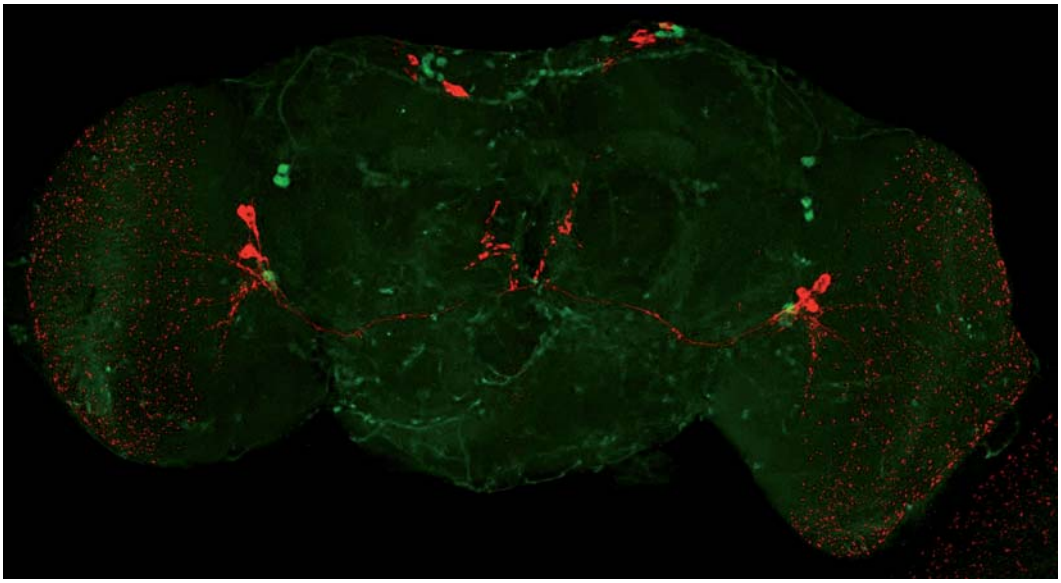
JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
253

Hannele Kauranen

The Role of the Circadian Clock in Adaptation in Seasonally Changing Environment in *Drosophila montana*



JYVÄSKYLÄN YLIOPISTO

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 253

Hannele Kauranen

The Role of the Circadian Clock in Adaptation in Seasonally Changing Environment in *Drosophila montana*

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
marraskuun 16. päivänä 2012 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in Auditorium S212, on November 16, 2012 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2012

The Role of the Circadian Clock in Adaptation in Seasonally Changing Environment in *Drosophila montana*

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 253

Hannele Kauranen

The Role of the Circadian Clock in Adaptation in Seasonally Changing Environment in *Drosophila montana*

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2012

Editors
Jari Haimi
The Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Sini Tuikka
Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science

URN:ISBN:978-951-39-4933-4
ISBN 978-951-39-4933-4 (PDF)

ISBN 978-951-39-4932-7 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2012

Tempus fugit

ABSTRACT

Kauranen, Hannele
The role of the circadian clock in adaptation in seasonally changing environment in *Drosophila montana*
Jyväskylä: University of Jyväskylä, 2012, 47 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 253)
ISBN 978-951-39-4932-7 (nid.)
ISBN 978-951-39-4933-4 (PDF)
Yhteenveto: Sirkadisen kellon rooli vaihteleviin ympäristöolosuhteisiin sopeutumisessa *Drosophila montana* -lajilla
Diss.

Adaptation to daily and seasonal changes in environmental conditions is crucially important for the survival and reproduction of all organisms, especially the ones living in the northern latitudes. One of the key factors in enhancing adaptation to this kind of environment is the evolution of two time-measuring systems; the circadian clock regulating daily variation and the photoperiodic timer regulating seasonal activities. In my thesis I have studied the role of the circadian clock in adaptation in northern environment and tried to find out whether and how it is connected with the photoperiodic timer in northern *Drosophila montana* species. My studies showed that *D. montana* possesses good entraining rhythms, displays only the evening activity peak and maintains its free-running rhythm better in constant light than in constant darkness differing in all these aspects e.g. from the more southern species *D. melanogaster*. I also found that the species differences in fly locomotor activity rhythms can be explained by the differences at the neuronal level. The function of the photoperiodic timer in *D. montana* seems to be based on either a non-circadian oscillatory hourglass timer or a rapidly damping circadian oscillator. The fact that the flies of this species shift both their evening activity peak and the expression level peaks of two circadian clock genes, *period* and *timeless,* in concert with the day length suggests that *D. montana* uses only one circadian oscillator to measure the day length. The lack of the morning activity peak in entrained conditions, the degradation of free-running activity rhythm in constant darkness and the lack of the expression of two studied neurotransmitter and photoreceptor proteins in specific brain neurons in *D. montana* suggest that the circadian clock has evolved in a different direction than that of *D. melanogaster*. The circadian clock of *D. montana* shows many features important for adaptation to northern environment and has undoubtedly been one of the key factors enabling this species to distribute to the North.

Keywords: Circadian clock; clock neurons; gene expression; eclosion rhythm; locomotor activity rhythm; seasonality.

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

Author's address

Hannele Kauranen
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
hannele.kauranen@jyu.fi

Supervisors

Professor Anneli Hoikkala
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Dr. Maaria Kankare
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Reviewers

Docent Tapio Heino, PhD
Department of Biosciences
P.O. Box 56
FI-00014 University of Helsinki
Finland

Professor Sakiko Shiga
Department of Biology and Geosciences
Graduate School of Science
University of Osaka city
3-3-138 Sugimoto, Sumiyoshi
Osaka 558-8585 Japan

Opponent

Dr. David Doležel
Faculty of Science
University of South Bohemia
Branišovvská 31
370 05 Českė Budějovice
Czech Republic

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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

I Kauranen, H., Menegazzi, P., Costa, R., Helfrich-Förster, C., Kankainen, AL. & Hoikkala, A. 2012. Flies in the North: Locomotor behavior and clock neuron organization of *Drosophila montana*. *Journal of Biological Rhythms*. 27: 377-387.

II Kauranen, H., Tyukmaeva, V. & Hoikkala, A. 2012. The flies of a northern *Drosophila* species with robust diapause lose their rhythmicity and ability to enter diapause in prolonged scotophases. Manuscript.

III Kauranen, H., Hoikkala, A. & Kankare, M. 2012. Daily and seasonal changes in the locomotor activity of *Drosophila montana* flies is accompanied by changes in the expression of two circadian genes, *period* and *timeless*. Manuscript.

IV Kauranen, H. & Hoikkala, A. 2012. Circadian control of entrained and free-running locomotor and eclosion rhythms in *Drosophila montana*. Manuscript.

The table shows the contributions to the original papers. HK = Hannele Kauranen, PM = Pamela Menegazzi, ALK = Annaliisa Kankainen, RC = Rodolfo Costa, CH-F = Charlotte Helfrich-Förster, VT = Venera Tyukmaeva, MK = Maaria Kankare, AH = Anneli Hoikkala.

	I	II	III	IV
Original idea	HK, AH	HK, VT	HK, MK, AH	HK, AH
Data	HK, PM	VT, HK	HK	HK
Analyses	HK, PM, ALK	VT, HK	HK, MK	HK
Writing	HK, RC, CH-F, AH	HK, VT, AH	HK, AH, MK	HK, AH

ABBREVIATIONS

Ct	circadian time
DD	constant darkness treatment
DN	Dorsal neurons
E	evening
FC	fold change
HLL	constant high light intensity treatment
LD	light: dark cycle
LL	constant light treatment
LLL	constant low light intensity treatment
LN_d*s*	dorsolateral neurons
LN	Lateral neurons
l-LN_v*s*	large ventrolateral neurons
LPNs	lateral-posterior neurons
M	morning
qPCR	quantitative real time PCR
s-LN_v*s*	small ventrolateral neurons
RNA	ribonucleic acid
Zt	Zeitgeber time
τ	period; the length of the intrinsic day of the flies in hours

1 INTRODUCTION

1.1 Importance of measuring time

From the beginning of life, all living organisms on Earth have been exposed to rhythmic fluctuations in environmental factors, such as day length, temperature and humidity, caused by the rotation of Earth around its axis and around the Sun. The only exceptions are the organisms living in the ocean basin or in underground caves. To be able to forecast daily and seasonal changes in their environment and to adjust their life cycle accordingly, the organisms have evolved different kinds of time-measuring mechanisms. These biological clocks help the individuals e.g. to coordinate their metabolic processes, to be active at the right time of day and/or to adjust physiological changes so that they occur at the right time of day or year (Saunders 2002).

One of the best studied biological clocks is the circadian clock, which has evolved as a response to the rotation of the Earth around its axis. As its' name reveals (in Latin, circa = about, dies = a day), this time keeping mechanism measures time in the cycles of approximate length of 24 h (Saunders 2002). Fossil evidence suggests that the circadian rhythms have existed already several hundred million years ago e.g. in corals and nautiloids (see Sharma 2003). Nowadays circadian clocks are found in a wide range of organisms from the cyanobacteria *Synechococcus* (Kondo & Ishiura 1999, Iwasaki & Kondo 2004), to plants (Johnson 2001), insects (Williams & Sehgal 2001) and mammals, including humans (Reppert & Weaver 2001). Regulation of daily changes in metabolism increases the intrinsic fitness value of organisms (Green et al. 2008), while synchronized changes in behavior and physiology increase their extrinsic adaptive value (Sharma 2003). Circadian control of daily changes in the above-mentioned traits is usually based on changes in photoperiod and/or temperature, but the circadian clock may function even in constant conditions. For example, some cave-dwelling millipedes have been shown to have functional circadian oscillators even though they live in constant environments (Koilraj et al. 2000).

Seasonal changes induced by the rotation of the Earth around the Sun are often even more drastic than daily variation. Adaptation of organisms to seasonally varying environment has been enhanced by the evolution of the photoperiod timer. This time measurement system is based on changes in day length, which is the most reliable environmental cue for detecting the seasonal changes (Tauber et al. 1986). The photoperiodic timer enables organisms to predict the forthcoming cold season early enough to prepare for it and it also regulates seasonal changes in various kinds of processes linked with development, survival and progeny production. Adaptation to drastic seasonal changes in environmental conditions is of crucial importance especially for species living in the northern environments.

1.2 Circadian clock

1.2.1 Evolution of the circadian clock

Daily light/dark cycles are thought to be the main force enhancing the evolution of circadian clocks (Hastings et al. 1991). These clocks may increase the adaptive value of organisms e.g. by gating their light-sensitive metabolic processes to occur during the dark period to avoid the harmful effects of light/UV radiation (see Sharma 2003). Also the high level of free oxygen in the atmosphere during early eukaryote evolution might have favoured the development of circadian rhythmicity in metabolic activities to minimize the deleterious effects of diurnal photo-oxidative exposures (Paietta 1982). Later on also rhythmic activities, such as prey-predator interactions (Fenn & Macdonald 1995) and the avoidance of competition (Gutman & Dayan 2005, Levy et al. 2007), are thought to have fine-tuned the circadian rhythms of different species (Sharma 2003). Circadian rhythmicity is likely to play an important role also in progeny production. For example, in *Drosophila melanogaster,* the reproductive success of flies with nonfunctional circadian clocks has been found to be markedly decreased compared to flies with functional circadian clocks (Beaver et al. 2002).

Circadian clock controls rhythmic changes in numerous traits in organisms. For example, in cyanobacteria this clock has been found to control cell division and in the fungus *Neurospora crassa* the production of asexual spores (Loros & Dunlap 2001). In plants the circadian system is known to synchronize e.g. leaf movements (Yakir et al. 2007). For example, in *Mimosa pudica* the persistence of circadian leaf opening and closing rhythms under constant conditions was discovered as early as 1729 (de Mairan 1729). In insects behavioral traits that are known to be under circadian regulation include locomotor activity (Klarsfeld et al. 2003), the timing of eclosion (Konopka & Benzer 1971), egg-laying rhythms (Howlader & Sharma 2006) and courtship behavior (Fujii et al. 2007). In humans, the circadian clock has been found to control e.g. the sleep-wake cycles, the maintenance of body temperature and the release of endocrine hormones (Haus 2007, Lack & Wright 2007, Refinetti 2010).

All circadian rhythms maintained by the circadian clock have some common basic characteristics. First, the circadian rhythms possess an ability to be entrained by environmental cues, Zeitgebers (time giver; in German, zeit = time, geber = giver), such as light and temperature. In diurnally changing environmental conditions the entrained period (τ) corresponds to the length of an extrinsic day, which is 24 h (entrained rhythms) (Dubruille & Emery 2008). Second, under constant conditions the circadian clock is able to maintain the circadian rhythms for a long time with a free-running period (τ), which is close to 24 h (free-running rhythms) (Dubruille & Emery 2008). The third well-known character of the circadian rhythms is that they are temperature-compensated, which means that the circadian clock is able to maintain a free-running period (τ) of the rhythm close to 24 h under a wide range of temperatures (Pittendrigh, 1960).

Changes in day length are extreme at high latitudes where the day length can vary from constant light in summer to constant darkness during winter. Some studies have suggested that these kinds of constant conditions may have favored weaker circadian regulation of different traits. For example, in the Svalbard ptarmigan, *Lagopus mutus hyperboreus,* the daily oscillating melatonin levels attenuate during the summer and winter (Reierth et al. 1999). Also, 2 reindeer subspecies, the northern *Rangifer tarandus platyrhynchus* and the southern *R. t. tarandus*, have been found to show interesting changes in their locomotor activity rhythms during the year. The northern *R. t. platyrhynchus* shows circadian activity rhythm only during the autumn and spring (van Oort et al. 2005), whereas the southern *R. t. tarandus* shows strong circadian activity rhythms for longer periods throughout the year (van Oort et al. 2007). However, even though the circadian regulation of some traits may be reduced at high latitudes, the clock can still control other circadian processes of the same species (Yerushalmi & Green 2009).

1.2.2 Genetic and neuronal background of the circadian clock

In *Drosophila* flies, as well as in many other organisms, circadian clock consists of three basic elements: the input pathways, the pacemaker/oscillator and the output pathways. Environmental signals, such as daily changes in light or temperature, are transmitted to the pacemaker through the input pathways to entrain it. The pacemaker itself generates molecular oscillations with a period (τ) approximately 24 h and controls several output pathways maintaining rhythmicity e.g. at metabolic, physiological and/or behavioural level.

