Flight metabolic rate and dispersal in the Glanville fritillary butterfly – from genes to populations

ANNIINA L. K. MATTILA

Metapopulation Research Group
Department of Biosciences
Faculty of Biological and Environmental Sciences
University of Helsinki
Finland

ACADEMIC DISSERTATION

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Auditorium 2 in Building B (Latokartanonkaari 7), on May 9th 2014 at 12 o'clock noon.

HELSINKI 2014

SUPERVISED BY: Prof. Ilkka Hanski
Department of Biosciences, University of Helsinki, Finland

REVIEWED BY: Prof. Juha Merilä
Department of Biosciences, University of Helsinki, Finland

Prof. Craig Primmer
Department of Biology, University of Turku, Finland

EXAMINED BY: Dr. Jon Bridle
School of Biological Sciences, University of Bristol, UK

CUSTOS: Prof. Liselotte Sundström
Department of Biosciences, University of Helsinki, Finland

MEMBERS OF THE THESIS ADVISORY COMMITTEE:

Prof. Kristian Donner
Department of Biosciences, University of Helsinki, Finland

Prof. Liselotte Sundström
Department of Biosciences, University of Helsinki, Finland

ISBN 978-952-10-9868-0 (paperback)
ISBN 978-952-10-9869-7 (PDF)
http://ethesis.helsinki.fi

Unigrafia
Helsinki 2014

The Glanville fritillary butterfly is named after

Lady Eleanor Glanville (c. 1654–1709),
one of the earliest female entomologists in 17th-century England.

People said about Lady Glanville:
"None but those who are deprived of their senses, would go in pursuit of butterflies."

To this she responded:
"They say I'm mad and perhaps it's true. But upon my oath, I never meant any harm. All I wanted was to be happy, and for my life to count for something. That is not madness, is it?"

CONTENTS

ABSTRACT 1

TIIVISTELMÄ 2

SUMMARY 4

1. Introduction 4

1.1. Dispersal and its key role for the persistence of natural populations in the face of habitat fragmentation and climate change 4

1.2. Evolution of movement and dispersal 7

1.3. The Glanville fritillary butterfly – a model for studying dispersal in fragmented populations 11

2. Aims of the thesis 13

3. Materials and methods 14

3.1. The study species and populations 14

3.2. Measurement and analysis of flight metabolic rate 15

3.3. Other measures of flight performance and dispersal capacity 16

3.4. Recording butterfly body temperature using infrared thermal imaging 17

3.5. Estimating heritability and phenotypic variance components using the animal model 18

3.6. Molecular methods 19

4. Results and discussion 20

4.1. Genetic basis and heritability of flight metabolic rate and dispersal capacity (I, II, III, V) 20

4.2. Cellular and physiological processes in flight (II, III) 23

4.3. Flight and temperature – linking individual variation in thermal flight performance with genetics and physiology (**I, III**) 26

4.4. Consequences of a complete lack of gene flow to a small population (**IV, II**) 28

4.5. Flight capacity and dispersal in fragmented and isolated populations (**II, IV, V**) 30

5. Conclusions and prospects 32

6. Acknowledgements 33

7. References 37

I Heritability of flight and resting metabolic rates in the Glanville fritillary butterfly 46

II Flight-induced changes in gene expression in the Glanville fritillary butterfly 62

III Changes in body temperature during flight in the Glanville fritillary butterfly: effects of genotype, flight metabolism and environmental conditions 84

IV High genetic load in an old isolated butterfly population 102

V Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly 116

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I **Mattila,** A. L. K. & Hanski, I. Heritability of flight and resting metabolic rates in the Glanville fritillary butterfly. – *Submitted manuscript*.

II Kvist, J.*, **Mattila, A. L. K.***, Somervuo, P., Ahola, V., Koskinen, P., Paulin, L., Salmela, L., Taipale, M., Holm, L., Auvinen, P., Frilander, M. J., Lehtonen, R. & Hanski, I. (*Authors share equal contribution). Flight-induced changes in gene expression in the Glanville fritillary butterfly. – *Submitted Manuscript*.

III **Mattila, A. L. K.** Changes in body temperature during flight in the Glanville fritillary butterfly: effects of genotype, flight metabolism and environmental conditions. – *Manuscript*.

IV **Mattila, A. L. K.,** Duplouy, A., Kirjokangas, M., Lehtonen, R., Rastas, P. & Hanski, I. (2012). High genetic load in an old isolated butterfly population. – *Proceedings of the National Academy of Sciences USA* 109(37): E2496-E2505.

V Niitepõld, K.*, **Mattila, A. L. K.** *, Harrison, P. J. & Hanski, I. (*Authors share equal contribution). (2011). Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. – *Oecologia* 165(4): 847–854

Contributions

	I	II	III	IV	V
Original idea	IH,AM	IH,RL	AM,IH	IH	KN,IH,PH
Study design	AM,IH	IH,AM,JK, RL,MF	AM	AM, AD, IH	KN, AM, PH,IH
Data collection	AM	AM	AM	AM,AD, MK,RL	KN,AM,PH
Contributing analytical tools or genomic data		VA,PK,LP, LS,MT,LH,PA		PR	
Analyses	AM,IH	PS,JK,AM, VA,IH	AM	AM,AD,MK, RL,PR	KN,AM,PH, IH
Manuscript preparation	AM,IH	AM,JK,PS, IH	AM	AM,AD,MK, RL,PR,IH	KN,AM,IH, PH

AM: Anniina L.K. Mattila
IH: Ilkka Hanski
RL: Rainer Lehtonen
JK: Jouni Kvist
MF: Mikko J. Frilander
VA: Virpi Ahola
PK: Patrik Koskinen
LP: Lars Paulin
LS: Leena Salmela
MT: Minna Taipale
LH: Liisa Holm
PA: Petri Auvinen
PS: Panu Somervuo
AD: Anne Duplouy
MK: Malla Kirjokangas
PR: Pasi Rastas
KN: Kristjan Niitepõld
PH: Philip J. Harrison

Sami Ojanen (Layout)
Juha Mattila (Cover illustration)
Mette Mattila, 5 y (Illustrations in-between chapters)

ABSTRACT

Loss and fragmentation of natural habitats and changing climate pose severe threats to biodiversity. The ability of populations and species to respond to these challenges by dispersing across landscapes is imperative for their long-term survival. Dispersal is also the main mechanism leading to gene flow, and dispersal is therefore essential for maintaining genetic diversity and adaptive potential of populations.

In this thesis, I build upon the vast knowledge gained during more than two decades of research on the Glanville fritillary butterfly (*Melitaea cinxia*), aiming towards a better understanding of the mechanisms and processes that shape dispersal in this model species. Previous studies have demonstrated a strong positive correlation between flight metabolic rate (FMR) and dispersal distances in the field. Here, I use FMR as a measure of flight and dispersal capacity. I study dispersal from multiple perspectives and use a variety of methods to address questions ranging from the genetic basis and heritability of flight capacity to interactions between genes, physiology and environment in affecting flight and dispersal. Variation in dispersal capacity and how it influences population and metapopulation-level processes are examined. Finally, I use a "natural experiment" to study the genetic and fitness consequences of complete lack of gene flow into a small isolated island population of the Glanville fritillary.

Key findings of the thesis include the demonstration of significant heritable genetic variation in FMR, indicating that FMR and therefore dispersal capacity has the potential to respond to selection due to e.g. habitat fragmentation and climate change. In a genome-wide gene expression study, 755 genes were significantly up- or down-regulated in response to an experimental flight treatment. Differences between sexes and two contrasting populations in flight-induced gene expression in major metabolic pathways were associated with differences in FMR, suggesting that similar molecular mechanisms influence both gender and population differences in flight performance. An experiment examining changes in butterfly body temperature during flight showed that FMR and tolerance of high temperatures may significantly influence flight performance in different thermal environments.

At the metapopulation level, male and female butterflies differed in the effects of flight capacity on realized dispersal rate between local populations, with likely consequences for the assortment of dispersive genotypes across fragmented landscapes. The small and completely isolated island population of the Glanville fritillary exhibited significant loss of genetic diversity and substantially reduced fitness. Complete and instant fitness recovery in hybrids strongly suggests that reduced population viability is due to high genetic load. This small isolated population serves as an example of the innumerable remnant populations in human-fragmented landscapes, in which extinction risk may increase due to lack of gene flow.

This work contributes to the mechanistic understanding of dispersal (and its importance) in fragmented and isolated populations and in changing environmental conditions in the Glanville fritillary butterfly. Many findings of this thesis are also likely to be applicable to other similar species, particularly those living in fragmented landscapes.

TIIVISTELMÄ

Luonnon elinympäristöjen häviäminen ja pirstoutuminen sekä muuttuva ilmasto ovat yhä enenevissä määrin uhka maapallon luonnon monimuotoisudelle. Eliökantojen ja lajien kyky ja mahdollisuus liikkua elinalueelta toiselle on välttämätön edellytys niiden pitkäaikaiselle selviytymiselle. Yksilöiden liikkuminen eliökantojen välillä mahdollistaa geenivirran, mikä on välttämätöntä populaatioiden geneettisen monimuotoisuuden ja niiden sopeutumiskyvyn säilymiselle.

Tässä väitöskirjassa tarkastellaan täpläverkkoperhosen (*Melitaea cinxia*) liikkumista ja lentokykyyn vaikuttavia tekijöitä. Tutkimus perustuu siihen laajaan tietämykseen, joka on kertynyt tämän ekologisen tutkimuksen ”mallilajiksi” muotoutuneen perhoslajin biologiasta yli kahden vuosikymmenen aikana. Aiemmat tutkimukset ovat osoittaneet, että perhosen lentoaineenvaihdunnan nopeus on vahvasti yhteydessä perhosten maastossa liikkumiin matkoihin. Käytän tässä tutkimuksessa lentoaineenvaihdunnan nopeutta perhosten lento- ja liikkumiskyvyn mittana. Perhosten liikkumista ja lentokykyä tutkitaan useasta eri näkökulmasta monilla eri menetelmillä. Tutkimusten tarkoituksena on selvittää liikkumisen perinnöllistä taustaa, liikkumiseen vaikuttavia geenejä sekä fysiologisia ja ympäristöstä johtuvia tekijöitä. Liikkumisen syy- ja seuraussuhteita tutkitaan myös täpläverkkoperhosen luonnonpopulaatioissa, sekä pirstoutuneessa elinympäristöverkostossa että täysin eristyneessä pienessä saaripopulaatiossa.

Tulokset osoittivat, että lentoaineenvaihdunnan nopeus periytyy sukupolvelta toiselle. Luonnon valinta voi siten muokata tätä ominaisuutta sellaiseen suuntaan, joka lisää yksilöiden kelpoisuutta muuttuneissa ympäristöolosuhteissa esimerkiksi elinympäristön pirstoutumisen tai ilmastonmuutoksen seurauksena. Hyönteisten lento kuluttaa erityisen paljon energiaa, ja tulokset osoittivat, että 755 geenin ilmentyminen muuttui merkitsevästi 15 minuutin aktiivisen lennon seurauksena. Solujen tärkeisiin aineenvaihdunta- ja stressivastetoimintoihin liittyvien geenien aktiivisuus lennon jälkeen oli yhteydessä lentoaineenvaihdunnan nopeuteen, mikä viittaa näiden toimintojen merkitykseen lentosuorituksen mahdollistavina ja sitä mahdollisesti rajoittavina tekijöinä. Perhosten lentoaineenvaihdunnan nopeuden ja korkeiden lämpötilojen kestokyvyn osoitettiin vaikuttavan lentokykyyn vaihtelevissa ympäristöoloissa. Lentokykyyn vaikuttavien tekijöiden ja lennon mekanismien parempi ymmärtäminen voi auttaa ennustamaan muuttuvien ympäristöolosuhteiden vaikutuksia perhosten ja muiden samankaltaisten lajien liikkumiskykyyn ja siten niiden populaatioiden menestymiseen.

Yksilöiden väliset erot lentokyvyssä vaikuttivat päinvastaisella tavalla koiraiden ja naaraiden liikkeisiin pirstoutuneessa elinympäristössä: naaraat joilla oli hyvä lentokyky liikkuivat enemmän populaatioiden välillä, kun taas lentokyvyltään parhaat koiraat jäivät todennäköisimmin syntymäkedolleen. Sukupuolten väliset erot johtunevat hyvän lentokyvyn erilaisista vaikutuksista koiraiden ja naaraiden kelpoisuuteen, mutta näillä eroilla on myös vaikutusta lentokyvyltään erilaisten yksilöiden jakautumiseen populaatioverkoston alueella. Pitkään täysin eristyksissä olleessa perhospopulaatiossa perimä oli köyhtynyt ja perhosten kelpoisuus merkittävästi alentunut, mikä korostaa populaatioiden välisten liikkeiden ja geenivirran merkitystä populaatioiden elinvoimaisuudelle. Tutkittu saaripopulaatio toimii esimerkkinä sellaisesta kasvaneesta sukupuuttoriskistä, joka uhkaa mitä luultavimmin lukemattomia maankäytössä tapahtuneiden muutosten seurauksena täysin eristyksiin jääneitä pieniä populaatioita.

SUMMARY

Anniina L. K. Mattila

Metapopulation Research Group, Department of Biosciences, PO Box 65 (Viikinkaari 1), 00014 University of Helsinki, Finland

1. INTRODUCTION

1.1. DISPERSAL AND ITS KEY ROLE FOR THE PERSISTENCE OF NATURAL POPULATIONS IN THE FACE OF HABITAT FRAGMENTATION AND CLIMATE CHANGE

The rapid loss and fragmentation of natural habitats due to human actions is the main cause of biodiversity loss and species extinctions (Baillie *et al.* 2004; IUCN 2014). At the same time, natural populations are threatened by changing climate (Parmesan 2006), with global mean temperatures expected to increase by as much as 6°C by the end of the 21st century and extreme weather events becoming more common (IPCC 2007). The ability of species and populations to respond to these rapid and major environmental changes by dispersing across landscapes is imperative for their survival (Hanski 1999; Ronce 2007; Clobert *et al.* 2012). Therefore, developing a better understanding of the causes, consequences and mechanisms of dispersal has become a key issue in population and conservation biology (Clobert *et al.* 2012).

What is dispersal?

Dispersal plays a major role in individual life histories, population dynamics, and in affecting species' ranges (Clobert *et al.* 2012). From the viewpoint of an individual, the multiple functions of dispersal include escaping unfavorable conditions, avoiding inbreeding and kin competition and distributing offspring over different geographical locations and conditions (Matthysen 2012). Importantly, at the population level, dispersal is the main mechanism leading to gene flow among populations. The term dispersal is often used differently in different contexts. Generally it can be distinguished from movements within a habitat by the outcome of displacing the individual away from an existing (natal) population (Clobert *et al.* 2012). Consequently, the dispersing individual will potentially reproduce at a different location than its mother. In short, dispersal means permanently leaving a local environment and settling in another environment, following movement across a more or less unfavourable habitat, often called the matrix. Dispersal can be active or passive, and it is a common phenomenon across all domains of life. Dispersal is often confused with migration, which is defined as (often two-way) seasonal or periodic movements of animals (Webster *et al.* 2002). Familiar examples of migration are the seasonal migrations of many bird species and the striking long-distance migrations of the Monarch butterfly. Patterns of migration are generally influenced by different processes than those of dispersal, and will not be discussed here.

Dispersal is a complex behaviour, influenced by genetic, morphological, physiological and behavioural factors, in concert with the environmental context (Clobert *et al.* 2012). Each of the different stages of dispersal (departure, transit and settlement) can be influenced by different processes and entail different costs (Bonte *et al.* 2012). In many cases, the ability or capacity of an individual to disperse can be expected to relate to its overall movement capacity (implying e.g. morphological or physiological properties affecting the ability to move) or propensity to move (implying the tendency or likelihood of movement, relating to e.g. other behavioural traits). Recently, a conceptual framework to integrate movement and dispersal has been developed (Nathan *et al.* 2008).

According to this framework, to fully understand the causes, mechanisms and spatiotemporal patterns of organismal movement and dispersal, as well as their role in ecological and evolutionary processes, dissecting dispersal into its components and investigating movement at different spatial scales is a useful approach (Nathan *et al.* 2008). To achieve this, it is important to gain knowledge on different mechanistic components of movement, including the internal state (motivation to move), movement capacity, navigational ability (when and where to move) as well as the external factors affecting movement.

The importance of dispersal in fragmented populations and the consequences of population isolation

The distributions of species and populations are rarely continuous, but more often they are to some extent spatially fragmented. Due to the rapid loss and degradation of natural habitats, populations are becoming increasingly fragmented, and severely fragmented populations are becoming more common. Although individual habitat fragments may be too small to support viable local populations, a network of such populations may persist in a dynamic balance between local extinctions and recolonizations, i.e. as a metapopulation (Levins 1969; Hanski 1999). The metapopulation theory has been widely applied to species living in fragmented habitats (Hanski 1999). As dispersal is the factor connecting the local populations in a metapopulation, sufficient dispersal is necessary for population viability in highly fragmented habitats (Hanski 1999; Ronce 2007). This makes dispersal an especially critical life-history trait for species inhabiting such habitats (Hanski 1999; Clobert *et al.* 2012).

The importance of dispersal is highlighted further in the extreme, but ever more frequent scenario of a small population left stranded on an isolated remnant habitat fragment with no opportunity for dispersal, and therefore no gene flow, between conspecific populations. In principle, population isolation can facilitate adaptation to local conditions because of the lack of disruptive gene flow (Slatkin 1985; Edmands 2007; Lopez *et al.* 2009). However, in the case of very small populations, it is more likely that such a population will be faced with challenges rather than rapid local adaptation. Demographic and environmental stochasticities increase the extinction risk of small and isolated populations, and the viability of such populations is further compromised by genetic processes. Prior to the 1970s, genetic factors were rarely mentioned as causes of extinction, but in the last couple of decades the role of genetic factors in extinction has been much discussed, and numerous theoretical and experimental studies have examined genetic threats to population persistence (Frankham 2005; Allendorf *et al.* 2013). Empirical studies of small and isolated natural populations give valuable information about whether theoretical expectations hold in nature, and which forces dominate in determining population viability and extinction probability of threatened populations.

Today, the generally accepted consensus is that although the demographic and environmental factors often dominate, the role of genetic deterioration in increasing the risk of extinction can be substantial (Spielman *et al.* 2004; Frankham 2005; Allendorf *et al.* 2013). Genetic factors are especially important in the case of extremely or completely isolated populations, because even a minimal amount of gene flow can greatly reduce the loss of genetic variation and hence make a difference for the long-term viability of populations. The introduction of only one or a few individuals into a small isolated population can be enough to result in "genetic rescue" at the population level (Spielman and Frankham 1992; Lopez *et al.* 2009). Such events have occurred and have been demonstrated in wild populations, e.g. in the greater prairie chicken (Westemeier *et al.* 1998), adders (Madsen *et al.* 1999; Madsen *et al.* 2004), gray wolf (Vila *et al.* 2003), deer mice (Schwartz and Mills 2005), Mexican wolves and Florida panthers (Hedrick and Fredrickson 2010), and plants (*Silene alba*; (Richards 2000).

There are different types of genetic threats to small and isolated populations: immediate inbreeding depression, the loss of adaptive genetic variation, and the fixation of deleterious mutations by genetic drift, causing mutation accumulation, high genetic load and even mutational meltdown (Lande 1994; Gaggiotti 2003; Frankham 2005). Inbreeding depression is most commonly caused by a load of

recessive deleterious alleles that segregate within a population and lead to fitness reduction when brought into homozygous state by inbreeding (mating among relatives) (Lynch and Walsh 1998a; Charlesworth and Charlesworth 1999; Keller and Waller 2002). Inbreeding typically causes reduced reproductive performance and survival in naturally outbreeding species (Keller and Waller 2002), and it can even cause the extinction of wild populations (Saccheri *et al.* 1998; Crnokrak and Roff 1999; Bijlsma *et al.* 2000; O'Grady *et al.* 2006). On the other hand, inbreeding exposes recessive highly deleterious and lethal mutations to selection, which may lead to them being purged from the population, thereby increasing population fitness (Byers and Waller 1999; Keller and Waller 2002). The purging process may at first appear a promising prospect for threatened populations. However, the efficiency and extent of purging depends strongly on the effective size of the population, becoming increasingly inefficient with decreasing population size (Keller and Waller 2002). Additionally, many genetic factors (e.g. the selective and dominance effects of the genetic variants) are likely to limit the efficiency of purging in natural populations (Wang *et al.* 1999; DeRose and Roff 1999; Wang 2000; Boakes *et al.* 2007). Because inbreeding depression is expressed already in the first inbred generation, it poses the most immediate genetic risk to small populations (Keller and Waller 2002).

In large populations, deleterious alleles are kept at low frequencies by selection. In small populations, on the other hand, chance effects (genetic drift) increasingly overwhelm selection. This is because the relative strengths of selection and random genetic drift are functions of population size. If there is no gene flow to the population to counteract the effects of genetic drift, some deleterious alleles of small effect will be lost while others become fixed, increasing fixation load and reducing fitness at the population level (Poon and Otto 2000; Whitlock *et al.* 2000; Frankham 2005). If mutational load in the ancestral population was high, small isolated populations can experience considerable fixation load even in the short term. Furthermore, if population size remains small and gene flow from outside is limited or nonexistent, new mutations of small effect may accumulate. Population fitness is cumulatively reduced by the resulting fixation load, which will ultimately reduce population size and further accelerate genetic drift. This negative feedback process, termed mutational meltdown, occurs at an accelerating rate and reduces population fitness and genetic variability in an irreversible way. Fitness decline due to mutation accumulation in small populations has been repeatedly demonstrated with models and observed in laboratory populations of model species, e.g. *E. coli* (Kibota and Lynch 1996), *D. melanogaster* (Fry *et al.* 1999), *C. elegans* (Vassilieva and Lynch 1999) and *A. thaliana* (Shaw *et al.* 2000). The ultimate consequence of accumulation of genetic load may be population extinction (Lande 1994; Lynch *et al.* 1995a; Lynch *et al.* 1995b) or even extinction of an entire metapopulation (Higgins and Lynch 2001). The likelihood of extinction due to mutation accumulation and mutational meltdown in natural populations is however debated (Charlesworth *et al.* 1993; Gilligan *et al.* 1997; Frankham 2005). This is because extinction due to mutation accumulation seems to require a high mutation rate producing mutations of small effect, very small population size and many generations (Zeyl *et al.* 2001; Garcia-Dorado 2003; Glemin *et al.* 2003). As genetic drift is a random process, independent populations are likely to differ in terms of which particular alleles have drifted to fixation. Therefore, crossing individuals from such independent populations is expected to mask the deleterious effects of recessive alleles and to increase fitness of the hybrid offspring, which is called heterosis (also referred to as hybrid vigor) (Whitlock *et al.* 2000). This is also the mechanism underpinning genetic rescue following gene flow to an isolated population.

