter diapause (II, III, IV), on which many insects' overwintering survival heavily depends. As many invasive species are also pests, they are repeatedly exposed to insecticides. This may not only lead to the evolution of insecticide resistance but may also influence various life-history, behavioral and physiological traits. The evolutionary history of invasive pest populations and current insecticide use as well as the interaction of the two may influence the range expansion potential of species. My aims were to decipher the genetic (I) and physiological/behavioral (IV, V) consequences of exposure to insecticides.

I found that successful overwintering was largely dependent on the ability to adjust physiology (lowered energy metabolism, large body mass) as well as diapause behavior (entering the soil) in response to photoperiod (III). Once these requirements were fulfilled, overwintering in the soil was not strongly dependent on physiology, except in males, whose winter survival increased with body mass. Overall, the beetles originating from the northern range limit of the species distribution were capable of fulfilling these physiological and behavioral requirements for successful overwintering in northern latitudes. They also presented adaptation to the long photoperiod of the north (IV) which is probably a crucial factor affecting successful range expansion to northern latitudes. However, even though they were able to adjust their diapause behavior in response to long day length, the beetles' physiological readiness to overwinter successfully was not high. Moreover, as pointed out by Hiiesaar et al. (2006), unexceptionally cold and harsh winters can result in high winter mortality and thereby slow the invasion of the beetle.

Whereas there was phenotypic variation in the physiological and behavioral traits measured, the evolutionary potential to respond to selection

i.e. heritability, in these traits was low (III), potentially limiting the beetles range expansion further north. Directional selection or founder events may have eroded the amount of heritable variation in the beetle during its spread across Europe. Nevertheless, the beetle seems to be very flexible or phenotypically plastic, especially for physiological traits such as energy metabolism. The pre-diapause beetles had the means to alter their energy metabolism (substantial increase followed by a decrease to low levels of diapause) so that they were physiologically ready to enter winter diapause within just 10 days of adult life (II). This is important in high latitude conditions where the time window available for entering diapause may be limited. Furthermore, the high overwintering survival (III) as well as high reproductive potential of the beetles suggests that range expansion further north is possible but is probably regulated by demographic stochasticity more than anything else.

European Colorado potato beetle populations possess genetic variation in the insecticide resistance conferring gene *AChE* (I) despite having experienced a population bottleneck during the invasion of Europe (Grapputo et al. 2005) and having been exposed to high selection pressure by organophosphate insecticides in the past. Recombination events and, possibly, a pre-adaptation to withstand plant alkaloids probably contributed to the genetic variation in this gene. The beetles likely already carried resistance to organophosphates when they invaded Europe and this may have contributed to the high prevalence of organophosphate resistance among European populations.

When a pest species arrives in a new environment, it can already be resistant to the commonly used insecticides. Therefore, subsequent exposure to insecticides may incur only stress. I found that exposure to pyrethroid insecticide stress had diverse, though subtle, effects on the life-history and physiological traits of (resistant) beetles which could either increase or decrease fitness. More importantly, the effects of tolerance to insecticide were dependent on the temperature conditions experienced by the parental generations (V). This complexity of the effects of several stressors (insecticide, temperature) can make it very difficult to predict both the outcome of management procedures and the invasion success of pest insect species to new environments.

Taken together, my results show that the range expansion potential of the Colorado potato beetle is not limited, though it may be reduced to some extent, by the genetic and physiological characteristics examined in this thesis. My results also underline that uncovering the evolutionary history of invasive populations can be important in understanding the evolutionary processes of invasions and determining the future of invasive pest populations (Keller & Taylor 2008).

It is suggested that many of the genetic and physiological characters contributing to this species' range expansion are pre-adaptations. A long history with plant alkaloids may have enabled beetle's high ability to evolve insecticide resistance. The environmental conditions at high latitudes, such as harsh winters, timing of diapause and cooler-and-shorter-than-average growing seasons, may pose some challenges to the Colorado potato beetles and slow its range expansion towards higher latitudes. However, its range may be limited

mostly by the occurrence of the preferred host plant, potato, on which it can sustain high population sizes. And it might be just a matter of chance and lapse of sufficient time for the beetle to reach all potato farming areas.

Acknowledgements

I would like, above all, to express my deepest gratitude to my supervisors Leena Lindström, Alessandro Grapputo and Johanna Mappes. I am especially grateful to Leena who gave valuable advice and support in planning and conducting experiments, thoughtful comments on this thesis and manuscripts, and encouragement in moments of despair and frustration. I am grateful to Ale for advice and help with the population genetics analyses, for ensuring that my visit to Padova, Italy, was successful, and for acting as Italian interpreter in situations in which no English was spoken. I am very grateful to Jonna for constructive comments on this thesis and manuscripts. I am also very grateful to my fellow research group member Anne Lyytinen for help and advice on experiments and valuable comments on manuscripts. Thanks to Sanna Boman, a past member of the research group, for support, inspiring discussion and friendship and to the newest member, Philipp Lehmann. I am very grateful to Andrew Suarez for accepting the invitation to be my opponent, and to Liselotte Sundström and Mats Björklund for evaluating this thesis.

Many other people have helped me along the way. I am grateful to Tarmo Ketola for co-authorship and for advice on the metabolic measurements. I would like to thank Maxine Iversen, Emily Knott, Mika Mökkönen, Zbyszek Boratynski, Jackson Jennings, Journal club leaders and members and all who read, commented on and improved the manuscripts in terms of both scientific content and language. Heartfelt thanks go to the summer workers and trainees for taking care of the beetles: Tanja Heikkilä, Sirpa Huttunen, Hannele Kauranen, Miia Koivula, Pilvi Koivuniemi, Uliana Kyrylenko, Lily Laine, Anu Luoto, Elina Manninen, Hanna Mappes, Marko Moilanen, Veera Pälvimäki, Kaisa Suisto, Jonna Timonen, Jurian Törnroth and Sampo Välimaa. I am also very grateful to Sari Viinikainen who performed most of the DNA laboratory work. I would like to thank Kirsi Liukkunen, Elina Virtanen, Michele Moretta and Sami Kyröläinen.

This work would have not been possible without the co-operation of Agrifood Research Finland who provided facilities for beetle rearing in Jokioinen. I would particularly like to thank Kari Tiilikkala, Irene Vänninen and Marjaana Virtanen. I am also grateful to Maria Pawinska, Kylli Hiessaar, Pekka Honkala, Whitney Cranshaw and Andrea Pilastro for providing beetle samples.

