

# **Dispersal and related life history traits in the Glanville fritillary butterfly**

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Academic dissertation

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Marjo Saastamoinen

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

- I. Hanski, I., Saastamoinen, M. & Ovaskainen, O. (2006). Dispersal-related life-history trade-offs in a butterfly metapopulation. – *Journal of Animal Ecology* 75: 91-100.
- II. Saastamoinen, M. (2007) Heritability of mobility and other life history traits in the Glanville fritillary butterfly. – *Heredity*, advance online publication, 5 September 2007, doi:10.1038/sj.hdy.6801056.
- III. Saastamoinen, M. (2007) Life-history, genotypic, and environmental correlates of clutch size in the Glanville fritillary butterfly. – *Ecological Entomology* 32: 235-242.
- IV. Saastamoinen, M. & Hanski, I. Genotypic and environmental effects on flight activity and oviposition in the Glanville fritillary butterfly. – Submitted manuscript.
- V. Saastamoinen, M. (2007). Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. – *Oecologia* 153: 569-578.

# Contributions

	I	II	III	IV	V
Original idea	IH	MS	MS, IH	IH, MS	MS, IH
Study design	IH, MS	MS	MS	MS, IH	MS
Data collection	MS	MS	MS	MS	MS
Analyses	MS, IH, OO	MS	MS	MS	MS
Manuscript preparation	IH, MS, OO	MS	MS	MS, IH	MS

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# Summary

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## 1. Introduction

Developing a mechanistic understanding of evolution by natural selection is a central aim of evolutionary biology. Several conditions are necessary for natural selection to occur. In order to have any response to selection, there has to be variation in the trait of interest, and this variation has to be heritable. Furthermore, individuals with different trait values need to differ in their performance, which leads to differences in fitness (Roff 1997). Life history of an individual is what lies between the birth and the death. Life history traits are numerous, including age at maturity, adult size, mating success, number of offspring, lifespan, and so forth. Researchers studying life history ecology are interested in analysing differences in fitness resulting from different life history trait variants, as well as understanding the factors that maintain trait variation. Variation in life history traits is typically greater between than within species. It is not surprising, therefore, that traditionally studies of life history ecology have focused on explaining variation between species. Recent studies have, however, shown that there is considerable variation in life histories also between populations of the same species (Roff 2002).

Another central concept in life history theory is that the phenotype of an individual consists of numerous traits that are more or less correlated with each other. Some of the traits may be correlated due to constraining relationships (Roff 2002; Stearns 1992). Correlations between traits can have both genotypic and phenotypic components, which can both be important in shaping the evolutionary trajectory (Roff 2002). Negative correlations, i.e. trade-offs, between traits are not only interesting due to their constraining effects but also because they can

be expected to maintain variation in life history traits in natural populations (Reznick 1985; Roff 2002; Stearns 1992). Trade-offs are not, however, the only mechanism that maintains variation in life history traits. For instance, spatial and temporal variation in selection pressures affecting life history evolution is likely to have significant effects on variation in natural populations (Olivieri and Ronce 2004). Studying such effects has proven to be logistically difficult, and therefore theory on the spatial processes shaping the evolution of life history traits far outweighs the empirical data and evidence.

The studies of life history evolution have traditionally focused on phenotypic variation in the traits of interest and how this variation varies across different environments. Recently, a more mechanistic approach has been developed for understanding how various morphological, behavioural and physiological traits combine to affect the life history of an individual, how the different traits may be correlated, and what are the mechanisms underlying the evolved changes in the life history traits (Prasad and Joshi 2003). This approach to life history ecology requires integration of various disciplines of biology, which is also occurring in many other fields of ecology and evolutionary biology.

In this thesis, my aim was to better understand the effect of spatial population structure on life history evolution and the maintenance of variation in some key life history traits in the Glanville fritillary butterfly. In these studies I combined ecological, genetic (heritability and candidate gene effects), and theoretical approaches. Here, I will first introduce the concept of metapopulation and the significance of dispersal in metapopulations, and discuss the

central assumptions of life history evolution including allocation trade-offs. I will then present the aims of the thesis, describe the ecological features of the study species and the methods used in the studies. Finally, the key results will be presented and discussed.