Genetic drift, in addition to leading to the accumulation of slightly deleterious mutations, is at the same time the main mechanism reducing genetic variability in small and isolated populations (Frankham 2005; Fauvergue *et al.* 2012). Genetic variation can be lost rapidly, especially in completely isolated small populations. Loss of genetic variation can affect populations adversely in two ways: by decreasing their fitness and by decreasing their adaptive potential due to shortage of material for natural selection (Amos and Balmford 2001). Sufficient genetic diversity is imperative for the capacity of species to evolve in response to changing conditions, e.g. changes in climatic conditions and

habitat (Le Galliard *et al.* 2012). This is especially true for species that show specialization to particular habitats (Buckley *et al.* 2012). For this reason, IUCN has designated genetic variation as one of the three levels of biodiversity requiring conservation (IUCN 1980). Genetic diversity in small populations is best preserved by sufficient dispersal among other conspecific populations.

Climate change and dispersal

In addition to the key role of dispersal for the persistence of small or fragmented populations, dispersal allows species to shift their ranges in changing environments, e.g. due to climate change (Berg *et al.* 2010). Under changing climatic conditions, the persistence of a species is mediated by the interplay between dispersal and local adaptive responses. In practice, a species may respond to warming climate by shifting its range through dispersal, adapting to changed conditions or by going extinct (Travis and Dytham 2012; Le Galliard *et al.* 2012). The distributions of many species have indeed been shown to be shifting towards the poles and higher altitudes (e.g. Parmesan *et al.* 1999; Wilson *et al.* 2005; Parmesan 2006), although generally the range adjustments lag behind climate change (Menendez *et al.* 2006). The spatial configuration of the available habitat and the ecological interactions between species may significantly affect the ability of species to track the suitable climate zone. In butterflies and many other insects, the evolution of herbivore-host relationships are expected to significantly influence possible range shifts, but contrary to previous understanding, species may not necessarily evolve towards being more generalist, but range shifts may also occur as dispersive specialists selectively spread into new regions (Bridle *et al.* 2014). In any case, dispersal ability is expected to have a major role in influencing the ability to respond to changing climate (Van Dyck 2012).

Dispersal rate can be subject to strong selection at shifting range margins, usually favoring increased dispersal rate, which can accelerate range shifting (Thomas *et al.* 2001; Travis and Dytham 2002; Brooker *et al.* 2007; Le Galliard *et al.* 2012; Travis and Dytham 2012). Evolutionary changes in dispersal traits at shifting range margins have been observed in many species (e.g. Hill *et al.* 1999; Simmons and Thomas 2004; Mitikka and Hanski 2010). Populations at range margins highlight a paradox in relation to gene flow and dispersal: gene flow may swamp local adaptation in adjacent populations, whereas a lack of it may prevent local adaptation because of reduced genetic variability and by preventing the spread and establishment of beneficial alleles (Bridle and Vines 2007).

Climatic conditions may also directly affect dispersal capacity, especially in ectothermic organisms in which body temperature is mostly governed by ambient temperature rather than metabolism. For instance, flight activity in butterflies and many other insects is strongly affected by thermoregulation (Box 1; Watt 1968; Heinrich 1993; Wickman 2009). Such temperature-dependent movement behavior may have significant consequences for climate-induced range shifts (Battisti *et al.* 2006). The influence of temperature on movements and dispersal may also vary within species and populations (Ovaskainen *et al.* 2008; Niitepõld *et al.* 2009; Niitepõld 2010), and climatic conditions may thus affect which dispersal phenotypes within populations are favoured.

1.2. EVOLUTION OF MOVEMENT AND DISPERSAL

Due to the pivotal role of dispersal in many population processes and in the resilience of species and populations against challenges posed by e.g. habitat fragmentation and climate change, it is of interest to ask how likely dispersal is to evolve to match new conditions? For dispersal to evolve, the prerequisites for evolution by natural selection have to be fulfilled. In addition to selective forces acting on dispersal, there has to be sufficient variation among individuals in dispersal-related traits. Moreover, to pass the favoured traits to the next generations, this variation has to be heritable. Here, I discuss within-species and within-population variation in dispersal capacity, and what is known about the genetic basis and heritability of dispersal-related traits. I also discuss the evolution of dispersal, paying special attention to fragmented and isolated populations.

Individual variation in dispersal capacity

Individual variation in dispersal can mostly be attributed to variation in the morphological, physiological and behavioural traits that affect individual movement capacity or propensity. Examples include body size, energy reserves, morphology of locomotory appendages, sensory and cognitive abilities, and metabolic rate (Matthysen 2012; Wheat 2012). Within-species and within-population variation in movement and dispersal capacity has been quite widely studied. Flight performance in butterflies and other insects is one example (Box 1). In some organisms, there are distinctive dispersive morphs, of which probably the most well-known is the case of wing-polymorphism in many insect species (Roff and Fairbairn 1991). Dispersing individuals may also exhibit specialised behaviours (Van Dyck and Baguette 2005), such as aerial "ballooning" in spiders (Bonte *et al.* 2009), or be of certain "personality", such as particularly exploratory birds (Dingemanse 2003).

Because dispersal behaviour is energetically very costly, trade-offs associated with dispersal are expected to be common (Bonte *et al.* 2012). Wing-dimorphism highlights a typical dispersal-associated trade-off caused by allocation of limited resources to either locomotory structures or reproductive investment (Roff and Fairbairn 2007b; Nespolo *et al.* 2008). However, trade-offs are not always apparent, as suggested by the existence of innate behavioural dispersal syndromes, in which dispersal behaviour shows strong phenotypic correlation with other life-history traits (Ronce and Clobert 2012). A characteristic of such dispersal syndromes is that they are often context-specific, i.e. highly influenced by the environmental conditions. An example of a dispersal syndrome is the "colonizer syndrome", where the more dispersive individuals can also be the most fecund (Bonte and Saastamoinen 2012).

The genetic basis and heritability of dispersal capacity

To a large extent, traits affecting dispersal capacity are genetically determined (Ronce 2007; Matthysen 2012; Zera and Brisson 2012). The genetic basis of variation in dispersal provides important information about the physiological mechanisms involved, shedding light on the nature of dispersal adaptations. Indeed, physiological aspects of movement and dispersal have been a major focus of recent in-depth studies on dispersal genetics (Zera and Brisson 2012; Wheat 2012). The genetic basis of migratory behaviour has also received considerable attention (Liedvogel *et al.* 2011). The rapid advance of molecular tools, especially the increasing applicability of next-generation genomics techniques to non-model species and natural populations (Ellegren and Sheldon 2008), has had a great impact on dispersal studies. Knowledge is accumulating on the associations of dispersal phenotypes with genotypes at candidate gene loci and with patterns of gene expression.

Some examples of transcriptomic studies on dispersal-related traits include studies investigating gene expression differences in winged and unwinged pea aphids (*Acyrthosiphon pisum*; (Brisson *et al.* 2007), in horses before and after exercise (Park *et al.* 2012) and in butterfly populations with dissimilar average dispersal capacity (Wheat *et al.* 2011, Section 1.3). Candidate genes with major effects on dispersal have been discovered in several study systems, confirming that single gene effects can contribute significantly to population-level heterogeneity in dispersal. One such gene is the *foraging* (*for*) gene in *Drosophila* fruit flies, which explains a large part of the bimodality in the distribution of the larval propensity to move in the presence of food (rovers vs. sitters; Sokolowski 2001; Edelsparre *et al.* 2014). The expression level of the *for* gene has also been shown to associate with exploratory and colonizing behaviour in pea aphids (Tares *et al.* 2013). In birds (*Parus major*), there is evidence for a connection between sequence variation in a dopamine-receptor gene (*Drd4*) and exploratory/dispersive behaviour (Fidler *et al.* 2007). Allelic variation in the genes encoding the glycolytic enzyme phosphoglucose isomerase (*Pgi*; Haag *et al.* 2005; Niitepõld *et al.* 2009) and another metabolic enzyme related to the cellular response to hypoxia, succinate dehydrogenase *(Sdhd;* (Marden *et al.* 2013), are associated with flight metabolic rate in the Glanville fritillary butterfly (*Melitaea cinxia*), and variation in these genes explains a large proportion of variation in dispersal rate in the field (Niitepõld *et al.* 2009, Box 1, Section 1.3).

BOX 1. BUTTERFLY FLIGHT – CAUSES OF VARIATION AND PROXIES FOR DISPERSAL CAPACITY

Butterflies are dependent on flight for most activities during their adult life, including foraging, escaping predation, mate location, searching for host plants and dispersal (Kingsolver 1983; Saastamoinen and Hanski 2008; Niitepõld *et al.* 2009; Gibbs 2010). However, insect flight is one of the most demanding and costly activities in the animal kingdom (Dudley 2000; Suarez 2000). Here, I summarize the major causes of the considerable inter- and intra-specific variation found in butterfly flight and dispersal capacity.

Genetics and physiology

Thoracic muscles of flying insects exhibit the highest rates of metabolism known for any locomotor tissue (Dudley 2000; Suarez 2000), being even several hundred times higher that metabolism at rest (Kammer and Heinrich 1978). Insect flight muscles are primarily aerobic (Dudley 2000) and rely heavily on tricarboxylic acid cycle (TCA) for energy production. In the Glanville fritillary butterfly, ***flight metabolic rate*** has been shown to explain a large proportion of the variation in dispersal rate in the field (Niitepõld *et al.* 2009, Section 1.3). Genotype in the ***phosphoglucose isomerase (Pgi) locus***, with an important role in energy metabolism (glycolysis), is associated with flight metabolism and flight performance in several butterfly species (Watt *et al.* 1983; Hughes and Zalucki 1993; Watt *et al.* 2003; Niitepõld *et al.* 2009; Mitikka and Hanski 2010). Allelic variation or gene expression differences at metabolic genes may influence the ***availability and use of fuels*** during flight. For instance, changes in metabolism increase fat stores used for flight during the long-distance migration of Monarch butterflies (Zhan 2011). The high rate of flight metabolism may also be limited by ***oxygen conductance to flight muscles***, highlighting the potential importance of the cellular ***response to hypoxia*** for variation in flight performance (Harrison 1998; Zhou *et al.* 2008; Marden *et al.* 2013).

Morphology

Many morphological features are considered to be associated with butterfly flight and dispersal capacity. ***Body size*** and ***flight muscle investment*** (relative thorax mass) are expected to be positively correlated with flight performance (Berwaerts *et al.* 2002; Almbro and Kullberg 2008; Berwaerts *et al.* 2008), and have been used as proxies of dispersal capacity (e.g. Hill *et al.* 1999). ***Wing loading***, i.e. body mass relative to wing surface area, influences flight performance in many butterflies (e.g. Almbro and Kullberg 2012), but may have differential effects for the speed and sustainability of flight (Berwaerts *et al.* 2002). ***Wing aspect ratio*** is a measure of wing shape, with high aspect ratio typically associated with fast flight (Berwaerts *et al.* 2002). ***Color and melanisation*** may influence flight especially through solar absorptivity and, thus, the dynamics of body temperature (Kingsolver and Watt 1984; Van Dyck and Matthysen 1998; Davis *et al.* 2012).

Thermoregulation

Flight in butterflies requires high muscle temperature (between 30-38°C in many species; Watt 1968; Heinrich 1993; Wickman 2009). The ***body temperature*** of butterflies is mostly governed by ambient temperature and solar radiation rather than metabolism (i.e. they are ectothermic), which makes flight activity sensitive to variation in temperature. In addition to the effects of ***color and melanisation*** (see above), thermoregulation can be influenced by ***body size*** through its consequences for ***convective cooling*** and the ***heat generated by flight metabolism*** (Gilchrist 1990; Kemp and Krockenberger 2004). In small temperate butterflies, body temperature quickly decreases and approaches ambient temperature during flight (Shreeve 1984; Heinrich 1986b), forcing them to land and bask at regular intervals. On the other hand, larger species generate enough heat by flight metabolism to stabilize body temperature even in low ambient temperatures (Heinrich 1986a).

Behavior, motivation and sex
Variation in flight activity can also result from ***behavioural differences***, e.g. due to differential ***motivation to fly***. For instance, the key ***functions of flight*** differ markedly in the two sexes. Females fly to locate a suitable place to lay their eggs (Kingsolver 1983), while for males, the primary function of flight is to locate mates and win matings (Scott 1974). Furthermore, male ***mate location strategy*** (´perchers´ vs. ´patrollers´; Scott 1974) has been linked to male flight performance. Successful territorial males are expected to perform short flight bouts but be able to accelerate fast (Berwaerts et al. 2002; Berwaerts et al. 2008).

The evolutionary response of species to changing selection pressures on dispersal can be a prerequisite for population survival in the face of habitat fragmentation and climate change. To assess whether, and how much, there is adaptive potential in dispersal-related traits, empirical studies are needed to describe the heritability of such traits (Zera and Brisson 2012). In fact, better knowledge about the heritability and genetic background of dispersal and movement behavior has been identified as one of the most pressing questions in ecology (Sutherland *et al.* 2013). Heritability (h^2) describes the degree of resemblance between relatives, and in its narrow definition is an estimate of the relative contribution of additive genetic factors to the total phenotypic variance in a population (Lynch and Walsh 1998b). Additive genetic variance is the variation that is relevant for the response to selection and, therefore, for short-term evolutionary potential. There is some controversy and misconceptions about the value and applications of measures of heritability, e.g. because of its context-dependent nature (Visscher *et al.* 2008; Hansen *et al.* 2011). However, heritabilities of similar traits have often shown to be very similar across populations and even species, and it continues to be a key parameter for predicting the response to selection (Visscher *et al.* 2008). In wild animal populations, reliable estimates of heritability are difficult to obtain with traditional quantitative genetic methods (parent-offspring regression and ANOVA-based analyses of full-sib and half-sib families), because of the general requirement for controlled and balanced breeding designs, and the difficulty of controlling for environmental variance and covariance (Lynch and Walsh 1998b). Additionally, failing to account for covariance among relatives due to a common environment or a shared parent inflates the estimates of heritability. To date, detailed quantitative-genetic studies of dispersal-related traits have been carried out in only a few species (Zera and Brisson 2012). Recently, application of "animal models" (for more details, see Section 3.5), which represent a powerful statistical approach to investigating the quantitative genetics of complex traits, is starting to yield more reliable estimates of heritability for wild animal populations (Kruuk 2004; Wilson *et al.* 2010).

Evolution of dispersal in fragmented and isolated populations

Spatially heterogeneous habitats can have a significant influence on the evolution of dispersal-related traits, potentially leading to complex eco-evolutionary dynamics of dispersal (Hanski and Mononen 2011). The question of how habitat fragmentation may affect the evolution of dispersal has been addressed both with theoretical models and empirical studies (reviewed by Clobert *et al.* 2012). Theoretical studies indicate that the evolutionary response to habitat fragmentation is very difficult to predict, as both increased and reduced dispersal may be selected for. A common assumption is that the increasing cost of dispersal with increasing fragmentation (Bonte *et al.* 2012) selects for reduced dispersal (Travis and Dytham 1999). On the other hand, increased dispersal with increasing fragmentation may evolve because of the benefits of colonizing new, unoccupied habitat (Heino and Hanski 2001; Zheng *et al.* 2009; Hanski and Mononen 2011). Such benefits arise from e.g. avoidance of resource- and sib-competition and inbreeding. Inconsistent model predictions are likely to be explained by the differences in model assumptions about the cost of dispersal, temporal stability of populations and the availability of unoccupied habitat (Hanski and Mononen 2011).

The above-mentioned factors probably explain the dissimilar responses to fragmentation that have been reported by empirical studies. For instance, speckled wood butterflies (*Pararge aegeria*; Merckx *et al.* 2003; Bergerot *et al.* 2012) and bog fritillary butterflies (*Proclossiana eunomia*; Schtickzelle *et al.* 2006) from more fragmented landscapes were found to have reduced dispersal propensity compared to butterflies from more continuous habitats. However, the opposite is the case in populations of the Glanville fritillary butterfly (Section 1.3; Hanski 2004; Duplouy *et al.* 2013). In fragmented populations in nature, the evolution of dispersal can be further complicated by interactions between dispersal and other life-history traits (dispersal syndromes), which are also under selection due to fragmentation (Ronce and Clobert 2012).

In the case of completely isolated populations surrounded by inhospitable habitat (e.g. oceanic island or extremely isolated habitat patches), and thus a very low probability of successful dispersal, selection for reduced dispersal is expected to be strong (Carlquist 1966; Cody and Overton 1996; Ronce 2007). Population isolation can further facilitate the evolution of reduced dispersal because of lack of disruptive gene flow (Slatkin 1985; Edmands 2007; Lopez *et al.* 2009). Many bird and insect species inhabiting islands have, in fact, become flightless (Carlquist 1966; Roff 1990). On the other hand, small and isolated populations are likely to suffer from reduced genetic variation (see Section 1.1), which can severely hinder adaptation (Amos and Balmford 2001; Frankham 2005; Swindell and Bouzat 2005). Insufficient genetic variation and the dominance of random genetic drift could impede the ability of such populations to respond to selection, including selection on dispersal.

1.3. THE GLANVILLE FRITILLARY BUTTERFLY – A MODEL FOR STUDYING DISPERSAL IN FRAGMENTED POPULATIONS

The Glanville fritillary butterfly (*Melitaea cinxia*) is an established ecological model species for the study of the consequences of habitat fragmentation and metapopulation dynamics (Hanski 1999; Ehrlich and Hanski 2004). In the Åland islands in Finland, the species has been closely monitored and studied since 1991 (Ojanen *et al.* 2013). This well-known study system has been used widely in studies of the ecology and evolution of dispersal in fragmented populations (Hanski 2012). With the availability of genomics resources, including the full transcriptome (Vera *et al.* 2008) and very soon the full genome (Ahola *et al.*, unpublished), the Glanville fritillary is also on the way to becoming a model in ecological and evolutionary genomics studies (e.g. Wheat *et al.* 2011; Kvist *et al.* 2013; Saastamoinen *et al.* 2013b).

In the Åland Islands, the habitat of the Glanville fritillary is highly fragmented, and the butterfly persists as a classic metapopulation in a large habitat patch network of dry meadows within an area of 50 x 70 km (Fig. 1; Hanski 1999; Nieminen *et al.* 2004; Ojanen *et al.* 2013). Complete population surveys take place biannually, enabling sampling of individuals for experiments and molecular studies (Ojanen *et al.* 2013). Of the around 4000 habitat patches, about 500-800 patches are occupied in a given year. The local populations are typically very small, consisting mostly of less than ten groups of full-sib larvae (Nieminen *et al.* 2004), and they have a high risk of extinction. Extinction risk is affected by inbreeding and allee effects (increased emigration and reduced population growth rate in low-density habitats) in addition to other reasons related to small population size (Hanski 1998; Kuussaari *et al.* 1998; Saccheri *et al.* 1998; Haikola 2003). The turnover rate of the local populations is very high, with around 100 local extinctions and re-colonizations taking place yearly (Nieminen *et al.* 2004; Hanski 2011; Ojanen *et al.* 2013). The total census size of the Åland metapopulation is around 5000 larval families (some tens of thousands of individuals), with the effective size being of the order of 10^4 (Ojanen *et al.* 2013).

Mark-release-recapture studies show that the Glanville fritillary is a relatively sedentary species, but in the case of small meadows most individuals emigrate at some point of their adult life (Kuussaari *et al.* 1996). The flight of the Glanville fritillary typically consists of short flight bouts, partly explained by the need to bask at regular intervals to gain sufficiently high body temperature for flight (Heinrich 1986b; Wickman 2009). In the experiment of Ovaskainen *et al.* (2008) on freely-flying butterflies followed with a harmonic radar, the average distance travelled during a flight bout was 32 m. Mean lifetime dispersal

distances, as estimated with mark-release-recapture studies, are some hundreds of meters, while the longest dispersal events are 1-2 km (Kuussaari *et al.* 1996; Niitepõld *et al.* 2011) and the longest recorded colonization distances are 4-5 km (van Nouhuys and Hanski 2002). Generally, females have a higher chance of dispersing, while some males remain in the natal population (Kuussaari *et al.* 1996). There is no evidence for morphological traits with significant effects on dispersal capacity in the Glanville fritillary (but see Breuker *et al.* 2007). However, there is evidence for considerable individual variation in dispersal capacity influenced by a range of environmental, phenotypic and genetic factors. The Glanville fritillary exhibits a dispersal syndrome that is associated with a single nucleotide polymorphism (SNP) in the gene *phosphoglucose isomerase* (*Pgi*) (Bonte and Saastamoinen 2012; Hanski 2012). Notably, *Pgi* genotype predicts flight metabolic rate and dispersal rate in the field (Niitepõld *et al.* 2009). Particularly, one *Pgi* genotype (AC heterozygote in the SNP *Pgi_111*) can have up to about 40% higher flight metabolic rate than the alternative common genotype AA (Niitepõld 2010). Furthermore, flight metabolic rate is positively correlated with dispersal rate in the field, explaining up to 30% of the variation in the distance moved in 1 h, making flight metabolic rate a good proxy for dispersal capacity in this species (Niitepõld *et al.* 2009). *Pgi* genotypes have been shown to differ in multiple other life-history and fitness traits, often so that dispersal capacity is positively linked with reproductive success and lifespan (Saastamoinen 2007a; Saastamoinen 2007b; Klemme and Hanski 2009; Bonte and Saastamoinen 2012; Niitepõld and Hanski 2013).

However, there are important trade-offs related to the *Pgi* polymorphism. For instance, the "inferior" *Pgi_111* AA genotype may do better when butterflies are limited by nutritional resources, as these genotypes appear to lose thorax mass at a slower rate in response to starvation stress (Saastamoinen *et al.* 2009). Further, there is typically a significant interaction with ambient temperature in the *Pgi* genotype-fitness associations, such that the genotype with superior fitness in low-intermediate thermal conditions (*Pgi_111* AC) is outperformed by the usually inferior genotype in high-temperature environments (Saastamoinen 2007b; Ovaskainen *et al.* 2008; Saastamoinen and Hanski 2008; Niitepõld *et al.* 2009; Niitepõld 2010). This is highlighted by the observation that the "inferior" *Pgi_111* AA genotype has a better tolerance of stressfully high and low temperatures (Kallioniemi and Hanski 2011; Luo *et al.* 2014). These differences in thermal performance may be linked to the kinetics and stability of the PGI enzyme at different temperatures (Watt *et al.* 1983).

Given that there is such marked individual variation in dispersal capacity, it can be expected that new populations are typically colonized by the more dispersive females. This is indeed the case: female butterflies originating from newly-established local populations have been shown to be on average more dispersive than those from old populations (persisting for more than five years). The new-population females have on average higher flight metabolic rate, higher dispersal rate between habitat patches, and higher within-habitat mobility (Hanski *et al.* 2002; Hanski *et al.* 2004; Haag *et al.* 2005; Saastamoinen 2007a; Ovaskainen *et al.* 2008; Wheat *et al.* 2011). The *Pgi* genotype associated with higher dispersal rate is also more common in newly-established than old populations (Hanski *et al.* 2004; Haag *et al.* 2005; Zheng *et al.* 2009; Hanski and Mononen 2011), affecting even population dynamics in these populations (Hanski and Saccheri 2006). Furthermore, in a fragmented landscape, any ecological process that increases local extinctions generates more opportunities for colonization, which strengthens selection for the more dispersive individuals at the metapopulation level. Such eco-evolutionary dynamics, involving the *Pgi* polymorphism and associated life-history and dispersal traits, has been documented in the Glanville fritillary metapopulation in the Åland islands (Hanski and Mononen 2011; Hanski 2012). Overall, increasing fragmentation leads to increasing dispersal rate in the Glanville fritillary in the Åland metapopulation (Heino and Hanski 2001; Hanski and Mononen 2011). A wider applicability of this result is indicated by a study comparing multiple fragmented and more continuous landscapes in northern Europe, which found that butterflies from the fragmented landscapes had higher flight metabolic rate than butterflies from continuous landscapes (Duplouy *et al.* 2013).