I thank the heads of the Ecology section as well as my colleagues at the Department of Biological and Environmental Science. I am especially very grateful to Juha Ahonen, Tarmo Halinen, Jari Haimi, Hannu Kautto, Harri Kovanen and Samuli Koskimaa for various help from transportation and logistics to technical advice. I would also like to thank Darwin group members and fellow PhD students as well as Sauna & Support. My very thanks go to past and present officemates, especially Sanna and Anne, who offered support and inspirational discussion; there were so many of you in the course of moving back and forth during the renovation, YAC422.1, YAC423.1, YAD412.1, YN321 and YAC420.2.

I thank my friends for support and delightful discussions, especially Elina, Sanna, Hanna, Henna, Jenni, Nina, Jaana and Veronica. I would also like to thank my parents, Päivi and Simo and my brothers, Risto, Aripetri and Topi.

This work was funded by the Academy of Finland, the Centre of Excellence in Evolutionary Research, Konnevesi Grant given by the University of Jyväskylä and by the Finnish Cultural Foundation.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Lajien levinneisyysalueen laajeneminen: koloradonkuoriaisen evolutiivinen fysiologia ja genetiikka

Lajien levinneisyysaluetta rajoittavat ja sitä muokkaavat tekijät ovat keskeinen tutkimusalue niin ekologiassa kuin evoluutiobiologiassa. Vieraslajien leviäminen uusille alueille tarjoaa erinomaisen tilaisuuden tutkia näitä tekijöitä. Vieraslajien leviämismenestyksen ymmärtäminen on erittäin tärkeää myös, koska nämä lajit muodostavat vakavan uhan luonnon monimuotoisuudelle, ekosysteemin toiminnalle, maataloudelle sekä ihmisten ja eläinten terveydelle. Näin ollen tieto leviämisen syistä edesauttaa myös leviämisen ja sen haittojen estämisessä.

Vieraslajien leviäminen etenkin pohjoisille alueille, kuten Suomeen, voi edellyttää useita samanaikaisia sopeumia elinkierto- ja käyttäytymisessä ja fysiologisissa ominaisuuksissa. Tällaisia ovat esimerkiksi hyönteisillä nopea kehitysaika ja hyvä energian varastoimiskyky, jotka mahdollistavat valmistautumisen talvehtimiseen hyvissä ajoin ennen lyhyen suotuisan kasvujakson päättymistä. Toisaalta, jotta vieraslajit selviytyisivät ankarasta ja pitkästä talvesta, talvehtimisen aloittaminen, kuten diapaussiin (hyönteisen horrosta syvempi lepovaihe) meno, on ajoitettava oikein. Yksilön on myös alennettava energia-aineenvaihdunnan tasoa riittävästi. Koska talvi saapuu pohjoisilla leveysasteilla aikaisemmin kuin etelämpänä, diapaussiin on osattava mennä käyttäen pidempää päivänpituutta merkinä oikeasta ajankohdasta. Alhainen aineenvaihdunnan taso taas mahdollistaa talvehtijoiden energiavarastojen käyttämisen sääste- liäästi, jotta ne riittävät koko talven.

Sopeutuminen uusiin oloihin edellyttää usein hyvää kykyä sietää erilaisia stressitekijöitä ja geneettistä vaihtelua niissä ominaisuuksissa, jotka ovat selviytymisen kannalta merkityksellisiä. Useat vieraslajit ovat kuitenkin levittäytyes- sään muutaman yksilön voimin uusille alueille usein läpikäyneet geneettisen pullonkaulan, mikä voi vähentää geneettisen vaihtelun määrää ja rajoittaa myöhemmin kykyä sopeutua uuden ympäristön oloihin. Lisäksi monet vieras- lajit ovat tuholaisia, joiden torjumiseksi käytetään paljon erilaisia torjunta- aineita. Näin ollen vieraslajien selviytymiseen ja leviämiseen vaikuttavat myös niiden kyky kehittää vastustuskyky torjunta-aineita vastaan sekä kyky sietää näiden aineiden aiheuttamaa stressiä.

Koloradonkuoriainen (*Leptinotarsa decemlineata*) on erinomainen kohdelaji tutkia vieraslajien leviämistä uusille alueille. Se on merkittävä perunan tuholai- nen ja lähtöisin Meksikosta, josta se on levinnyt kaikkialle USA:han. Lajia on tavattu 1920-luvulta lähtien melkein koko Euroopasta lukuun ottamatta Brittein saaria ja Skandinaviaa. Nykyään koloradonkuoriainen on leviämässä edelleen itään ja pohjoiseen. Tällä hetkellä pohjoisimmat kuoriaispopulaatiot ovat Venä- jän Karjalassa, josta kuoriaisia on levinnyt Suomeen useina vuosina (2002-2007). Kaikkein tehokkain keino torjua koloradonkuoriaista on torjunta-aineiden käyt- tö. Torjumisen tekee ongelmalliseksi kuitenkin se, että koloradonkuoriaisella on

erinomainen kyky kehittää lyhyessä ajassa torjunta-aineresistenssi. Koloradonkuoriaisella on myös havaittu olevan geneettistä vaihtelua elinkierto-*piirteissä* sekä neutraalia geneettistä vaihtelua huolimatta siitä, että se on läpikäynyt pulonkaulailmiön levitessään Eurooppaan. Kuoriaisten levinneisyysalueen laajeneminen pohjoiseen on hidastunut jonkin verran. Aikaisemmat tutkimukset ovat osoittaneet, ettei kuoriaisen leviämistä pohjoiseen rajoita kesäolosuhteet vaan mahdollisesti talvi.