Genetic studies on *D. melanogaster* rhythms have shown that the pacemaker is constructed of molecular transcriptional-translational feedback loops. These feedback loops, which involve a number of genes, lead to self-sustained circadian oscillations (Williams & Sehgal 2001). This kind of molecular feedback loop mechanism has been found in the circadian clocks of several organisms ranging from bacteria to *Neurospora* (Loros & Dunlap 2001, Lakin-Thomas et al. 2011), plants (Alabadi et al. 2001) and mammals, including humans (King & Takahashi

2000, Okamura et al. 2002). Interestingly, many circadian clock genes show striking homology between the species (Young & Kay 2001, Stanewsky 2003).

In *D. melanogaster*, the main pacemaker consists of numerous genes, including *Clock (Clk)*, *cycle (cyc)*, *period (per)* and *timeless (tim)* (Peschel & Helfrich-Förster 2011). To create the oscillating pacemaker, *Clk* and *cyc* activate the transcription of other circadian genes, including *period* (*per*) and *timeless* (*tim*). Due to this activation, the transcription levels of *per* and *tim* start to increase, reaching their peak expression at the end on the day or during the early night (Hardin et al. 1990, Sehgal et al. 1995). Increased mRNA production of these genes leads to oscillations of PER and TIM proteins, which lag behind the RNA oscillations by about 6 h (reviewed in Hardin 2004, Nitabach & Taghert 2008). Subsequent light-induced degradation of TIM during the next morning, mediated by an intracellular photoreceptor *cryptochrome* (Konopka et al. 1989, Stanewsky et al. 1998, Emery et al. 2000a), leads to a degradation of PER and permits CLK-CYC mediated transcriptional activation to start again (see Nitabach & Taghert 2008).

The location of the pacemaker running the circadian rhythms varies between the species. For example, in the sea slug *Aplysia* the pacemaker has been found to locate in the eye (Block et al. 1993), while in mammals the circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus (see Harrington 1992). Some species have also been found to have peripheral oscillators, which can be under the control of the central pacemaker or be directly entrained by environmental cues (Hege et al. 1997, Giebultowicz 2001, Myers et al. 2003). In plants, almost all cells have their own autonomous circadian system (Thain et al. 2002), whereas mammals have peripheral oscillators that are located in many tissues, such as the liver, but they are coordinated by the central pacemaker (Yamazaki et al. 2000).

In *D. melanogaster,* the neural network behind the circadian pacemaker is located in the central neuronal system consisting of ~150 lateral (LN) and dorsal (DN) neurons, which express particular circadian clock genes (reviewed in (Taghert & Shafer 2006, Nitabach & Taghert 2008). LNs and DNs can further be divided into several subgroups: the small ventrolateral neurons (s-LN_vs), the large ventrolateral neurons (l-LN_vs), the dorsolateral neurons (LN_ds) and three groups of dorsal neurons (DN_1s, DN_2s and DN_3s) (reviewed in Helfrich-Förster 2003), as well as lateral-posterior neurons (LPNs) (Taghert & Shafer 2006). *D. melanogaster* have also peripheral oscillators, which can be directly entrained by environmental signals. In this species, light-sensitive autonomous peripheral oscillators have been found to be located e.g. in the head (other than central pacemaker), thorax, gut, excretory system and testes (Giebultowicz 2001, Beaver et al. 2002, Glossop & Hardin 2002, Myers et al. 2003).

1.2.3 Locomotor activity

The locomotor activity rhythm is the best studied circadian rhythm in *D. melanogaster*. The flies of this species are crepuscular showing 2 activity peaks in laboratory conditions, the morning (M) and evening (E) peak (Hamblencoyle et

al. 1992, Rieger et al. 2003). When *D. melanogaster* flies are released into constant darkness, they show only the evening activity peak and free-run with a period of ~ 24 h (Helfrich-Förster 2000, Allada & Chung 2010). In constant light these flies lose their rhythmicity at light intensities over 10 lux (Aschoff 1979, Konopka et al. 1989, Helfrich-Förster et al. 2001), but show free-running rhythms at low light intensities (~ 0.03 lx) (Bachleitner et al. 2007). Contrary to *D. melanogaster*, some *Drosophila* species living at higher latitudes, such as *D. suboccidentalis, D. subquinaria* and *D. virilis*, have been found to show only an evening activity peak (Simunovic & Jaenike 2006, Bahn et al. 2009).

In *D. melanogaster*, 4 out of 5 s-LNvs and all l-LNvs express neuropeptide *Pigment-dispersing factor* (*Pdf)* (see Yoshii et al. 2009), but the 5th s-LNv does not express *Pdf* (Kaneko et al. 1997, Rieger et al. 2006). PDF-positive s-LNvs are necessary for the morning activity of *D. melanogaster* flies under LD conditions, which forms a neuronal basis for the morning (M) oscillator, while the 5th PDF-negative s-LNv and LNds control the evening activity of the flies and are defined as the evening (E) oscillator under LD conditions (Grima et al. 2004, Stoleru et al. 2004, Rieger et al. 2006, Nitabach & Taghert 2008). s-LNvs are also the most important clock neurons for the maintenance of persistent locomotor activity rhythms in constant darkness (Renn et al. 1999).

Pittendrigh and Daan suggested already in 1976 that the morning (M) and evening (E) activity peaks are controlled by separate circadian oscillators and that by adjusting the phase-relationship between them the circadian clock enables organisms to synchronize their behaviour with seasonal changes in day length (Allada & Chung 2010). The phase-relationship between (M) and (E) - oscillators has indeed been found to change according to the season in several species, including *D. melanogaster* (Aschoff 1966, Majercak et al. 1999). In *D. melanogaster* the interval between the morning (M) activity and the evening activity (E) peaks is longer under long day conditions than under short day conditions, which helps the flies to keep track of the changing seasons (Rieger et al. 2003, Beer et al. 2010, Rieger et al. 2012). Based on the current view, the neurons of the M-oscillators maintain circadian rhythm under constant darkness and during the short winter days (Picot et al. 2007, Stoleru et al. 2007), whereas those of the E-oscillator control the rhythm in constant light and under long summer days (Stoleru et al. 2004, Picot et al. 2007, Stoleru et al. 2007).

Studies on the rhythmicity of organisms in natural or semi-natural conditions can give a more reliable picture of their rhythmicity than do studies performed in laboratory conditions with regularly changing LD and / or temperature cycles (Boulos & Macchi 2005, Rieger et al. 2007, Vanin et al. 2012). For example, simulated twilight increases the ability of these flies to adapt their locomotor activity rhythms to long photoperiods (Rieger et al. 2012).

1.2.4 Eclosion rhythm

Eclosion is the developmental stage, when insects emerge from their pupal case. The role of the circadian clock in regulating the timing of eclosion is to gate it to occur at a particular time of day, usually in the morning when the relative

humidity is high (Pittendrigh 1954, Qiu & Hardin 1996). The first circadian clock gene, *period*, was found in *D. melanogaster* by Konopka and Benzer (1971), when these researchers were studying eclosion rhythm of the wild-type and mutant flies of this species.

The eclosion rhythm of *D. melanogaster* flies is regulated by a peripheral clock in the prothoracic glands (Myers et al. 2003). These glands are known to assess the growth and the size of developing pupae and determine when the pupae are ready to eclose (Allada & Chung 2010). Evidence for a partly independent control of the locomotion and eclosion rhythms comes from the differences between these rhythms detected in various *Drosophila* species, including *D. pseudoobscura* (Engelmann & Mack 1978), *D. rajasekari* (Joshi 2001) and *D. melanogaster* (Myers et al. 2003). Further evidence comes from the findings that *ebony* (Newby & Jackson 1991) and tim^{blind} (Wulbeck et al. 2005) mutations change the locomotor activity rhythm, but not the eclosion rhythm, of *D.melanogaster* flies. The function of the peripheral clock in *D. melanogaster* may, however, still be partly regulated by the central circadian clock neurons as ablation of PDF-expressing lateral neurons (LN_vs) and a null mutation of *Pdf* have been found to disrupt the PG clock and change the eclosion rhythm of the flies (Myers et al. 2003). Also, some mutations in the circadian clock genes (e.g. *period, timeless* and *doubletime*) have been found to have the same kind of effects on both locomotor activity and eclosion rhythms of the flies (Sehgal et al. 1994, Sehgal et al. 1996, Rothenfluh et al. 2000).

1.3 Photoperiodic timer

Photoperiodic timer enables the organisms to measure seasonal changes in day length and forecast the forthcoming changes in environmental conditions on the basis of this information. As the photoperiod is the most reliable cue for the changing seasons, many organisms living in the north control their physiology and behavior on the basis of this cue.

Photoperiodic timer consists of light receptors, the photoperiodic counter and the photoperiodic clock. Light receptors differ between species, but e.g. *D. melanogaster* flies sense light with 3 different photoreceptive organs, the ocelli, the compound eyes and the Hofbauer-Buchner eyelets (HB-eyelets) containing different rhodopsins (Peschel & Helfrich-Förster 2011). Photoperiodic counter counts and accumulates information on LD cycles and delivers this information to the photoperiodic clock, which interprets the information and evokes specific behavioral or physiological changes when the day length goes below (or the night above) the critical day/night length.

In insects many traits are controlled by the photoperiod timer. Good examples are the dormancy (quiescence/diapause) e.g. in *Drosophila auraria* (Pittendrigh & Takamura 1987) and linden bug *Pyrrhocoris apterus* (Hodek 1968, Hodkova 1977), seasonal changes in the morphological forms of silk moth *Bombyx mori* (Tsurumaki et al. 1999), the growth of cutworm *Agrostis occulta*

larvae (Danilevskii 1965) and the migration of the monarch butterfly *Danaus plexippus* (Brower 1977). One of the best studied dormancies in insects is adult reproductive diapause, during which the females postpone their sexual maturation and reproduction until a more favourable season (Saunders 2002). Photoperiodically regulated diapause responses have been found to occur in more than 500 insect species (Nishizuka et al. 1998).

1.4 Connection between the circadian clock and photoperiodic timer

1.4.1 Models describing the possible connection

In 1936 Bünning proposed that the photoperiodic timer requires cooperation with the circadian clock, but the role of this clock in the photoperiodic control of seasonal rhythms is still under intense debate. In theory, the length of the photoperiod could be measured without the involvement of the circadian clock, but studies on several species have suggested that the circadian clock takes part in this process (Imaizumi & Kay 2006, reviewed in Foster & Kreizman 2009). Although the genetic regulation of circadian clock is well-described in *D. melanogaster* (reviewed in Peschel & Helfrich-Förster 2011), the genetic and physiological mechanisms underlying the photoperiodic timer are poorly understood, which is partly due to the fact that the above-mentioned species does not show robust photoperiodic responses.

Many theoretical models have been developed for the function of photoperiodic timer with or without the involvement of the circadian clock in photoperiodic time measurement. In an hour-glass model, the photoperiodic timer has been suggested to be based on a non-circadian mechanism and to be driven only by external LD cycles, so that it needs to be reset every day (Lees 1973). The hour-glass model has been suggested to explain e.g. the induction of adult reproductive diapause in the spider mite *Tetranychus urticae* (Veerman & Vaz Nunes 1987) and larval diapause in the rice stem borer *Chilo suppressalis* (Chen et al. 2011).

Bünning (1936) suggested a model, in which the photoperiod time measurement is based on the oscillation of organisms' circadian clock. In this model, the photoperiodic timer is assumed to consist of 1 or more circadian oscillators, which are entrainable by light and which restart time measurement spontaneously during prolonged dark phases. Thus, the light signals perceived by the organisms should entrain the circadian oscillator and evoke (or do not evoke) photoperiodic responses, when the light period coincides with the organisms' photosensitive phase (Bünning 1936). Bünning presented also a damped circadian oscillator model, where both hourglass–like and oscillatory photoperiodic timers are thought to be based on the circadian system of the organisms and to differ from each other only in their propensity to dampen

(Bünning 1969, Lewis & Saunders 1987, Saunders & Lewis 1987a, Saunders & Lewis 1987b).

Bünning's original hypothesis was further developed in external (Pittendrigh & Minis 1964, Pittendrigh 1966) and internal (Pittendrigh 1972) coincidence models. The external coincidence model is based on a single circadian oscillator in which phase is set by the LD cycle in such a way that a particular light-sensitive phase (φi) is restricted to a short period during the latter half of the subjective night. Here, a short-day/long-night response is thought to be evoked when φi regularly coincides with darkness during the long autumn nights (see Vaz Nunes & Saunders 1999). In the internal coincidence model, on the other hand, the photoperiodic time measurement has been suggested to be based on changes in phase relationship between the morning (M) and evening (E) oscillators so that an increase in the day length leads to an increase in the phase angle difference between the oscillators triggering a long-day response, and vice versa (Pittendrigh 1972).

Finally, Pittendrigh (1972) presented a third model of how the circadian clock could be involved in the photoperiodic timer: a circadian resonance model. This model was further developed by Vaz Nunes and Veerman (1982) and named an hourglass timer-oscillator counter model. In this model the night length is suggested to be measured through a non-circadian hourglass timer, but the photoperiodic counter mechanism still requires the involvement of the circadian clock (Vaz Nunes & Veerman 1982).

Even though researchers have found evidence for all above-mentioned models while studying the function of photoperiodic timer in different species (Saunders 1990, Claret & Arpagaus 1994, Tauber & Kyriacou 2001), the final conclusion on whether the circadian clock is or is not necessary for the function of the photoperiodic timer is still unresolved.

1.4.2 Candidate genes for the circadian clock and photoperiodic timer

Information on the molecular basis of the circadian clock of *D. melanogaster* offers a good possibility to find out whether any of the circadian clock core genes (e.g. *clock*, *cycle*, *timeless* and *period*) play a role in the photoperiodic timer. *timeless* (*tim*) is one of the key genes that could play an important role in both time measuring systems through its sensitivity to night length. Indeed, mRNA and protein levels of *tim* have been found to decrease under long-day conditions compared to short day conditions e.g. in *Sarcophaga crassipalpis* and *D. melanogaster* flies (Goto & Denlinger 2002, Shafer et al. 2004). *period* is a second clock gene that has been suggested to play a role in seasonal adaptation (Saunders et al. 2004). Mutations in this gene have been found to disrupt e.g. the females' ability to discriminate between long and short days in *D. melanogaster* (Saunders et al. 1989). As Emerson et al. (2009) have noticed, mutations or allelic variants in clock genes do not need to modify the effects of the entire circadian clock on photoperiodism, but they can also have direct effects on diapause.