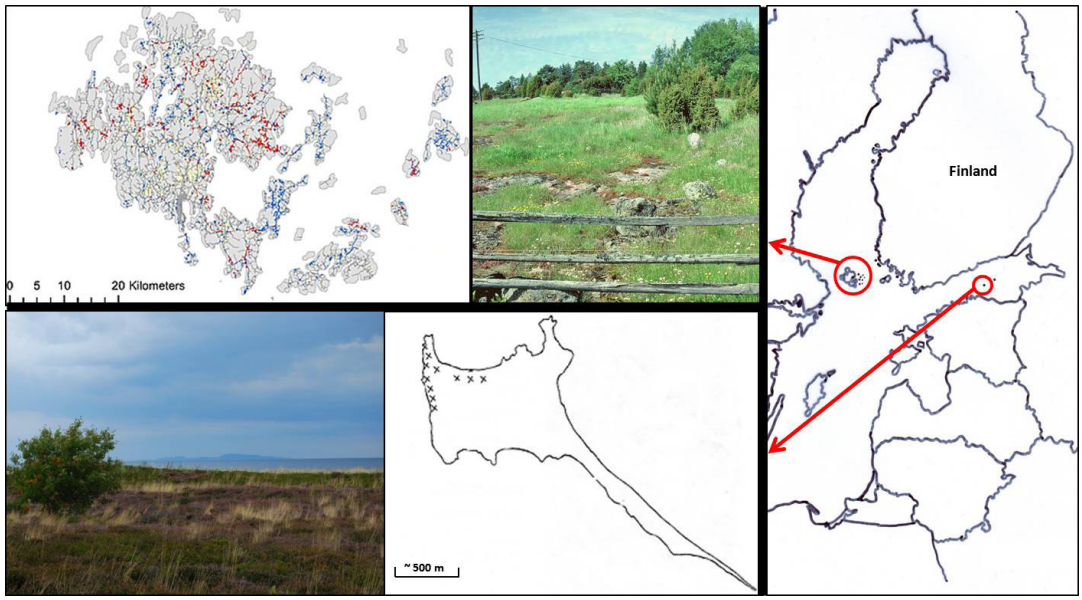

Figure 1. Maps and geographical locations of the study populations. The upper row shows the Åland metapopulation (left; Chapters **I**-**V**), with meadows occupied in 2012 shown in red and unoccupied meadows in blue, and a typical Glanville fritillary habitat patch in Åland (center; images with permission from Ojanen *et. al.* 2013). The bottom row shows the shoreline meadow inhabited by the Glanville fritillary on the island of Pikku Tytärsaari (left; Chapters **II** and **IV**), and a map of the island with the main distribution area marked with crosses (center; images courtesy of Elena Glazkova). The geographical locations of the two populations are marked on the map representing the Baltic Sea region (far right; map courtesy of Anne Duplouy).

2. AIMS OF THE THESIS

Building a better knowledge of organismal movements and dispersal is crucial for understanding the response of natural populations to the imminent global challenges threatening biodiversity, especially the loss and fragmentation of habitats and climate change (Section 1.1). For a comprehensive understanding, it is important to consider multiple facets of dispersal, ranging from the genetic basis and mechanisms of dispersal to individual and population level processes, as well as how these may be affected by variation in the environment (Section 1.2). Before a unified cross-species framework for the ecology and evolution of dispersal may be developed, empirical knowledge on a range of different model species is required.

In this thesis, I build on the vast knowledge gained during the more than two decades of research on the Glanville fritillary butterfly (Section 1.3), aiming towards a more comprehensive understanding of the mechanisms and processes involved in dispersal in this ecological model species. A variety of different methods are used (Section 3) to address questions ranging from the genetic basis and heritability of flight and dispersal capacity to the interplay between genes, physiology and environment in affecting movements. Finally, the consequences of variation in dispersal capacity to population and metapopulation level processes are investigated. Common to all studies is the use of flight metabolic rate as a measure of butterfly flight and dispersal capacity (Box 1, Sections 1.3 and 3.2). More specifically, the thesis aims to address the following main questions:

- How is flight metabolic rate inherited from one generation to the next, and does heritability differ in different environmental conditions (**I**)?
- Which genes and functional groups of genes primarily influence flight metabolic rate and flight capacity (**II**, **III**)?

- How significant are single-gene effects of the phosphoglucose isomerase (*Pgi*) gene on different aspects of flight and dispersal behavior, and how much does *Pgi* genotype contribute to the heritability of flight metabolism (**I**, **III**, **V**)?
- Which cellular and physiological processes are important in a flying butterfly, and can they predict variation in flight capacity (**II**, **III**)?
- How is butterfly flight influenced by environmental conditions, especially temperature, and what are the mechanisms behind the dissimilar responses of different individuals to variation in the environment (**I**, **III**)?
- Do the two sexes differ in the characteristics of flight and dispersal physiology and behavior (**II**, **III**, **IV**, **V**)?
- What are the consequences of long-term complete population isolation on life-history and fitness and the genetic viability of a small butterfly population (**IV**)?
- Is there evidence for selection on reduced dispersal capacity in a completely isolated small population, and which evolutionary forces determine average dispersal capacity in such a setting (**IV**)?
- What are the consequences of variation in flight metabolic rate, flight performance and *Pgi* genotype for dispersal at the habitat patch network (metapopulation) level (**V**)?

3. MATERIALS AND METHODS

3.1. THE STUDY SPECIES AND POPULATIONS

The Glanville fritillary butterfly, *Melitaea cinxia* (*Melitaeini: Nymphalidae*), is widely distributed in the Palaearctic region from western continental Europe to southern Siberia and NW China (Nieminen *et al.* 2004). The butterfly is declining in numbers in many parts of its range due to habitat loss and alteration. In Finland, it occurs at its northern range margin and is listed as an endangered species (Rassi *et al.* 2010). The Finnish mainland populations have gone extinct, and the present-day distribution is limited to the Åland Islands southwest of mainland Finland (Fig. 1).

The Åland metapopulation

In the northern parts of its range, including the Åland Islands, the Glanville fritillary has an obligate diapause and one generation per year (Kuussaari *et al.* 2004). It inhabits dry meadows with one of the two host plants: the ribwort plantain *Plantago lanceolata* and the spiked speedwell *Veronica spicata*. Caterpillars live in sib-groups and diapause gregariously in a densely spun winter nest. The size of the larval group has a positive effect on winter survival (Boggs and Nieminen 2004). In the spring, larvae continue feeding and pupate in May. The flight season lasts from early June to mid-July, with males emerging on average 2-3 days earlier than females (Boggs and Nieminen 2004). Males usually locate females using the "perching" tactic, by establishing mating territories where they wait for passing females and chase away intruding males, which requires repeated rapid take-off flights. "Patrolling" males, on the other hand, fly more continuously around the habitat in search of females (Scott 1974; Boggs and Nieminen 2004). Females usually mate only once soon after eclosion and lay eggs in clusters on leaves of the host plants, often after having dispersed to a new habitat patch. Details of the metapopulation structure and dynamics, as well as characteristics of dispersal in the Glanville fritillary butterfly, are presented in Section 1.3.

In Chapters **I**-**V**, samples collected as larvae during the Åland autumn surveys and reared in common garden conditions at the Lammi biological station butterfly laboratory were used in the experiments. In Chapter **V**, second generation laboratory-reared butterflies originating from Åland were also used (tethered flight experiment, Section 3.3), as well as individuals captured as adults in the wild (mark-recapture experiment, Section 3.3). To avoid data interdependence due to family structure, samples in each experiment originated from different families and local populations around the Åland metapopulation. In Chapter **IV**, the large Åland metapopulation served as a reference when investigating the population genetic, life-history and fitness effects of long-term complete isolation in the small island population of Pikku Tytärsaari (below). In Chapter **II**, butterflies from the Åland metapopulation exemplified individuals with high

flight capacity, in comparison with individuals from Pikku Tytärsaari, which served as examples of poor flyers (based on the results of Chapter **IV**).

The isolated island population of Pikku Tytärsaari

In addition to the well-studied large metapopulation in the Åland islands, which has been used as a model system throughout this thesis, Chapters **II** and **IV** additionally investigated a small and completely isolated population of the Glanville fritillary on the island of Pikku Tytärsaari (hereafter denoted as PT). The island of PT is located in the Gulf of Finland in the Baltic Sea (Fig. 1). The nearest mainland in Estonia is about 30 km away, greatly exceeding the maximum colonization distances of the butterfly (4-5 km; van Nouhuys and Hanski 2002). The distance to the Åland islands is about 400 km (Fig. 1).

The Glanville fritillary was first recorded on PT in 1936 and again in 1994, and hence the population has persisted for at least 75 years (last surveyed in 2011). The island is very small, only about 1.5 km^2 in area, and there is a single meadow of around 10 ha with the host plant Veronica spicata. The butterfly was most likely unintentionally introduced with plant material brought from the Estonian coast by people using the uninhabited island as a base for fishing and travel. The introduction as larvae from one or several larval families is supported by the presence of the specialist parasitoid Cotesia melitaearum, which is an even worse disperser than the butterfly (van Nouhuys and Hanski 2002), but could have arrived as parasitized host larvae. The Russian island has been seldom visited by people after the World War II. Because of this and the great distances to nearest conspecific populations, it is practically certain that there has been no further gene flow to the island.

PT was exhaustively surveyed in 2009 and 2011, during which 111 and 198 larval family groups were recorded, respectively. Assuming that the expected genetic heterozygosity was initially equal to the current heterozygosity in the large Estonian population on the island of Saaremaa (used as a population genetic reference), knowing the current level of genetic variation (Chapter IV), and assuming that the age of the PT population is 100 y (=100 generations), the effective population size of PT is currently estimated to be N_e = 95 (following the method of Hartl and Clark 2007). This estimate is consistent with the numbers of family groups found during the surveys.

3.2. MEASUREMENT AND ANALYSIS OF FLIGHT METABOLIC RATE

Previous studies on the Glanville fritillary have shown that metabolic rate during flight correlates positively with dispersal rate in field conditions, explaining up to one third of the variation in distances travelled (Niitepõld *et al.* 2009). In this thesis, flight metabolic rate (FMR) is thus considered a proxy of butterfly dispersal capacity. In insects, metabolic rates can be measured using the rates of gas exchange. Aerobic respiration, which is the primary source of energy used in insect flight (Dudley 2000), requires oxygen molecules (O_2) to generate ATP (energy). In this reaction, carbon dioxide (CO_2) is released in proportion to the oxygen used and energy consumed. The amount of CO_2 produced thus gives a reliable estimate of the rate of metabolism, and it can be measured using flow-through respirometry (for a detailed description of the method used to measure metabolic rate with respirometry in the Glanville fritillary, see Niitepõld *et al.* 2009; Niitepõld 2009).

Here, flight metabolic rate was measured from butterflies of the age of 2-4 days, which has allowed the butterflies to use their flight muscles for a minimum of one day in favourable conditions in a flight cage. To equalize activity level and nutritional state, on the day before the measurement butterflies were taken to conditions which discourage flight activity (room temperature, dim light) and were provided with water only. Preceding the measurement, the butterfly was weighed and acclimatized in the measurement chamber under a black cloth for ~30 min. FMR was measured using flow-through respirometry (Fig. 2), where CO_2-free dry air was pumped through a 1-l transparent jar at the rate of ~1.0 l/min in the constant temperature of 30°C. The measurement temperature was chosen based on previous studies of FMR in the Glanville fritillary (Niitepõld *et al.* 2009; Niitepõld 2010). Individuals were stimulated to fly inside the respirometry chamber by gently shaking or tapping the chamber, with the aim of forcing

individuals to use their maximal flight performance. In Chapters **I**, **II** and **IV**, FMR was measured during a period of 15 mins of flight, while somewhat shorter flight durations were used in Chapters **III** (7 mins) and **V** (10 mins).

Fifteen min of active flight during the FMR measurement reflects both the maximal flight performance and flight endurance of butterflies. However, as metabolic rate reaches a peak within the first few minutes of active flight and thereafter usually declines rapidly (Fig. 2A), shorter measurements also give accurate estimates of the maximal flight performance. The 7-15 min period of active flight is demanding for this butterfly species, mimicking a long dispersal event in the field. The treatment is not excessively harsh, however, because butterflies recover and e.g. their longevity is not affected (Saastamoinen and Rantala 2013). Furthermore, FMR has been shown to be significantly repeatable in individual butterflies (r = 0.46-0.91; Niitepõld and Hanski 2013).

Four different measures of FMR were studied: peak FMR (the maximum rate of CO_2 emission during the flight, describes maximal flight performance), integrated FMR (the total volume of CO_2 produced during the duration of the flight experiment, describes maximal flight performance and endurance of flight), and peak FMR_{END} and integrated FMR_{END} (the peak CO_2 emission and the total CO_2 volume produced during the last 5 min of the experiment, respectively, describe endurance of flight). The average CO_2 emission rate during 60 s of stable baseline before the flight experiment was used as a measure of resting metabolic rate (RMR; **I**, **IV**). Because of the well-established positive correlation between body size and metabolic rate (Kleiber 1947; Gillooly *et al.* 2001), measures of FMR and RMR were corrected for variation in body mass by using residuals from the linear regression of metabolic rate against body weight. Further, because of the sexual size dimorphism and possibly differential scaling of metabolic rate with body mass in female and male butterflies (Fig. 2B), residual metabolic rates were calculated separately for the two sexes. In Chapter **II**, mass-specific metabolic rate (FMR per unit of body weight) was used, as the relatively small sample size did not allow a reliable regression analysis. Butterflies were allowed to recover from the metabolic measurement for a minimum of 24 h under favorable conditions before any possible further experimental treatments.

3.3. OTHER MEASURES OF FLIGHT PERFORMANCE AND DISPERSAL CAPACITY

Besides flight metabolic rate, thermal performance of flight (**III**; Section 3.4) and molecular characteristics (**I-V**; Section 3.6), several other types of dispersal-related traits were studied. In Chapter **IV** many morphological traits expected to influence flight performance were measured to investigate whether

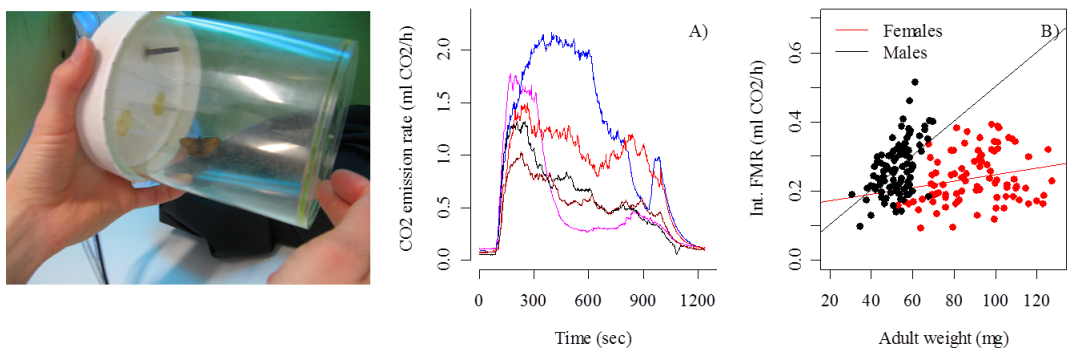

Figure 2. The image on the left shows a butterfly placed in a respirometry chamber for a flight metabolic rate measurement. **A.** Examples of CO_2 output (ml CO_2/h) during 15 min of flight, with colors representing different individuals. These examples demonstrate typical patterns of flight metabolism in the course of time as well as the amount of variation among individuals. **B.** An example of scaling of metabolic rate with body mass in the two sexes. The figure shows the regression of integrated flight metabolic rate (the total amount of CO_2 produced during 15 min of flight) against adult body mass in females (red) and males (black). Modified from Chapter **I**.

complete population isolation had influenced dispersal capacity. The traits included relative thorax mass, wing area, wing aspect ratio and wing loading, which are often used as proxies for butterfly flight performance (Box 1).

In Chapter **V**, three very different measures of flight and dispersal performance were examined. In addition to measurements of flight metabolism, the endurance of flight was measured in a tethered flight experiment, and dispersal rate between habitat patches was studied in the natural habitat of the butterfly with mark-release-recapture (MRR) studies. In the tethered flight experiment, the butterfly was attached from the dorsal side of its thorax to the end of a vertical rod, and was placed inside a cylinder with controlled lighting and temperature. The butterfly was stimulated to fly by directing an air flow at the butterfly. The total duration of flight during a period of five min was used as a measure of flight endurance. Two MRR studies were performed, one in a natural habitat patch network in the Åland islands, and the other one on an uninhabited small island in the eastern Åland archipelago with a network of naturally occurring habitat patches, where common-garden reared butterflies were released for the experiment. In the MRR studies, all habitat patches were searched for butterflies daily during the flight season. During the first capture or before the initial release, butterflies were individually marked with a number written on their wing. At each capture, the location and identity of the captured butterfly were recorded before releasing it at the same location. Two measures of dispersal were calculated from the MRR data: the observed number of movements between habitat patches and the sum of the respective movement distances. MRR studies have the advantage of allowing the observation of dispersal under natural environmental conditions and in the natural habitat of the study species.

3.4. RECORDING BUTTERFLY BODY TEMPERATURE USING INFRARED THERMAL IMAGING

Infrared (IR) thermal imaging provides an accurate and non-invasive method for recording the surface body temperature of butterflies. The thermal image camera is a thermometer with automatic functions, high accuracy and spatial resolution, and the possibility to store thermal data for subsequent analysis. In Chapter **III**, an IR thermal image camera (DIAS PYROVIEW 380L compact) was used to record body temperature (T_b) of butterflies at the time of flight take-off and immediately after a known duration of flight, allowing the calculation of the rate of cooling (due to air convection) during flight (Box 1).

The flight experiments were carried out in a large outdoor population cage covered with a mesh that prevents butterflies from escaping but allows practically natural environmental conditions (Hanski *et al.* 2006). The weather conditions during the experiments were recorded with a weather station data logger placed inside the population cage. To start the experiment, the butterfly was placed on a platform covered with white cardboard in sunshine and protected from two sides by windshields. The butterfly was allowed to bask while being simultaneously photographed with the IR camera at one sec intervals until it took off on its own. The flying butterfly was followed on foot, recording the time in flight with a stopwatch. Because the flight of the Glanville fritillary typically consists of short flight bouts (Ovaskainen *et al.* 2008), in about half of the flight experiments the butterfly was chased to continue its flight immediately after landing to enable the study of T_b changes during longer flights. At the end of the flight experiment, the butterfly was captured and T_b was immediately recorded with the IR camera.

The images were analyzed using the PYROSOFT Compact program (DIAS Infrared Systems). Figure 3 shows an example thermal image series of a butterfly at the beginning of basking, at the time of take-off and immediately after flight. The average surface temperature at five random points within the outline of the thorax was used as a measure of thoracic temperature. The surface temperature underestimates the actual body temperature, but the measurements are comparable between individuals and experiments (see also Saastamoinen and Hanski 2008). The last image of the basking butterfly before take-off, and the first image of the butterfly after flight, were analyzed to obtain thoracic temperature (T_b) at take-off and after flight, respectively. The

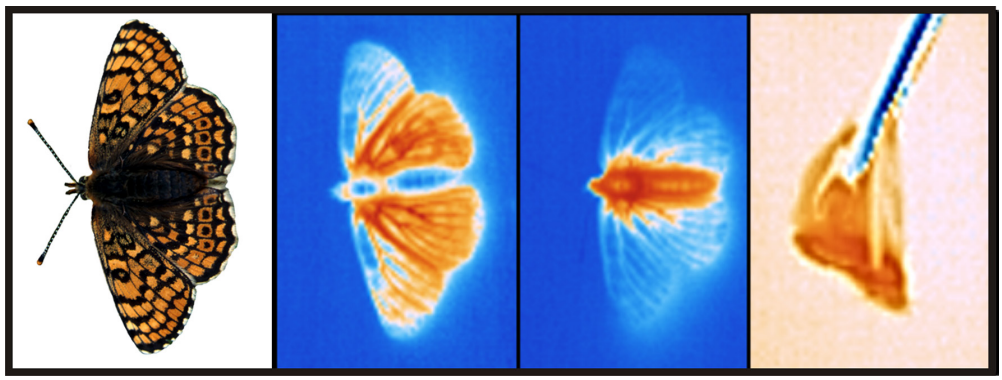

Figure 3. A thermal image series of a butterfly (Chapter **III**) in the beginning of basking, at the time of take-off and immediately after flight (held by tweezers). The colors represent relative temperature (blue=cold – red=warm).

amount of cooling during each flight experiment (Δ) was calculated as the difference between take-off T_b and flight T_b, and the cooling rate as the amount of cooling per second of flight (Δ / duration of flight).

3.5. ESTIMATING HERITABILITY AND PHENOTYPIC VARIANCE COMPONENTS USING THE ANIMAL MODEL

Factors including genetics, environment and random effects can all contribute to phenotypic variation between individuals. Heritability (h^2) describes the degree of resemblance between relatives, and in its narrow definition is an estimate of the relative contribution of additive genetic factors to the total phenotypic variance in a population (Lynch and Walsh 1998b, Chapter 1.2). "Animal models" provide a powerful statistical approach to investigating the quantitative genetics of complex traits (Kruuk 2004; Wilson *et al.* 2010). The animal model is a linear mixed effects model that can make use of information from all family relationships in a complex, unbalanced and incomplete pedigree to estimate quantitative-genetic parameters. It can be fitted with either restricted maximum likelihood (REML) or Bayesian inference (Wilson *et al.* 2010). Given sufficient pedigree data, the method can partition phenotypic variance into different components, including additive genetic variance as well as maternal and environment influences. Failing to account for covariance among relatives due to maternal and common environment effects would inflate estimates of heritability (Kruuk and Hadfield 2007).

In Chapter **I**, we investigated additive genetic variance (heritability), maternal effects and common environment effects in the flight and resting metabolic rates of the Glanville fritillary. We fitted an animal model using the software Wombat (Meyer 2007), which uses the REML method. Heritability and variance components were estimated separately for butterflies reared in two different temperature treatments: the standard (benign) and high temperature (stressful) treatments, which formed two separate two-generation pedigrees. The objective of this experimental setup was to investigate whether heritability differs in different environmental conditions. The high-temperature treatment represents environmental (thermal) stress. The pedigrees include parents and their offspring as well as full-sib and half-sib relationships. The model was fitted separately for four different measures of flight metabolic rate and the resting metabolic rate. The phenotypic variance (V_p) components included additive genetic variance (V_a), yearly variance (V_{year}; corresponds to generation), maternal effects (V_{mat}; describes the similarity of the offspring of the same mother), and a common environment effect (the "box effect", V_{box}; describes the similarity of full-sibs reared in separate rearing boxes). The narrow-sense heritability (h^2) was calculated as the proportion of phenotypic variance explained by additive genetic variance (i.e. V_a/V_p) (Kruuk 2004; Wilson *et al.* 2010).