Väitöskirjatyössäni tutkin fysiologiaa ja evolutiivisia tekijöitä ja prosesseja, jotka vaikuttavat koloradonkuoriaisen leviämismenestykseen, etenkin pohjoisille alueille. Tutkin, kuinka paljon koloradonkuoriaisen eurooppalaisissa populaatioissa on geneettistä vaihtelua torjunta-aineresistenssiä aiheuttavassa *AChE* geenissä, ja mitkä eri tekijät ovat vaikuttaneet geneettisen vaihtelun määrään. Tutkin etenkin perusaineenvaihdunnan ja painon vaikutusta kuoriaisten diapaussikäyttäytymiseen (aikuiset kuoriaiset aloittavat talvehtimisen ja diapaussin kaivautumalla maan alle) ja talvehtimismenestykseen sekä selvitin periytyvän vaihtelun määrää näissä ominaisuuksissa. Tutkin lisäksi, kuinka päivänpituus ja torjunta-ainestressi vaikuttavat kuoriaisen fysiologiaan ominaisuuksiin ja elinkierto-*piirteisiin* sekä diapaussikäyttäytymiseen ja talvehtimismenestykseen.

Tutkimukseni osoitti, että kuoriaisten fysiologisella valmiudella oli merkittävä vaikutus diapaussikäyttäytymiseen, mikä taas vaikutti kuoriaisten talvehtimismenestykseen. Toisin sanoen kuoriaisten täytyi ensin saavuttaa tarpeeksi alhainen perusaineenvaihdunnan taso ja korkea paino kaivautuakseen maan alle diapaussiin. Tämän saavutettuaan kuoriaisten talvehtimismenestys oli hyvä, eikä fysiologisilla ominaisuuksilla ollut enää mainittavaa merkitystä itse selviytymiseen maan alla. Painavat kuoriaiset selviytyivät talvesta hyvin, koska niillä oli luultavasti myös paljon rasvaa, mikä on pääasiallinen energianlähde talvehtimisen aikana. Alhainen energia-aineenvaihdunnan taso taas kuluttaa energiavaroja hitaammin. Vaikka fysiologisissa ominaisuuksissa ja diapaussikäyttäytymisessä oli paljon fenotyypistä eli ilmiasun vaihtelua, periytyvän geneettisen vaihtelun määrä oli näissä *piirteissä* alhainen. Tämä voi mahdollisesti rajoittaa kuoriaisten sopeutumiskykyä pohjoisiin oloihin näiden ominaisuuksien suhteen.

Huomattavaa kuitenkin oli, että kuoriainen pystyy valmistautumaan onnistuneesti talvehtimiseen hyvin lyhyessä ajassa, kymmenen ensimmäisen aikuispäivän aikana. Tämä heijastui myös kuoriaisten iän suhteen tapahtuviin muutoksiin perusaineenvaihdunnan tasossa. Kyky valmistautua onnistuneesti talvehtimiseen lyhyessä ajassa voi olla hyvinkin tärkeää pohjoisessa, missä suotuisan kasvujakson pituus on paljon lyhyempi kuin etelämpänä. Tutkimukseni osoitti myös, että pohjoinen kuoriaispopulaatio oli sopeutunut menemään diapaussiin pidemmän päivän oloissa.

Tutkimuksessani selvisi myös että, monet eri tekijät ovat vaikuttaneet geneettisen vaihtelun määrään torjunta-aineresistenssiä aiheuttavassa *AChE* geenissä. Rekombinaatio sekä mahdollisesti sopeutuminen kestävään perunan sukulaislajien sisältämiä kasvialkaloideja ovat ylläpitäneet geneettisen vaihte-

lun määrää tässä geenissä ennen kuin kuoriainen saapui Eurooppaan. Sitä vastaan torjunta-ainealtistuksen aiheuttama valinta ja geneettinen pullonkaula, jonka kuoriaiset ovat läpikäyneet levitessään Eurooppaan, ovat vähentäneet geneettisen vaihtelun määrää. Näiden tekijöiden yhteisvaikutuksen seurauksena eurooppalaisissa kuoriaispopulaatioissa on edelleen oletettua enemmän geneettistä vaihtelua, vaikkakin se on vähäisempää kuin Pohjois-Amerikassa.

Tutkimukseni osoitti myös, että kuoriaisilla on hyvä kyky sietää torjunta-ainestressiä. Torjunta-ainestressillä oli vain vähäisiä haitallisia vaikutuksia kuoriaisen fysiologisiin ominaisuuksiin, kuten kehitysaikaan ja rasvavarastojen määrään. Torjunta-ainestressin vaikutukset ominaisuuksiin riippuivat siitä, millaisissa lämpöoloissa kuoriaisten emot oli kasvatettu. Kaiken kaikkiaan tulokset kertoivat siitä, että torjunta-ainestressin sietokykyyn voivat vaikuttaa useat eri tekijät, joiden vaikutukset elinkierto- ja fysiologisiin ominaisuuksiin voivat olla moninaisia lisäten tai vähentäen yksilöiden kelpoisuutta. Tämän vuoksi torjuntatoimenpiteiden vaikutuksia tuholaisvieraslajien leviämismenestykseen voi olla vaikea ennustaa.

Tulokseni osoittavat, etteivät tutkimani fysiologiset ja evolutiiviset tekijät välttämättä rajoita, vaikka voivat jossain määrin hidastaa, kuoriaisen leviämisen alueen laajenemista pohjoiseen. Vieraslajien aikaisemmalla evolutiivisella historialla ja emojen kokemalla historialla voi olla huomattava merkitys vieraslajien leviämismenestyksessä.