1.5 Aims of the thesis

The main aim of my thesis was to trace the role of the circadian clock in adaptation to northern environment and to find out whether and how its function is connected with the photoperiodic timer. To address these questions, I studied how the circadian clock regulates daily and seasonal rhythms in the locomotor activity and eclosion of northern *D. montana* flies and traced the genetic and neuronal background of the circadian clock in this species.

In the first paper I studied the function of the circadian clock of *D. montana* at phenotypic (locomotor activity) and neuronal level to find out whether the clock shows features that could be adaptive to seasonally varying conditions at high latitudes. Both the entrained and free-running locomotor activity rhythms of the female flies were monitored in different LD cycles and temperatures to see whether and how these rhythms differ from those of a more southern species, *D. melanogaster*. In addition, I studied the neuronal background of the circadian clock of *D. montana* by tracing the expression of PDF neuropeptide and CRY protein in its neural cells of the brains to find out whether the two species show differences also at the neuronal level.

The connection between the circadian clock and the photoperiodic timer in *D. montana* was studied in more detail by using the Nanda-Hamner protocol as described in paper II. The purpose of this study was to find out whether the induction of the photoperiodic reproductive diapause of *D. montana* females requires the involvement of the circadian oscillator or not. In this experiment the flies were kept in 13 chambers in LD cycles with the same duration of the light period (12 h) followed by unique dark periods ranging from 12 to 60 h, so that the total LD cycle in different chambers varied between 24 and 72h. The general assumption of this experiment is that if the circadian clock takes part in the photoperiodic response, then the underlying sensitivity to light should cycle between light insensitivity and light sensitivity during the long dark periods. In our experiment this means that only the females that were maintained in LD cycles of 24, 48 or 72 h were expected to give a photoperiodic response (enter diapause).

In paper III I traced daily and seasonal rhythms in the locomotor activity of the flies and in the expression level of 2 key circadian clock genes, *per* and *tim,* when flies were reared in seminatural conditions during summer and autumn where females are expected to develop ovaries or enter reproductive diapause, respectively. The main purpose of this study was to see how seasonal changes in light and temperature conditions affect the locomotor activity patterns of the females and also to find out whether the expression level of *per* and *tim* show daily and/or seasonal rhythms. I also studied whether the reproductive stage of the females has an effect on their locomotor activity and/or on the daily changes in the expression level of the above mentioned genes.

Finally, I studied the effects of different constant condition treatments (constant darkness and constant high and low light intensity) on the flies' free-

running locomotor activity rhythm (IV) to see whether flies are more active and retain their locomotor activity better in constant high and/or low light intensity than in constant darkness and whether their rhythms differ under these conditions. I also measured the free-running eclosion rhythm of *D. montana* larvae in constant darkness to find out whether the eclosion and the locomotor activity rhythms of *D. montana* are under different neuronal control in different circadian oscillators (IV).

2 MATERIALS AND METHODS

2.1 Study species and populations

Drosophila montana belongs to the *Drosophila virilis* species group. It is originated in Asia from where it has spread on different continents at high latitudes (Throckmorton 1982). *D. montana* has diverged from *D. virilis* approximately 10 million years ago (Spicer & Bell 2002), and the divergence time between *D. virilis* (genus *Drosophila*) and *D. melanogaster* groups (genus *Sophophora*) is about 63 million years (Tamura et al. 2004).

In my thesis work I have used the females of *D. montana* isofemale strains and a mass-bred population originating from Oulanka (66 °N, 29 °E) (I, III, IV) and isofemale strains collected from Pelkosenniemi (67 °N, 27 °E) and from Lahti (60 °N, 25 °E) (II). Isofemale strains were established from the progenies of wild-caught females inseminated in the wild and the mass-bred population was created by combining F_3 progenies of 20 isofemale strains. Isofemale strains were maintained in bottles containing malt medium (Lakovaara 1969) and the mass-bred population was reared in a wooden population cage attached to six malt bottles. Both isofemale strains and the mass-bred population have been maintained since their establishment (2008 or 2009) in the laboratory in constant light of ~ 300 lux at 19 °C and with relative humidity of 60 %.

In Finland, *D. montana* flies are exposed to very long days or continuous light during their mating season in the early summer, and thus maintaining them in continuous light in our "fly room" enabled the flies to produce about 7-8 generations per year. When the day length starts to shorten in late summer, practically all females of this species enter adult reproductive diapause (Lumme 1978, Tyukmaeva 2011) and they spend the long and dark winter under constant darkness, probably under snow cover.

2.2 Phenotypic measurements

2.2.1 The locomotor activity experiments (I, III, IV)

I studied the entrained locomotor activity rhythms of *D. montana* females under different photoperiods either in constant or fluctuating temperature. In addition, I traced their free-running locomotor activity rhythms in constant light or darkness and constant temperature (see Table I for the experimental conditions).

Females were collected within 1 day after eclosion for the locomotor activity experiments (I, IV). Only in studies concerning the females' entrained locomotor activity rhythms in seminatural conditions (III) the age of the females differ between the study groups: non-diapausing females and diapausing females. The locomotor activity rhythms of the females were measured by placing the females in glass tubes connected with either Trikinetics *Drosophila* Activity Monitors (I, III) or Trikinetics *Drosophila* High Resolution Activity Monitors (IV) (Waltham, MA, USA).

2.2.2 The eclosion rhythm experiments (IV)

Free-running eclosion rhythms of fly pupae in study IV were monitored in constant darkness at 19 °C using recording equipment based on the "falling ball" principle (Lankinen & Lumme 1982). Sexually mature females and males of the parental generation were allowed to mate and lay eggs in malt bottles for one week and, once most of their progeny had pupated, the pupae were rinsed from the walls of the bottles with water (at 19 °C), washed and dried on absorbing paper. The pupae were placed individually into the holes in an acryl plate (80 x 100 mm), whose opening was closed with a stainless steel ball. The plates were then placed in an eclosion rhythm monitor and maintained in constant darkness at 19 °C for 21 days.

2.2.3 Reproductive diapause (I, II, III, IV)

In the Nanda-Hamner experiment (II), the females were collected within 1 day after their emergence, sexed and transferred into vials containing yeast-sucrose-agar media (Rosato & Kyriacou, 2006) with some dry yeast on the top. Female vials were transferred into an air-conditioned room with automatically controlled temperature (16 °C) and divided into 13 wooden experimental chambers with specific LD cycles. All chambers had a light period of 12 h followed by unique dark periods ranging from 12 h to 60 h. In addition, we transferred females of same strains into constant darkness in the same room. All females were kept in a given LD cycle or constant darkness for 21 days.

After all the locomotor experiments (I, III, IV) and the Nanda-Hamner experiment (II) either whole females (I, II) or their abdomens (III, IV) were stored until their reproductive stage was determined on the basis of the size and developmental stage of their ovaries (Tyukmaeva et al. 2011).

2.3 Neurogenetic and genetic methods

2.3.1 Immunohistochemistry (I)

I performed immunohistochemistry experiments with 1 *D. montana* isofemale line using antibodies designed against PDF and CRY (I). Prior to PDF and CRY staining, female flies were kept in constant darkness (DD) at 20 °C for 72 h. At Zeitgeber time (ZT) 0, the females were fixed in 4 % paraformaldehyde in a phosphate buffer (PB, pH 7.4) with 0.5 % Triton-X in darkness for 4 h at room temperature and rinsed 3 times for 15 min in PB, after which their brains were dissected in the same buffer. Brains were collected in PB with 0.5 % Triton X and subsequently blocked in 5 % normal goat serum (NGS) in PB with 0.5 % Triton X overnight at 4 °C before treating them with primary and secondary antibodies. Brains were then incubated in PDF or CRY antibody solution for 48 h at 4 °C and rinsed 5 times for 10 min in PB with 0.5 % Triton X-100. The primary rabbit anti-CRY was diluted by 1:1000 and the primary mouse anti-PDF by 1:2500 in PB containing 5 % NGS and 0.5 % Triton X-100.

The secondary fluorescence-conjugated antibody PDF and CRY stainings were performed for 2 different groups of brains. As secondary antibodies in these stainings, we used Alexa Fluor 546 (goat anti-mouse; Invitrogen) for PDF and Alexa Fluor 488 (goat anti-rabbit; Invitrogen) for CRY. The secondary antibodies were diluted 1:200 in PB containing 5 % NGS and 0.5 % Triton X-100. After washes the brains were mounted on Vectashield mounting medium (Vector Laboratories, Burlingame, CA). More detailed information on the protocol is given in paper I. The fluorescence signals of the whole brains were detected using a confocal microscope (Olympus FV1000). We examined at least 10 brains for PDF and CRY immunostaining. Images were processed using the program ImageJ.

2.3.2 Gene expression (III)

When studying both daily and seasonal variation in the expression levels of *timeless* and *period* genes (III), fresh samples of females for RNA extractions were collected from the experimental photoperiod every 6 h over the 24 h period after performing the locomotor activity experiments. The samples for non-diapausing females were collected at photoperiods 24LL, 16:8 LD and 14:10 LD and the ones for diapausing females at photoperiods 16:8 LD and 14:10 LD. In each of these photoperiods the first RNA sample was collected immediately before the lights-on transition (ZT 0) and the 2nd, 3rd and 4th samples 6, 12 and 18 h after lights-on transition (ZT 6, ZT 12 and ZT 18). The females of all samples were flash-frozen in liquid nitrogen and stored at – 84 °C.

2.3.3 RNA extraction and cDNA synthesis (III)

Prior to RNA extractions the flies were put in pre-cooled (2 h in –84°C) RNAlaterICE solution and kept overnight or for at least 16 h in –20 °C, after which their heads were cut off using a scalpel and then used individually for RNA extractions.

Total RNA was extracted with the ZR RNA Microprep kit with DNase treatment according to the manufacturer's protocol. After extraction, the purity and concentration of each sample was measured with a NanoDrop spectrophotometer and the integrity of RNA in some samples was checked with an Agilent 2100 Bioanalyzer.

Before cDNA synthesis, RNA samples were diluted to equal concentrations (10-15 ng/µl) and 2 µl of total RNA of each sample was used as template for cDNA synthesis using iScript Reverse Transcription Supermix (Bio-Rad Laboratories) following the manufacturer's protocol. 20 µl of the reaction mixture included in addition to RNA, 4 µl of 5 x iScript reaction mixture, 1 µl of reverse transcriptase enzyme and dH_2O. The PCR cycling conditions were: 5 min at 25 °C, 30 min at 42 °C and 5 min at 85 °C for cDNA reactions.

2.3.4 Quantitative real time PCR (qPCR) (III)

Primers for *timeless* and *period* genes and candidate control genes for quantative real time PCR (qPCR) were designed using NetPrimer and Geneious programs (III). Candidates for the control genes were chosen on the basis of their stability in our earlier studies (Kankare et al. 2010, Kankare et al. unpublished, Salminen et al. unpublished). Amplification efficiency values of all the primer pairs were checked using 2-fold serial dilutions of pooled cDNA (from all the treatments) with 3 technical replicates and 7-9 dilution points.

Expression patterns of experimental and control genes were traced with qPCR using 6 biological replicates and 3 technical replicates from all the treatments. qPCR reactions were run using the following mixture: 10 µl 2x Power SYBR Green PCR Master Mix (Bio-Rad Laboratories), 0.3 µl of each gene-specific primer and 1 µl of cDNA solution; the total volume of reaction was 20 µl. qPCR reactions were run with a Bio-Rad CFX96 instrument with the following cycling conditions: initiation at 95 °C for 3 min, denaturation at 95 °C for 10 seconds, annealing at 55 °C for 10 seconds and extension at 72 °C for 30 seconds. Denaturation, annealing and extension phases of the PCR were repeated 40 times and they were followed by a melting curve analysis to check the purity of the qPCR reaction.

2.4 Statistical analysis

2.4.1 Analysis of fly locomotor activity (I, III, IV)

The raw locomotor activity data of the flies were displayed as double-plotted actograms (48-h plots) for experimental days under different entraining conditions (LD cycles and temperatures) (I, III, IV) and constant conditions (I, IV). The primary analysis of these actograms was done with the program ActogramJ (Schmid et al., 2011). The rhythmicity and the length of the period (τ) of the flies were traced by analyzing the actograms using the Lomb-Scargle periodogram method; when the periodogram analysis detected significant periodicity in fly's activity rhythm across consecutive days, the fly was determined to be rhythmic. The power of the Lomb-Scargle periodogram analysis was defined as the amplitude of the peak only for the rhythmic flies from the Lomb Scargle periodogram with significance level $p < 0.05$.

The mean activity level of the females was calculated under different entraining (I, III, IV) and constant conditions (I, IV) over experimental days in five-minute bins. Prior to performing these tests, normality assumptions of the distributions of fly activity levels were tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests and homogeneity of variance with Levene's test. If the data did not fulfill the criteria of normal distribution and/or homogeneity of variances, non-parametric tests were used. All the analyses were done with PASW Statistics 18.0 (SPSS Inc.).

The effects of temperature and photoperiod on the mean activity levels of the flies in entraining conditions (I) were analyzed with 2-way analysis of variance (ANOVA) with temperature and photoperiod as fixed factors. In cases, whenthe data were not normally distributed and/or the variances were not equal, we used Kruskal-Wallis and Mann-Whitney U –tests. Pairwise comparisons between the mean activity levels of the flies in constant light or darkness were performed with Mann-Whitney U –tests (I).

When tracing the factors (strain, temperature, entraining photoperiod, and/or constant condition) affecting fly rhythmicity (I), the data were analyzed with hierarchical logit models. For this analysis the locomotor activity data were made binary, with a value of 1 for each rhythmic fly and a value of 2 for each arrhythmic fly. The goodness of fit of the final model including all significant variables and their interaction terms was tested by using the likelihood-ratio $\chi 2$-test.