3.6. MOLECULAR METHODS

Three main molecular methods were used in this thesis: SNP (single nucleotide polymorphism) genotyping of candidate gene loci such as *phosphoglucose isomerase* (*Pgi*) (**I**, **III** and **V**), assessment of neutral genetic variability using microsatellite markers (**IV**), and whole-genome transcriptome sequencing (RNA-seq; **II**).

The candidate genes and SNPs were selected on the basis of previous association and expression studies on the Glanville fritillary (Vera *et al.* 2008; Niitepõld *et al.* 2009; Orsini *et al.* 2009; Fischer and Karl 2010; Wheat *et al.* 2011; Saastamoinen *et al.* 2013; Kvist *et al.* 2013; for detailed information on the SNPs and their selection criteria, see de Jong *et al.* 2014). SNPs were genotyped from DNA extracted from butterfly thorax samples using the Sequenom iPLEX Gold chemistry genotyping platform (Sequenom Inc., CA, USA; **I**, **III**) or by running primer extension reactions (SNuPe kit, GE Healthcare) with Megabase 1000 (GE Healthcare) as described in Orsini *et al.* (2009; **V**). In Chapter **III**, I studied the associations between flight metabolic rate and genotype for 11 SNPs in six genes: *Pgi*, *Flightin*, *Glucose-6-phosphate 1-dehydrogenase* (*G6PD*), *Heat shock protein 70kDa (Hsp70)*, *Succinate dehydrogenase complex subunit D (Sdhd)* and *Troponin-T*. Studies by Wheat *et al.* (2011) and Marden *et al.* (2012) have shown that *Sdhd* genotype is related to flight metabolic rate, flight endurance and hypoxia tolerance in the Glanville fritillary. *G6PD* has a major role in aerobic metabolism, as it is a rate-limiting enzyme of the pentose phosphate pathway (Eanes *et al.* 2006), whereas *Flightin* has a function specific to insect flight muscles (Barton *et al.* 2005). The isoform composition of *Troponin-T* is known to affect muscle force and power output in other animals, and to be associated with the rate of energy consumption during insect flight (Marden *et al.* 2008). Heat shock proteins (*Hsps*) protect cells against stress-induced damage (Sorensen *et al.* 2003). In the case of SNPs with a significant association with FMR (*Pgi*, *Hsp70* and *Flightin*), I examined their associations with butterfly body temperature dynamics during flight. The role of *Pgi* was also studied in relation to its contribution to the heritability of flight metabolic rate (**I**), its gene expression response to an experimental flight treatment (**II**), as well as its association with dispersal rate of individual butterflies within a natural habitat patch network in the Åland islands (**V**).

The consequences of long-term small population size and complete lack of gene flow for the genetic variability of the PT island population (Section 3.1) was assessed in Chapter **IV** using microsatellite markers. Samples from PT and the reference populations in Åland and Saaremaa were genotyped for seven microsatellite loci. Based on these microsatellite genotype data, several population genetic parameters were calculated, including the allele distributions, expected and observed heterozygosities (mean for individual loci), gene diversity (average over all data), F_{st} values, the numbers of shared alleles with PT, inbreeding coefficients and average relatedness. To estimate the number of founding individuals of the PT population, the decline in heterozygosity in time was simulated using different founding propagule sizes. The expected heterozygosity values of the simulated data were compared with the value calculated for the actual sample.

In Chapter **II**, the full 393 Mbp draft genome of the Glanville fritillary with its 16,667 gene models (12,410 gene models with a functional annotation; (Ahola *et al.*, unpublished) was utilized to study gene expression changes induced by intensive flight activity by transcriptome sequencing (RNA-seq). RNA samples were taken from butterflies that had experienced a 15 min flight treatment (during which flight metabolic rate was measured) either 1 h or 20 h previous to sampling. RNA was additionally extracted from control butterflies that had not experienced the flight treatment. Total RNA was extracted from butterfly thorax samples. Two RNA-seq libraries (separated by size into two pools: ~450 bp and ~650 bp libraries) were prepared for each individual and were sequenced with Illumina Hiseq2000 (Illumina Inc., CA, USA). After quality control, RNA reads were mapped against genomic scaffolds. The expression level of a gene was calculated based on reads that were mapped within gene models. The gene models that yielded more than 10 read counts in at least 10 samples (8,258 genes) were used in the analyses of differential expression. Differentially expressed genes were recorded using generalized linear models applied to each gene, with the read

count as the response variable and population (PT / Åland), sex and flight treatment category (control / 1 h after flight / 20 h after flight), and their interactions as factors. Enrichment of particular gene functions within the genes that showed differential expression in the flight treatment vs. control butterfly samples (755 genes), as well as those genes that showed differences between the two studied populations were studied with Gene Ontology (GO) and KEGG functional pathway enrichment analyses. Gene expression changes in gene groups of special interest, i.e. hypoxia, glycolysis and TCA (tricarboxylic acid) cycle genes, were studied by finding linear models that best explained the gene expression changes in each gene group.

4. RESULTS AND DISCUSSION

4.1. GENETIC BASIS AND HERITABILITY OF FLIGHT METABOLIC RATE AND DISPERSAL CAPACITY (I, II, III, V)

For dispersal to evolve, there has to be variation in dispersal-related traits, and this variation has to have a genetic basis and be heritable from one generation to the next. Because of the crucial role of dispersal in adapting to, or escaping from, challenges posed by habitat fragmentation and climate change (Section 1.1.), empirical studies are needed on the genetic basis and heritability of dispersal-related traits (Zera and Brisson 2012; Sutherland *et al.* 2013). I begin this section by assessing the extent to which flight metabolic rate and dispersal capacity have a heritable genetic basis in the Glanville fritillary. I then discuss the role of *Pgi* genotype for different aspects of flight and dispersal behavior, as well as other observed single-gene effects on flight capacity.

In the Glanville fritillary, heritability of mobility (small-scale movements) under semi-natural conditions has been previously estimated using parent-offspring regression (Saastamoinen 2008) and full-sib analyses (Klemme and Hanski 2009), which both found significant heritability of flight activity (h^2 = 0.61 and h^2 = 0.29, respectively). However, the extent to which maternal effects contributed to these heritability estimates is unknown. Maternal effects may play a large role, as genetically or environmentally determined maternal quality, in addition to the additive genetic background, may influence the quality of the offspring (Pakkasmaa *et al.* 2003; Kruuk 2004; Kruuk and Hadfield 2007). In fact, Saastamoinen (2008) found mobility to be heritable from mother to daughter only, suggesting that the heritability estimate from mother to her female offspring could be inflated by maternal effects that are expressed in females only. Here, however, using a two-generation design with parent-offspring, full-sibling and half-sibling family relationships, and employing the animal model method (Kruuk 2004; Wilson *et al.* 2010), maternal effects on flight metabolism were found to be negligible, but flight metabolic rate had significant heritability with additive genetic variance accounting for about 40% of total phenotypic variance (h^2 = 0.38, P = 0.039 for Integrated FMR; Fig. 4, Chapter **I**). According to this result, flight metabolic rate thus has a significant heritable genetic basis and the potential to respond to selection, highlighting its importance for the evolution of dispersal capacity in this species. In comparison, there was no heritability in resting metabolic rate, which was instead strongly influenced by maternal effects.

Notably, however, heritability of flight metabolism was found to be context-dependent, as in stressful high-temperature conditions environmentally induced variation dominated over additive genetic effects. This result is in line with studies comparing heritabilities in favourable and stressful environments in wild populations which have shown that, in general, estimates of additive genetic variance are lower in unfavourable conditions (Charmantier and Garant 2005), whereas laboratory studies in Drosophila and other invertebrates have indicated even an opposite effect (Hoffmann and Merilä 1999; Saastamoinen *et al.* 2013a). The absence of a significant heritability for flight metabolism in individuals reared as larvae in high temperature suggests that the significant heritability measured under the standard conditions may not be realized under stressful thermal conditions in nature. Temperatures as high as the ones used for the high temperature treatment (35°C) are rarely experienced by these temperate butterflies in the field, but such conditions may become more common as a result of climate change. The resulting potential dominance of environmental effects over additive genetic

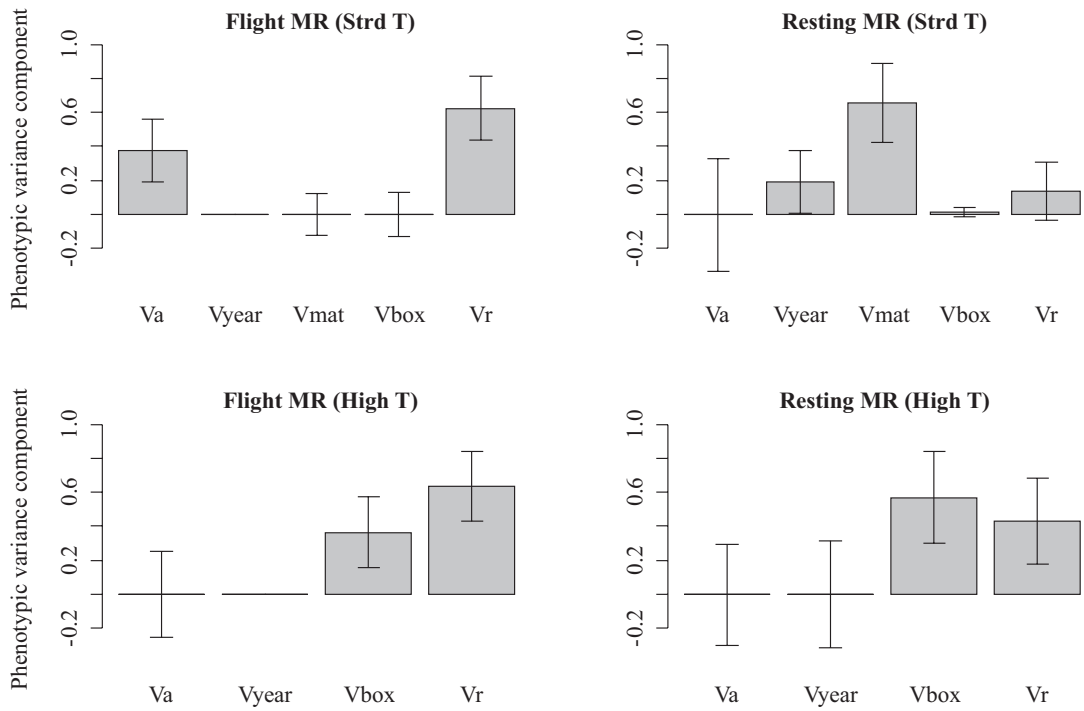

Figure 4. Variance components of flight metabolic rate (Integrated FMR) and resting metabolic rate (RMR) (Chapter **I**). Integrated FMR is the total amount of CO_2 produced during 15 min of flight. RMR is average CO_2 emission rate at rest. The upper panels show results for standard temperature treatment, the lower panels for the high-temperature treatment. The variance components are: additive genetic variance V_a, yearly variance V_{year}, maternal effects V_{mat}, common environment effect V_{box}, and residual variance V_r. Whiskers on the bars show the standard error of the estimate.

effects may act as a constraint for the evolution of flight capacity. Heritability of dispersal-related traits has been studied in e.g. the tropical butterfly *Bicyclus anynana* (Saastamoinen *et al.* 2012), crickets (Roff and Fairbairn 2007a; Ketola and Kotiaho 2009), wind-dispersed insects (Gatehouse 1997), and moths (Gu and Danthanarayana 1992; Han *et al.* 2009), but these studies have typically been performed on laboratory populations and without accounting for sources of phenotypic variance other than genetic effects, nor the possible effects of dissimilar conditions on heritability. The results presented here thus represent one of the most comprehensive assessments of heritability of dispersal capacity, and in a more natural setup than in most heritability studies on insects to date.

There is thus a significant heritable genetic basis in flight metabolism. Is it possible to find genes contributing to the genetic basis of flight metabolism and dispersal capacity? Previous studies on the Glanville fritillary have indicated significant effects of the phosphoglucose isomerase (*Pgi*) genotype on multiple life-history and fitness traits, including an association with flight metabolic rate (FMR) and dispersal rate in the field (Niitepõld *et al.* 2009, Section 1.3.). *Pgi* serves an important function in glycolysis, i.e. the metabolic pathway by which glucose and fructose, the main components of flower nectar ingested by butterflies, are transformed into high-energy molecules (ATP and NADH) in glycolysis and downstream pathways such as aerobic respiration (the citric acid/TCA cycle in mitochondria) providing energy for e.g. flight. More specifically, the PGI enzyme catalyzes the conversion of glucose-6-phospate into fructose-6-phosphate. There is evidence for the association of *Pgi* genotype with dispersal capacity for several other butterfly and insect species (Watt *et al.* 1983; Hughes and Zalucki 1993; Watt *et al.* 2003; Rank *et al.* 2007; Niitepõld *et al.* 2009; Mitikka and Hanski 2010, reviewed in Wheat 2010). Studies on the *Colias*

butterflies exhibiting similar *Pgi* - fitness associations have shown that the observed differences in fitness and flight performance can be largely explained by dissimilar metabolic capacity of the different PGI enzyme isoforms, which differ in their thermal stability and kinetic properties (Watt *et al.* 1983; Watt 1992). These enzymatic properties can thus explain the phenotype-temperature interactions, with different *Pgi* genotypes performing better at different temperatures (Watt *et al.* 1983; Saastamoinen and Hanski 2008; Niitepõld 2010).

In this thesis, the significance of *Pgi* genotype on several different aspects of flight and dispersal behavior in the Glanville fritillary was examined. At the individual level, I found a significant association of *Pgi* genotype with flight metabolic rate (Chapter **III**), but only in male butterflies. In Chapter **I**, *Pgi* genotype was not significantly associated with flight metabolism, although there was a trend parallel to results found in previous studies on the Glanville fritillary (Niitepõld *et al.* 2009; Niitepõld 2010). *Pgi* did not contribute any significant amount to the total heritable variation in FMR (Chapter **I**). Moreover, unlike expected, there was only weak indication of differential flight behavior in different thermal conditions of the *Pgi* genotypes, such that the *Pgi_111* AC/CC genotypes (associated with high FMR) appeared to maintain body temperature during flight somewhat better than the other genotypes (Chapter **III**). In the study of Saastamoinen & Hanski (2008), the *Pgi_111* AC genotype was found to have a significant advantage in low ambient temperatures, based on the observation that in such conditions, they had on average higher body temperature during flight. Results of the genome-wide RNA-seq study indicated that many genes related to glycolysis showed a significant expression difference between the two studied populations, including *Pgi*, which was significantly more highly expressed in ÅL than PT (Chapter **II**). This result suggests that the expression of *Pgi* may play a role in influencing flight capacity, additionally to the allelic effects demonstrated by previous studies. Finally, at the metapopulation level, there was indication that females with the *Pgi_111* AC genotype moved somewhat more between local habitat patches than *Pgi_111* AA (Chapter **V**, discussed in more detail in Section 4.5).

The effects of *Pgi* genotype found in Chapters **I**, **III** and **V** were thus generally similar, but overall less significant than what has been previously found in many studies on the Glanville fritillary. Variation in the results may be due to acclimatization to different thermal conditions prior to the experiments, as the differences between *Pgi* genotypes appear to be greatest in individuals acclimatized to low ambient temperatures (Oksanen & Hanski, unpublished). Here, butterflies were maintained for most of their adult life in warm conditions (max. 28°C) in the laboratory compared to e.g. the study of Niitepõld *et al.* (2009), in which butterflies were mostly kept in cool field conditions. These observations highlight the temperature-dependent effects of *Pgi* genotype on dispersal capacity and fitness reported by many previous studies (Saastamoinen 2007a; Saastamoinen and Hanski 2008; Niitepõld 2010; Kallioniemi and Hanski 2011). The complex associations involving temperature-genotype interactions, differences between the sexes (Chapters **III** and **V**), and heterozygote advantage (Orsini *et al.* 2009) contribute to non-additive genetic variance and can lead to departures from the simple additive model, probably also explaining why *Pgi* genotype was not found to contribute to the heritability of flight metabolic rate (Chapter **I**). The reason why a genotype-FMR association in *Pgi* and in other studied genes was only found in male but not female butterflies is not clear, but could be related to the overall higher repeatability of FMR measures in males (Niitepõld and Hanski 2013), allowing for more precise association testing. The result of differential expression of *Pgi* in populations greatly differing in flight capacity (PT vs. ÅL, Chapter **II**) warrants more study on the expression of this important glycolytic gene.

In addition to the *Pgi* genotype, I examined the associations of flight metabolic rate with 8 SNPs in 5 other genes (Chapter **III**). The genes included *Flightin*, *Glucose-6-phosphate 1-dehydrogenase* (*G6PD*), *Heat shock protein 70kDa* (*Hsp70*), *Succinate dehydrogenase complex subunit D* (*Sdhd*) and *Troponin-T*, which were chosen as candidates based on indication of possible significant effects on flight performance shown in previous gene expression and association studies in the Glanville fritillary and other flying insects (Section 3.6.). Of these, genotype at one of the studied SNPs in the

Heat shock protein 70kDa (*Hsp70*) locus showed a highly significant association with flight metabolic rate in male butterflies. Heat shock proteins (Hsps) have an important function in protecting cells against stress-induced damage (Sorensen *et al.* 2003), including physiological stress caused by intensive exercise (Sorensen *et al.* 2003; Fittipaldi *et al.* 2014). One important form of exercise-induced stress is oxidative damage, which is associated with energy use and can reduce lifespan (Nilsson 2002; Alonso-Alvarez *et al.* 2004; Hulbert *et al.* 2007; Ketola and Kotiaho 2009). Taking into account that butterfly flight is one of the most energy-consuming activies, with flight metabolic rates greatly exceeding metabolism at rest (Suarez 2000; Dudley 2000), it is perhaps not unexpected that *Hsp*s may have a role in influencing the rates of metabolism during flight. *Hsp70* expression is also known to act as a buffer against the differential thermal tolerance of individuals (Rutherford 2003), such as that due to *Pgi* genotype. The influence of *Pgi* genotype on the tolerance of extreme temperatures has been demonstrated in several studies on insects (Watt *et al.* 1983; Dahlhoff and Rank 2000; Neargarder *et al.* 2003; Rank *et al.* 2007; Luo *et al.* 2014). Studies of willow beetles (*Chrysomela aeneicollis*) have shown that genetic variation in *Pgi* leads to differential expression of *Hsp70* in response to thermal stress (Dahlhoff and Rank 2000; Neargarder *et al.* 2003; McMillan *et al.* 2005). The less thermally tolerant *Pgi* genotypes upregulate *Hsp70* to a greater extent, possibly buffering differences in the thermal tolerance of different *Pgi* genotypes (Dahlhoff and Rank 2000; Rank *et al.* 2007). The role of *Hsp*s in the flight of the Glanville fritillary is further highlighted by the observed association of *Hsp70* genotype with body temperature at flight take-off (Chapter **III**, discussed further in Section 4.3.).

Another candidate gene found in this thesis to be related to flight metabolism was *Flightin*, which showed an association with flight metabolic rate in male butterflies, such that the *GG* homozygotes had significantly elevated FMR compared to other genotypes (Chapter **III**). *Flightin* has a function specific to insect flight muscles (Barton *et al.* 2005), and its significance in flight performance and flight metabolism could be worthy of further investigation. I did not find any FMR differences between the genotypes at the *Sdhd* locus, which has been shown in studies of Wheat *et al.* (2011) and Marden *et al.* (2012) to associate with flight metabolic rate, endurance of flight, and hypoxia tolerance in the Glanville fritillary. One possible reason for these conflicting results is that the previous studies examined an indel polymorphism in *Sdhd* (Wheat *et al.* 2011; Marden *et al.* 2013), whereas I investigated variation in a SNP which is not fully linked with the corresponding indel variation. In Chapter **II**, in which flight-induced gene expression changes were studied, the RNA-seq data did not allow investigation of SNP-FMR associations, because the coverage of RNA-seq reads was not sufficient to obtain reliable genotypes for most SNPs. However, the study setup was well-suited to study the cellular and physiological processes important in flight, and how these may be related to flight metabolism. This approach yielded evidence for some individual candidate genes, the expression of which were found to be significantly associated with average flight capacity. Probably the most promising of these was *citrate synthase*, which has an important function in aerobic respiration (next section).

4.2. CELLULAR AND PHYSIOLOGICAL PROCESSES IN FLIGHT (II, III)

The associations of flight capacity with genes related to energy metabolism (e.g. *Pgi*) and stress responses (e.g. *Sdhd* and *Hsp70*) indicate that these cellular processes are fundamental in determining flight performance in butterflies (Chapter **III**, Niitepõld *et al.* 2009; Marden *et al.* 2012; Wheat *et al.* 2011) and other insect species (Eanes *et al.* 2006). To investigate in more detail and at the whole-genome level which cellular and physiological processes influence flight, we studied the genes and functional groups of genes that are activated or suppressed in response to flight activity (Chapter **II**). This was done by studying flight-induced changes in gene expression. The flight treatment employed was a 15-minute flight metabolic rate experiment. This allowed both the examination of the gene expression response to flight at the maximal capacity level of butterflies, as well as relating these gene expression changes to individual variation in flight performance. The flight treatment mimicked a long and demanding flight bout during e.g. a dispersal event between distant habitat fragments, giving thus indication of

the cellular processes that may act as limiting factors in such situations.

Altogether 755 genes changed their expression significantly in response to the flight treatment. A great majority of the changes were evident only 20 h after flight, indicating a long-lasting effect of the 15 min of active flight. Most of the flight-responsive genes were related to various cellular metabolic processes. Among the most significantly enriched functional gene groups (in total 266 significantly enriched Gene Ontology (GO) groups) were ATP metabolic process, stress response and defense response genes, including genes related to the response to hypoxia. Additionally, fifteen functional pathway categories (KEGG pathways) were significantly overrepresented among the flight-responsive genes. These included e.g. glycolysis, TCA (tricarboxylic/citric acid) cycle, and spliceosome pathways, indicating a cellular response to stressful and energy-consuming circumstances. Also many categories related to immunity were overrepresented among the 755 genes responding to flight. In a previous study on the Glanville fritillary using an experimental immune response assessment (encapsulation rate), Saastamoinen & Rantala (2013) showed that intensive flight activity enhanced the immune response. Immune genes are known to respond to intensive exercise also in humans (Nieman 1997; Fragala *et al.* 2011).