REFERENCES

- Addo-Bediako, A., Chown, S. L. & Gaston, K. J. 2000. Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. Lond., Ser. B, Biol. Sci.* 267: 739-345.
- Addo-Bediako, A., Chown, S. L. & Gaston, K. J. 2002. Metabolic cold adaptation in insects: a large-scale perspective. *Funct. Ecol.* 16: 332-338.
- Allendorf, F. W. & Lundquist, L. L. 2003. Introduction: population biology, evolution, and control of invasive species. *Conser. Biol.* 17: 24-30.
- Alyokhin, A. 2009. Colorado potato beetle management on potatoes: current challenges and future prospects. *Fruit, Veg. Cereal Sci. Biotech.* 3: 10-19.
- Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G. & Grafius, E. 2008. Colorado potato beetle resistance to insecticides. *Am. J. Pot. Res.* 85: 395-413.
- Artacho, P. & Nespolo, R. F. 2009. Natural selection reduces energy metabolism in the garden snail, *Helix aspersa* (*Cornu aspersum*). *Evolution* 63: 1044-1050.
- Baker, H. G. 1974. The evolution of weeds. *Annu. Rev. Ecol. Syst.* 5: 1-24.
- Bale, J. S. & Hayward, S. A. L. 2010. Insect overwintering in a changing climate. *J. Exp. Biol.* 213: 980-994.
- Bernard, L. & Lagadic, L. 1993. Sublethal effects of dietary cyfluthrin on nutritional performance and gut hydrolase activity in larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. *Pestic. Biochem. Physiol.* 46: 171-180.
- Blanckenhorn, W. U. & Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr. Comp. Biol.* 44: 413-424.
- Blossey, B. & Notzold, R. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *J. Ecol.* 83: 887-889.
- Blows, M. W. & Hoffmann, A. A. 1993. The genetics of central and marginal populations of *Drosophila serrata*. I. Genetic variation for stress resistance and species borders. *Evolution* 47: 1255-1270.
- Blows, M. W. & Hoffmann, A. A. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86: 1371-1384.
- Boman, S. 2008. Ecological and Genetic Factors Contributing to Invasion Success: The Northern Spread of the Colorado Potato Beetle (*Leptinotarsa Decemlineata*). 50 p., Studies in Biological and Environmental Science, University of Jyväskylä.
- Boman, S., Grapputo, A., Lindström, L., Lyytinen, A. & Mappes, J. 2008. Quantitative genetic approach for assessing invasiveness: geographic and genetic variation in life-history traits. *Biol. Invasions* 10: 1135-1145.
- Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* 13: 115-155.
- Bridle, J. R. & Vines, T. H. 2007. Limits to evolution at range margins: when and why does adaptation fail? *Trends Ecol. Evol.* 22: 140-147.

- Brock, M. T., Weinig, C. & Galen, C. 2005. A comparison of phenotypic plasticity in the native dandelion *Taraxacum ceratophorum* and its invasive congener *T. officinale*. *New Phytol.* 166: 173-183.
- Campero, M., Slos, S., Ollevier, F. & Stoks, R. 2007. Sublethal pesticide concentrations and predation jointly shape life history: Behavioral and physiological mechanisms. *Ecol. Appl.* 17: 2111-2122.
- Cano, L., Escarre, J., Fleck, I., Blanco-Moreno, J. M. & Sans, F. X. 2008. Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. *J. Ecol.* 96: 468-476.
- Casagrande, R. A. 1987. The Colorado potato beetle: 125 years of mismanagement. *Bull. Entomol. Soc. Am* 33: 142-150.
- Cheverud, J. M. & Routman, E. J. 1996. Epistasis as a source of increased additive genetic variance at population bottlenecks. *Evolution* 50: 1042-1051.
- Chown, S. L. & Gaston, K. J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biol. Rev. Camb. Philos. Soc.* 74: 87-120.
- Chown, S. L. & Nicolson, S. W. 2004. *Insect Physiological Ecology, Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S. L., Slabber, S., McGeoch, M. A., Janion, C. & Leinaas, H. P. 2007. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. Lond. , Ser. B, Biol. Sci.* 274: 2531-2537.
- Clark, J. M., Lee, S. H., Kim, H. J., Yoon, K. S. & Zhang, A. G. 2001. DNA-based genotyping techniques for the detection of point mutations associated with insecticide resistance in Colorado potato beetle *Leptinotarsa decemlineata*. *Pest Manag. Sci.* 57: 968-974.
- Clement, M., Posada, D. & Crandall, A. K. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9: 1657-1659.
- Crill, W. D., Huey, R. B. & Gilchrist, G. W. 1996. Within- and between-generation effects of temperature on the morphology and physiology of *Drosophila melanogaster*. *Evolution* 50: 1205-1218.
- Crnokrak, P. & Roff, D. A. 2002. Trade-offs to flight capability in *Gryllus firmus*: the influence of whole-organism respiration rate on fitness. *J. Evol. Biol.* 15: 388-398.
- Daloze, D., Braekman, J. C. & Pasteels, J. M. 1986. A toxic dipeptide from the defense glands of the Colorado beetle. *Science* 233: 221-223.
- Davis, M., Grime, J. & Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *J. Ecol.* 88: 528-534.
- Dawson, M. N., Grosberg, R. K., Stuart, Y. E. & Sanford, E. 2010. Population genetic analysis of a recent range expansion: mechanisms regulating the poleward range limit in the volcano barnacle *Tetraclita rubescens*. *Mol. Ecol.* 19: 1585-1605.
- de Kort, C. A. D. 1990. Thirty-five years of diapause research with the Colorado potato beetle. *Entomol. Exp. Appl.* 56: 1-13.

- de Wilde, J. & Hsiao, T. H. 1981. Geographic diversity of the Colorado potato beetle and its infestation in Eurasia. In: Lashomb, J. & Casagrande, R. (eds), *Advances in Potato Pest Management*. Hutchinson Ross Publishing Company, Stroudsburg, Pennsylvania.
- de Wilde, J. & Stegwee, D. 1958. Two major effects of the corpus allatum in the adult Colorado beetle. *Arch. Neerlandaises Zool.* 13: 277-289.
- DeRose, M. A. & Roff, D. A. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* 53: 1288-1292.
- Desneux, N., Decourtye, A. & Delpuech, J. M. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52: 81-106.
- Dlugosch, K. M. & Parker, I. M. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 17: 431-449.
- Dolezal, P., Habustova, O. & Sehnal F. 2007. Effects of photoperiod and temperature on the rate of larval development, food conversion efficiency, and imaginal diapause in *Leptinotarsa decemlineata*. *J. Insect Physiol.* 53: 849-857.
- Duckworth, R. A. 2009. Maternal effects and range expansion: a key factor in a dynamic process? *Phil. Trans. Royal Soc. Lond. , Ser. B, Biol. Sci.* 364: 1075-1086.
- Dyer, A. R., Brown, C. S., Espeland, E. K., McKay, J. K., Meimberg, H. & Rice, K. J. 2010. The role of adaptive trans-generational plasticity in biological invasions of plants. *Evol. Appl.* 3: 179-192.
- Ellstrand, N. C. & Schierenbeck, K. A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci. USA* 97: 7043-7050.
- Elton, C. S. 1985. *The Ecology of Invasions by Animals and Plants*. London: Methuen.
- EPPO 2006. Distribution maps of quarantine pests for Europe, *Leptinotarsa decemlineata*. EPPO-European and Mediterranean plant protection organization.
- Excoffier, L., Laval, G. & Schneider, S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinf.* 1: 47-50.
- Falconer, D. S. & Mackay, T. F. C. 1996. *Introduction to Quantitative Genetics*. Longman, Essex.
- Ferro, D. N., Logan, J. A., Voss, R. H. & Elkinton, J. S. 1985. Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. *Environ. Entomol.* 14: 343-348.
- Ferro, D. N., Tuttle, A. F. & Weber, D. C. 1991. Ovipositional and flight behavior of overwintered Colorado potato beetle (Coleoptera: Chrysomelidae). *Environ. Entomol.* 20: 1309-1314.
- French-Constant, R. H., Daborn, P. J. & Goff, G. L. 2004. The genetics and genomics of insecticide resistance. *Trends Genet.* 20: 163-170.
- Ficetola, G. F., Bonin, A. & Miaud, C. 2008. Population genetics reveals origin and number of founders in a biological invasion. *Mol. Ecol.* 17: 773-782.