### *Metapopulation structure and importance of dispersal*

Species rarely consist of populations that do not interact with other nearby populations of the same species, and therefore considering the spatial population structure of species while studying their ecological and evolutionary dynamics is essential (Hanski 1999). A metapopulation consists of a group of spatially separated conspecific populations that interact through dispersal. The term metapopulation was first introduced by Richard Levins (1969), who used it to model population dynamics of insect pests in agricultural fields. Since then, the metapopulation theory has been widely applied to species living in fragmented habitats (Hanski 1999).

In a classic metapopulation, each local population has relatively independent dynamics and eventually goes extinct due to one of the many processes threatening local populations (Hanski 1999). The smaller and more isolated the local population is, the more prone it is to extinction (Hanski and Gaggiotti 2004). However, even if all individual local populations have finite lifespans the metapopulation as a whole may be quite stable as long as immigrants from existing populations are able to re-colonize approximately the same number of habitat patches that were left empty by local extinctions (Hanski 1999). The metapopulation theory emphasizes the importance of connectivity among local populations and habitat patches (Hanski and Gilpin 1997; Hanski and Gaggiotti 2004). The only way individuals can re-colonize empty habitat patches and hence contribute to the survival of the metapopulation is via dispersal.

Dispersal is one of the most important processes affecting species living in fragmented landscapes, and it contributes to metapopulation spatial structure, local dynamics, and metapopulation dynamics (e.g. Clobert *et al.* 2004; Hanski and Gilpin 1997). Dispersal ability is an

evolved character and many species have evolved distinct adaptations to increase their dispersal ability, e.g. wing dimorphism in insects (Zera and Harshman 2001). Dispersal also allows species and populations to occupy more of the available habitat, and helps individuals to cope with spatial as well as temporal heterogeneity (Clobert *et al.* 2001). Furthermore, dispersal allows species to expand and shift their ranges in changing environments, for example due to climate change (Hill *et al.* 1999a; Travis and Dytham 2002).

The term dispersal is somewhat vague and it is often used differently in different contexts (Box 1). In general, it implies movement, either movement away from an existing population or away from the parent individual (Clobert *et al.* 2001). Dispersal is not necessarily a single but a complex trait, the components of which may evolve rapidly. These different components are likely to be coupled to at least some extent. High dispersal rate in insects typically relies on flight ability, which is a fundamental prerequisite for successful life history in most insects, as individuals typically need to fly in order to feed, mate, and lay eggs (Kingsolver 1983a, b). Selection favouring increased flight ability, which is likely to be related to increased flight metabolic rate (Haag *et al.* 2005; Niitepõld *et al.* in prep.), is therefore likely to affect dispersal rate. There may also be different kinds of movement behaviours based on flight, and Van Dyck and Bagueette (2005) have pointed out the possibility of uncoupled selection on within-patch movements and dispersal movements between habitat patches.

How increased loss and fragmentation of natural habitats may affect the evolution of dispersal has received a great deal of attention in recent years (e.g. Hanski *et al.* 2004; Heino and Hanski 2001; Travis and Dytham 1999; Van Dyck and Matthysen 1999). Increased dispersal may be selected against due to e.g. increased mortality during dispersal (Ronce and Olivieri 2004). Furthermore, selection against dispersal may arise due to intrinsic cost of dispersal as flight in general is energetically very expensive and requires high metabolic capacity (Harrison and Roberts 2000). On the other hand, there are other factors, especially at the among-population level, that select for increased dispersal. These factors



### **Box 1. Glossary of terms related to dispersal, and how they are applied in this thesis**

**Dispersal:** Process of movement, either movement away from an existing population or away from the parent. Dispersal refers to between rather than within patch movements, and is most often used to describe one-way movement.

**Mobility:** Refers to within-patch movements and search of resources (e.g. foraging, ovipositing, and mate locating). Sexes are likely to move within a habitat patch for different reasons, as male movement is mainly affected by females whereas in females the availability of host plants for oviposition is likely to determine the amount of movements. Mobility is likely to be correlated with dispersal ability. In this thesis mobility was measured in a large outdoor cage, and I investigated whether it was correlated with dispersal rate as inferred from other types of data.

**Migration:** Movement of an organism from one location to another either permanently (similar to dispersal) or on a seasonal cycle. In this thesis used as a synonym of dispersal.