This largely descriptive study of cellular and physiological processes induced by butterfly flight activity sets the background for closer investigation of the roles of the various flight-responsive gene groups. Here, we focused in more detail on genes in the TCA cycle, glycolysis and hypoxia pathways, many of which have been identified by previous studies on the Glanville fritillary and other insects to be among the key factors influencing flight performance due to their important functions in energy metabolism and aerobic respiration (Box 1; Eanes *et al.* 2006; Niitepõld *et al.* 2009; Wheat *et al.* 2011; Marden *et al.* 2012). That these three pathways were among the most significantly overrepresented gene groups within the 755 flight-responsive genes provides strong evidence for their central roles in insect flight. In each of the three pathways, there was a striking bimodality of genes with low versus high basal expression level (expression without the flight treatment), and these genes showed different patterns of expression changes in response to flight (Figure 5). For instance, low-expression hypoxia genes exhibited, on average, fast up-regulation after flight activity, but returned relatively rapidly back to the basal level, suggesting that these genes respond to momentary incidences of oxygen shortage in the cells, such as that caused by markedly elevated aerobic respiration levels during flight (Suarez 2000). In contrast, the response was more variable and on average longer-lasting in those hypoxia genes with basally high expression.

Comparisons of gene expression changes in the hypoxia, glycolysis and TCA cycle genes also demonstrated significant differences in flight-induced responses between the two sexes (Figure 5). We attempted to link these expression differences to differences between the sexes in flight metabolic rate (males have significantly higher mass-specific FMR than females). For instance, there was a very clear difference in the gene expression response to flight between female and male butterflies in the glycolysis and TCA cycle genes, especially in the genes with a high basal expression level. In fact, utilizing the recently constructed genome-wide linkage map of the Glanville fritillary (Rastas *et al.* 2013), we found that the first and rate-limiting enzyme of the TCA cycle, citrate synthase (*CS*), which plays a key role in regulating energy production (Cheng *et al.* 2009), was located in the sex chromosome (Z). As males have two Z chromosomes but females have only one, and as there are no dosage compensation mechanisms in Lepidoptera (Mank 2009), one could expect that males have roughly two-fold higher expression in Z chromosome genes. This was observed for *CS* expression in the present experiment. In previous studies, the levels of *CS* expression and enzymatic activity have been shown to increase with muscle exercise (Leek *et al.* 2001). Thus the on average higher mass-specific rates of flight metabolism in males compared to females could be partly explained by higher expression of *CS*. *CS* is located in the Z chromosome also in other Lepidoptera (*Bombyx mori* and *Heliconius melpomene*), which suggests that *CS* expression may be an important factor contributing to sex differences in flight performance in butterflies and moths in general.

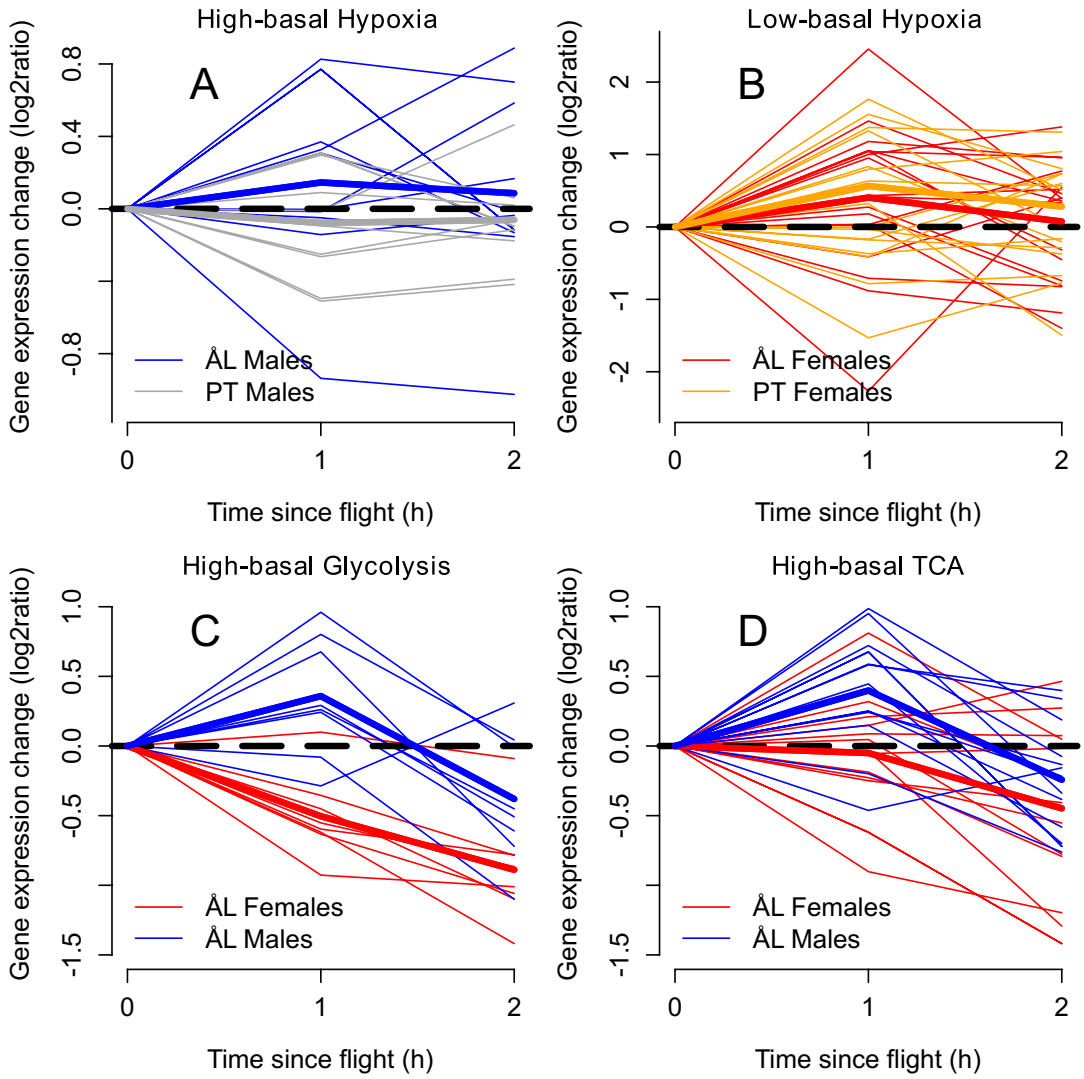

Figure 5. Examples of flight-induced changes in gene expression at 1 h and 20 h after the flight treatment (Chapter II). Results are shown for hypoxia, glycolysis and TCA cycle genes with high or low basal expression level. The values are gene averages within population-sex groups (Åland females: red, Åland males: blue, PT females: orange, PT males: grey); averages of all genes are shown as thick lines. Panel A shows expression changes in hypoxia genes with a high basal expression level in Åland (blue) and PT (grey) males. Panel B shows expression changes in hypoxia genes with a low basal expression level in Åland (red) and PT (orange) females. Panels C and D show expression changes in glycolysis (C) and TCA cycle (D) genes with a high basal expression level in females (red) and males (blue) from Åland.

The two populations, which represented "good flyers" (Åland) and "poor flyers" (PT; Chapter **IV**), showed marked differences in their gene expression responses to flight (Figure 5). Many groups of metabolism genes, including TCA cycle and oxygen transport genes showed substantially higher expression in Åland compared to PT, including the expression of *CS*. This implies that the expression of *CS* has consequences extending beyond the sex difference, and that overall, the expression of *CS* may be a key regulator of flight metabolic rate. In contrast, stress and immune response genes showed higher expression in PT, indicating that butterflies originating from this population may be more susceptible to stress or oxygen damage in response to intensive flight, which may help to explain their

significantly reduced flight metabolic rate and performance (Wheat *et al.* 2011; Marden *et al.* 2013, Chapters **II** and **IV**, see also Sections 4.4. and 4.5.).

Finally, one aim of the gene expression experiment was to link flight-induced changes in gene expression to flight metabolic rate to assess whether flight and dispersal capacity could be predicted from variation in gene expression. Indeed, we found that differences in flight-induced expression changes were associated with differences in flight metabolic rate between the two sexes and the two populations, suggesting that similar underlying mechanisms may influence both gender and population differences in flight performance. For instance, the male butterflies originating from Åland had the highest average rate of mass-specific flight metabolic rate and also the strongest up-regulation of the flight-responsive genes, including the genes related to glycolysis, TCA cycle and the response to hypoxia. This resulted in a significant correlation between mass-corrected flight metabolic rate and flight-induced gene expression change: the higher the FMR, the more these genes were up-regulated (Fig. 6). However, one needs to observe that the correlation is mostly driven by differences between the sexes and the two populations, whereas gene expression and flight metabolism were not correlated within the population-sex groups. Inferring causality is also complicated by the fact that the correlation is between flight metabolism and metabolism-induced gene expression. It is thus not possible to conclude whether the positive correlation actually explains the differences in flight capacity. Considering the evidence for the key role of these gene groups in influencing flight capacity, such a correlation could be well-explained biologically.

4.3. FLIGHT AND TEMPERATURE – LINKING INDIVIDUAL VARIATION IN THERMAL FLIGHT PERFORMANCE WITH GENETICS AND PHYSIOLOGY (I, III)

It is important to understand how the genetic and physiological basis of flight influences variation in flight capacity, but equally important is to increase knowledge of how individuals differ in their responses to varying environmental conditions, and how this is reflected in their flight and dispersal capacity. The need to predict the consequences of climate change makes the influence of temperature of special interest. Butterfly flight is an extremely energy-demanding activity (Dudley 2000) and it requires high muscle temperatures (Box 1; Heinrich 1993). The flight of butterflies, especially those inhabiting temperate environments, is thus strongly affected by thermoregulation. A previous study on the Glanville fritillary showed that butterflies with the *Pgi_111 AC* genotype associated with high flight metabolic rate had on average higher body temperature when caught in flight in low ambient temperatures (Saastamoinen and Hanski 2008). This observation indicates the existence of significant variation among individuals in thermal flight performance, which may interact with flight metabolism.

In the experiment recording butterfly body temperature at take-off and after a known duration of flight, I found that cooling of body temperature was negatively correlated with flight metabolism in male butterflies (Fig. 7; Chapter **III**). In other words, males with lower than average FMR cooled down about 1.5 times faster than those with high FMR. Thus, during 30 seconds of flight, males with low FMR cooled on average 8.7°C, whereas males with high FMR cooled on average only 5.7°C. Such a magnitude of difference in cooling within a single short flight bout can be expected to have significant consequences in the life-time of a butterfly, during which it performs thousands of such flight bouts (see Ovaskainen *et al.* 2008). In variable temperate environments, the ability to fly in low ambient temperatures can yield significant fitness advantages, as more time for flight allows more time for e.g. reproduction and dispersal (Kingsolver 1983). Butterflies have mostly been considered to be ectothermic, i.e. their body temperature is governed by ambient air temperature and solar radiation. In large butterflies, heat produced by flight metabolism has been shown to facilitate flight even in low temperatures (Heinrich 1986a), but such internal heat production has been considered to have a negligible effect on flight in small butterflies (Shreeve 1984; Heinrich 1986b; Wickman 2009). The present result indicates that this assumption may not hold. At the same time, this result is possibly the first to link within-species variation in flight metabolism to flight thermal dynamics, and demonstrate the significance

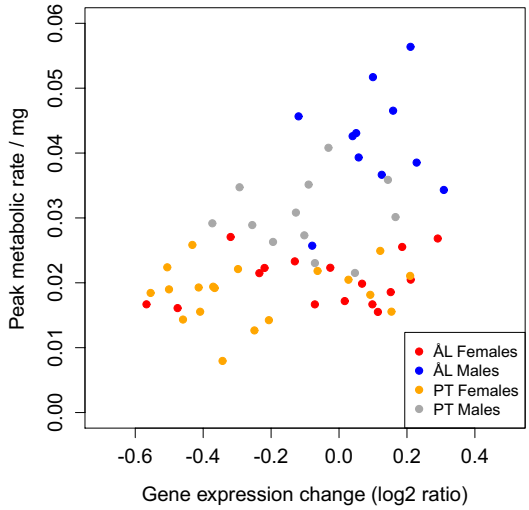

Figure 6. Correlation between gene expression change induced by flight and mass-corrected peak flight metabolic rate (P = 0.002; Chapter **II**). Gene expression change for each individual is an average over hypoxia genes with high basal expression level in the two sexes and populations.

of such variation to butterfly movements in natural conditions.

Another finding of Chapter **III** indicated that *heat shock protein* (*Hsp70*) genotype is associated with butterfly body temperature at take-off. Considering that the function of *Hsp70* is related to protecting against cellular damage, especially such caused by exposure to extreme temperatures (Sorensen *et al.* 2003), the result suggests that tolerance of high temperatures may be important for flight performance. Indeed, previous studies indicate that basking butterflies have a risk of overheating, which can result in reduced survival and fecundity (Rawlins 1980; Kingsolver and Watt 1983). For example, *Colias* butterflies avoid further heating when their body temperature exceeds 40-42°C (Kingsolver and Watt 1983). Here, the thoraces of basking butterflies reached surface temperatures as high as 40.5°C, which is likely to be close to the upper thermal tolerance limit. The association of *Hsp70* genotype with take-off body temperature was found in the same *Hsp70* SNP which was also related to flight metabolism in males (see Section 4.1.), so that the genotypes with elevated FMR took off with a lower body temperature on average. Therefore, individuals that have an advantage in low ambient temperatures can be susceptible to overheating at higher temperatures, where thermally more tolerant butterflies perform better (see also Niitepõld 2010).

The factors affecting body temperature dynamics during flight differed markedly in female and male butterflies. For instance, body temperature cooling during flight was not affected by flight metabolism in females, but was instead largely governed by environmental conditions. The strongest effect on take-off T_b in males came from environmental variation, while in females there were no significant environmental effects. These differences can be related to behavioral, morphological and physiological differences between the sexes. Firstly, the functions of flight, which influence the motivation to fly, are very different for females and males: mated females fly to find a suitable location for laying eggs, whereas males fly to keep a territory and to find mates (see Section 4.5. and Chapter **V**). Secondly, the body size

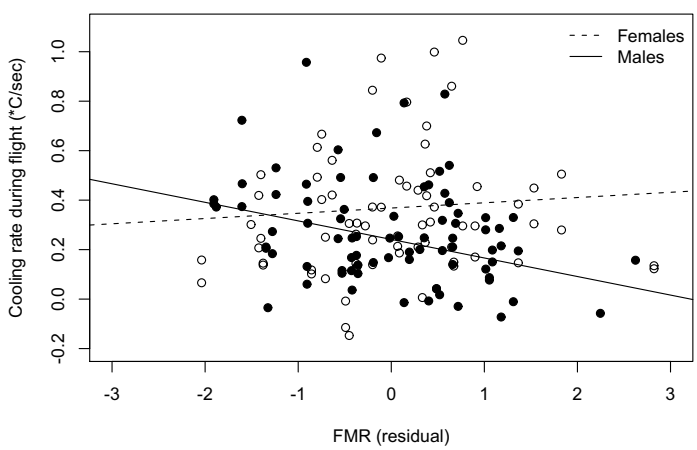

Figure 7. Thorax cooling rate (Δ/sec; $P_{females}$ = 0.552, $R^2_{females}$=0, P_{males} = 0.005, R^2_{males} = 0.080) in relation to flight metabolic rate (Chapter **III**). Cooling rate is calculated as Δ (take-off body temperature – flight body temperature) divided by flight duration (sec), and FMR is the residual from a linear model of FMR against adult body mass. Results are shown for females (open circles, dotted line) and males (black circles, black line).

and allocation of resources to body parts differ greatly between the sexes. Females are significantly heavier than males (here 75% heavier) and they allocate most of their mass to reproductive functions in the abdomen rather than to flight muscles in the thorax (see also Saastamoinen *et al.* 2009). Thirdly, males have significantly higher FMR per unit of body mass than females (here 79% higher). It is thus possible that the heating of flight muscles caused by FMR is greater in males, and this may override the effect of faster convective cooling due to their greater surface-to-area ratio (Gilchrist 1990). Females, on the other hand, may be more constrained by environmental conditions also because they need to acquire higher body temperature for flight (Heinrich 1974; Pivnick and McNeil 1986; Gilchrist 1990).

Previous studies on the Glanville fritillary have demonstrated marked consequences of ambient temperatures experienced during larval development for life-history traits and fitness (Kallioniemi and Hanski 2011; Kvist *et al.* 2013). Here, the results of Chapter **I** showed that butterflies reared as larvae in stressfully high temperatures had significantly reduced flight metabolic rate. Considering the effect of FMR for flight thermal dynamics (Chapter **III**), the result suggests that thermal conditions experienced during larval development can have consequences for adult flight metabolism, and thus may not only influence the dispersal capacity of adult butterflies but also their performance in varying thermal conditions. Furthermore, challenging environmental conditions may constrain the evolution of traits such as dispersal capacity, if environmental variation overrides additive genetic effects. Such an effect seems to be another consequence of the exposure of larvae to high temperatures during development: significant heritability of flight metabolic rate was only found in standard temperature conditions, whereas variation in flight metabolism was dominated by environmental effects in butterflies reared in stressfully high temperatures (Chapter **I**, Section 4.1.). Variation in environmental temperatures can therefore have far-reaching consequences over life stages and even generations, which may additionally differ greatly between the sexes. Consideration of the mechanistic details of variation in thermal performance will increase our understanding of the impacts of climate change on dispersal and its evolution (Helmuth *et al.* 2005).

4.4. CONSEQUENCES OF A COMPLETE LACK OF GENE FLOW TO A SMALL POPULATION (IV, II)

So far, I have discussed individual-level variation in flight and dispersal capacity, its genetic basis and heritability, as well as the effects of variation in environmental conditions on butterfly flight. Because of the central role of dispersal to the persistence of populations, these topics are relevant for understanding dispersal and its evolution. In this section, I take a population-level view, and discuss a case study which demonstrates reasons why gene flow created by dispersal between populations can be imperative for the viability of small populations. In addition to the demographic and environmental stochastic factors which threaten such populations, small and extremely isolated populations can be faced with genetic threats, such as inbreeding depression, high genetic load and loss of genetic diversity (Section 1.1.). The relative importance of these different forces in small and isolated natural populations is beginning to draw more attention (e.g. Paland and Schmid 2003; Luquet *et al.* 2011; Willi *et al.* 2013), but such studies continue to be relatively rare (Lopez *et al.* 2009).

A small butterfly population ($N_e \approx 100$) discovered inhabiting a very small island, the island of Pikku Tytärsaari (PT), in the middle of the Gulf of Finland in the Baltic Sea serves here as a rare "natural experiment" of the genetic and fitness consequences of long-term (~75-100 years) complete population isolation (Section 3.1., Chapter **IV**). We used the large metapopulation in Åland as a reference, and compared life-history and measures of fitness in the two populations in 58 traits related to behavior, development, morphology, metabolism, reproductive performance, and survival in common garden experiments in the laboratory and under semi-natural conditions in a large outdoor cage. Butterflies from the PT population showed inferior performance in the majority of the traits studied. For instance, reproductive fitness was substantially reduced, as lifetime production of larvae was only 31 % in PT females compared with females from Åland. Also, flight capacity was compromised in PT, with flight metabolic rate showing a reduction by about 50% compared with Åland (Figs. 8-9; Chapters **IV**, **II**). In contrast, resting metabolic rate

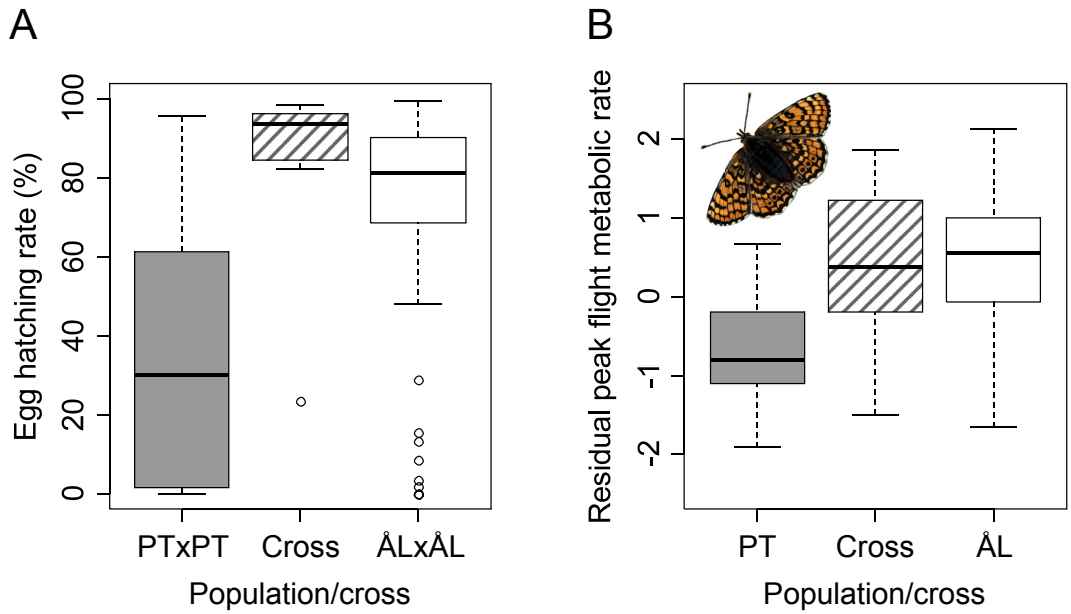

Figure 8. Genetic load and heterosis in the small isolated population (PT) of the Glanville fritillary butterfly (Chapter **IV**). **A**. Egg-hatching rate (%) in PT (gray) and Åland (white) and in crosses between PT females and males from elsewhere (hatched gray lines). **B**. Peak flight metabolic rate (residual) in field-collected PT (gray) and Åland (white) females and in the female offspring of crosses between PT females and males from elsewhere (hatched gray lines). These results show instant and complete fitness recovery in the crosses. (Figure © Mattila A L K *et al.* PNAS 2012; 109: E2496-E2505, URL: http://www.pnas.org.)

was significantly elevated in PT males, implying an increased energetic demand for maintenance functions and possibly related to the observed shorter lifespan of PT males (Nilsson 2002; Hulbert *et al.* 2007). Examination of gene expression patterns in PT and Åland revealed extensive differences between the two populations (Chapter **II**), which suggested that butterflies in PT have less efficient energy metabolism and that they may be more susceptible to stress and oxygen damage in response to intensive flight, which may contribute to their significantly reduced flight metabolic rate and performance (Wheat *et al.* 2011; Marden *et al.* 2013).