- Fonseca, D. M., Widdel, A. K., Hutchinson, M., Spichiger, S. & Kramers, L. D. 2010. Fine-scale spatial and temporal population genetics of *Aedes japonicus*, a new US mosquito, reveal multiple introductions. *Mol. Ecol.* 19: 1559-1572.
- Forgash, A. J. 1985. Insecticide resistance in the Colorado potato beetle. In: Ferro, D. N. & Voss, R. H. (eds), *Proceedings of the Symposium on the Colorado Potato Beetle. XVII International Congress of Entomology.*
- Gaston, K. J. 2003. *The Structure and Dynamics of Geographic Ranges.* 266 p., Oxford University Press, New York.
- Gaston, K. J. 2009. Geographic range limits of species. *Proc. R. Soc. Lond. , Ser. B, Biol. Sci.* 276: 1391-1393.
- Genovesi, P. 2005. Eradications of invasive alien species in Europe: a review. *Biol. Invasions* 7: 127-133.
- Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21: 394-407.
- Gilchrist, G. W. & Lee, C. E. 2007. All stressed out and nowhere to go: does evolvability limit adaptation in invasive species? *Genetica* 129: 127-132.
- Gilchrist, G. W., Jeffers, L. M., West, B., Folk, D. G., Suess, J. & Huey, R. B. 2008. Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura*. *Evol. Appl.* 1: 513-523.
- Grapputo, A., Boman, S., Lindström, L., Lyytinen, A. & Mappes, J. 2005. The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. *Mol. Ecol.* 14: 4207-4219.
- Grewal, P. S., Power, K. T. & Shetlar, D. J. 2001. Neonicotinoid insecticides alter diapause behaviour and survival of overwintering white grubs (Coleoptera: Scarabaeidae). *Pest Manag. Sci.* 57: 852-857.
- Hahn, D. A. & Denlinger, D. L. 2007. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. *J. Insect Physiol.* 53: 760-773.
- Hard, J. J., Bradshaw, W. E. & Holzapfel, C. M. 1993. The genetic basis of photoperiodism and its evolutionary divergence among populations of the pitcher-plant mosquito, *Wyeomyia smithii*. *Am. Nat.* 142: 457-473.
- Hardin, M. R., Benrey, B., Coll, M., Lamp, W. O., Roderick, G. K. & Barbosa, P. 1995. Arthropod pest resurgence: An overview of potential mechanisms. *Crop Protection* 14: 3-18.
- Hare, J. D. 1990. Ecology and management of the Colorado potato beetle. *Annu. Rev. Entomol.* 35: 81-100.
- Heikkilä, J. 2006. *Economics of Invasive Alien Species: Pre-Emptive Versus Reactive Control.* 162 p., MTT Discussion Papers 9, Licentiate Thesis.
- Hersteinsson, P. & Macdonald, D. W. 1992. Interspecific competition and the geographical distribution of red and arctic foxes *Vulpes vulpes* and *Alopex lagopus*. *Oikos* 64: 505-515.
- Hiisaar, K., Metspalu, L., Joudu, J. & Jogar, K. 2006. Over-wintering of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in field conditions

- and factors affecting its population density in Estonia. *Agron. Res.* 4: 21-30.
- Hoffmann, A. A. & Blows, M. 1994. Species borders: Ecological and evolutionary perspectives. *Trends Ecol. Evol.* 9: 223-227.
- Hoffmann, A. A. & Parsons, P. A. 1989. Selection for increased desiccation resistance in *Drosophila melanogaster*: Additive genetic control and correlated responses for other stresses. *Genetics* 122: 837-845.
- Hoffmann, A. A., Hallas, R. J., Dean, J. A. & Schiffer, M. 2003. Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* 301: 100-102.
- Holt, R. D. 2003. On the evolutionary ecology of species' ranges. *Evol. Ecol. Res.* 5: 159-178.
- Hough-Goldstein, J. A., Geiger, J., Chang, D. & Saylor, W. 1993a. Palatability and toxicity of the Colorado potato beetle (Coleoptera: Chrysomelidae) to domestic chickens. *Ann. Entomol. Soc. Am.* 86: 158-164.
- Hough-Goldstein, J. A., Heimpel, G. E., Bechmann, H. E. & Mason, C. E. 1993b. Arthropod natural enemies of the Colorado potato beetle. *Crop Protection* 12: 324-334.
- Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D. & Serra, L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287: 308-309.
- Jenkins, N. L. & Hoffmann, A. A. 1999. Limits to the southern border of *Drosophila serrata*: Cold resistance, heritable variation, and trade-offs. *Evolution* 53: 1823-1834.
- Jeschke, J. M. & Strayer, D. L. 2005. Invasion success of vertebrates in Europe and North America. *Proc. Natl. Acad. Sci. USA* 102: 7198-7202.
- Keane, R. M. & Crawley, M. J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* 17: 164-170.
- Keller, S. R. & Taylor, D. R. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecol. Lett.* 852-866.
- Kellermann, V., Van Heerwaarden, B., Sgro, C. M. & Hoffmann, A. A. 2009. Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* 325: 1244-1246.
- Kenis, M., Auger-Rozenberg, M. A., Roques, A., Timms, L., Péré, C., Cock, M. J. W., Settele, J., Augustin, S. & Lopez-Vaamonde, C. 2009. Ecological effects of invasive alien insects. *Biol. Invasions* 11: 21-45.
- Kennedy, T. A., Naeem, S., Howe, K. M., Knops, J. M. H., Tilman, D. & Reich, P. 2002. Biodiversity as a barrier to ecological invasion. *Nature* 417: 636-638.
- Ketola, T. & Kotiaho, J. S. 2009. Inbreeding, energy use and condition. *J. Evol. Biol.* 22: 770-781.
- Kinlan, B. P. & Hastings, A. 2005. Rates of population spread and geographical range expansion. what exotic species tell us. In: Sax, D. F., Stachovicz, J. J. & Gaines, S. D. (eds), *Species Invasions; Insights into Ecology, Evolution and Biogeography*: 381-419. Sinauer Associates, Inc., Sunderland.