2.4.2 Analysis of pupal eclosion data (IV)

Pupal eclosion rhythms were traced by plotting the number of eclosions per 1 h bin for each strain. The free-running period (τ) of fly eclosion rhythms was estimated by periodogram analysis using the Lomb-Scargle periodogram method. The data for the first 12 circadian hours after the pupae had been

transferred to the eclosion monitors in constant darkness were excluded from the periodogram analysis to avoid possible effects of the transfer.

2.4.3 Analysis of qPCR data (III)

The relative gene expression values of *tim* and *per* were calculated using a relative expression method (Δ(Ct)) with real efficiency values (see study III for more details of the analysis). Statistical significance of the expression changes in these genes between the studied time points within the LD cycles, as well as between the non-diapausing and diapausing females in short photoperiods, were calculated with REST 2009 program using randomization with 10 000 iterations and bootstrapping methods.

3 RESULTS AND DISCUSSION

3.1 Function of the circadian clock at phenotypic level: regulation of the locomotor activity and eclosion rhythms in *Drosophila montana*

I have studied the role of the circadian clock in adaptation to environmental conditions prevailing at high latitudes by tracing the locomotor activity and eclosion rhythms of the flies of a northern *D. montana* population. The flies of this species appeared to possess good entrained locomotor activity rhythm in all LD cycles used in studies I, III and IV. Interestingly, these flies displayed only an evening activity peak in all studied photoperiods and temperatures, both in fluctuating (III) and constant (I, IV) temperature. Even though most insect species studied so far, including *D. melanogaster*, have been found to show bimodal locomotor activity with clear morning and evening peaks (e.g. Hamblencoyle et al. 1992), unimodal activity is not exceptional. This kind of activity has been detected e.g. in the Japanese honeybee *Apis cerana japonic* (Fuchikawa & Shimizu 2007) and in 2 *Drosophila* species, *D. subquinaria* (Simunovic & Jaenike 2006) and *D. virilis* (Bahn et al. *2009).*

One of the main characters of circadian clock rhythms is their self-sustainability, which means that the circadian rhythms keep cycling (free-run) even in the absence of environmental cues. However, the conditions in which free-running rhythms can be seen vary between the species. For example, *D. melanogaster* flies have been shown to retain their rhythmicity in constant darkness (Helfrich-Förster 2000, Dubruille & Emery 2008), but become rapidly arrhythmic in constant light (Konopka et al. 1989, Stanewsky et al. 1998), whereas *D. virilis* flies lose their rhythmicity in constant darkness (Bahn et al. 2009). *D. montana* flies were found to lose their locomotor activity rhythm when released into constant darkness after entrainment (like *D. virilis)*, but not when released into constant high and low light intensities (I, IV). In addition, the flies were more active in constant high and low intensity light than in constant darkness (IV).

Differences between the daily locomotor activity rhythms and activity levels of *D. montana* and *D. melanogaster* flies in constant light and darkness might be explained by species differences at the neuronal level. The difference in the activity rhythms is likely to be due to the fact that *D. montana* flies lack the expression of PDF in s-LN_v neurons, which have been shown to be necessary for the morning activity of *D. melanogaster* (more about this in chapter 1.2). Furthermore, in *D. melanogaster* the M oscillator has been shown to periodically rouse the fly in constant darkness, but not in constant light, whereas the E oscillator can do the same in constant light but not in constant darkness (Picot et al. 2007). The ability of *D. montana* flies to retain their locomotor activity rhythm in constant light is likely to be adaptive as these flies are exposed to long (even continuous) days during their mating season in early summer in the northern latitudes. On the other hand, also the loss of rhythmicity in constant darkness can be an evolutionary adaptation, as these flies are exposed to constant darkness during the long and dark winters, which they generally spend under a snow cover.

Contrary to showing weak locomotor activity rhythm in darkness, *D. montana* flies possessed persistent free-running eclosion rhythms in this condition (IV), which suggests that the locomotor activity and eclosion rhythms are at least partly under different neuronal control in this species. This kind of discrepancy has earlier been detected also in *D. montana*'s sister species *D. littoralis* (Lankinen, 1985). The presence of two or more circadian oscillators, which control independently or cooperatively the locomotor activity and eclosion rhythms, has been postulated also for *D. pseudoobscura* (Engelmann & Mack 1978), *D. rajasekari* (Joshi 2001) and *D. melanogaster* (Myers et al. 2003). In the last-mentioned species, the peripheral oscillator acting in the prothoracic gland controlling the eclosion rhythm has been found to be under the control of a central clock (Myers et al. 2003).

3.2 The neuronal background of the circadian clock of *D. montana*

In addition to differences in their locomotor activity rhythms, *D. montana* flies were found to differ from *D. melanogaster* also in the number and location of specific circadian clock neurons regulating these rhythms. In *D. montana* the expression of PDF neuropeptide, which is essential for synchronizing the oscillations of the circadian clock neurons and in transferring the circadian signals from the pacemaker to downstream neurons, was totally lacking (or showed very low expression) in small ventrolateral neurons (s-LN_vs) in the fly brains (I). Based on recent studies, the same seems to be true also in the other studied species of the *D. virilis* group; in *D. virilis, D. littoralis* and *D. ezoana* (Bahn et al. 2009, Hermann et al. 2012). Thus, the situation of all these species resembles that of *D. melanogaster* Pdf^{01} -mutants, which do not express PDF in their s-LN_v neurons and also lack the morning activity peak (Renn et al. 1999). Furthermore, ablation of PDF positive s-LN_v neurons in *D. melanogaster* has been found to

result in arrhythmic behavior in constant darkness and the loss of the morning activity peak in laboratory conditions (Shafer & Taghert 2009).

In *D. melanogaster*, the circadian photoreceptor gene *cryptochrome* (*cry*) plays an important role in entraining the flies to LD cycles and contributes also to the adjustment of the evening activity peak (Stanewsky et al. 1998, Emery et al. 2000b) *D. montana* differed from *D. melanogaster* in that this protein was not expressed in its large ventrolateral neurons (l-LN_vs) (I), again resembling the situation in *D. virilis, D. littoralis* and *D. ezoana* (Bahn et al. 2009, Hermann et al. 2012). In *D. melanogaster*, the expression of CRY in different neuron groups, including l-LN_vs, has been suggested to be responsible for the arrhythmicity of the flies in LL (Emery et al. 2000a, Benito et al. 2008, Yoshii et al. 2008). This is supported by the finding that cry^b and cry^0 mutants of this species retain their rhythmicity in LL (Emery et al. 2000a, Dolezelova et al. 2007) due to the reduced action of CRY in their dorsal neurons (Dubruille et al. 2009, Zhang et al. 2010a, Zhang et al. 2010b) and possibly also in l-LN_vs (Emery et al. 2000b, Shang et al. 2008, Fogle et al. 2011).

Neurogenetic differences detected in this study (I) between *D. montana* and *D. melanogaster* are not likely to be due to a lack of immunoreactivity of *D. melanogaster* antibodies used in *D. montana.* The comparisons of the amino acid sequences for PDF antibody region between *D. melanogaster, D. virilis* and *D. montana* showed this region to be identical between these 3 species and also the CRY antibody region showed high sequence similarities between the species (I). The differences found in the expression of PDF in s-LN_vs in *D. montana* compared to *D. melanogaster* could explain, at least partly, the lack of the morning activity and the attenuated function of the circadian clock in this species in constant darkness. In addition, the absence of CRY expression in l-LN_vs in *D. montana* may enable the flies to retain their rhythmicity in continuous light during northern summers.

3.3 The role of the circadian clock in controlling seasonal rhythms and its possible connection with the photoperiodic timer

The role of the circadian clock in controlling seasonal changes in insect behavior and development, alone or together with a photoperiodic timer, is still unclear (Schiesari et al. 2011) and many theoretical models and experimental designs have been developed to find answers to this question (Bünning 1936, Pittendrigh & Minis 1964, Pittendrigh 1972, Lees 1973). To find out whether the circadian clock is involved in the function of the photoperiodic timer in *D. montana* I traced photoperiodic responses of the females of this species (diapause/reproduction) under different LDs using the classical Nanda-Hamner protocol (II). If *D. montana* females used circadian clock in photoperiodic time measurement, their diapause response would be predicted to rise and fall with a period ~24 h during the prolonged nights. On the other hand, if they showed non-rhythmic diapause

response during those prolonged nights, their photoperiodic time measurement could be expected to be based on an hourglass-like mechanism or a damped oscillator. The study revealed no cycling in the females' diapause response, thus favoring the latter option. The fact that *D. montana* females did not show a 'short day response' and enter diapause in prolonged nights could be due to the fact that their light-sensitivity did not show rhythmic fluctuation in darkness (circadian clock is not involved or it is rapidly damped). Another possibility would be that the females had problems in the counter mechanism of the photoperiodic timer (they did not succeed in collecting information on the required number of LD cycles for diapause induction). The finding on low diapause incidence of these flies is intriguing, as usually insects that have given negative results in the Nanda-Hamner protocol have shown short and not long day response (e.g. high diapause incidence) during the prolonged nights.

Pittendrigh and Daan (1976) proposed for the maintenance of seasonal changes in behavior a general model, in which the morning (M) and evening (E) activity peaks are assumed to be induced by the morning and evening oscillators. Seasonal changes in the phase relationship between these peaks enable the organisms to anticipate environmental changes and to synchronize their behavior accordingly (Allada & Chung 2010). As *D. montana* flies do not show a morning peak in their locomotor activity, they cannot measure changes in day length by comparing the distance between morning and evening peaks (I, III, IV). The same is true for some other species with unimodal activity, such as the house fly *Musca domestica* (Helfrich et al. 1985), Japanese honeybee *Apis cerana japonica* (Fuchikawa & Shimizu 2007) and *D. virilis* (Bahn et al. 2009). However, Potdar and Sheeba (2012) have recently demonstrated that in *D. melanogaster* l-LN_vs may set the phase of the evening peak in a wide variety of photoperiods, ranging from extreme short days (4:20 LD) up to extreme long days (20:4 LD). Thus the evening activity peak and its' phase-shifting during a day in different day lengths might enable *D. montana* and other species showing unimodal activity to detect seasonal changes in day length. The measurement of the day length in these species could follow the external coincidence hypothesis proposed by Bünning in 1936, where only one circadian oscillator (in this case the evening oscillator) is thought to be responsible for measuring the length of the day.

Seasonal changes in photoperiod and temperature are known to modify the daily activity patterns of organisms. In *D. montana* the position of the single activity peak (evening peak) phase-shifted earlier in respect to the lights-off transition along with an increase in day length (I). Simultaneous changes in photoperiod and temperature in seminatural conditions advanced the females' evening activity peak even more than photoperiodic changes alone (III). In addition, the activity level of the females decreased towards the autumn; diapausing females being less active than the non-diapausing females in the shortest photoperiod (III). Photoperiod and temperature are known to affect the phase-shift of the flies' activity peaks also in *D. melanogaster,* in which the evening activity of the flies is delayed under long day conditions relative to the prior sunrise and the flies become progressively more nocturnal (Chen et al. 2006). In colder temperatures, *D. melanogaster* flies phase-shift their evening

activity to an earlier time of day (Majercak et al. 1999). Temperature cycles have been found to entrain the circadian clock of the flies of this species also without any changes in the photoperiod (Wheeler et al. 1993). The same seems to be true also in *D. montana*, as I have found a 3 °C difference in the temperature to increase the flies' free-running rhythmicity in constant darkness (Kauranen, unpublished). Futhermore, Vanin et al. (2012) have shown that in natural conditions *D. melanogaster* flies show a third activity peak, the afternoon peak. We did not detect this kind of 'extra' peak in *D. montana* in seminatural conditions. An advancement of the evening activity peak and a decrease in the activity level of *D. montana* flies towards the autumn, as well as the lower activity of diapausing females compared to the non-diapausing females in late summer, are expected to be adaptive features in northern latitudes.

3.4 Daily and seasonal changes in the expression level of *timeless* and *period* genes

The expression of the circadian clock genes has been found to show both daily and seasonal changes in several insect species (Majercak et al. 1999, Warman et al. 2000, Goto & Denlinger 2002). For example, in *Sargophaga crassipalpis*, the expression level of *tim,* but not that of *per*, has been found to dampen under long day conditions, and the expression levels of both genes has been shown to be sensitive to cold temperature (Goto & Denlinger 2002). In addition, the expression peaks of both of these genes have been shown to phase-shift in concert with the onset of scotophase in this species (Kostal et al. 2009). However, the species seem to vary in this, since in other species, such as *Protophormia terraenovae* (Muguruma et al. 2010), neither of these genes show expression differences under short- and long-day conditions.

In *D. montana*, the expression levels of *per* and *tim* cycled in all photoperiods showing a mutual phase relationship with each other (III). The peak expression of both of these genes phase-shifted towards the light-on transition as the day length and temperature decreased (III). The peak expression of *per* and *tim* have been found to phase-shift to an earlier time of day in shorter photoperiods also in the silkmoth *Bombyx mori* (Iwai et al. 2006). In *D. melanogaster* the phases of *tim* and *per* are advanced at lower temperatures and in *per* this phase advance has been found to be caused by its differential splicing (Majercak et al. 1999). Co-occurrence of the evening activity peak and the highest levels of *tim* and *per* expression varied in *D. montana* between the photoperiods / temperatures in seminatural conditions (III). In the shortest photoperiod the expression peaks of these genes phase-shifted to an earlier time of day so that they occurred earlier than the evening activity peak of the females, both in diapausing and non-diapausing females. The cyclic expression of these genes suggests the action of the circadian component in these oscillations. In addition, phase-shifting of the peak expression of *per* and *tim* indicate the plasticity of the circadian clock in *D. montana*, which is an important character for the species living in northern hemisphere.