The great fitness reductions in PT are best explained by a high load of deleterious mutations drifted to high frequency or fixation. This is supported by the result showing that the PT population had substantially reduced neutral genetic variation compared to two large reference populations (Åland and a continuous population in Saaremaa, Estonia), consistent with large-scale fixation of alleles by genetic drift (Lande 1994; Whitlock *et al.* 2000; Frankham 2005). Also, one generation of full-sib mating had little or no effect on most traits in the PT population, while in the Åland population this resulted in significant inbreeding depression. The reduction in inbreeding depression in the PT population is another sign of increased homozygosity, as fixed loci show no inbreeding depression (Whitlock 2002). Part of the most deleterious mutations causing inbreeding depression may also have been purged from PT (Hedrick 1994; Keller and Waller 2002; Glemin *et al.* 2003). Finally, the best evidence supporting the claim that the great reduction of fitness in the PT population is caused mainly by high genetic load comes from crossing experiments, where butterflies from PT were crossed with butterflies from elsewhere. These hybrid offspring showed instant and complete fitness recovery in the two traits studied, egg viability and flight metabolic rate (Fig. 8). Such a fitness increase in hybrid offspring is caused by heterosis (hybrid vigor), which is the result of the masking of the deleterious effects of recessive alleles when brought into heterozygous state (Whitlock *et al.* 2000). Heterosis is also the mechanism of genetic rescue following gene flow to

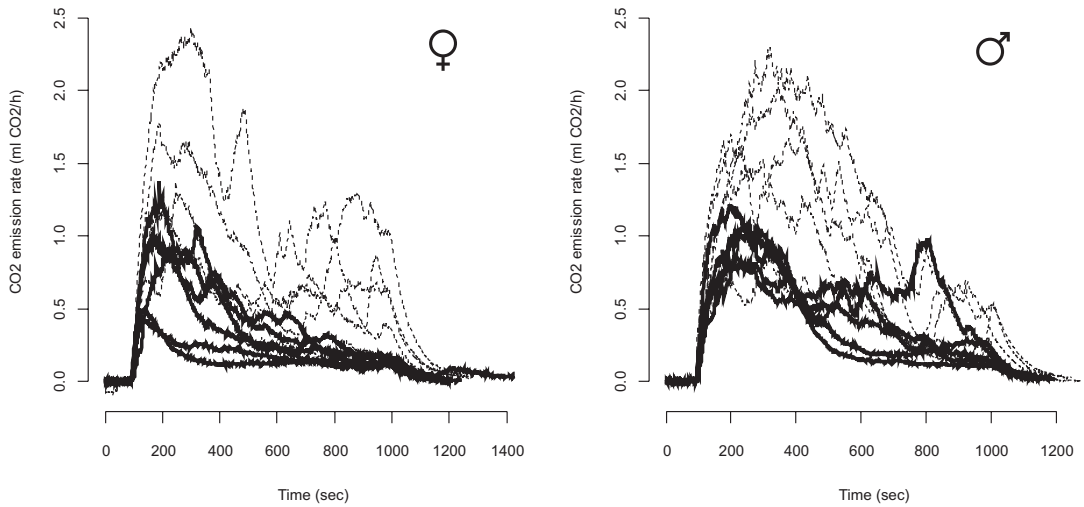

Figure 9. Examples of CO_2 output (ml CO_2/h) during 15 min of flight in females (A) and males (B) from the **Åland** (thin dashed lines) and PT (thick solid lines) populations (Chapter **IV**). (Figure © Mattila A L K *et al.* PNAS 2012; 109: E2496-E2505, URL: http://www.pnas.org.)

an isolated population (e.g. Westemeier *et al.* 1998; Vila *et al.* 2003; Madsen *et al.* 2004; Hedrick and Fredrickson 2010), and is strong evidence of high fixation load (Whitlock *et al.* 2000).

There are other studies reporting reduced fitness and genetic deterioration in small, isolated populations (e.g. Madsen *et al.* 1999; Rowe and Beebee 2003; Puurtinen *et al.* 2004; Kawamura 2005; Gelatt *et al.* 2010; Clark *et al.* 2011; Luquet *et al.* 2011). Our results on the Glanville fritillary represent one of the greatest recorded fitness declines for a natural population. One possible reason for this is that we investigated a comprehensive set of traits over the entire life cycle, compared to previous studies which typically studied only a few fitness components, which can lead to an underestimate of the true fitness effects (Szulkin *et al.* 2007). The substantial fitness decline reported here for a population with knowledge of its population history, as well as the available evidence of the mechanism of the fitness decline (fixation load), are in agreement with models that have predicted a high risk of extinction due to mutational meltdown within an order of 100 generations in populations with an effective size smaller than 100 individuals (Lynch *et al.* 1995a; see also Frankham *et al.* 2014). Our results thus indicate that the PT population exemplifies populations with a high risk of extinction due to fitness decline caused by high genetic load, an increasingly common situation in fragmented landscapes.

4.5. FLIGHT CAPACITY AND DISPERSAL IN FRAGMENTED AND ISOLATED POPULATIONS (II, IV, V)

In the absence of disruptive gene flow, population isolation can facilitate local adaptation (Section 1.2.; Slatkin 1985; Edmands 2007; Lopez *et al.* 2009). If successful dispersal away from the population has become very unlikely or impossible, reduced dispersal rate is expected to be selected for (Carlquist 1966; Cody and Overton 1996; Ronce 2007). In the case of the Glanville fritillary population on the PT island, we found no clear signals of local adaptation in dispersal-related or other traits. Flight metabolic rate was greatly reduced in butterflies from PT (Fig. 9, Chapters **IV**, **II**), indicating reduced dispersal capacity. However, the reduction in flight metabolism is best explained as another consequence of high genetic load (Section 4.4.). This claim is strongly supported by the finding that flight metabolic rate showed complete and immediate recovery in PT hybrids, i.e. heterosis (Chapter **IV**). The gene expression differences between the populations (Chapter **II**) suggested that PT butterflies are prone to cellular stress and inefficient energy metabolism, explaining partly the reduced flight metabolism

but probably also contributing to the reductions observed in other fitness proxy traits. This is not likely to be adaptive in any population, regardless of isolation. Additionally, reduction in dispersal capacity was not supported by measures of flight morphology: thorax mass was on average smaller in PT, but PT females had a higher wing aspect ratio (associated with fast flight; Berwaerts *et al.* 2002) and smaller wing loading (generally associated with higher capacity for sustained flight; Berwaerts *et al.* 2002). Flight activity was reduced especially in PT males, but this can hardly be considered adaptive, as reduced flight activity of males can greatly decrease their mating success (Watt *et al.* 1996; De Block and Stoks 2007). In fact, male mating success was significantly compromised in PT.

The seeming lack of local adaptation in dispersal rate, which is likely to be selected against in a completely isolated population such as PT, can probably be explained by the dominance of random genetic drift over natural selection and the fact that butterflies still need to fly for other reasons than dispersal in the PT population. Drift has resulted in high genetic load and markedly reduced genetic variation in the PT population (Chapter **IV**), which is likely to hinder adaptation (Amos and Balmford 2001; Frankham 2005; Swindell and Bouzat 2005). Nonetheless, a subsequent study demonstrated that given strong enough selection, local adaptation is possible even in a genetically deteriorated population such as PT (Duplouy and Hanski 2013). Given its location in the middle of the Baltic Sea and its low topography, the PT island is highly exposed to strong sea winds. Butterflies therefore have a high risk of being unwillingly blown off to the sea with dire consequences. Duplouy & Hanski (2013) found that butterflies originating from PT were significantly better able to hold on to substrate because they had evolved more curved claws in their legs. Thus, the PT population may represent another example of selection on reduced emigration rate from isolated populations (Cody and Overton 1996; Ronce 2007), although the mechanism is not reduced flight capacity but enhanced ability to avoid accidental emigration. Such a mechanism is plausible in butterflies for which losing flight capacity would be detrimental and thus not likely to be selected for even in a completely isolated population.

Finally, turning to dispersal of the Glanville fritillary at the metapopulation scale, we studied the effects of flight capacity on realized dispersal between local populations in a natural habitat patch network (Chapter **V**). Here, we used flight metabolic rate, *Pgi* genotype and endurance of tethered flight as measures of flight capacity. Pooling the results for these different measures, flight capacity was found to have opposite associations with dispersal rate in the two sexes: females with high flight capacity moved more between habitat patches, whereas males showing superior flight capacity were less likely to disperse. These contrasting results between the sexes are likely to be explained by the different roles of flight in females and males. Previous studies have shown that female mobility can be predicted from population demography and landscape structure, as females from newly-established and isolated populations are particularly mobile (Hanski *et al.* 2004; Saastamoinen 2007a; Ovaskainen *et al.* 2008), and more likely to be of the *Pgi* genotype associated with high dispersal capacity (Haag *et al.* 2005). This is because females with high flight capacity are expected to be the ones that most frequently establish new populations. In contrast, the studies have failed to detect similar associations in males (Hanski 2004; Hanski *et al.* 2006). Indeed, the mobility of males is more likely to be related to the mating system (Merckx and Van Dyck 2005). Male butterflies usually adopt either a perching (territorial) strategy, or a patrolling strategy (employing within-population searching flights) to acquire mates (Scott 1974), and these alternative strategies have been shown to be reflected in e.g. morphology and thermoregulation in other butterfly species (Van Dyck *et al.* 1997; Van Dyck and Matthysen 1998). Successful territorial males appear to be adapted to perform fast and agile but short flight bouts (Berwaerts *et al.* 2008). Therefore, males that have inferior ability to keep a territory may be forced to disperse elsewhere to find mates (Bergman and Wiklund 2009), resulting in the inverse relationship between male flight performance and realized dispersal.

These contrasting effects of flight capacity on dispersal in females and males can result in patterns of dispersal evolution in fragmented populations deviating from those expected given consistent effects in the two sexes. Considering that flight metabolic rate is significantly heritable (Chapter

I), an especially dispersive mother will produce especially dispersive female offspring, but also male offspring that are especially unlikely to disperse. This could enable the persistence of dispersive genes in a local population following establishment. However, previous studies show that the average mobility of females decreases within a few years following population establishment (Hanski *et al.* 2004). This has been explained by the loss of the most dispersive females from the population through emigration (Hanski and Saccheri 2006; Zheng *et al.* 2009). The present results suggest that another contributing factor to this decline in average female mobility could be the immigration of males that carry genes which are expressed as a sedentary phenotype in females. At the same time, this example highlights how behavioral differences in the sexes can contribute to maintenance of variation in dispersal rates in metapopulations. Other important factors maintaining variation in dispersal include spatially varying selection pressures and interactions with thermal conditions (which may further differ between the sexes; Chapter **III**), also the main factors maintaining variation in the *Pgi* gene (Hanski and Mononen 2011). These results highlight the importance of studying dispersal in both sexes to gain a better understanding of the evolution of dispersal in fragmented populations.

5. CONCLUSIONS AND PROSPECTS

In this thesis, I have investigated flight capacity and dispersal in the Glanville fritillary butterfly from multiple perspectives, building upon the vast knowledge available for this ecological model species. The causes, consequences, mechanisms and evolution of dispersal is a timely topic due to the key role of dispersal for the persistence of populations in the face of habitat fragmentation and climate change. I have approached these questions from the gene to the population and the metapopulation levels, attempting to contribute to the understanding of the molecular and physiological basis of intraspecific variation in flight and dispersal capacity, as well as to our knowledge of how dispersal rate may evolve in response to population isolation, habitat fragmentation and changes in environmental conditions. A completely isolated small population of the Glanville fritillary served as a natural experiment demonstrating the detrimental genetic and fitness consequences of long-term complete lack of gene flow. A multilevel approach and empirical knowledge from a range of different model species will contribute to developing a unified cross-species framework for the ecology and evolution of dispersal (Nathan *et al.* 2008). Many of the processes that influence dispersal in the Glanville fritillary are also likely to be applicable to many other species of insects and invertebrates.

As could be readily expected based on the complexity of dispersal as a trait, the results of this thesis show that the genetic basis of flight and dispersal capacity extends greatly beyond limited single gene effects, such as those of the *Pgi* genotype. Associations of flight metabolic rate with genes of very different functions and part of various cellular processes highlight this complexity. The present studies add to evidence showing that sugar metabolism, aerobic respiration, stress responses, the immune system and susceptibility to hypoxia all play significant roles in defining flight capacity of the Glanville fritillary butterfly. Both allelic variation and variation in gene expression patterns were demonstrated to be important. The results indicate that differences in flight capacity between the sexes and populations may at least to some extent be predictable from levels of flight-induced changes in gene expression in major metabolic pathways. Furthermore, flight metabolic rate, an important proxy of dispersal rate, was shown to have a significant heritable genetic basis, demonstrating that flight metabolic rate has the potential to respond to selection on dispersal capacity. It remains a challenge for future studies to find out how much of the extensive variation in the expression of the flight-responsive genes contributes to this heritability. The gene groups here identified can also be utilized for assessing the relative importance of different genes and cellular functions for flight capacity using experimental approaches. The fully sequenced genome of the Glanville fritillary (Ahola *et al.*, unpublished) will facilitate association studies with extensive scans of large numbers of SNPs, which are likely to elucidate further the genetic basis of flight and dispersal.

Because of changing climate, gaining knowledge on the effects of temperature on movement and dispersal of different species is imperative. Temperature has

been known to be a major factor influencing the flight activity of ectothermic butterflies especially in temperate climates. In this thesis, I show that the rate of metabolism during flight can have significant but sex-specific consequences for the thermal performance of individual butterflies. Male butterflies with high flight metabolic rate were shown to have an advantage in low ambient temperatures because their body temperature cooled down at a lower rate during flight. Although such an effect is known from among-species comparisons, the present study is possibly the first to demonstrate the significance of intraspecific variation in flight metabolism on flight performance in relation to thermal conditions. The results also suggest that individuals having an advantage in low ambient temperatures can be susceptible to overheating at higher temperatures, and that high temperatures experienced during earlier developmental stages can have deleterious consequences for adult flight capacity. Thus, in the future, selection may favor individuals with tolerance of high temperatures over individuals with overall higher flight capacity. Evolution of dispersal may become constrained in stressful thermal conditions if environmentally induced variation overrides heritable genetic effects, as was suggested by the present heritability estimates in different thermal regimes. These results highlight mechanisms by which changes in climatic conditions are likely to significantly influence the evolution of butterfly flight and dispersal capacity in the future.

In the case where a population becomes completely isolated with no possibility of successful dispersal, reduction in dispersal rate is expected to be selected for. Investigating flight capacity from the molecular level to the physiology and morphology of individuals originating from a small isolated island in the Baltic Sea, we found no clear adaptive reduction of dispersal rate. This may be explained by the fact that flight is crucial for practically all activities during the adult life of a butterfly, and hence flight capacity is unlikely to be strongly selected against even in completely isolated populations. A follow-up study suggested that reduced probability of accidental emigration from a small windy island can be under strong selection, and local adaptation may hence take place even in genetically compromised populations (Duplouy and Hanski 2013). These studies reflect the relative importance of natural selection and genetic drift on the evolution of dispersal in small and isolated populations. At the level of population networks (metapopulations), flight capacity was found to have contrasting effects on realized dispersal between habitat patches in the two sexes. Thus, females with high flight capacity were more dispersive, but the opposite was true for males. This result highlights a common theme throughout the thesis: females and males differ greatly in flight- and dispersal-related traits at the molecular, physiological and morphological levels, and in the way in which they respond to environmental conditions, which is reflected in their dissimilar dispersal behaviors. Therefore, selection can be expected to act differently in the two sexes. To understand the evolution of dispersal, these differences need to be accounted for.

Finally, the crucial importance of dispersal as a mechanism maintaining gene flow between populations is demonstrated by the studies on the consequences of long-term complete isolation in the small island population of the Glanville fritillary. The results showing significant loss of genetic diversity, substantially reduced fitness and its complete and instant recovery in hybrids (heterosis) present strong evidence for reduced population viability due to a high load of deleterious mutations that have become fixed or increased to high frequency. This butterfly population serves as an example of the increasingly common situation in human-fragmented landscapes, in which small and completely isolated populations may become more vulnerable to extinction due to high genetic load. This risk should be acknowledged as a particularly serious concern for conservation as genetic load may erode population viability in an irreversible way. Maintaining gene flow between small populations is imperative for their long-term persistence.

6. ACKNOWLEDGEMENTS

When I found myself in an interview for a LUOVA graduate school position straight from a year of changing diapers and talking baby talk, I´m not sure I knew what I was getting myself into. Yet, here I am now, enriched with so many unforgettable experiences, illustrating my nearly finished PhD thesis with butterfly drawings of my already 5-year old daughter. I have been able to realize my

dream of doing exciting science with the ultimate goal of making a difference, however small, for helping to preserve biodiversity, and I wouldn´t change a day of it. The only part still missing is this acknowledgements section, and yes, do I have great many people to thank. They are people without whom this work would not have been possible.

The first person to thank is, without question, my supervisor Ilkka Hanski. Thank you for giving me this unparalleled opportunity to test my wings (along with those of butterflies) in science. You have been an incredible supervisor. People often ask me: "does he have any time for a mere PhD student, with directing a group of 70 researchers, writing books, publishing a list of papers each year, and traveling the world giving invited speeches, collecting awards and dung beetles alike?" And I don´t know how he does it, but yes, he has been the most dedicated, reachable supervisor. The familiar sound of your wooden clogs on the University hallway tell me you are in your office to be found, and hardly ever have you had to turn me away from your door. Otherwise, I could count on speedy replies to e-mails be it evening, weekend, summer cottage or field work in the jungles of Madagascar. The combination of reachability and the space to let me begin to develop into an independent researcher has suited me better than well. Receiving edits (usually within hours!) for manuscripts has always resulted in moments of epiphany. I am overwhelmingly thankful for all I have learned from you, not just about the technicalities of scientific work, but from your whole outlook on science: the way you find interesting questions in everything, and are not afraid to think outside the box or to pursue something completely novel, and how you see it all as a great adventure. You have inspired me tremendously.

I am deeply indebted to all my coauthors for your essential contributions to this thesis. I have been lucky to be a part of both large and smaller teams of collaborators including people from a variety of different backgrounds. This has been often fun, sometimes challenging, and always a great learning process. In the end, I think everything has come together quite nicely. Of the coauthors, I would especially like to mention Phil Harrison, who was the dedicated supervisor of my master´s work, and who has played a significant role in encouraging me to pursue a PhD. The success of my experimental work was greatly influenced by Kristjan Niitepõld and everything he taught me about insect respirometry. To this day, I receive "respirometry news" from Kristjan, when he has come up with some cool trick related to butterfly shaking. Besides, the work of this thesis is based on many of the findings of his own PhD in MRG (metapopulation research group). Anne Duplouy, you have been a great fellow butterfly lady, role model and friend. Thank you also for asking me to co-coordinate the CBFL-course, which was a good experience. Continue to dance away! I thank Rainer Lehtonen, the only restaurant geneticist in the world, for his rocking attitude towards science, the legendary MRG song (yes, it is in YouTube) and encouragement. Pasi Rastas, Panu Somervuo and Virpi Ahola have been pleasant company both to work with and talk to at coffee break. Malla Kirjokangas did an amazing job with essential PT experiments, and offered her friendly help with many practical matters. I admire Jouni Kvist for his versatile knowledge on molecular mechanisms, which was imperative for making sense out of the mess of RNA-seq results. I commend the *M. cinxia* genome team for nearly being there.

I would like to sincerely thank Jon Bridle for taking the time to read my thesis and for coming all the way to Finland to be my opponent. I have great faith that it will be a good experience for us both. I am most grateful to Juha Merilä and Craig Primmer for their thorough examination of this thesis, and for the insightful comments that have greatly improved it. Lotta Sundström has been both a part of my thesis advisory group and now my custos, and I am very thankful of all the invaluable advice you have given, and for making sure that I have all the doctoral degree pieces together as they should be. Kristian Donner, also a part of the advisory group, deserves my warmest gratitude for your calm and friendly encouragement. Veijo Kaitala is acknowledged for the smooth running of the department, and the patient advice on how to finish up this degree. I thank Anni Tonteri, the coordinator of LUOVA graduate school, for your excellent work in arranging courses and workshops, and for being available to answer any questions about the practicalities of doing a PhD. You have also been a most friendly and compassionate cheerleader.

In addition to my official supervisor, I have had an unofficial one in Marjo Saastamoinen. Marjo has been the one to ask about many practical matters starting from planning of experimental design to which conferences to attend and who to talk to there. She has commented on many of my manuscripts, posters and practice talks. Marjo, I am deeply grateful to you for being a mentor, role model and confidant. You have always been willing to listen to my ideas, problems and worries, always willing to help. Thank you also for introducing me to many friends and colleagues during the conference trips we made together. Thank you for your unequalled encouragement and belief in me. I´m looking forward to possibly some more collaboration in the future. Helena W. and Katja B. have also encouraged me greatly in pursuing a career in science. Your support means a lot to me.

This work would not have been possible without the many research assistants that contributed tremendously to the tedious practical tasks that have been crucial in collecting all of the data. Suvi Ikonen, the research director of the butterfly lab, I don´t have enough words to tell you how important you have been, both in keeping the ensemble of different experiments and rearings at Lammi running and organized, and for being a great friend. It has always been fun to come to the butterfly hall. I sincerely thank Coong Lo, Tuomas Kankaanpää, Linda Peltola, Alma Oksanen, Elli Lappalainen, Mari Kekkonen, Pave Väisänen, Juha Kallio, Annukka Ruokolainen, Toshka Nyman, Suvi Saarnio, Swee Wong, Pia Välitalo and others for your much-appreciated work in the experiments, in the field and in the molecular ecology lab. Elena Glazkova is thanked for sailing to the PT island to collect the very important samples. Evgeniy Meyke has always found the correct data for me from the huge cinxia database. I thank you all from the bottom of my heart. I´m also deeply grateful for the great working and living environment at the Lammi biological station. I particularly thank the director of the station, Janne Sundell, for allowing me and my family, including the dog, to stay in the cabin accommodation during my visits. It was like having a little summer cabin holiday.

I cannot imagine a better place for doing a PhD than the Metapopulation research group. I have gotten so much energy and perspective from the inspiring, talented, dedicated, supportive, international and fun group of people that makes up MRG. Thank you again, Ilkka, for building and keeping together this wonderland of the science world. Working together with a mix of people from such diverse backgrounds (both culturally and scientifically) has been a great richness. Other advantages of this diversity are that there has always been someone to ask about almost any matter, and it has given an opportunity to keep track of the state-of-the-art in a wider field of environmental sciences. The weekly MRG seminars, "obligatory" coffee breaks and lunchtimes play a great role in keeping this rapidly growing group coherent. Not to mention the annual meetings which have been memorable. Thank you also to Ilkka and Eeva for welcoming this huge group of people to your home for the end-of-summer party each year. I am extremely grateful to everyone in the group for creating this truly exceptional working environment. My special thanks go to Viia and Sami in the office for doing such an amazing job taking care of the bureaucracy and practical matters. Your contributions have been essential. Sami is acknowledged also for making the expert layout of this thesis.

I have made many life-long friends during my time in MRG. Combining motherhood of small children and a PhD is possibly not the easiest task in the world. I couldn´t have made it through without the office 5618 fellow PhD student moms. Johanna, Hanna and Aija, I cannot express how much your support has meant to me. After a week of chicken pox, or a morning of dressing ten layers on a protesting child, ramming the strollers through knee-high piles of snow, going through the heartache of leaving behind a crying child in daycare, only to miss the next bus, it has been possible (each at our turn) to come to this office and offload it all to understanding ears, and then get back to being a scientist. Our children have grown alongside our theses, and conversations in the office have been a happy mix of conservation effectiveness, ArcGIS, butterflies, mildew, models in R, planning of experiments, conferences, and the new skills and undertakings of Emil, Taavetti, Annika, Seela, Otso and Mette, of Little Daughter islands and little daughter humans. Not to mention any other topics of conversation related to careers in science and life in general… Not one day has been dull in your company, and I will miss all of you terribly.