- Kirkpatrick, M. & Barton, N. H. 1997. Evolution of a species' range. *Am. Nat.* 150: 1-23.
- Klemme, I. & Hanski, I. 2009. Heritability of and strong single gene (*Pgi*) effects on life-history traits in the Glanville fritillary butterfly. *J. Evol. Biol.* 22: 1944-1953.
- Kolar, C. S. & Lodge, D. M. 2001. Progress in invasion biology: predicting invaders. *Trends Ecol. Evol.* 16: 199-204.
- Kolbe, J. J., Glor, R. E., Rodriguez Schettino, L., Chamizo Lara, A., Larson, A. & Losos, J. B. 2004. Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431: 177-181.
- Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H. & Frost, S. D. W. 2006. GARD: a genetic algorithm for recombination detection. *Bioinformatics* 22: 3096-3098.
- Kostál, V. 2006. Eco-physiological phases of insect diapause. *J. Insect Physiol.* 52: 113-127.
- Krieger, M. J. B. & Ross, K. G. 2002. Identification of a major gene regulating complex social behavior. *Science* 295: 328-332.
- Küster, E. C., Kühn, I., Bruelheide, H. & Klotz, S. 2008. Trait interactions help explain plant invasion success in the German flora. *J. Ecol.* 96: 860-868.
- Labbé, P., Berticat, C., Berthomieu, A., Unal, S., Bernard, C., Weill, M. & Lenormand, T. 2007. Forty years of erratic insecticide resistance evolution in the mosquito *Culex pipiens*. *PLoS Genetics* 3: 2190-2199.
- Lambrinos, J. G. 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology* 85: 2061-2070.
- Lardies, M. A., Catalan, T. P. & Bozinovic, F. 2004. Metabolism and life-history correlates in a lowland and highland population of a terrestrial isopod. *Can. J. Zool.* 82: 677-687.
- Lavergne, S. & Molofsky, J. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc. Natl. Acad. Sci. USA* 104: 3883-3888.
- Lee, C. E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17: 386-391.
- Lee, C. E., Remfert, J. L. & Gelembiuk, G. W. 2003. Evolution of physiological tolerance and performance during freshwater invasions. *Integr. Comp. Biol.* 43: 439-449.
- Lee, J. E., Janion, C., Marais, E., Van Vuuren, B. J. & Chown, S. L. 2009. Physiological tolerances account for range limits and abundance structure in an invasive slug. *Proc. R. Soc. Lond., Ser. B, Biol. Sci.* 276: 1459-1468.
- Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* 17: 183-189.
- Levine, J. M. 2000. Species diversity and biological invasions: Relating local process to community pattern. *Science* 288: 852-854.
- Levine, J. M. & D'Antonio, C. M. 2003. Forecasting biological invasions with increasing international trade. *Conserv. Biol.* 17: 322-326.
- Lighton, J. R. B. 2008. *Measuring Metabolic Rates*. 201 p., Oxford University Press, New York.

- Lodge, D. M. 1993. Biological invasions: Lessons for ecology. *Trends Ecol. Evol.* 8: 133-137.
- Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Genetics*. 980 p., Sinauer Associates, Inc, Sunderland.
- Lyytinen, A., Lindström, L. & Mappes, J. 2008. Genetic variation in growth and development time under two selection regimes in *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* 127: 157-167.
- Lyytinen, A., Boman, S., Grapputo, A., Lindström, L. & Mappes, J. 2009. Cold tolerance during larval development: effects on the thermal distribution limits of *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* 133: 92-99.
- Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M. & Bazzaz, F. A. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecol. Appl.* 10: 689-710.
- Maron, J. 2006. The relative importance of latitude matching and propagule pressure in the colonization success of an invasive forb. *Ecography* 29: 819-826.
- Maron, J. L., Vila, M., Bommarco, R., Elmendorf, S. & Beardsley, P. 2004. Rapid evolution of an invasive plant. *Ecol. Monogr.* 74: 261-280.
- May, M. L. 1989. Oxygen consumption by adult Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *J. Insect Physiol.* 35: 797-804.
- Maynard-Smith, J. & Haigh, J. 1974. Hitch-hiking effect of a favorable gene. *Genet. Res.* 23: 23-35.
- McKenzie, J. A. 2001. Pesticide resistance. In: Fox, C. A., Rolf, D. A. & Fairbairn, D. J. (eds), *Evolutionary Ecology; Concepts and Case Studies*. Oxford University Press, New York.
- Michaelides, P. K., Clevery, A. L. & Wright, D. J. 1997. Sub-lethal effects of tefluthrin on *Diabrotica undecimpunctata howardi*, Barber: plant protection and larval development. *Crop protection* 16: 423-429.
- Mitchell, C. E. & Power, A. O. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421: 625-627.
- Mousseau, T. A. & Fox, C. W. 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* 13: 403.
- Mousseau, T. A. & Roff, D. A. 1987. Natural selection and the heritability of fitness components. *Heredity* 59: 181-197.
- Munyiri, F. N., Shintani, Y. & Ishikawa, Y. 2004. Evidence for the presence of a threshold weight for entering diapause in the yellow-spotted longicorn beetle, *Psacotheta hilaris*. *J. Insect Physiol.* 50: 295-301.
- Nei, M., Maruyama, T. & Chakraborty, R. 1975. The bottleneck effect and genetic variability in population. *Evolution* 29: 1-10.
- Nespolo, R. F., Castañeda, L. E. & Roff, D. A. 2007. Quantitative genetic variation of metabolism in the nymphs of the sand cricket, *Gryllus firmus*, inferred from an analysis of inbred-lines. *Biol. Res.* 40: 5-12.
- Newcomb, R. D., Gleeson, D. M., Yong, C. G., Russell, R. J. & Oakeshott, J. G. 2005. Multiple mutations and gene duplications conferring