4 CONCLUSIONS

An ability to predict forthcoming changes in environmental conditions is crucial for the survival and reproduction of organisms living in a seasonally changing environment, as their fitness depends largely on their capability to utilize available energy resources and to synchronize their metabolic, developmental and behavioural processes along with the changing seasons. One of the key factors in enhancing adaptation to this kind of environment is the evolution of two time-measuring systems: the circadian clock regulating daily variation and the photoperiodic timer regulating seasonal activities. The genetic background of the circadian clock is well-known, but the genetic and physiological mechanisms behind the photoperiodic timer and the role of the circadian clock in this timer are still unclear. In my thesis I have studied the role of the circadian clock in adaptation to seasonally varying environment in a northern *Drosophila* species, *D. montana,* by tracing the locomotor activity and eclosion rhythms of the flies of this species, as well as by studying the function of their circadian clock at the genetic and neuronal level. In addition, I have tried to find out whether and how the circadian clock plays a role in the function of the photoperiodic timer regulating the diapause behaviour of this species.

My studies show that the circadian clock of *D. montana* possesses features that are adaptative in seasonally varying northern environments. First, I found that *D. montana* flies show good entrained activity rhythms and display only a single, evening activity peak in different photoperiods (I, III, IV). Second, the flies of this species were found to maintain their free-running locomotor activity rhythm better in constant light (both high and low light intensity) than in constant darkness (I, IV). *D. montana* flies differ in both above-mentioned characters from the more southern species *D. melanogaster,* which is one of the most important model species used in studies of the circadian clock. Study I showed that explanations for the species differences in fly locomotor activity rhythms can be found at the neuronal level. In this study *D. montana* was found to differ from *D. melanogaster* in the number and location of specific circadian neurons expressing the neuropeptide PDF and the photoreceptor CRY protein, both of which play an important role in regulating circadian rhythms e.g. in the

locomotor activity (I). Study IV also showed that contrary to the situation with the locomotor activity rhythms, *D. montana* flies show persistent free-running eclosion rhythm in constant darkness (IV). This difference is likely explained by a difference in their control: the locomotor activity of the flies is directly under the control of central circadian clock whereas the eclosion rhythm is regulated by the circadian oscillators in peripheral ganglia, which might function more independently in this species than e.g. in *D. melanogaster*. To study the possible role of the circadian clock in the function of photoperiodic timer, I traced the females' photoperiodic diapause responses under different LDs using a classical Nanda-Hamner protocol (II). The study revealed no circadian rhythmicity in females' diapause response, which suggests that the function of the photoperiodic timer in *D. montana* is based on either an non-oscillatory hourglass timer or a rapidly damping circadian oscillator. *D. montana* flies can use only one circadian oscillator (evening oscillator) to measure the length of day, as they do not show a morning activity peak (I), hence their time measurement could be described using the external coincidence model proposed by Bünning in 1936. Indeed, I found in study IV that *D. montana* females shift their evening activity towards an earlier time of the day in seasonally changing environmental conditions and also decrease their activity towards the autumn, with diapausing females being less active than non-diapausing females (III). In this study also the expression peaks of two circadian clock genes *per* and *tim,* were found to phase-shift in concert with a decrease in the day length, so that in the shortest photoperiod the highest expression peak of these genes occurred earlier than did the females' evening activity peak.

Circadian rhythms of *D. montana* are not exceptional among insect species and recent studies on some other species of the *D. virilis* species group have detected the same kinds of phenomena (Bahn et al. 2009, Hermann et al. 2012). Differences in the function of the circadian clock between the species of *D. virilis* group (genus *Drosophila*) and *D. melanogaster* (genus *Sophophora*) suggest that the circadian clock has evolved in different directions in the different evolutionary lineages. The lack of the morning activity peak in entrained rhythms, the degradation of free-running activity rhythm in constant darkness and the findings on the expression of PDF and CRY in the brain neurons of *D. montana* flies strongly suggest that the morning oscillator of this species does not function in the same way as it does in *D. melanogaster* and that the evening oscillator plays a more important role in the circadian clock of *D. montana.* This kind of circadian clock may have been one of the key factors enabling *D. montana* flies to distribute to high latitudes and altitudes, where it is beneficial to be rhythmic during the long summer days and non-rhythmic and less active during the short winter days.

Altogether, the studies included in my thesis expand the knowledge on the function of the circadian clock as well as on its role in seasonal adaptation. However, further studies at the behavioural, genetic and neuronal levels are needed to find out whether and how the circadian clock is involved in the function of the photoperiodic timer. For example, studies on the expression of the circadian clock genes at the protein level in the brains of *D. montana* flies could

give more detailed information on their direct or indirect role in photoperiodism, especially if the studies would be performed on females at different developmental stages (non-diapausing/diapausing) and in different environmental conditions. In addition, the use of other kinds of techniques, such as gene silencing, would help to trace the importance of the circadian clock genes in evoking photoperiodic responses in *D. montana*. In efforts to understand the function and interaction between the two clock mechanisms, selection of suitable model species with clear free-running and entrained rhythms controlled by the circadian clock, and robust photoperiodic responses determined by the photoperiodic timer, will be the key factor. Clearly, *D. montana* offers a good object for these studies.

Acknowledgements

First of all I would like to express my gratitude to my supervisors Anneli Hoikkala and Maaria Kankare. Anneli, I am grateful that you have always helped, given support and offered a shoulder, sometimes even literally, for me where to lean on whenever I have needed it. I would like to thank you for that your door has always been open and I appreciate a lot that you have let me shape my thesis into the direction that has felt most interesting for me. I would also like to thank Maaria for being my supervisor and helping me with lots of things, especially with things considering DNA laboratory. I would also like to express my gratitude for Professor Rodolfo Costa and Professor Charlotte Helfrich-Förster for all your help and collaboration and letting me spent some time in your laboratories during these years to learn skills which have been essential for finishing this thesis. In addition, I would like to thank Dr. Pamela Menegazzi for all your help, support and last, but not least, for being one of my best friends. Sometimes you just have to go faraway to meet great people. We have shared many great moments and now, when I am one step closer our Plan A, we will hopefully have more of them in the future. I would also like to thank Annaliisa Kankainen for statistical analysis in one of the papers included my thesis.

I am thankful also for Professor Sakiko Shiga and docent Tapio Heino for evaluating this thesis and Dr. David Doležel for accepting the invitation to be my opponent. I would also like to thank Jari Hami for the help during the editorial process and Emily Knott for correcting the language in some parts of my thesis. It has also been nice to work in the Ecology section here in the University of Jyväskylä and I would like to thank all of you, especially other PhD students in our "corridor", for creating a great atmosphere for working. Most of all, I want to thank other fly girls and a fly boy; Laura, Tiina, Venera, Nina, Jenni and Jackson. It has been a privilege to share the working place with very good friends and I am deeply grateful for that. Laura, we shared the student life over ten years and we had so many great moments together. Thank you for all of that. Tiina, thank you for all the moments we have shared during these years too. Venera, it has been great to have you in our group, you are always so happy and bake such excellent cakes! Thank you, to Jackson, for being a great colleague, our one and only fly boy and a good friend. I would also like to thank Nina for being such a good friend and sharing some of the most awful moments in my life besides many great ones! And Jenni, thank you for sharing same kind of view on life and the sense of humour; we have had so many unforgettable moments and I hope to have more of them during our lives, so that we will have many memories to reminisce together in the retirement home. If there is no one else who could understand me, I know you can. Thank you for that. In the end, I would also like to thank all of you fly girls for all those very important conversations in the little coffee room where we have wondered many things from the very scientific perspective and have done the world little bit better place to be. In addition, I would like to thank Mikko "DF" Merisalo and Darren "Sweetie" J. Parker for the company and support during almost the most awful times in this project. Mikko, I am happy that you beat your head against the wall at the same time as I did. I

am also very grateful for you Darren for expanding my English vocabulary. And now when I am almost The Doctor I am waiting for to learn more about your clock-wise/anti-clockwise stuff. I have also been privileged to meet great people around the world during these years, and I would like to thank all of you for sharing nice moments together. Paola Cusumano, Laura Caccin, Elena Carbognin and Francesca Baccio, thank you for being so nice and helpful for me when I was visiting in Italy. Damiano Persian, you have been such a good company and I am grateful for you and Pamela for carrying me through some difficult moments in my life. Friends are needed when your legs cannot carry you anymore. In addition, I would like to thank Christiane Hermann for all your help.

I have also enjoyed sharing the office with many great people during these years and I would like to thank all of you making every day at work easier and more fun. Especially I would like to thank Anne Laita; thanks for sharing the last moments of this thesis project with me and becoming a good friend. This thesis would not been able to done without the help from many people and I would like to thank you all. I would like to acknowledge Elisa Kiukkanen and Laura Pitkänen for the help in the fly room and Elina Virtanen with all the things considering DNA laboratory. Sari Viinikainen and Anssi Lipponen, I am happy that I have always had possibility to ask questions, even very stupid ones, and still you have always helped me. I would also like to thank "Rengit" for all the help that I have got from you during these years. Also our helpers; Mikko Merisalo, Antti Miettinen, Ville Hoikkala, Anne Lehtovaara, Jaana Haka, Taru Kuparinen, Lily Laine, Marina Mustonen and Jenni Marjakangas, your work has been essential for both our group and for my thesis. Thank you all for your work and devotion to the flies. I would like also thank my students Eija Lönn, Hanna Häkkinen and Tinja Pitkämäki, you have all been great. I would also like to thank the Centre of Excellence in Evolutionary Research, Jenny and Antti Wihuri Foundation, Finnish Concordia Fund, Societas Entomologia Fennica, Biological Interactions Graduate School, Societas Pro Fauna et Flora Fennica for funding this work.

I would also like to thank my family and friends outside the university for all your help and support. Niina, you have always been my best friend since we were babies and I know that I can always trust on and count on you. Thank you for being so great. Thank you also Elisa for all your friendship during these years. Mom and dad, thank you for supporting me always whatever I have decided to do. In addition, thanks for both of you for all the help with my zoo during these years. Mom and grand mom, thank you for the food, the cleaning help and also taking care a lot of Vilma; without that this would not have been possible. Johanna and Sami, thank you for giving me moments to think something else than just work. Anssi, I would not have been able to do this without you. Thank you for always being there for me. Finally, I would like to thank my sweet, lovely daughter Vilma, who has gave me strength to carry on and has reminded me what the most important thing in my life is.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Sopeutuminen ympäristöoloissa tapahtuviin vuorokauden ja vuoden aikaisiin muutoksiin on tärkeää erityisesti pohjoisessa eläville eliölajeille. Tällaisissa oloissa yksilöiden on kyettävä mittaamaan aikaa ja ennakoimaan tulevat ympäristön muutokset, jotta ne voivat muuttaa niin aineenvaihduntaansa, fysiologiaansa kuin käyttäytymistäänkin vallitseviin ympäristöoloihin sopiviksi. Tässä ”ennustamisessa” ovat keskeisessä roolissa vuorokausirytmejä ylläpitävä sirkadinen kello ja vuodenaikaisrytmejä säätelevä valojaksoinen kello.

Maapallon pyöriminen oman akselinsa ympäri 24 tunnin sykleissä on johtanut eliöiden sirkadisen kellon evoluutioon. Tämä aikaa mittaava biologinen kellomekanismi ylläpitää eliöiden noin 24 tunnin pituista sisäistä vuorokautta ja lisää eliöiden sekä sisäistä että ulkoista kelpoisuutta säätelemällä esimerkiksi syanobakteereilla solun jakautumista, kasveilla lehtien liikkeitä, *Drosophila*-kärpäsillä liikeaktiivisuutta ja kuoriutumisrytmiä sekä ihmisillä uni-valverytmiä ja ruumiinlämpötilaa. Sirkadisen kellon ylläpitämille rytmisille ominaisuuksille on tyypillistä se, että ne tahdistuvat ympäristön antamien signaalien, kuten valo- tai lämpötilamuutosten mukaan. Lisäksi ko. ominaisuuksien rytmisyys säilyy vaikka eliöt laitetaan vakaaseen ympäristöön. Maapallon pyörimisliike auringon ympäri vuoden kuluessa on vastaavasti johtanut valojaksoisen kellon evoluutioon. Valojaksoisen kellon tiedetään mittaavan lähinnä päivän pituudessa tapahtuvia muutoksia, joiden avulla se auttaa eliöitä luotettavasti ennakoimaan ympäristön tulevat vuodenaikojen vaihtelut ja mahdollistaa siten niiden sopeutumisen tällaisiin muuttuviin olosuhteisiin. Valojaksoisen kellon tiedetään säätelevän mm. *Drosophila*-naaraiden lisääntymislepokauteen eli diapaussiin siirtymistä ja monarkkiperhosten vuodenaikaisvaellusta. Sirkadisen kellon geneettisestä ja neurologisesta taustasta tiedetään jo paljon, mutta valojaksoisen kellon taustalla vaikuttavat geenit sekä neurologiset kytkennät ovat vielä suurelta osin tuntemattomia. Lisäksi on vielä epäselvää nojautuuko valojaksoisen kellon toiminta sirkadiseen kelloon, vai perustuvatko kyseiset biologiset kellot täysin eri mekanismeihin.

Väitöskirjatutkimukseni tavoitteena oli selvittää kuinka sirkadisen kellon evoluutio on voinut edesauttaa *Drosophila montana* -lajin kärpästen sopeutumista pohjoiseen ympäristöön. Lisäksi tutkin, onko sirkadisen kellon toiminta mahdollisesti yhteydessä valojaksoiseen kelloon ja jos on, niin miten. *D. montana* -laji on levittäytynyt ympäri pohjoista pallonpuoliskoa sopeutuen erilaisiin oloihin ja lisäksi sille on kehittynyt talvehtimisstrategiaksi valojakson säätelemä lisääntymislepokausi, joten se tarjoaa mielenkiintoisen tutkimuskohteen. Vastatakseni väitöskirjatutkimukseni kysymyksiin tutkin kuinka sirkadinen kello säätelee *D. montana* -naaraiden vuorokausi- ja vuodenaikaisrytmejä sekä liikeaktiivisuudessa että kuoriutumisen ajoittumisessa, ja kuinka tämä kello toimii geneettisellä ja neurologisella tasolla. Lisäksi pyrin selvittämään sirkadisen kellon osuutta valojaksoisen kellon toiminnassa tutkimalla *D. montana* -naarailla esiintyvää valojaksoista lisääntymislepokautta eri koeoloissa.