Keep up the *fresh* citrus smell! Other office mates through the years, my warmest thanks go also to you. Heini was for several years an inseparable part of the spirit of this office, who has been deeply missed after her departure to Australia. Thank you Heini for your friendly and positive vibe. Coming to the office felt like coming home, when the light was always glowing at the end of the hallway, and Heini was greeting you from her spot. Veera, thanks for letting me inherit your window spot, and for the fun times singing together. Johanna K., it was nice to have you here for that short while, your friendly ways were much appreciated. Riikka, you weren´t here for long before your maternity leave, but I wish you all the best for your PhD, for being a new parent, and their combination. You can do it! Rachel, I wish you could have kept your spot in our office for longer. Thank you so much for your support and help during the crucial stages of finalizing my work. It was also great to have Alvaro, the most recent addition to the office, as my cheerful neighbor in these very final weeks.

I want to also give my warmest thanks to other fellow PhD students, friends and colleagues in MRG and Biocenter 3. Your peer support has been invaluable. Henna, you have inspired so many of us with your perseverance. Tanjona, thank you for bringing so much spirit and character to the group. I am honoured to have the title of being your BFFFF (Best Finnish Female Friend Forever). Victoria, you have been a great friend and excellent company at lunchtime. Andreia, thank you for all your encouragement. Ayco, thank you for providing advice in data analysis issues. It was always nice to also bump into you in the coffee room. Toby, I admire your skills and outlook. You are also fun to be around, and have been an outstanding addition to our cinxia group. Wolfgang is thanked for delicious cakes, which brought a crowd into the coffee room. I thank Anna-Liisa, Anni, Mar, Saskya, Maaike and Elizabeth for setting such great examples for us beginning female scientists. I admire your courage, professionalism, expertise and skill to also make it all work in the context of life outside science. I would also like to especially mention Peter, Timo, Laura, Ulisses, Krista, Markku, Aili, Tuuli, Tommi, Silvija, Erin, Marissa, Kristina, Joona, Otso and many others for the countless lunch and coffee times, which always provided an essential break in the working day. I apologize for my slow eating habits... Heikki H. and Jenni L., you have always been nice to bump into in the hallway, and have given me you friendly support, which I am most thankful for. I have had the privilege of travelling to many conferences with amazing company. Thank you for the memorable times especially in Ottawa and Lisbon. Hanna L., you were the most fun roommate in Tuebingen. The most recent trip to the movement and dispersal conference in Aberdeen was a great experience shared with Elena and Luisa. The best of luck with your theses!

Many friends from outside the MRG bubble have been crucially important in widening my horizons, and a great source of joy. Outi and Maija, our Wednesday lunch meetings and other extra-curricular activities have meant so much to me. You have also provided me with a wider perspective on academic careers outside our department. Annika, I have greatly enjoyed meeting with you every once in a while in Viikki premises. I thank Marja, Tuija, Anne, Irina and Hansku for our long-lasting friendship, and the invigorating get-togethers we have continued to regularly arrange. Paula and Jari, after the kids were born, our weekend visits have turned a bit less relaxing, but have been definitely fun and important in taking my mind away from any work-related matters. Our neighbors Katja and Atte have rescued us many times with their help. Anni, Heli, Sini and Emmi, my beautiful and lovely cousins, you inspire me with your spirit and personalities, and our life-long friendship has been invaluable. Päivi, Arto, Leni and Hannu, thank you for your friendship and encouragement through the years. Sini, my beloved goddaughter, I´m sorry that we haven´t been able to meet very often lately. I hope that you will also be inspired to follow your dreams in life. Juha, thank you so much for the beautiful drawing (hand-drawn with pencil and marker) of *M. cinxia* on the cover of this thesis. I am most grateful to my parents-in law, Marke and Jokke for all the support and help during these years. Marke spent many many weeks in Lammi taking care of Mette so that I wouldn´t have to be away from her while doing my experiments. I thank you from the bottom of my heart.

Finally, I want to express my deepest gratitude to my parents Merja and Markku for always believing in me, encouraging me, helping me to find my way, and supporting me in everything I do. Your love has given me the wings to pursue my dreams. Iskä, I guess you

realize now what effect it had when you carried all the cool bugs for us to see, and Äiti, when you took us out on lake Ylisjärvi to see the nesting gray herons and when you let us run wild in the forests and fields: you now have three biologist daughters. Thank you for also giving me a place where I can come to be cared for and to experience Finnish nature at its best. Listening to the song of the kuikka echoing on the lake while a Saimaa seal lies on the smooth rocky shore drying its winter fur has given me great inspiration also for my work. My lovely sisters Elina and Katriina, thank you for being my soul mates, confidants and best friends, and for the encouragement especially during these last years. Katriina deserves an extra special thanks for babysitting many weeks at Lammi biological station and for being the best godmother Mette could have. It has been particularly important during these most recent times leading up to the defense. I cherish both of you sisters. Walter, my best brother-in-law, thank you also for your support. Saima has been the most cheerful little member of the family, bringing so much joy to everyone around. Paju the dog is an important part of my family, and she has many times rescued my sanity by her calm unconditional faithfulness and love and by taking me out in the forests and fields of Sipoo. Finally, I send my love to my husband Jari. We met during my first year as a biology student, and you have stood by me every step of the way. We have come far, even though Lammi has "stolen" me many times. The recent years, and especially the most recent months have not always been easy, but I always knew we would make it. Thank you for taking care of our home the last months, and for putting your arm around me each night, so that I could sleep calmly no matter how stressed I was. I will always love you.

I dedicate this thesis to my daughter Mette, who has inspired me, challenged me, and brought me to a whole new world outside work filled with fairies, soap bubbles, giggles and tears. Mette-rakas, nyt on äidin kirja valmis! Kiitos että jaksoit odottaa, ja annoit paljon haleja ja pusuja vaikka äiti olikin paljon töissä. Ja kiitos hienoista perhosista jotka piirsit. Olette isin kanssa äidille kaikkein tärkeintä maailmassa!

This work was supported by the Finnish Doctoral Programme in Wildlife Biology Research (LUOVA), the Academy of Finland and the European Research Council.

7. REFERENCES

Ahola V., R. Lehtonen, *et. al.* Glanville fritillary genome retains ancient karyotype and reveals selective chromosomal fusions in Lepidoptera. in prep.

Allendorf F. W., G. Luikart, and S. N. Aitken. 2013. Conservation and the Genetics of Populations. Wiley-Blackwell, West Sussex, UK.

Almbro M., and C. Kullberg. 2008. Impaired escape flight ability in butterflies due to low flight muscle ratio prior to hibernation. J. Exp. Biol. 211:24-28.

Almbro M., and C. Kullberg. 2012. Weight loading and reproductive status affect the flight performance of *Pieris napi* butterflies. J. Insect Behav. 25:441-452.

Alonso-Alvarez C., S. Bertrand, G. Devevey, J. Prost, B. Faivre, and G. Sorci. 2004. Increased susceptibility to oxidative stress as a proximate cost of reproduction. Ecol. Lett. 7:363-368.

Amos W., and A. Balmford. 2001. When does conservation genetics matter? Heredity 87:3:257-265.

Baillie J. E. M., C. Hilton-Taylor, and S. N. Stuart, eds. 2004. 2004 IUCN Red List of Threatened Species: A Global Species Assessment. The IUCN Species Survival Commission.

Barton B., G. Ayer, N. Heymann, D. Maughan, F. Lehmann, and J. Vigoreaux. 2005. Flight muscle properties and aerodynamic performance of *Drosophila* expressing a *flightin* transgene. J. Exp. Biol. 208:549-560.

Battisti A., M. Stastny, E. Buffo, and S. Larsson. 2006. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. Global Change Biol. 12:662-671.

Berg M. P., E. T. Kiers, G. Driessen, M. van der Heijden, B. W. Kooi, F. Kuenen, M. Liefting, H. A. Verhoef, and J. Ellers. 2010. Adapt or disperse: understanding species persistence in a changing world. Global Change Biol. 16:587-598.

Bergerot B., T. Merckx, H. Van Dyck, and M. Baguette. 2012. Habitat fragmentation impacts mobility in a common and widespread woodland butterfly: do sexes respond differently? BMC Ecol. 12:5.

Bergman M., and C. Wiklund. 2009. Differences in mate location behaviours between residents and nonresidents in a territorial butterfly. Anim. Behav. 78:1161-1167.

Berwaerts K., E. Matthysen, and H. Van Dyck. 2008. Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: A quantitative genetic assessment. Evolution 62:2525-2533.

Berwaerts K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An

experimental test with the butterfly *Pararge aegeria*. Funct. Ecol. 16:484-491.

Bijlsma R., J. Bundgaard, and A. Boerema. 2000. Does inbreeding affect the extinction risk of small populations? predictions from *Drosophila*. J. Evol. Biol. 13:502-514.

Boakes E. H., J. Wang, and W. Amos. 2007. An investigation of inbreeding depression and purging in captive pedigreed populations. Heredity 98:172-182.

Boggs C., and M. Nieminen. 2004. Checkerspot reproductive biology. Pp. 92-111 *in* P. R. Ehrlich and I. Hanski, eds. On the Wings of Checkerspots: A Model System for Population Biology. Oxford University Press, New York.

Bonte D., N. De Clercq, I. Zwertvaegher, and L. Lens. 2009. Repeatability of dispersal behaviour in a common dwarf spider: evidence for different mechanisms behind short- and long-distance dispersal. Ecol. Entomol. 34:271-276.

Bonte D., and M. Saastamoinen. 2012. Dispersal syndromes in butterflies and spiders. Pp. 161-170 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Bonte, D., Van Dyck, H., Bullock, J. M., Coulon, A., Delgado, M., Gibbs, M. *et al.* (2012). Costs of dispersal. Biological Reviews, 87:290-312.

Breuker C. J., P. M. Brakefield, and M. Gibbs. 2007. The association between wing morphology and dispersal is sex-specific in the Glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera : Nymphalidae). Eur. J. Entomol. 104:445-452.

Bridle, J. R., Buckley, J., Bodsworth, E. J., and Thomas, C. D. 2014. Evolution on the move: specialization on widespread resources associated with rapid range expansion in response to climate change. Proc. R. Soc. B-Biol. Sci. 281:20131800.

Bridle J. R., and T. H. Vines. 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends Ecol. Evol. 22:140-147.

Brisson J. A., G. K. Davis, and D. L. Stern. 2007. Common genome-wide patterns of transcript accumulation underlying the wing polyphenism and polymorphism in the pea aphid (*Acyrthosiphon pisum*). Evol. Dev. 9:338-346.

Brooker R. W., J. M. J. Travis, E. J. Clark, and C. Dytham. 2007. Modelling species' range shifts in a changing climate: The impacts of biotic interactions, dispersal distance and the rate of climate change. J. Theor. Biol. 245:59-65.

Buckley J., R. K. Butlin, and J. R. Bridle. 2012. Evidence for evolutionary change associated with the recent range expansion of the British butterfly, *Aricia agestis*, in response to climate change. Mol. Ecol. 21:267-280.

Byers D., and D. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu. Rev. Ecol. Syst. 30:479-513.

Carlquist S. 1966. Biota of long-distance dispersal.I. Principles of dispersal and evolution. Q. Rev. Biol. 41:247-270.

Charlesworth B., and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genet. Res. 74:329-340.

Charlesworth D., M. Morgan, and B. Charlesworth. 1993. Mutation accumulation in finite outbreeding and inbreeding populations. Genet. Res. 61:39-56.

Charmantier A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 272:1415-1425.

Cheng T., C. Liao, W. Tsai, C. Lin, C. Yeh, C. Teng, and W. Chang. 2009. Identification and characterization of the mitochondrial targeting sequence and mechanism in human citrate synthase. J. Cell. Biochem. 107:1002-1015.

Clark R. W., M. N. Marchand, B. J. Clifford, R. Stechert, and S. Stephens. 2011. Decline of an isolated timber rattlesnake (*Crotalus horridus*) population: Interactions between climate change, disease, and loss of genetic diversity. Biol. Conserv. 144:886-891.

Clobert J., M. Baguette, T. G. Benton, and J. M. Bullock, eds. 2012. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Cody M. L., and J. M. Overton. 1996. Short-term evolution of reduced dispersal in island plant populations. J. Ecol. 84:53-61.

Crnokrak P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity 83:260-270.

Dahlhoff E., and N. Rank. 2000. Functional and physiological consequences of genetic variation at phosphoglucose isomerase: Heat shock protein expression is related to enzyme genotype in a montane beetle. Proc. Natl. Acad. Sci. U. S. A. 97:10056-10061.

Davis A. K., J. Chi, C. Bradley, and S. Altizer. 2012. The redder the better: wing color predicts flight performance in monarch butterflies. PLoS One 7:e41323.

De Block M., and R. Stoks. 2007. Flight-related body morphology shapes mating success in a damselfly. Anim. Behav. 74:1093-1098.

de Jong M., S. Wong, R. Lehtonen, and I. Hanski. 2014. Cytochrome P450 gene Cyp337 and heritability of

fitness traits in the Glanville fritillary butterfly. Mol. Ecol. in press.

DeRose M. A., and D. A. Roff. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. Evolution 53:1288-1292.

Dingemanse N. J. 2003. Natal dispersal and personalities in great tits (*Parus major*). Proc. R. Soc. B-Biol. Sci. 270:741-747.

Dudley R. 2000. Energetics and flight physiology. Pp. 159-202 *in* The biomechanics of insect flight: form, function, evolution. Princeton University Press, Princeton, New Jersey.

Duplouy A., and I. Hanski. 2013. Butterfly survival on an isolated island by improved grip. Biology letters 9:20130020.

Duplouy A., S. Ikonen, and I. Hanski. 2013. Life history of the Glanville fritillary butterfly in fragmented versus continuous landscapes. Ecol. Evol. 3:5141-5156.

Eanes W. F., T. J. S. Merritt, J. M. Flowers, S. Kumagai, E. Sezgin, and C. Zhu. 2006. Flux control and excess capacity in the enzymes of glycolysis and their relationship to flight metabolism in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. A. 103:19413-19418.

Edelsparre A. H., A. Vesterberg, J. H. Lim, M. Anwari, and M. J. Fitzpatrick. 2014. Alleles underlying larval foraging behaviour influence adult dispersal in nature. Ecol. Lett. in press

Edmands S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Mol. Ecol. 16:463-475.

Ehrlich P. R., and I. Hanski. 2004. On the wings of checkerspots: a model system for population biology. Oxford University Press, New York.

Ellegren H., and B. C. Sheldon. 2008. Genetic basis of fitness differences in natural populations. Nature 452:169-175.

Fauvergue X., E. Vercken, T. Malausa, and R. A. Hufbauer. 2012. The biology of small, introduced populations, with special reference to biological control. Evolutionary Applications 5:424-443.

Fidler A. E., K. van Oers, P. J. Drent, S. Kuhn, J. C. Mueller, and B. Kempenaers. 2007. *Drd4* gene polymorphisms are associated with personality variation in a passerine bird. Proc. R. Soc. B-Biol. Sci. 274:1685-1691.

Fittipaldi S., I. Dimauro, N. Mercatelli, and D. Caporossi. 2014. Role of exercise-induced reactive oxygen species in the modulation of heat shock protein response. Free Radic. Res. 48:52-70.

Fragala M. S., W. J. Kraemer, C. R. Denegar, C. M. Maresh, A. M. Mastro, and J. S. Volek. 2011. Neuroendocrine-immune interactions and responses to exercise. Sports Medicine 41:621-639.

Frankham R. 2005. Genetics and extinction. Biol. Conserv. 126:131-140.

Frankham R., C. J. A. Bradshaw, and B. W. Brook. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biol. Conserv. 170:56-63.

Fry J., P. Keightley, S. Heinsohn, and S. Nuzhdin. 1999. New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U. S. A. 96:574-579.

Gaggiotti O. 2003. Genetic threats to population persistence. Ann. Zool. Fenn. 40:155-168.

Garcia-Dorado A. 2003. Tolerant versus sensitive genomes: The impact of deleterious mutation on fitness and conservation. Conserv. Genet. 4:311-324.

Gatehouse A. 1997. Behavior and ecological genetics of wind-borne migration by insects. Annu. Rev. Entomol. 42:475-502.

Gelatt T. S., C. S. Davis, I. Stirling, D. B. Siniff, C. Strobeck, and I. Delisle. 2010. History and fate of a small isolated population of Weddell seals at White Island, Antarctica. Conserv. Genet. 11:721-735.

Gilchrist G. 1990. The consequences of sexual dimorphism in body size for butterfly flight and thermoregulation. Funct. Ecol. 4:475-487.

Gilligan D., L. Woodworth, M. Montgomery, D. Briscoe, and R. Frankham. 1997. Is mutation accumulation a threat to the survival of endangered populations? Conserv. Biol. 11:1235-1241.

Gillooly J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Glemin S., J. Ronfort, and T. Bataillon. 2003. Patterns of inbreeding depression and architecture of the load in subdivided populations. Genetics 165:2193-2212.

Gu H., and W. Danthanarayana. 1992. Quantitative genetic analysis of dispersal in *Epiphyas postvittana*.1. Genetic variation in flight capacity. Heredity 68:53-60.

Haag C. R., M. Saastamoinen, J. H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. Proc. R. Soc. Lond. B: Biol. Sci. 272:2449-2456.

Haikola S. 2003. Effects of inbreeding in the Glanville fritillary butterfly (*Melitaea cinxia*). Ann. Zool. Fenn. 40:483-493.

Han L. Z., H. N. Gu, B. P. Zhai, and X. X. Zhang. 2009. Genetic effects on flight capacity in the beet armyworm, *Spodoptera exigua* (Lep., Noctuidae). J. Appl. Entomol. 133:262-271.

Hansen T. F., C. Pelabon, and D. Houle. 2011. Heritability is not evolvability. Evol. Biol. 38:258-277.

Hanski, I. 1998. Metapopulation dynamics. Nature 396:41-49.

Hanski I. 1999. Metapopulation Ecology. Academic Press, London.

Hanski I. 2011. Eco-evolutionary spatial dynamics in the Glanville fritillary butterfly. Proc. Natl. Acad. Sci. U. S. A. 108:14397-14404.

Hanski I. 2012. Dispersal and eco-evolutionary dynamics in the Glanville fritillary butterfly. Pp. 290-303 *in* J. Clobert, M. Baguette, T. Benton and J. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford.

Hanski, I., Breuker, C. J., Schöps, K., Setchfield, R., and Nieminen, M. 2002. Population history and life history influence the migration rate of female Glanville fritillary butterflies. Oikos 98:87-97.

Hanski, I., Erälahti, C., Kankare, M., Ovaskainen, O., and Sirén, H. 2004. Variation in migration propensity among individuals maintained by landscape structure. Ecol. Lett. 7:958.

Hanski I., and T. Mononen. 2011. Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. Ecol. Lett. 14:1025-1034.

Hanski, I., Saastamoinen, M., and Ovaskainen, O. 2006. Dispersal-related life-history trade-offs in a butterfly metapopulation. J. Anim. Ecol. 75:91-100.

Hanski, I., & Saccheri, I. 2006. Molecular-level variation affects population growth in a butterfly metapopulation. PLoS Biology 4:719-726.

Harrison J. F. 1998. Oxygen-sensitive flight metabolism in the dragonfly *Erythemis simplicicollis*. J. Exp. Biol. 201:1739.

Hartl D. L., and A. G. Clark. 2007. Pp. 121-128 *in* Principles of Population Genetics, Fourth Edition. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Hedrick P. W. 1994. Purging inbreeding depression and the probability of extinction - Full-sib mating. Heredity 73:363-372.

Hedrick P. W., and R. Fredrickson. 2010. Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Cons. Genet. 11:615-615-626.

Heino M., and I. Hanski. 2001. Evolution of migration rate in a spatially realistic metapopulation model. Am. Nat. 157:495-511.

Heinrich B. 1974. Thermoregulation in endothermic insects. Science 185:747-756.

Heinrich B. 1986a. Comparative thermoregulation of 4 montane butterflies of different mass. Physiol. Zool. 59:616-626.

Heinrich B. 1986b. Thermoregulation and flight activity of a satyrine, *Coenonympha inornata* (Lepidoptera, Satyridae). Ecology 67:593-597.

Heinrich B. 1993. Butterflies and Wings. Pp. 76-116 *in* The Hot-Blooded Insects: Strategies and Mechanisms of Thermoregulation. Harvard University Press.

Helmuth B., J. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: Does mechanism matter? Annu. Rev. Physiol. 67:177-201.

Higgins K., and M. Lynch. 2001. Metapopulation extinction caused by mutation accumulation. Proc. Natl. Acad. Sci. U. S. A. 98:2928-2933.

Hill J. K., C. D. Thomas, and D. S. Blakeley. 1999. Evolution of flight morphology in a butterfly that has recently expanded its geographic range. Oecologia 121:165-170.

Hoffmann A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14:96-101.

Hughes J., and M. Zalucki. 1993. The relationship between the *Pgi* locus and the ability to fly at low temperatures in the Monarch butterfly *Danaus plexippus*. Biochem. Genet. 31:521-532.

Hulbert A. J., R. Pamplona, R. Buffenstein, and W. A. Buttemer. 2007. Life and death: Metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87:1175-1213.

IPCC. 2007. Climate Change 2007: Synthesis Report. *in* C. W. Team, R. K. Pachauri and A. Reisinger, eds. Contribution of Working Groups I,II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

IUCN. 1980. World Conservation Strategy. International Union for Conservation of Nature and Natural Resources (IUCN).

IUCN. 2014. IUCN Red List of Threatened Species, International Union for Conservation of Nature and Natural Resources-The World Conservation Union, Species Survival Commission.

Kallioniemi E., and I. Hanski. 2011. Interactive effects of *Pgi* genotype and temperature on larval growth and survival in the Glanville fritillary butterfly. Funct. Ecol. 25:1032-1039.

Kammer A. E., and B. Heinrich. 1978. Insect flight metabolism. Pp. 133-228 *in* J. E. Treherne, ed. Advances in Insect Physiology. Academic Press Inc., London.

Kawamura K. 2005. Low genetic variation and inbreeding depression in small isolated populations of the Japanese rosy bitterling, *Rhodeus ocellatus kurumeus*. Zool. Sci. 22:517-524.

Keller L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17:230-241.

Kemp D., and A. Krockenberger. 2004. Behavioural thermoregulation in butterflies: the interacting effects of body size and basking posture in *Hypolimnas bolina* (L.) (Lepidoptera : Nymphalidae). Aust. J. Zool. 52:229-236.

Ketola T., and J. S. Kotiaho. 2009. Inbreeding, energy use and condition. J. Evol. Biol. 22:770-781.

Kibota T., and M. Lynch. 1996. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature 381:694-696.

Kingsolver J. 1983. Ecological significance of flight activity in *Colias* butterflies - Implications for reproductive strategy and population structure. Ecology 64:546-551.