- organophosphorus insecticide resistance have been selected at the *Rop-1* locus of the sheep blowfly, *Lucilia cuprina*. *J. Mol. Evol.* 60: 207-220.
- Nilsson, J.-Å. 2002. Metabolic consequences of hard work. *Proc. R. Soc. Lond. , Ser. B, Biol. Sci.* 269: 1735-1739.
- Noronha, C. & Cloutier, C. 2006. Effects of potato foliage age and temperature regime on prediapause Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae). *Environ. Entomol.* 35: 590-599.
- Novak, S. J. & Mack, R. N. 2005. Genetic bottlenecks in alien plant species, influence of mating systems and introduction dynamics. In: Sax, D. F., Stachowicz, J. J. & Gaines, S. D. (eds), *Species Invasions, Insights into Ecology, Evolution, and Biogeography*: 201-228. Sinauer Associates, Inc., Sunderland.
- Östman, Ö. 2005. Asynchronous temporal variation among sites in condition of two carabid species. *Ecol. Entomol.* 30: 63-69.
- Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Von Holle, B., Moyle, P. B., Byers, J. E. & Goldwasser, L. 1999. Impact: toward a framework for understanding the ecological effects of invaders. *Biol. Invasions* 1: 3-19.
- Parsons, P. A. 1991. Evolutionary rates: stress and species boundaries. *Annu. Rev. Ecol. Syst.* 1-18.
- Paterson, A. H., Schertz, K. F., Lin, Y. R., Liu, S. C. & Chang, Y. L. 1995. The weediness of wild plants: Molecular analysis of genes influencing dispersal and persistence of johnsongrass, *Sorghum halepense* (L.) Pers. *Proc. Natl. Acad. Sci. USA* 92: 6127-6131.
- Peterson, A. T. & Vieglais, D. A. 2001. Predicting species invasions using ecological niche modeling: new approaches from bioinformatics attack a pressing problem. *Bioscience* 51: 363-371.
- Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. 2000. Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50: 53-64.
- Pimentel, D., Zuniga, R. & Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52: 273-288.
- Porter, S. D., Williams, D. F., Patterson, R. S. & Fowler, H. G. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? *Environ. Entomol.* 26: 373-384.
- Poulet, N., Balaresque, P., Aho, T. & Björklund, M. 2009. Genetic structure and dynamics of a small introduced population: the pikeperch, *Sander lucioperca*, in the Rhône delta. *Genetica* 135: 77-86.
- Preisser, E. L., Elkinton, J. & Abell, K. 2008. Evolution of increased cold tolerance during range expansion of the elongate hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae). *Ecol. Entomol.* 33: 709-715.
- Prevosti, A., Ribo, G., Serra, L., Aguade, M., Balana, J., Monclus, M. & Mestres, F. 1988. Colonization of America by *Drosophila subobscura*: Experiment in

- natural populations that supports the adaptive role of chromosomal-inversion polymorphism. *Proc. Natl. Acad. Sci. USA* 85: 5597-5600.
- Quinlan, R. J. & Gatehouse, A. G. 1981. The effect of low doses of nedosulfan on lipid reserves of the tsetse fly *Glossina morsitans morsitans*. *Entomol. Exp. Appl.* 29: 29-38.
- Rantala, M. J. & Roff, D. A. 2006. Analysis of the importance of genotypic variation, metabolic rate, morphology, sex and development time on immune function in the cricket, *Gryllus firmus*. *J. Evol. Biol.* 19: 834-843.
- Räsänen, K. & Kruuk, L. E. B. 2007. Maternal effects and evolution at ecological time-scales. *Funct. Ecol.* 21: 408-421.
- Reed, D. H., Lowe, E. H., Briscoe, D. A. & Frankham, R. 2003. Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage. *Evolution* 57: 1822-1828.
- Reichard, S. H. & Hamilton, C. W. 1997. Predicting invasions of woody plants introduced into North America. *Conserv. Biol.* 11: 193-203.
- Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Funct. Ecol.* 13: 217-224.
- Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J. & Pigliucci, M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9: 981-993.
- Roff, D. A. 1997. *Evolutionary Quantitative Genetics*. 512 p., Chapman & Hall, New York.
- Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X. & Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497.
- Ruiz, G. M., Carlton, J. T., Grosholz, E. D. & Hines, A. H. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *Am. Zool.* 37: 621-632.
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., Neil, P., Parker, I. M., Thompson, J. N. & Weller, S. G. 2001. The population biology of invasive species. *Ann. Rev. Ecol. Syst.* 32: 305-332.
- Sanford, E., Holzman, S. B., Haney, R. A., Rand, D. M. & Bertness, M. D. 2006. Larval tolerance, gene flow, and the northern geographic range limit of fiddler crabs. *Ecology* 87: 2882-2894.
- Sax, D. F., Stachovicz, J. J. & Gaines, S. D. (eds) 2005. *Species Invasions; Insights into Ecology, Evolution, and Biogeography*. Sinauer Associates, Inc., Sunderland.
- Schmidt, P. S., Matzkin, L., Ippolito, M. & Eanes, W. F. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila Melanogaster*. *Evolution* 59: 1721-1732.
- Sexton, J. P., McKay, J. K. & Sala, A. 2002. Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecol. Appl.* 12: 1652-1660.
- Sexton, J. P., McIntyre, P. J., Angert, A. L. & Rice, K. J. 2009. Evolution and ecology of species range limits. *Annu. Rev. Ecol. Evol. Syst.* 40: 415-436.