Tutkimukseni osoittavat, että *D. montana* -lajin sirkadinen kello omaa ominaisuuksia, jotka ovat tärkeitä ko. lajin sopeutumiselle pohjoiseen elinympäristöön. *D. montana* -naaraiden liikeaktiivisuus tahdistuu hyvin erilaisiin ympäristöoloihin kuten valojaksoihin ja lämpötiloihin, naaraiden aktiivisuustason saavuttaessa huippunsa alkuillan aikana. Vakaissa oloissa *D. montana* ylläpitää liikeaktiivisuusrytminsä paremmin jatkuvassa valossa kuin jatkuvassa pimeydessä. Täten *D. montana* -kärpäsen liikeaktiivisuusrytmi poikkeaa eteläisemmästä, sirkadisen kelloon liittyvän tutkimuksen yhtenä mallilajina pidetystä *D. melanogaster* -lajista, jolla liikeaktiivisuuden on havaittu jakautuvan sekä aamu- että ilta-aktiivisuushuippuun. Viimeksi mainitun lajin kärpäset myös säilyttävät liikeaktiivisuusrytminsä jatkuvassa pimeydessä, mutta menettävät sen jatkuvassa valossa. Tutkimuksessani havaitsin *D. montana*- ja *D. melanogaster* -kärpästen välillä myös neuronitason eroja, jotka voivat selittää liikeaktiivisuudessa havaittuja eroavaisuuksia ko. lajien välillä. *D. montana* poikkesi sekä *Pigment-dispersing factor*- ja *cryptochrome* -geenejä ekspressoivien neuronien lukumäärän että niiden sijainnin suhteen *D. melanogaster* -lajista. Vaikka *D. montana* -kärpäset menettävät liikeaktiivisuusrytminsä jatkuvassa pimeydessä, tutkimukseni osoittaa, että ne säilyttävät silti kuoriutumisrytminsä ko. oloissa, mikä voi johtua siitä, että nämä rytmit toimivat osittain eri säätelymekanismien alla.

Väitöskirjatyöhöni sisältyi myös Nanda-Hamner -koe, jossa tutkin sirkadisen kellon yhteyttä lisääntymislepokauden laukeamiseen *D. montana* -naarailla 13 eri valojaksossa, joissa valoisan ajan määrä oli vakio ja pimeän ajan pituus vaihteleva. Mikäli sirkadinen kello olisi yhteydessä valojaksoisen kellon toimintaan, naaraiden valosensitiivisyyden ja samalla myös niiden herkkyyden siirtyä lisääntymislepokauteen tulisi vaihdella noin 24 tunnin sykleissä. Tämän kokeen tulokset eivät antaneet selkeää vastausta sirkadisen kellon osallisuudesta valojaksoisen kellon toimintaan *D. montana* -naarailla. Kokeen tulokset kuitenkin osoittivat valojaksoisen kellon toimivan *D. montana* -lajilla joko itsenäisesti ilman sirkadisen kellon osallisuutta tai pohjautuvan sellaiseen sirkadisen kellon ylläpitämään oskillaattoriin, joka menettää rytmisyytensä vakaissa oloissa. Tutkimukseni mukaan päivän pituuden mittaus perustuu *D. montana* -lajilla lähinnä ilta-aktiivisuushuipun ajoittumiseen: päivänpituuden lyhentyessä *D. montana* -kärpästen ilta-aktiivisuushuippu siirtyi aikaisemmaksi ja niiden liikeaktiivisuus väheni. Myös kahden sirkadiseen kelloon liittyvien *period*- ja *timeless*-geenien ekspressiohuiput siirtyivät vastaavasti aikaisempaan ajankohtaan.

D. montana-lajin lisäksi myös kolmen muun saman *D. virilis* -ryhmän lajin sirkadisen kellon toiminnan on havaittu poikkeavan niille kaukaista sukua olevan *D. melanogaster* -lajin kellon toiminnasta. Löydetyt erot sirkadisen kellon toiminnassa eri lajiryhmien välillä kuvastavat todennäköisesti eri suuntaan edennyttä sirkadisten kellojen evoluutiota lajien sopeutuessa erilaisiin elinympäristöihin. *D. montana* -lajin sirkadinen kello omaa selkeästi piirteitä, jotka mahdollistavat ko. lajin sopeutumisen pohjoisiin elinympäristöihin. Tämän lajin kärpästen liikeaktiivisuusrytmin säilyminen jatkuvassa valossa kuvastaa ko. lajin sopeutumista pohjoisiin kesiin, jolloin pohjoisessa elävät lajit altistuvat

jatkuvalle valolle. Myös rytmisyyden katoaminen jatkuvan pimeyden aikana ja liikeaktiivisuuden väheneminen lyhyissä päivänpituuksissa ovat sopeutumia pohjoisiin talvioloihin, joissa ylimääräinen aktiivisuus tai sen rytmisyys kuluttaisi vain yksilöiden resursseja ja heikentäisi niiden talvesta selviytymistä.

Väitöskirjatutkimukseni tuo lisää tietoa sirkadisen kellon roolista eliöiden sopeutumisessa pohjoisiin oloihin niin fenotyyppisellä, geneettisellä kuin neurologisellakin tasolla. Lisäksi tutkimukseni valottaa evoluutiota, jota on tapahtunut eri lajien välillä niiden sopeutuessa erilaisiin elinympäristöihin. Tekemäni tutkimukset antavat hyvän pohjan jatkotutkimuksille, joissa erilaisten molekyyligeneettisten menetelmien, kuten proteiinitason tutkimuksien avulla voidaan tutkia tarkemmin sirkadisen kellon osuutta sekä eliöiden sopeutumisessa erilaisiin elinympäristöihin että erityisesti valojaksoisen kellon toiminnassa.

REFERENCES

Alabadi D., Oyama T., Yanovsky M., Harmon F., Mas P. & Kay S. 2001. Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science* 293: 880-883.

Allada R. & Chung B.Y. 2010. Circadian organization of behavior and physiology in *Drosophila*. *Annu. Rev. Physiol.* 72: 605-624.

Aschoff J. 1979. Circadian rhythms - influences of internal and external factors on the period measured in constant conditions. *Zeitschrift Fur Tierpsychologie-J. Comp. Ethology* 49: 225-249.

Aschoff J. 1966. Circadian activity pattern with 2 peaks. *Ecology* 47: 657-662.

Bachleitner W., Kempinger L., Wuelbeck C., Rieger D. & Helfrich-Foerster C. 2007. Moonlight shifts the endogenous clock of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 104: 3538-3543.

Bahn J.H., Lee G. & Park J.H. 2009. Comparative analysis of *Pdf*-mediated circadian behaviors between *Drosophila melanogaster* and *D. virilis*. *Genetics* 181: 965-975.

Beaver L., Gvakharia B., Vollintine T., Hege D., Stanewsky R. & Giebultowicz J. 2002. Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 99: 2134-2139.

Beer K., Hermann C., Helfrich-Foerster C. & Rieger D. 2010. *Drosophila* with *period* gene expression restricted to subsets of Lateral Neurons show either morning or evening activity under short and long days. *J. Neurogenet.* 24: 19-19.

Benito J., Houl J.H., Roman G.W. & Hardin P.E. 2008. The blue-light photoreceptor CRYPTOCHROME is expressed in a subset of circadian oscillator neurons in the *Drosophila* CNS. *J. Biol. Rhythms* 23: 296-307.

Block G., Khalsa S., McMahon D., Michel S. & Guesz M. 1993. Biological clocks in the retina: cellular mechanisms of biological timekeeping. *International Review of Cytology - a Survey of Cell Biology, Vol 146* 146: 83-144.

Boulos Z. & Macchi M. 2005. Season- and latitude-dependent effects of simulated twilights on circadian entrainment. *J. Biol. Rhythms* 20: 132-144.

Brower L. 1977. Monarch migration. *Natural History* 86: 41-53.

Bünning E. 1936. Die endogene tagesrhythmik als grundlage der photoperiodischen reaction. *Berl. Dtsh. Bot. Ges.* 54: 590-607.

Bünning E. 1969. Common features of photoperiodism in plants and animals. *Photochem. Photobiol.* 9: 219-228.

Chen F., Han Y., Hu Q. & Hou M. 2011. Diapause induction and photoperiodic clock in *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae). *Entomol. Sci.* 14: 283-290.

Chen W., Majercak J. & Edery I. 2006. Clock-gated photic stimulation of *timeless* expression at cold temperatures and seasonal adaptation in *Drosophila*. *J. Biol. Rhythms* 21: 256-271.

Claret J. & Arpagaus M. 1994. Evidence for dawn and dusk hourglasses in *Pimpla* photoperiodic clock. *Biol. Rhythm Res.* 25: 106-118.

Danilevskii A.S. 1965. Photoperiodism and seasonal development of insects. In Oliver & Boyd (eds.), Edinburgh & London.

de Mairan J. 1729. Observation botanique. *Hist. Acad. Roy. Sci.* 35–36.

Dolezelova E., Dolezel D. & Hall J.C. 2007. Rhythm defects caused by newly engineered null mutations in *Drosophila*'s *cryptochrome* gene. *Genetics* 177: 329-345.

Dubruille R. & Emery P. 2008. A plastic clock: how circadian rhythms respond to environmental cues in *Drosophila*. *Mol. Neurobiol.* 38: 129-145.

Dubruille R., Murad A., Rosbash M. & Emery P. 2009. A constant light-genetic screen identifies KISMET as a regulator of circadian photoresponses. *Plos Genetics* 5: e1000787.

Emerson K.J., Bradshaw W.E. & Holzapfel C.M. 2009. Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. *Trends Genet.* 25: 217-225.

Emery P., Stanewsky R., Hall J. & Rosbash M. 2000a. *Drosophila* cryptochromes - A unique circadian rhythm photoreceptor. *Nature* 404: 456-457.

Emery P., Stanewsky R., Helfrich-Förster C., Emery-Le M., Hall J. & Rosbash M. 2000b. *Drosophila* CRY is a deep brain circadian photoreceptor. *Neuron* 26: 493-504.

Engelmann W. & Mack J. 1978. Different oscillators control circadian rhythm of eclosion and activity in *Drosophila*. *J. Comp. Physiol.* 127: 229-237.

Fenn M. & Macdonald D. 1995. Use of middens by red foxes - risk reverses rhythms of rats. *J. Mammal* 76: 130-136.

Fogle K.J., Parson K.G., Dahm N.A. & Holmes T.C. 2011. CRYPTOCHROME is a blue-light sensor that regulates neuronal firing rate. *Science* 331: 1409-1413.

Foster R.G. & Kreitzman L. 2009. Seasons of life, Profile books Ltd, London, UK.

Fuchikawa T. & Shimizu I. 2007. Circadian rhythm of locomotor activity in the Japanese honeybee, *Apis cerana japonica*. *Physiol. Entomol.* 32: 73-80.

Fujii S., Krishnan P., Hardin P. & Amrein H. 2007. Nocturnal male sex drive in *Drosophila*. *Curr.t Biol.* 17: 244-251.

Giebultowicz J. 2001. Peripheral clocks and their role in circadian timing: insights from insects. *Philos. Trans. R. Soc. Lond., Ser.B* 356: 1791-1799.

Glossop N. & Hardin P. 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. *J. Cell Sci.* 115: 3369-3377.

Goto S. & Denlinger D. 2002. Short-day and long-day expression patterns of genes involved in the flesh fly clock mechanism: *period*, *timeless*, *cycle* and *cryptochrome*. *J. Insect Physiol.* 48: 803-816.

Green C.B., Takahashi J.S. & Bass J. 2008. The meter of metabolism. *Cell* 134: 728-742.

Grima B., Chelot E., Xia R. & Rouyer F. 2004. Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. *Nature* 431: 869-873.

Gutman R. & Dayan T. 2005. Temporal partitioning: An experiment with two species of spiny mice. *Ecology* 86: 164-173.

Hamblencoyle M., Wheeler D., Rutila J., Rosbash M. & Hall J. 1992. Behavior of period altered circadian rhythm mutants of *Drosophila* in light - dark cycles (Diptera, Drosophilidae). *J. Insect Behav.* 5: 417-446.

Hardin P. 2004. Transcription regulation within the circadian clock: The E-box and beyond. *J. Biol. Rhythms* 19: 348-360.

Hardin P., Hall J. & Rosbash M. 1990. Feedback of the *Drosophila period* gene product on circadian cycling of its messenger-RNA Levels. *Nature* 343: 536-540.

Hastings JW., Rusak B. & Boulos Z. 1991. Circadian rhythms: the physiology of biological timing. In Prosser CL. (ed.), *Neural and intergrative animal physiology*, Wiley-Liss, Inc., New York, pp. 435-546.

Haus E. 2007. Chronobiology in the endocrine system. *Adv. Drug. Deliv. Rev.* 59: 985-1014.

Hege D., Stanewsky R., Hall J. & Giebultowicz J. 1997. Rhythmic expression of a PER-reporter in the malpighian tubules of decapitated *Drosophila*: Evidence for a brain-independent circadian clock. *J. Biol. Rhythms* 12: 300-308.

Helfrich C., Cymborowski B. & Engelmann W. 1985. Circadian activity rhythm of the house fly continues after optic tract severance and lobectomy. *Chronobiol. Int.* 2: 19–32.

Helfrich-Förster C. 2000. Differential control of morning and evening components in the activity rhythm of *Drosophila melanogaster* - sex-specific differences suggest a different quality of activity. *J. Biol. Rhythms* 15: 135-154.

Helfrich-Förster C. 2003. The neuroarchitecture of the circadian clock in the brain of *Drosophila melanogaster*. *Microsc. Res.Tech.* 62: 94-102.

Helfrich-Förster C., Winter C., Hofbauer A., Hall J. & Stanewsky R. 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 249-261.

Hermann C., Saccon R., Sethilan P.R., Domnik L., Dircksen H., Yoshii T. & Helfrich-Förster C. 2012. The circadian clock network in the brain of different *Drosophila* species. *J. Comp. Neurol.* in press.

Hodek I. 1968. Diapause in females of *Pyrrhocoris apterus L* (Heteroptera). *Acta Entomologica Bohemoslovaca* 65: 422-435.

Hodkova M. 1977. Function of Neuroendocrine Complex in Diapausing *Pyrrhocoris apterus* females. *J. Insect Physiol.* 23: 23-28.

Howlader G. & Sharma V.K. 2006. Circadian regulation of egg-laying behavior in fruit flies *Drosophila melanogaster*. *J. Insect Physiol* 52: 779-785.