Kingsolver, J. G., and Watt, W. B. 1983. Thermoregulatory strategies in *Colias* butterflies - Thermal stress and the limits to adaptation in temporally varying environments. Am. Nat. 121:32-55.

Kingsolver, J. G., and Watt, W. B. 1984. Mechanistic constraints and optimality models - Thermoregulatory strategies in *Colias* butterflies. Ecology 65:1835-1839.

Kleiber M. 1947. Body size and metabolic rate. Physiol. Rev. 27:511-541.

Klemme I., and I. Hanski. 2009. Heritability of and strong single gene (*Pgi*) effects on life-history traits in the Glanville fritillary butterfly. J. Evol. Biol. 22:1944-1953.

Kruuk L. E. B. 2004. Estimating genetic parameters in natural populations using the 'animal model'. Phil. Trans. R. Soc. London B-Biol. Sci. 359:873-890.

Kruuk L. E. B., and J. D. Hadfield. 2007. How to separate genetic and environmental causes of similarity between relatives. J. Evol. Biol. 20:1890-1903.

Kuussaari M., M. Nieminen, and I. Hanski. 1996. An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. J. Anim. Ecol. 65:791-801.

Kuussaari, M., Saccheri, I., Camara, M., and Hanski, I. 1998. Allee effect and population dynamics in the Glanville fritillary butterfly. Oikos 82:384-392.

Kuussaari M., S. van Nouhuys, J. J. Hellmann, and M. C. Singer. 2004. Larval biology of checkerspots. Pp. 138-160 *in* P. R. Ehrlich and I. Hanski, eds. On the Wings of Checkerspots: A Model System for Population Biology. Oxford University Press, New York.

Kvist J., C. W. Wheat, E. Kallioniemi, M. Saastamoinen, I. Hanski, and M. J. Frilander. 2013. Temperature treatments during larval development reveal extensive heritable and plastic variation in gene expression and life history traits. Mol. Ecol. 22:602-619.

Lande R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48:1460-1469.

Le Galliard J. F., M. Massot, and J. Clobert. 2012. Dispersal and range dynamics in changing climates: a review. Pp. 317-336 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Leek B., S. Mudaliar, R. Henry, O. Mathieu-Costello, and R. Richardson. 2001. Effect of acute exercise on citrate synthase activity in untrained and trained human skeletal muscle. Am. J. Physiol. -Regul. Integr. Comp. Physiol. 280:R441-R447.

Levins R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. Bull. ESA 15:237-240.

Liedvogel M., S. Akesson, and S. Bensch. 2011. The genetics of migration on the move. Trends Ecol. Evol. 26:561-569.

Lopez S., F. Rousset, F. H. Shaw, R. G. Shaw, and O. Ronce. 2009. Joint effects of inbreeding and local adaptation on the evolution of genetic load after fragmentation. Conserv. Biol. 23:1618-1627.

Luo S., S. Chong Wong, C. Xu, I. Hanski, R. Wang, and R. Lehtonen. 2014. Phenotypic plasticity in thermal tolerance in the Glanville fritillary butterfly. J. Therm. Biol. 42:33-39.

Luquet E., P. David, J. Lena, P. Joly, L. Konecny, C. Dufresnes, N. Perrin, and S. Plenet. 2011. Heterozygosity-fitness correlations among wild populations of European tree frog (*Hyla arborea*) detect fixation load. Mol. Ecol. 20:1877-1887.

Lynch M., J. Conery, and R. Burger. 1995a. Mutation accumulation and the extinction of small populations. Am. Nat. 146:489-518.

Lynch M., J. Conery, and R. Burger. 1995b. Mutational meltdowns in sexual populations. Evolution 49:1067-1080.

Lynch M., and B. Walsh. 1998a. Inbreeding depression. Pp. 251-292 *in* Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, MA.

Lynch M., and B. Walsh. 1998b. Resemblance between relatives. Pp. 131-176 *in* Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, MA.

Madsen, T., Shine, R., Olsson, M., & Wittzell, H. 1999. Conservation biology - Restoration of an inbred adder population. Nature 402:34-35.

Madsen T., B. Ujvari, and M. Olsson. 2004. Novel genes continue to enhance population growth in adders (*Vipera berus*). Biol. Conserv. 120:145-147.

Mank J. E. 2009. The W, X, Y and Z of sex-chromosome dosage compensation. Trends Genet. 25:226-233.

Marden, J. H., Fescemyer, H. W., Saastamoinen, M., MacFarland, S. P., Vera, J. C., Frilander, M. J., & Hanski, I. 2008. Weight and nutrition affect pre-mRNA splicing of a muscle gene associated with performance, energetics and life history. J. Exp. Biol. 211:3653-3660.

Marden J. H., H. W. Fescemyer, R. J. Schilder, W. R. Doerfler, J. C. Vera, and C. W. Wheat. 2013. Genetic variation in *Hif* signaling underlies quantitative variation in physiological and life-history traits within lowland butterfly populations. Evolution 67:1105-1115.

Matthysen E. 2012. Multicausality of dispersal: a review. Pp. 3-18 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

McMillan D., S. Fearnley, N. Rank, and E. Dahlhoff. 2005. Natural temperature variation affects larval survival, development and *Hsp70* expression in a leaf beetle. Funct. Ecol. 19:844-852.

Menendez R., A. Megias, J. Hill, B. Braschler, S. Willis, Y. Collingham, R. Fox, D. Roy, and C. Thomas. 2006. Species richness changes lag behind climate change. Proc. R. Soc. B-Biol. Sci. 273:1465-1470.

Merckx T., and H. Van Dyck. 2005. Mate location behaviour of the butterfly *Pararge aegeria* in woodland and fragmented landscapes. Anim. Behav. 70:411-416.

Merckx, T., Van Dyck, H., Karlsson, B., & Leimar, O. 2003. The evolution of movements and behaviour at boundaries in different landscapes: a common arena experiment with butterflies. Proc. R. Soc. B-Biol. Sci. 270:1815-1821.

Meyer K. 2007. WOMBAT—A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). J. Zhejiang Univ. Sci. B 8:815-821.

Mitikka V., and I. Hanski. 2010. *Pgi* genotype influences flight metabolism at the expanding range margin of the European map butterfly. Ann. Zool. Fenn. 47:1-14.

Nathan R., W. M. Getz, E. Revilla, M. Holyoak, R. Kadmon, D. Saltz, and P. E. Smouse. 2008. A movement ecology paradigm for unifying organismal movement research. Proc. Natl. Acad. Sci. U. S. A. 105:19052-19059.

Neargarder G., E. Dahlhoff, and N. Rank. 2003. Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. Funct. Ecol. 17:213-221.

Nespolo R. F., D. A. Roff, and D. J. Fairbairn. 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). Funct. Ecol. 22:624-631.

Nieman D. C. 1997. Immune response to heavy exertion. J. Appl. Physiol. 82:1385-1394.

Nieminen M., M. Siljander, and I. Hanski. 2004. Structure and dynamics of *Melitaea cinxia* metapopulations. Pp. 63-91 *in* P. R. Ehrlich and I. Hanski, eds. On the Wings of Checkerspots: a Model System for Population Biology. Oxford University Press, New York.

Niitepõld K. 2009. Flight metabolic rate in the Glanville fritillary butterfly. University of Helsinki, Helsinki. Dissertation: http://urn.fi/URN:ISBN:978-952-10-5902-5.

Niitepõld K. 2010. Genotype by temperature interactions in the metabolic rate of the Glanville fritillary butterfly. J. Exp. Biol. 213:1042-1048.

Niitepõld, K., and Hanski, I. 2013. A long life in the fast lane: positive association between peak metabolic rate and lifespan in a butterfly. J. Exp. Biol. 216:1388-1397.

Niitepõld, K., Mattila, A. L. K., Harrison, P. J., and Hanski, I. 2011. Flight metabolic rate has contrasting effects on dispersal in the two sexes of the Glanville fritillary butterfly. Oecologia 165:847-854.

Niitepõld K., A. D. Smith, J. L. Osborne, D. R. Reynolds, N. L. Carreck, A. P. Martin, J. H. Marden, O. Ovaskainen, and I. Hanski. 2009. Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field. Ecology 90:2223-2232.

Nilsson J. A. 2002. Metabolic consequences of hard work. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 269:1735-1739.

O'Grady J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biol. Conserv. 133:42-51.

Ojanen S. P., M. Nieminen, E. Meyke, J. Pöyry, and I. Hanski. 2013. Long-term metapopulation study of the Glanville fritillary butterfly (*Melitaea cinxia*): survey methods, data management, and long-term population trends. Ecol. Evol. 3:11:3713-3737.

Orsini L., C. Wheat, C. Haag, J. Kvist, M. Frilander, and I. Hanski. 2009. Fitness differences associated with *Pgi* SNP genotypes in the Glanville fritillary butterfly (*Melitaea cinxia*). J. Evol. Biol. 22:367-375.

Ovaskainen O., A. D. Smith, J. L. Osborne, D. R. Reynolds, N. L. Carreck, A. P. Martin, K. Niitepõld, and I. Hanski. 2008. Tracking butterfly movements with harmonic

radar reveals an effect of population age on movement distance. Proc. Natl. Acad. Sci. U. S. A. 105:19090-19095.

Pakkasmaa S., J. Merila, and R. O'Hara. 2003. Genetic and maternal effect influences on viability of common frog tadpoles under different environmental conditions. Heredity 91:117-124.

Paland S., and B. Schmid. 2003. Population size and the nature of genetic load in *Gentianella germanica*. Evolution 57:2242-2251.

Park K., J. Park, J. Ko, B. C. Kim, H. Kim, K. Ahn, *et al.* 2012. Whole transcriptome analyses of six thoroughbred horses before and after exercise using RNA-Seq. BMC Genomics 13:473.

Parmesan C., N. Ryrholm, C. Stefanescu, J. Hill, C. Thomas, H. Descimon, *et al.* 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399:579-583.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst. 37:637-669.

Pivnick K., and J. McNeil. 1986. Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera, Hesperiidae). Ecology 67:1024-1035.

Poon A., and S. Otto. 2000. Compensating for our load of mutations: Freezing the meltdown of small populations. Evolution 54:1467-1479.

Puurtinen M., K. Knott, S. Suonpää, T. van Ooik, and V. Kaitala. 2004. Genetic variability and drift load in populations of an aquatic snail. Evolution 58:749-756.

Rank N. E., D. A. Bruce, D. M. McMillan, C. Barclay, and E. P. Dahlhoff. 2007. Phosphoglucose isomerase genotype affects running speed and heat shock protein expression after exposure to extreme temperatures in a montane willow beetle. J. Exp. Biol. 210:750-764.

Rassi P., E. Hyvärinen, A. Juslén, and I. Mannerkoski, eds. 2010. Suomen lajien uhanalaisuus 2010 - The 2010 Red List of Finnish Species. Ympäristöministeriö & Suomen ympäristökeskus, Helsinki.

Rastas P., L. Paulin, I. Hanski, R. Lehtonen, and P. Auvinen. 2013. Lep-MAP: fast and accurate linkage map construction for large SNP datasets. Bioinformatics 29:3128-3134.

Rawlins J. 1980. Thermoregulation by the Black swallowtail butterfly, *Papilio polyxenes* (Lepidoptera, Papilionidae). Ecology 61:345-357.

Richards C. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. Am. Nat. 155:383-394.

Roff D. A. 1990. The evolution of flightlessness in insects. Ecol. Monogr. 60:389-421.

Roff D., and D. Fairbairn. 1991. Wing dimorphisms and the evolution of migratory polymorphisms among the Insecta. Am. Zool. 31:243-251.

Roff D. A., and D. J. Fairbairn. 2007a. Laboratory evolution of the migratory polymorphism in the sand cricket: Combining physiology with quantitative genetics. Physiol. Biochem. Zool. 80:358-369.

Roff D. A., and D. J. Fairbairn. 2007b. The evolution and genetics of migration in insects. Bioscience 57:155-164.

Ronce O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Ann. Rev. Ecol. Evol. Syst. 38:231-253.

Ronce O. and J. Clobert. 2012. Dispersal syndromes. Pp. 120-138 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Rowe G., and T. Beebee. 2003. Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. Evolution 57:177-181.

Rutherford S. 2003. Between genotype and phenotype: Protein chaperones and evolvability. Nat. Rev. Genet. 4:263-274.

Saastamoinen M. 2007a. Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. Oecologia 153:569-578.

Saastamoinen M. 2007b. Life-history, genotypic, and environmental correlates of clutch size in the Glanville fritillary butterfly. Ecol. Entomol. 32:235-242.

Saastamoinen M. 2008. Heritability of dispersal rate and other life history traits in the Glanville fritillary butterfly. Heredity 100:39-46.

Saastamoinen, M., Brakefield, P. M., and Ovaskainen, O. 2012. Environmentally induced dispersal-related life-history syndrome in the tropical butterfly, *Bicyclus anynana*. J. Evol. Biol. 25:2264-2275.

Saastamoinen M., J. E. Brommer, P. M. Brakefield, and B. J. Zwaan. 2013a. Quantitative genetic analysis of responses to larval food limitation in a polyphenic butterfly indicates environment and trait-specific effects. Ecol. Evol. 3:3576-3589.

Saastamoinen, M., and Hanski, I. 2008. Genotypic and environmental effects on flight activity and oviposition in the Glanville fritillary butterfly. Am. Nat. 171:701-712.

Saastamoinen, M., Ikonen, S., and Hanski, I. 2009. Significant effects of *Pgi* genotype and body reserves on lifespan in the Glanville fritillary butterfly. Proc. R. Soc. B-Biol. Sci. 276:1313-1322.

Saastamoinen, M., Ikonen, S., Wong, S. C., Lehtonen, R., and Hanski, I. 2013b. Plastic larval development in a

butterfly has complex environmental and genetic causes and consequences for population dynamics. J. Anim. Ecol. 82:529-539.

Saastamoinen M., and M. J. Rantala. 2013. Influence of developmental conditions on immune function and dispersal-related traits in the Glanville fritillary (*Melitaea cinxia*) butterfly. PLoS One 8:e81289.

Saccheri I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491-494.

Schtickzelle N., G. Mennechez, and M. Baguette. 2006. Dispersal depression with habitat fragmentation in the bog fritillary butterfly. Ecology 87:1057-1065.

Schwartz M., and L. Mills. 2005. Gene flow after inbreeding leads to higher survival in deer mice. Biol. Conserv. 123:413-420.

Scott J. 1974. Mate-locating behavior of butterflies. Am. Midl. Nat. 91:103-117.

Shaw R., D. Byers, and E. Darmo. 2000. Spontaneous mutational effects on reproductive traits of *Arabidopsis thaliana*. Genetics 155:369-378.

Shreeve T. 1984. Habitat selection, mate location, and microclimatic constraints on the activity of the speckled wood butterfly *Pararge aegeria*. Oikos 42:371-377.

Simmons A., and C. Thomas. 2004. Changes in dispersal during species' range expansions. Am. Nat. 164:378-395.

Slatkin M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. 16:393-430.

Sokolowski M. 2001. Drosophila: Genetics meets behaviour. Nat. Rev. Genet. 2:879-890.

Sorensen J., T. Kristensen, and V. Loeschcke. 2003. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6:1025-1037.

Spielman, D., Brook, B. W., and Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proc. Natl. Acad. Sci. U. S. A. 101:15261-15264.

Spielman D., and R. Frankham. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: improvement of reproductive fitness due to immigration of one individual into small partially inbred populations. Zoo Biol. 11:343-351.

Suarez R. 2000. Energy metabolism during insect flight: Biochemical design and physiological performance. Physiol. Biochem. Zool. 73:765-771.

Sutherland W. J., R. P. Freckleton, H. C. J. Godfray, S. R. Beissinger, T. Benton, D. D. Cameron, *et al.* 2013. Identification of 100 fundamental ecological questions. J. Ecol. 101:58-67.

Swindell W., and J. Bouzat. 2005. Modeling the adaptive potential of isolated populations: Experimental simulations using *Drosophila*. Evolution 59:2159-2169.

Szulkin M., D. Garant, R. H. McCleery, and B. C. Sheldon. 2007. Inbreeding depression along a life-history continuum in the great tit. J. Evol. Biol. 20:1531-1543.

Tares S., L. Arthaud, M. Amichot, and A. Robichon. 2013. Environment exploration and colonization behavior of the pea aphid associated with the expression of the *foraging* gene. PLoS One 8:e65104.

Thomas C., E. Bodsworth, R. Wilson, A. Simmons, Z. Davies, M. Musche, and L. Conradt. 2001. Ecological and evolutionary processes at expanding range margins. Nature 411:577-581.

Travis J., and C. Dytham. 1999. Habitat persistence, habitat availability and the evolution of dispersal. Proc. R. Soc. B-Biol. Sci. 266:723-728.

Travis J., and C. Dytham. 2002. Dispersal evolution during invasions. Evol. Ecol. Res. 4:1119-1129.

Travis J. and C. Dytham. 2012. Dispersal and climate change: a review of theory. Pp. 337-348 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Van Dyck H. 2012. Dispersal under global change - the case of the Pine processionary moth and other insects. Pp. 355-365 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Van Dyck H., and M. Baguette. 2005. Dispersal behaviour in fragmented landscapes: Routine or special movements? Basic Appl. Ecol. 6:535-545.

Van Dyck H., and E. Matthysen. 1998. Thermoregulatory differences between phenotypes in the speckled wood butterfly: hot perchers and cold patrollers? Oecologia 114:326-334.

Van Dyck, H. V., Matthysen, E., Windig, J. J., and Dhondt, A. A. 1997. Seasonal phenotypic variation in the speckled wood butterfly (*Pararge aegeria* L.): Patterns in and relationships between wing characters. Belg. J. Zool. 127:167-178.

van Nouhuys S., and I. Hanski. 2002. Colonization rates and distances of a host butterfly and two specific parasitoids in a fragmented landscape. J. Anim. Ecol. 71:639-650.

Vassilieva L., and M. Lynch. 1999. The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. Genetics 151:119-129.

Vera J. C., C. W. Wheat, H. W. Fescemyer, M. J. Frilander, D. L. Crawford, I. Hanski, and J. H. Marden. 2008. Rapid

transcriptome characterization for a nonmodel organism using 454 pyrosequencing. Mol. Ecol. 17:1636-1647.

Vila C., A. Sundqvist, O. Flagstad, J. Seddon, S. Bjornerfeldt, I. Kojola, *et al.* 2003. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proc. R. Soc. Lond. Ser. B-Biol. Sci. 270:91-97.

Visscher P. M., W. G. Hill, and N. R. Wray. 2008. Heritability in the genomics era - concepts and misconceptions. Nat. Rev. Genet. 9:255-266.

Wang J. L. 2000. Effects of population structures and selection strategies on the purging of inbreeding depression due to deleterious mutations. Genet. Res. 76:75-86.

Wang J., W. Hill, D. Charlesworth, and B. Charlesworth. 1999. Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. Genet. Res. 74:165-178.

Watt W. B. 1968. Adaptive significance of pigment polymorphisms in *Colias* butterflies .I. Variation of melanin pigment in relation to thermoregulation. Evolution 22:437-458.

Watt W. B. 1992. Eggs, enzymes, and evolution - Natural genetic variants change insect fecundity. Proc. Natl. Acad. Sci. U. S. A. 89:10608-10612.

Watt, W. B., Cassin, R. C., & Swan, M. S. 1983. Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from in vitro biochemistry. Genetics 103:725-739.

Watt W. B., K. Donohue, and P. A. Carter. 1996. Adaptation at specific loci.6. Divergence vs parallelism of polymorphic allozymes in molecular function and fitness-component effects among *Colias* species (Lepidoptera, Pieridae). Mol. Biol. Evol. 13:699-709.

Watt W. B., C. W. Wheat, E. H. Meyer, and J. F. Martin. 2003. Adaptation at specific loci. VII. Natural selection, dispersal and the diversity of molecular-functional variation patterns among butterfly species complexes (*Colias* : Lepidoptera, Pieridae). Mol. Ecol. 12:1265-1275.

Webster M., P. Marra, S. Haig, S. Bensch, and R. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends Ecol. Evol. 17:76-83.

Westemeier R. L., J. D. Brawn, S. A. Simpson, T. L. Esker, R. W. Jansen, J. W. Walk, *et al.* 1998. Tracking the long-term decline and recovery of an isolated population. Science 282:1695-1698.

Wheat C. W. 2010. Phosphoglucose isomerase (*Pgi*) performance and fitness effects among Arthropods and its potential role as an adaptive marker in conservation genetics. Conserv. Genet. 11:387-397.

Wheat C. W. 2012. Dispersal genetics: emerging insights from fruitflies, butterflies and beyond. Pp. 95-115 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal Ecology and Evolution. Oxford University Press, Oxford, UK.

Wheat, C. W., Fescemyer, H. W., Kvist, J., Tas, E. V. A., Vera, J. C., Frilander, M. J., *et al.* 2011. Functional genomics of life history variation in a butterfly metapopulation. Mol. Ecol. 20:1813-1828.

Whitlock M. 2002. Selection, load and inbreeding depression in a large metapopulation. Genetics 160:1191-1202.

Whitlock, M. C., Ingvarsson, P. K., and Hatfield, T. 2000. Local drift load and the heterosis of interconnected populations. Heredity 84:452-457.

Wickman P. 2009. Thermoregulation and habitat use in butterflies. Pp. 55-61 *in* J. Settele, T. Shreeve, M. Konvicka and H. Van Dyck, eds. Ecology of Butterflies in Europe. Cambridge University Press.

Willi Y., P. Griffin, and J. Van Buskirk. 2013. Drift load in populations of small size and low density. Heredity 110:296-302.

Wilson A. J., D. Reale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, *et al.* 2010. An ecologist's guide to the animal model. J. Anim. Ecol. 79:13-26.

Wilson R., D. Gutierrez, J. Gutierrez, D. Martinez, R. Agudo, and V. Monserrat. 2005. Changes to the elevational limits and extent of species ranges associated with climate change. Ecol. Lett. 8:1138-1146.

Zera A. J., and J. A. Brisson. 2012. Quantitative, physiological, and molecular genetics of dispersal/migration. Pp. 63-82 *in* J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock, eds. Dispersal ecology and evolution. Oxford University Press, Oxford, UK.

Zeyl C., M. Mizesko, and J. de Visser. 2001. Mutational meltdown in laboratory yeast populations. Evolution 55:909-917.

Zhan S., C. Merlin, J. L. Boore, and S. M. Reppert. 2011. The monarch butterfly genome yields insights into long-distance migration. Cell 147:1171-1185.

Zheng C., O. Ovaskainen, and I. Hanski. 2009. Modelling single nucleotide effects in phosphoglucose isomerase on dispersal in the Glanville fritillary butterfly: coupling of ecological and evolutionary dynamics. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 364:1519-1532.

Zhou D., J. Xue, J. C. K. Lai, N. J. Schork, K. P. White, and G. G. Haddad. 2008. Mechanisms underlying hypoxia tolerance in *Drosophila melanogaster*: *hairy* as a metabolic switch. PLoS Genet. 4:e1000221.