**Dispersal ability or capacity:** Ability of an individual to disperse between habitat patches. Implies some morphological or physiological differences between individuals that have or do not have high ability to disperse.

**Dispersal propensity:** Tendency or likelihood of an individual to disperse.

**Dispersal rate:** Often refers to the proportion of individuals that disperse away from a population (synonym of emigration rate), but also means the speed of dispersal (distance moved in unit time).

**Flight ability (similar to dispersal ability):** A physiological measure of the ability to fly. This can be e.g. a measure of flight metabolic rate or some morphological surrogate (e.g. wing loading, aspect ratio etc.).

**Colonization:** Movement of an organism to an empty habitat patch and the successful establishment of a new local population.

include, among others, avoidance of sib-competition and inbreeding in the natural populations as well as the potential of finding more favourable conditions elsewhere via dispersal (Clobert *et al.* 2001). Numerous though mainly theoretical studies have shown that, depending on the species (life history) and the degree of habitat loss, fragmentation may select for either increased or decreased rate of dispersal (Heino and Hanski 2001). The two opposing selection pressures, selection for dispersal during recolonization and selection against dispersal once a population has been established, act on dispersal rate when local extinctions are the sole source of environmental variation (Olivieri and Gouyon 1997). These two antagonistic selective forces create the so-called “metapopulation effect” (Olivieri and Gouyon 1997; Van Valen 1971), and as pointed out by Ronce and Olivieri (2004), should be taken into account while studying the evolution of life history traits and genetic systems in metapopulations consisting of extinction-prone local populations.

Importantly, other life history traits apart from dispersal may experience variable selection due to the metapopulation effect (Ronce and Olivieri 2004). Furthermore, selection on dispersal may interact with selection on other life history traits through phenotypic or genotypic correlations. These correlations may therefore cause variation in other life history traits among local populations with dissimilar selection on dispersal. The consequences of spatial population structure for the evolution of life history traits and syndromes have been studied with theoretical models (e.g. Bach *et al.* 2006; Poethke *et al.* 2003; Ronce *et al.* 2000), but empirical studies are still relatively rare. Furthermore, studies on the consequences of metapopulation structure on the evolution of dispersal far outnumber those on other life history traits.

#### *Life history evolution and allocation trade-offs*

Life history theory postulates that many of the ecological and evolutionary questions about

individuals are best understood in terms of the key reproductive traits that define the individual's course of life (Stearns 1992; Roff 2002). These traits include, among others, age at maturity and first reproduction, interval between reproductive events, clutch size and lifespan (Roff 2002). Variation in the life history characteristics among species and among populations of the same species reflect dissimilar allocations of individuals' resources, such as time, effort, and energy, to competing life history functions, especially growth, body maintenance, and reproduction. Allocation is required as available resources are finite (van Noordwijk and de Jong 1986). As time, effort, and energy used for one purpose diminish the time, effort, and energy available for another, one of the key assumptions of optimality models of life history evolution is that traits are often constrained by these inherent allocation trade-offs (Zera and Harshman 2001).

Studies of life history trade-offs have traditionally focused on differential allocation of limiting internal resources to reproduction, maintenance, and growth among species (Calow 1979). More recently, studies of life history evolution and trade-offs have been extended to variation among phenotypes and/or genotypes between and within populations of the same species (e.g. Zera and Huang 1999; Zera and Cisper 2001). Most comprehensive physiological studies of life history trade-offs have been conducted on wing-polymorphic crickets (*Gryllus firmus* and *G. assimilis*; reviewed by Zera and Denno 1997 and Zera and Harshman 2001), which have become a model system in this field of evolutionary biology. In these crickets, comparisons are often performed between flight-capable (macropter) and flightless (brachypter) morphs of the same species (review by Zera and Denno 1997). Trade-offs between numerous life history traits has been demonstrated, with differential allocation of internal reserves into ovarian development versus somatic growth or into maintenance versus storage to name a few (review in Zera and Harshman 2001). In general, the most common life history trade-offs include those between offspring size and offspring number, body size and development time, and flight and reproduction (Roff 2002). Numerous experiments on life history trade-offs have also

been conducted on *Drosophila melanogaster*, some species of lizards and birds (e.g. Sgro and Partridge 1999; Sinervo and DeNardo 1996; Prasad and Joshi 2003).