Imaizumi T. & Kay S.A. 2006. Photoperiodic control of flowering: not only by coincidence. *Trends Plant Sci.* 11: 550-558.

Iwai S., Fukui Y., Fujiwara Y. & Takeda M. 2006. Structure and expressions of two circadian clock genes, *period* and *timeless* in the commercial silkmoth, *Bombyx mori*. *J. Insect Physiol.* 52: 625-637.

Iwasaki H. & Kondo T. 2004. Circadian timing mechanism in the prokaryotic clock system of cyanobacteria. *J. Biol. Rhythms* 19: 436-444.

Johnson C. 2001. Endogenous timekeepers in photosynthetic organisms. *Annu. Rev. Physiol.* 63: 695-728.

Joshi D. 2001. Rhythm-specific mutations in *Drosophila rajasekari* altered adult locomotor activity rhythm but not eclosion rhythm. *Biol. Rhythm Res.* 32: 35-43.

Kaneko M., Helfrich-Förster C. & Hall J. 1997. Spatial and temporal expression of the *period* and *timeless* genes in the developing nervous system of *Drosophila*: newly identified pacemaker candidates and novel features of clock gene product cycling. *J. Neurosci.* 17: 6745-6760.

Kankare M., Salminen T.S., Laiho A., Vesala L. & Hoikkala A. 2010. Changes in gene expression linked with adult reproductive diapause in northern malt fly species: a candidate gene microarray study. *BMC Ecol* 10: 3.

King D. & Takahashi J. 2000. Molecular genetics of circadian rhythms in mammals. *Annu. Rev. Neurosci.* 23: 713-742.

Klarsfeld A., Leloup J. & Rouyer F. 2003. Circadian rhythms of locomotor activity in *Drosophila*. *Behav. Processes* 64: 161-175.

Klein D.C., Moore R.Y. & Reppert S.M. 1992. Suprachiasmatic nucleus: the mind's clock. In Harrington M. (ed.), Oxford University Press, New York.

Koilraj A., Sharma V., Marimuthu G. & Chandrashekaran M. 2000. Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae, Spirostreptida). *Chronobiol. Int.* 17: 757-765.

Kondo T. & Ishiura M. 1999. The circadian clocks of plants and cyanobacteria. *Trends Plant Sci.* 4: 171-176.

Konopka R. & Benzer S. 1971. Clock mutants of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 68: 2112-2116.

Konopka R., Pittendrigh C. & Orr D. 1989. Reciprocal behavior associated with altered homeostasis and photosensitivity of *Drosophila* clock mutants. *J. Neurogenet.* 6: 1-10.

Kostal V., Zavodska R. & Denlinger D. 2009. Clock genes *period* and *timeless* are rhythmically expressed in brains of newly hatched, photosensitive larvae of the fly, *Sarcophaga crassipalpis*. *J. Insect Physiol.* 55: 408-414.

Lack L.C. & Wright H.R. 2007. Chronobiology of sleep in humans. *Cellular and Molecular Life Sciences* 64: 1205-1215.

Lakin-Thomas P.L., Bell-Pedersen D. & Brody S. 2011. The genetics of circadian rhythms in *Neurospora*. *Gen. Circ. Rhythms* 74: 55-103.

Lakovaara S. 1969. Malt as a culture medium for *Drosophila* species. *Drosoph. Inf. Serv.* 44: 128.

Lankinen P. 1985. Genetic variation of circadian eclosion rhythm, and its relation to photoperiodism in *Drosophila littoralis*. Doctoral dissertation, University of Oulu.

Lankinen P. & Lumme J. 1982. An improved apparatus for recording the eclosion rhythm in *Drosophila*. *Dros. Inf. Serv.* 58: 161-163.

Lees AD. 1973. Photoperiodic time measurement in the aphid *Megoura viciae*. *J. Insect Physiol.* 19: 2279-2316.

Levy O., Dayan T. & Kronfeld-Schor N. 2007. The relationship between the golden spiny mouse circadian system and its diurnal activity: an

experimental field enclosures and laboratory study. *Chronobiol. Int.* 24: 599-613.

Lewis R. & Saunders D. 1987. A damped circadian oscillator model of an insect photoperiodic clock .1. Description of the model based on a feedback-control yystem. *J. Theor. Biol.* 128: 47-59.

Loros J. & Dunlap J. 2001. Genetic and molecular analysis of circadian rhythms in *Neurospora*. *Annu. Rev. Physiol.* 63: 757-794.

Lumme J. 1978. Phenology and photoperiodic diapause in northern populations of *Drosophila*. In Dingle H. (ed.), *Evolution of insect migration and diapause*, Springer Verlag, New York, pp. 145-170.

Majercak J., Sidote D., Hardin P. & Edery I. 1999. How a circadian clock adapts to seasonal decreases in temperature and day length. *Neuron* 24: 219-230.

Muguruma F., Goto S.G., Numata H. & Shiga S. 2010. Effect of photoperiod on clock gene expression and subcellular distribution of PERIOD in the circadian clock neurons of the blow fly *Protophormia terraenovae*. *Cell Tissue Res* 340: 497-507.

Myers E., Yu J. & Sehgal A. 2003. Circadian control of eclosion: interaction between a central and peripheral clock in *Drosophila melanogaster*. *Curr. Biol.* 13: 526-533.

Newby L. & Jackson F. 1991. *Drosophila ebony* mutants have altered circadian activity rhythms but normal eclosion rhythms. *J Neurogenet* 7: 85-101.

Nishizuka M., Azuma A. & Masaki S. 1998. Diapause response to photoperiod and temperature in *Lepisma saccharina linnaeus* (Thysanura: Lepismatidae). *Ent. Science* 1: 7-14.

Nitabach M.N. & Taghert P.H. 2008. Organization of the *Drosophila* circadian control circuit. *Curr. Biol.* 18: R84-R93.

Okamura H., Yamaguchi S. & Yagita K. 2002. Molecular machinery of the circadian clock in mammals. *Cell Tissue Res.* 309: 47-56.

Paietta J. 1982. Photo-oxidation and the evolution of circadian rhythmicity. *J. Theor. Biol.* 97: 77-82.

Peschel N. & Helfrich-Förster C. 2011. Setting the clock by nature: circadian rhythm in the fruitfly *Drosophila melanogaster*. *FEBS Lett.* 585: 1435-1442.

Picot M., Cusumano P., Klarsfeld A., Ueda R. & Rouyer F. 2007. Light activates output from evening neurons and inhibits output from morning neurons in the *Drosophila* circadian clock. *Plos Biology* 5: 2513-2521.

Pittendrigh C.S. 1954. On temperature independence in the clock system controlling emergence time in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 40: 1018-1029.

Pittendrigh C.S. 1960. Circadian rhythms and the circadian organization of living systems. Cold Spring Harb Symp Quant Biol 25: 159-184.

Pittendrigh C.S. 1966. Circadian oscillation in *Drosophila pseudoobscura* pupae - a model for photoperiodic clock. *Zeitschrift Fur Pflanzenphysiologie* 54: 275-307.

Pittendrigh C.S. 1972. Circadian surfaces and diversity of possible roles of circadian organization in photoperiodic induction. *Proc. Natl. Acad. Sci. USA* 69: 2734-2737.

Pittendright C.S. & Daan S. 1976. A functional analysis of pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. *J. Comp. Physiol.* 106: 333–355.

Pittendrigh C.S. & Takamura T. 1987. Temperature dependence and evolutionary adjustment of critical night length in insect photoperiodism. *Proc. Natl. Acad. Sci. USA* 84: 7169-7173.

Pittendrigh C.S. & Minis D. 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Am. Nat.* 98: 261-294.

Potdar S. & Sheeba V. 2012. Large ventral lateral neurons determine the phase of evening activity peak across photoperiods in *Drosophila melanogaster*. *J. Biol. Rhythms* 27: 267-279.

Qiu J. & Hardin P. 1996. Developmental state and the circadian clock interact to influence the timing of eclosion in *Drosophila melanogaster*. *J. Biol. Rhythms* 11: 75-86.

Refinetti R. 2010. The circadian rhythm of body temperature. *Frontiers in Bioscience-Landmark* 15: 564-594.

Reierth E., Van't Hof T. & Stokkan K. 1999. Seasonal and daily variations in plasma melatonin in the high-arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *J. Biol. Rhythms* 14: 314-319.

Renn S., Park J., Rosbash M., Hall J. & Taghert P. 1999. A *pdf* neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila*. *Cell* 99: 791-802.

Reppert S. & Weaver D. 2001. Molecular analysis of mammalian circadian rhythms. *Annu. Rev. Physiol.* 63: 647-676.

Rieger D., Stanewsky R. & Helfrich-Förster C. 2003. *Cryptochrome*, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. *J. Biol. Rhythms* 18: 377-391.

Rieger D., Shafer O., Tomioka K. & Helfrich-Förster C. 2006. Functional analysis of circadian pacemaker neurons in *Drosophila melanogaster*. *J. Neurosci.* 26: 2531-2543.

Rieger D., Peschel N., Dusik V., Glotz S. & Helfrich-Förster C. 2012. The ability to entrain to long photoperiods differs between 3 *Drosophila melanogaster* wild type strains and is modified by twilight simulation. *J. Biol. Rhythms* 27: 37-47.

Rieger D., Fraunholz C., Popp J., Bichler D., Dittmann R. & Helfrich-Förster C. 2007. The fruit fly *Drosophila melanogaster* favors dim light and times its activity peaks to early dawn and late dusk. *J. Biol. Rhythms* 22: 387-399.

Rosato E. & Kyriacou C.P. 2006. Analysis of locomotor activity rhythms in *Drosophila*. *Nature Protocols* 1: 559 - 568.

Rothenfluh A., Abodeely M., Price J. & Young M. 2000. Isolation and analysis of six *timeless* alleles that cause short- or long-period circadian rhythms in *Drosophila*. *Genetics* 156: 665-675.

Saunders D. 1990. The circadian basis of ovarian diapause regulation in *Drosophila melanogaster* - is the *period* gene causally involved in photoperiodic time measurement. *J. Biol. Rhythms* 5: 315-331.

Saunders DS. 2002. Insect clocks. In Steel, CGH., Vafopoulou, X. & Lewis RD. (eds.), 3rd ed, Elsevier Science, Amsterdam, pp. 560.

Saunders D. & Lewis R. 1987a. A damped circadian oscillator model of an insect photoperiodic clock .2. Simulations of the shapes of the photoperiodic response curves. *J. Theor. Biol.* 128: 61-71.

Saunders D. & Lewis R. 1987b. A damped circadian oscillator model of an insect photoperiodic clock .3. Circadian and hourglass responses. *J. Theor. Biol.* 128: 73-85.

Saunders D., Lewis R. & Warman G. 2004. Photoperiodic induction of diapause: opening the black box. *Physiol. Entomol.* 29: 1-15.

Saunders D., Henrich V. & Gilbert L. 1989. Induction of diapause in *Drosophila melanogaster* - Photoperiodic regulation and the impact of arrhythmic clock mutations on time measurement. *Proc. Natl. Acad. Sci. USA* 86: 3748-3752.

Schiesari L., Kyriacou C.P. & Costa R. 2011. The hormonal and circadian basis for insect photoperiodic timing. *FEBS Lett.* 585: 1450-1460.

Schmid B., Helfrich-Förster C. & Yoshii T. 2011. A new ImageJ plug-in "ActogramJ" for chronobiological analyses. *J. Biol. Rhythms* 26: 464-467.

Sehgal A., Ousley A. & HunterEnsor M. 1996. Control of circadian rhythms by a two-component clock. *Mol. Cell. Neurosci.* 7: 165-172.

Sehgal A., Price J., Man B. & Young M. 1994. Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. *Science* 263: 1603-1606.

Sehgal A., Rothenfluhhilfiker A., Hunterensor M., Chen Y., Myers M. & Young M. 1995. Rhythmic expression of *timeless* - a basis for promoting circadian cycles in *period* gene autoregulation. *Science* 270: 808-810.

Shafer O.T. & Taghert P.H. 2009. RNA-Interference knockdown of *Drosophila Pigment-dispersing factor* in neuronal subsets: the anatomical basis of a neuropeptide's circadian functions. *Plos One* 4: e8298.

Shafer O., Levine J., Truman J. & Hall J. 2004. Flies by night: effects of changing day length on *Drosophila*'s circadian clock. *Curr. Biol.* 14: 424-432.

Shang Y., Griffith L.C. & Rosbash M. 2008. Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the *Drosophila* brain. *Proc. Natl. Acad. Sci. USA* 105: 19587-19594.

Sharma V. 2003. Adaptive significance of circadian clocks. *Chronobiol. Int.* 20: 901-919.

Simunovic A. & Jaenike J. 2006. Adaptive variation among *Drosophila* species in their circadian rhythms. *Evol. Ecol. Res.* 8: 803-811.

Spicer G. & Bell C. 2002. Molecular phylogeny of the *Drosophila virilis* species group (Diptera: Drosophilidae) inferred from mitochondrial 12S and 16S ribosomal RNA genes. *Ann. Entomol. Soc. Am.* 95: 156-161.

Stanewsky R. 2003. Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. *J. Neurobiol.* 54: 111-147.

Stanewsky R., Kaneko M., Emery P., Beretta B., Wager-Smith K., Kay S., Rosbash M. & Hall J. 1998. The *cry(b)* mutation identifies *cryptochrome* as a circadian photoreceptor in *Drosophila*. *Cell* 95: 681-692.

Stoleru D., Peng Y., Agosto J. & Rosbash M. 2004. Coupled oscillators control morning and evening locomotor behaviour of *Drosophila*. *Nature* 431: 862-868.

Stoleru D., Nawathean P., Fernandez M.d.l.P., Menet J.S., Ceriani M.F. & Rosbash M. 2007. The *Drosophila* circadian network is a seasonal timer. *Cell* 129: 207-219.

Taghert P.H. & Shafer O.T. 2006. Mechanisms of clock output in the *Drosophila* circadian pacemaker system. *J. Biol. Rhythms* 21: 445-457.

Tamura K., Subramanian S. & Kumar S. 2004. Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. *Mol. Biol. Evol.* 21: 36-44.