- Shea, K. & Chesson, P. 2002. Community ecology theory as a framework for biological invasions. *Trends Ecol. Evol.* 17: 170-176.
- Sih, A., Bell, A. M. & Kerby, J. L. 2004. Two stressors are far deadlier than one. *Trends Ecol. Evol.* 19: 274-276.
- Steigenga, M. J. & Fischer, K. 2007. Within- and between-generation effects of temperature on life-history traits in a butterfly. *J. Therm. Biol.* 32: 396-405.
- Stephens, M., Smith, N. I. & Donnelly, P. 2001. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* 68: 978-989.
- Suarez, A. V. & Tsutsui, N. D. 2008. The evolutionary consequences of biological invasions. *Mol. Ecol.* 17: 351-360.
- Suarez, A. V., Holway, D. A. & Ward, P. S. 2005. The role of opportunity in the unintentional introduction of nonnative ants. *Proc. Natl. Acad. Sci. USA* 102: 17032-17035.
- Tanaka, K. 1992. Photoperiodic control of diapause and climatic adaptation of the house spider, *Achaearanea tepidariorum* (Araneae, Theridiidae). *Funct. Ecol.* 6: 545-552.
- Tauber, M. J. & Tauber, C. A. 2002. Prolonged dormancy in *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae): a ten-year field study with implications for crop rotation. *Environ. Entomol.* 31: 499-504.
- Tauber, M. J., Tauber, C. A. & Masaki, J. P. 1986. *Seasonal Adaptations in Insects*. Oxford University Press, Oxford.
- Tauber, M. J., Tauber, C. A., Obrycki, J. J., Gollands, B. & Wright, R. J. 1988. Voltinism and the induction of aestival diapause in the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* 81: 748-754.
- Tower, W. L. 1906. *An Investigation of Evolution in Chrysomelid Beetles of the Genus Leptinotarsa*. Carnegie Institution, Washington, D.C.
- Treier, U. A., Broennimann, O., Normand, S., Guisan, A., Schaffner, U., Steinger, T. & Müller-Schärer, H. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* 90: 1366-1377.
- Tsutsui, N. D. & Suarez, A. V. 2003. The colony structure and population biology of invasive ants. *Conserv. Biol.* 17: 48-58.
- Tsutsui, N. D., Suarez, A. V., Holway, D. A. & Case, T. J. 2000. Reduced genetic variation and the success of an invasive species. *Proc. Natl. Acad. Sci. USA* 97: 5948-5953.
- Valosaari, K., Aikio, S. & Kaitala, V. 2008. Spatial simulation model to predict the Colorado potato beetle invasion under different management strategies. *Ann. Zool. Fenn.* 45: 1-14.
- van Heerwaarden, B., Kellermann, V., Schiffer, M., Blacket, M., Sgro, C. M. & Hoffmann, A. A. 2010. Testing evolutionary hypotheses about species borders: patterns of genetic variation towards the southern borders of two rainforest *Drosophila* and a related habitat generalist. *Proc. R. Soc. Lond., Ser. B, Biol. Sci.* 276: 1517-1526.

- van Kleunen, M., Weber, E. & Fischer, M. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol. Lett.* 13: 235-245.
- Vermeij, G. J. 1996. An agenda for invasion biology. *Biol. Conserv.* 78: 3-9.
- Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D. & Van Tienderen, P. H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.* 10: 212-217.
- Walsh, B. D. 1865. The new potato-bug and its natural history. *Pract. Entomol.* 1: 1-4.
- Wares, J. P., Hughes, A. R. & Grosberg, R. K. 2005. Mechanisms that drive evolutionary change, insights from species introductions and invasions. In: Sax, D. F., Stachovicz, J. J. & Gaines, S. D. (eds), *Species Invasions, Insights into Ecology, Evolution, and Biogeography*: 229-257. Sinauer Associates, Inc., Sunderland.
- Weber, D. C. & Ferro, D. N. 1996. Flight and fecundity of Colorado potato beetles (Coleoptera: Chrysomelidae) fed on different diets. *Ann. Entomol. Soc. Am.* 89: 297-306.
- Wei, H. & Du, J. 2004. Sublethal effects of larval treatment with deltamethrin on moth sex pheromone communication system of the Asian corn borer, *Ostrinia furnacalis*. *Pestic. Biochem. Physiol.* 80: 12-20.
- Wierenga, J. M. & Hollingworth, R. M. 1992. Inhibition of insect acetylcholinesterase by the potato glycoalkaloid alpha-chaconine. *Nat. Toxins* 1: 96-99.
- Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. 1998. Quantifying threats to imperiled species in the United States. *Bioscience* 48: 607.
- Williams, D. G., Mack, R. N. & Black, R. A. 1995. Ecophysiology of introduced *Pennisetum setaceum* on Hawaii: The role of phenotypic plasticity. *Ecology* 76: 1569-1580.
- Williamson, M. & Fitter, A. 1996a. The varying success of invaders. *Ecology* 77: 1661-1666.
- Williamson, M. & Fitter, A. 1996b. The characters of successful invaders. *Biol. Conserv.* 78: 163-170.
- Yocum, G. D., Rinehart, J. P., Chirumamilla-Chapara, A. & Larson, M. L. 2009. Characterization of gene expression patterns during the initiation and maintenance phases of diapause in the Colorado potato beetle, *Leptinotarsa decemlineata*. *J. Insect Physiol.* 55: 32-39.
- Zalapa, J. E., Brunet, J. & Guries, R. P. 2010. The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae). *Evol. Appl.* 3: 157-168.
- Zalewski, A., Pierny, S. B., Zalewska, H. & Lambin, X. 2009. Landscape barriers reduce gene flow in an invasive carnivore: geographical and local genetic structure of American mink in Scotland. *Mol. Ecol.* 18: 1601-1615.
- Zhu, K. Y. & Clark, J. M. 1995. Comparisons of kinetic properties of acetylcholinesterase purified from azinphosmethyl-susceptible and resistant strains of Colorado potato beetle. *Pestic. Biochem. Physiol.* 51: 57-67.

- Zhu, K. Y. & Clark, J. M. 1997. Validation of a point mutation of acetylcholinesterase in Colorado potato beetle by polymerase chain reaction coupled in enzyme inhibition assay. *Pestic. Biochem. Physiol.* 57: 28-35.
- Zhu, K. Y., Lee, S. H. & Clark, J. M. 1996. A point mutation of acetylcholinesterase associated with azinphosmethyl resistance and reduced fitness in Colorado potato beetle. *Pestic. Biochem. Physiol.* 55: 100-108.

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^α-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 SIIKAMÄKI, 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 hartsihappoporaisten 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. - Metasäteilyllisyyden 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. - Ultraviolettisäteilyn vedenalainen 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.), ravinnonkäytön erityispiirteet: sopeumia pohjaelämään? 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. - Ultraviolettisäteilyn 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-biotiinteknologiaan. 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.) habitaahtinvalinta 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. - Epätyyppinen *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. - Kanaintukantojen 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, elinkiertopiirteisiin 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. - Ultravioletti 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 PIHROINEN, 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.