Tauber E. & Kyriacou B. 2001. Insect photoperiodism and circadian clocks: models and mechanisms. *J. Biol. Rhythms* 16: 381-390.

Tauber M.J., Tauber C.A. & Masaki S. 1986. Seasonal adaptations of insects. In Taylor F. (ed.) Oxford University press, Oxford, pp. 411.

Thain S., Murtas G., Lynn J., McGrath R. & Millar A. 2002. The circadian clock that controls gene expression in *Arabidopsis* is tissue specific. *Plant Physiol.* 130: 102-110.

Throckmorton L.H. 1982. The *virilis* species group. In Ashburner M., Carson H.L., & Thompson J.N. jr (eds.), *The genetics and biology of Drosophila*, Academic Press, London pp. 227–296.

Tsurumaki J., Ishiguro J., Yamanaka A. & Endo K. 1999. Effects of photoperiod and temperature on seasonal morph development and diapause egg oviposition in a bivoltine race (Daizo) of the silkmoth, *Bombyx mori L. J. Insect Physiol.* 45: 101-106.

Tyukmaeva V.I., Salminen T.S., Kankare M., Knott K.E. & Hoikkala A. 2011. Adaptation to a seasonally varying environment: a strong latitudinal cline in reproductive diapause combined with high gene flow in *Drosophila montana*. *Ecol. and Evol.* 1: 160-168.

van Oort B.E.H., Tyler N.J.C., Gerkema M.P., Folkow L., Blix A. & Stokkan K. 2005. Circadian organization in reindeer. *Nature* 438: 1095-1096.

van Oort B.E.H., Tyler N.J.C., Gerkema M.P., Folkow L. & Stokkan K. 2007. Where clocks are redundant: weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften* 94: 183-194.

Vanin S., Bhutani S., Montelli S., Menegazzi P., Green E.W., Pegoraro M., Sandrelli F., Costa R. & Kyriacou C.P. 2012. Unexpected features of *Drosophila* circadian behavioural rhythms under natural conditions. *Nature* 484: 371-108.

Vaz Nunes M. & Veerman A. 1982. Photoperiodic time measurement in the spider mite *Tetranychus urticae* - a novel concept. *J. Insect Physiol.* 28: 1041-1053.

Vaz Nunes M. & Saunders D. 1999. Photoperiodic time measurement in insects: a review of clock models. *J. Biol. Rhythms* 14: 84-104.

Veerman A. & Vaz Nunes M. 1987. Analysis of the operation of the photoperiodic counter provides evidence for hourglass time measurement in the spider mite *Tetranychus urticae*. *J. Comp. Physiol., A.* 160: 421-430.

Warman G., Newcomb R., Lewis R. & Evans C. 2000. Analysis of the circadian clock gene *period* in the sheep blow fly *Lucilia cuprina*. *Genet. Res.* 75: 257-267.

Wheeler D., Hamblencoyle M., Dushay M. & Hall J. 1993. Behavior in light dark cycles of *Drosophila* mutants that are arrhythmic, blind, or both. *J. Biol. Rhythms* 8: 67-94.

Williams J. & Sehgal A. 2001. Molecular components of the circadian system in *Drosophila*. *Annu. Rev. Physiol.* 63: 729-755.

Wulbeck C., Szabo G., Shafer O., Helfrich-Förster C. & Stanewsky R. 2005. The novel *Drosophila tim*(*blind*) mutation affects behavioral rhythms but not periodic eclosion. *Genetics* 169: 751-766.

Yakir E., Hilman D., Harir Y. & Green R.M. 2007. Regulation of output from the plant circadian clock. *Febs Journal* 274: 335-345.

Yamazaki S., Numano R., Abe M., Hida A., Takahashi R., Ueda M., Block G., Sakaki Y., Menaker M. & Tei H. 2000. Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682-685.

Yerushalmi S. & Green R.M. 2009. Evidence for the adaptive significance of circadian rhythms. *Ecol. Lett.* 12: 970-981.

Yoshii T., Todo T., Wuelbeck C., Stanewsky R. & Helfrich-Förster C. 2008. *Cryptochrome* is present in the compound eyes and a subset of *Drosophila*'s clock neurons. *J. Comp. Neurol.* 508: 952-966.

Yoshii T., Wuelbeck C., Sehadova H., Veleri S., Bichler D., Stanewsky R. & Helfrich-Förster C. 2009. The neuropeptide *Pigment-dispersing factor* adjusts period and phase of *Drosophila*'s clock. *J. Neurosci.* 29: 2597-2610.

Young M. & Kay S. 2001. Time zones: A comparative genetics of circadian clocks. *Nature Reviews Genetics* 2: 702-715.

Zhang L., Chung B.Y., Lear B.C., Kilman V.L., Liu Y., Mahesh G., Meissner R., Hardin P.E. & Allada R. 2010a. DN1(p) circadian neurons coordinate acute light and PDF inputs to produce robust daily behavior in *Drosophila*. *Curr. Biol.* 20: 591-599.

Zhang Y., Liu Y., Bilodeau-Wentworth D., Hardin P.E. & Emery P. 2010b. Light and temperature control the contribution of specific DN1 neurons to *Drosophila* circadian behavior. *Curr. Biol.* 20: 600-605.

TABLE 1 The experimental conditions and treatments used in the studies I, III & IV. Study strains as well as mass-bred population refer to the type of the strains vs. mass-bred population used in the experiments. Entraining photoperiod refers to the photoperiod used for the entrainment of the flies at the beginning of each experiment. Entraining temperature refers to the temperature in each experiment. Lashes used refer to the particular day vs. night temperatures if the temperature has been cycling during the experiment. Constant condition treatment refers to the constant condition where the flies in each experiment have been released after entrainment. LD = light: dark cycle, DD = constant darkness, LL = constant light, HLL = constant high light intensity, LLL = constant low light intensity.

	I	III	IV
Study lines/ mass-bred population	Isofemale lines, Oulanka	Mass-bred population, Oulanka	Isofemale lines, Oulanka
Entraining photoperiod	22:2 LD, 20:4 LD, 16:8 LD	24:0 LD, 22:2 LD, 18:6 LD, 16:8 LD, 14:10 LD	20:4 LD
Entraining temperature	20 °C, 16 °C	19 °C, 19 °C/13 °C, 17 °C/13 °C, 16 °C/12 °C, 14 °C/11 °C	20 °C
Constant condition treatment	DD, LL	–	DD, HLL, LLL

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., Keurusselän kalastosta ja sen rakenteesta. - On the fish fauna of Lake Keurusselkä, 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 capricornuturn 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 inacrophyte 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, ectomycorrhizal 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 "-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, JUKKA, Predation risk and competition in mixed species tit flocks. 29 p. Yhteenveto 2 p. 1991.

25 SUOMEN MUUTTUVA LUONTO. Mikko Raatikaiselle omistettu juhlakirja. 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.

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 KIHLSTRÖM, 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, PIRKKO, 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 theresponses of fish to environmental stress. 39 p. Yhteenveto 2 p. 1995.
44 LAPPALAINEN, PEKKA, The dinuclear Cu_A 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 cycteine 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 oligode-oxyribonucleotides 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, KATJA, 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 hartsihappoperäisten 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. - Metsäteollisuuden 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 mäkitervakkopopulaatioiden elinkykyyn. 33 p. (124 p.). Yhteenveto 3 p. 2000.

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 ultraviolettinäön 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 pohjaeläinyhteisöille - 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. - Kreosootilla 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 vuorovaiku-tukset 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 streptavi-diinin kvaternäärirakenteen ja fysioke-miallisten ominaisuuksien muokkaus sekä avidiinin kaltaisten proteiinien karakteri-sointi. 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 forrest soil. A disturbance oriented study. - Maaperän hajottajaeliöstön monimuotoisuuden merkitys metsäekosysteemin toiminnassa ja häiriönsiedossa. 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. Sellobiohydrolaasi I:n, ohran kysteiiniproteinaasin 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. - Ultraviolettinäön ekologinen merkitys hedelmiä syövien eläinten ja hedelmäkasvien välisissä vuorovaikutussuhteissa. 27 p. (95 p.) Yhteenveto 2 p. 2002.

112 MARTTILA, ARI, Engineering of charge, biotin-binding and oligomerization of avidin: new tools for avidin-biotin technology. - Avidiinin varauksen, biotiininsitomisen sekä oligomerisaation muokkaus: uusia työkaluja avidiini–biotiiniteknologiaan. 68 p. (130 p.) Yhteenveto 2 p. 2002.

113 JOKELA, JARI, Landfill operation and waste management procedures in the reduction of methane and leachate pollutant emissions from municipal solid waste landfills. - Kaatopaikan operoinnin ja jätteen esikäsittelyn vaikutus yhdyskuntajätteen biohajoamiseen ja typpipäästöjen 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. - Pohjaeläinyhteisöjen 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 yhteisörakenne 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 signaloinnin kuntoriippuvuus 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, kestävyyden 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.

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 sivuttaissuuntaisella 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.*) habitaatinvalinta 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 järvissä 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 nieriälle (*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, PIIA, Immunological characterization and engineering of the major latex allergen, hevein (Hev b 6.02). - Luonnonkumiallergian pääallergeenin, heveiinin (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. - Lajienvälisten suhteiden vaikutus puukiipijän pesintämenestykseen metsäympäristössä. 39 p. (104 p.) Yhteenveto 2 p. 2005.

151 TYNKKYNEN, 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.

175 Toivanen, Tero, Short-term effects of forest restoration on beetle diversity. - Metsien ennallistamisen merkitys kovakuoriaislajiston monimuotoisuudelle. 33 p. (112 p.) Yhteenveto 2 p. 2007.

176 Ludwig, Gilbert, Mechanisms of population declines in boreal forest grouse. - Kanalintukantojen laskuun vaikuttavat tekijät. 48 p. (138 p.) Yhteenveto 2 p. 2007.

177 Ketola, Tarmo, Genetics of condition and sexual selection. - Kunnon ja seksuaalivalinnan genetiikka. 29 p. (121 p.) Yhteenveto 2 p. 2007.

178 Seppänen, Janne-Tuomas, Interspecific social information in habitat choice. - Lajienvälinen sosiaalinen informaatio habitaatinvalinnassa. 33 p. (89 p.) Yhteenveto 2 p. 2007.

179 Bandilla, Matthias, Transmission and host and mate location in the fish louse *Argulus coregoni* and its link with bacterial disease in fish. - *Argulus coregoni* -kalatäin siirtyminen kalaisäntään, isännän ja parittelukumppanin paikallistaminen sekä loisinnan yhteys kalan bakteeritautiin. 40 p. (100 p.) Yhteenveto 3 p. Zusammenfassung 4 p. 2007.

180 Meriläinen, Päivi, Exposure assessment of animals to sediments contaminated by pulp and paper mills. - Sellu- ja paperiteollisuuden saastuttamat sedimentit altistavana tekijänä vesieläimille. 79 p. (169 p.) Yhteenveto 2 p. 2007.

181 Routtu, Jarkko, Genetic and phenotypic divergence in *Drosophila virilis* and *D. montana*. - Geneettinen ja fenotyyppinen erilaistuminen *Drosophila virilis* ja *D. montana* lajien mahlakärpäsillä. 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 järviekosysteemin 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 varoitussignaloinnissa. 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.

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 Trebatická, 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. - Arbuskelimykorritsa, resurssien saatavuus ja maanalaiset kasvien ja mikrobien väliset vuorovaikutukset. 38 p. (119 p.) Yhteenveto 2 p. 2009.

201 Aroviita, Jukka, 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 Kuntu, 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 Piiroinen, Saija, Range expansion to novel environments: evolutionary physiology and genetics in *Leptinotarsa decemlineata*. - Lajien levinneisyysalueen laajeneminen: koloradonkuoriaisen evolutiivinen fysiologia ja genetiikka. 51 p. (155 p.) Yhteenveto 3 p. 2010.

210 Niskanen, Einari, On dynamics of parvoviral replication protein NS1. - Parvovirusten replikaationproteiini NS1:n dynamiikka. 81 p. (154 p.) Yhteenveto 3 p. 2010.

211 Pekkala, Satu, Functional characterization of carbomoyl phosphate synthetase I deficiency and identification of the binding site for enzyme activator.- Karbamyylifosfaatti syntetaasi I:n puutteen patologian toiminnallinen karakterisaatio ja entsyymin aktivaattorin sitoutumiskohdan identifikaatio. 89 p. (127 p.) Yhteenveto 2 p. 2010.

212 Halme, Panu, Developing tools for biodiversity surveys - studies with wood-inhabiting fungi.- Työkaluja monimuotoisuustutkimuksiin - tutkimuskohteina puulla elävät sienet. 51 p. (125 p.) Yhteenveto 2 p. 2010.

213 Jalasvuori, Matti, Viruses are ancient parasites that have influenced the evolution of contemporary and archaic forms of life. - Virukset ovat muinaisia loisia, jotka ovat vaikuttaneet nykyisten ja varhaisten elämänmuotojen kehitykseen. 94 p. (192 p.) Yhteenveto 2 p. 2010.

214 Postila, Pekka, Dynamics of the ligand-binding domains of ionotropic glutamate receptors. - Ionotrooppisten glutamaattireseptoreiden ligandin-sitomisdomeenien dynamiikka. 54 p. (130 p.) Yhteenveto 3 p. 2010.

215 Poikonen, Tanja, Frequency-dependent selection and environmental heterogeneity as selective mechanisms in wild populations. - Frekvenssistä riippuva valinta ja ympäristön heterogeenisyys luonnonvalintaa ohjaavina tekijöinä luonnonpopulaatiossa. 44 p. (115 p.) Yhteenveto 4 p. 2010.

216 kekäläinen, Jukka, Maintenance of genetic variation in sexual ornamentation – role of precopulatory and postcopulatory sexual selection. - Seksuaaliornamenttien geneettisen muuntelun säilyminen – parittelua edeltävän ja sen jälkeisen seksuaalivalinnan merkitys. 52 p. (123 p.) Yhteenveto 3 p. 2010.

217 Syrjänen, Jukka, Ecology, fisheries and management of wild brown trout populations in boreal inland waters. - Luontaisten taimenkantojen ekologia, kalastus ja hoito pohjoisilla sisävesillä. 43 p. (108 p.) Yhteenveto 3 p. 2010.