A trade-off that is commonly assumed to exist in insects and that is most relevant in the context of the present thesis is the one between dispersal and reproduction (i.e. flight-oogenesis-syndrome). Both of these traits are energetically demanding processes and could therefore compete for the same resources (Baguette and Schtickzelle 2006; Roff 2002). In insects, support for a trade-off between reproduction and dispersal mainly comes from studies on wing-dimorphic species (Mole and Zera 1993; 1994; Zera and Denno 1997). In these species, the flightless morph can be easily distinguished from the dispersive morph as the former is either wingless or has highly reduced wings (Zera and Denno 1997). Several studies on wing-dimorphic insects have demonstrated that flight capacity is negatively correlated with reproductive performance (e.g. Mole and Zera 1993; see also Zera and Denno 1997 for a review). Cost of increased dispersal ability in terms of reduced reproduction is less well documented for wing-monomorphic species, in which dispersal is a continuously varying trait (but see Baguette and Schtickzelle 2006). Measuring dispersal capacity or rate is more difficult in wing-monomorphic than wing-dimorphic species, and therefore surrogates such as thorax weight, aspect ratio (wing span<sup>2</sup>/wing area), and wing loading (total dry mass/wing area) are often used (Hill *et al.* 1999b; Hughes *et al.* 2003). Hughes *et al.* (2003) demonstrated that in the speckled wood butterfly (*Pararge aegeria*) increased dispersal, as indicated by a relatively large and broad thorax, is associated with reduced investment in reproduction in expanding populations. Using such surrogates as a measure of dispersal is not, however, straightforward and therefore new methods of measuring dispersal more directly in wing-monomorphic species are needed. Importantly, cost of increased dispersal or mobility can also be something else than a purely physiological cost. For example, for a species living in a fragmented landscape the increased time spent outside the habitat patches by the more dispersive individuals is likely to be costly, as this may decrease the lifetime fecundity

due to less time for oviposition. Leaving the natal habitat patch may also increase mortality due to various factors.

In general, trade-offs between life history traits are most evident under more stressful environmental conditions, especially in the case of physiological trade-offs involving resource allocation (van Noordwijk and de Jong 1986; Zera and Harshman 2001). An increment of resources allocated to one function necessitates a decrement of resources to another function, commonly called as the “Y” model of resource allocation (van Noordwijk and de Jong 1986). However, a positive correlation can exist between traits linked by a functional trade-off if variability in nutrient input among individuals is greater than variability in nutrient allocation (Roff and Fairbairn 2007). Therefore, when interested in functional trade-offs between two traits or function, studies should be conducted not only by using phenotypic or genetic approaches but also by direct physiological (experimental) studies. Additionally, the importance of functional trade-offs should be validated by research on natural populations, as trade-offs observed under constant conditions created in the laboratory may not match those that individuals experience in the wild. Therefore, a comprehensive understanding of the evolution of life history trade-offs requires combination of physiological studies of allocation, ecological studies of nutrient acquisition as well as life history studies in the field.

Life history trade-offs are not only interesting due to the constraining relationships, but also because they are one of the mechanism maintaining variation in life history traits in natural populations. Other mechanisms include mutation-selection balance, differential selection in patchy environments, genotype x environment (G x E) interactions, and pleiotropy (Gillespie and Turelli 1989; Roff 1997). According to the mutation-selection balance concept the amount of variation in a trait reflects the balance between mutations, creating new variation, and selection, decreasing variation (Charlesworth and Hughes 2000). Genotype x environment interactions maintain variation in life history traits due to different trait variants being favored differently under different environmental conditions (Leroi *et al.* 1994).

Finally, pleiotropy, which describes the effect of a single gene on multiple phenotypic traits, has been proposed as a plausible mechanism maintaining variation in life history traits, though conclusive empirical evidence is still scarce (Roff 1997).

Finally, as selection can only work on traits that are genetically determined, understanding of the evolutionary processes in life history evolution is facilitated by knowing the variances and heritabilities of life history traits. Lande (1982) pointed out that a dynamic theory of life history evolution is derived by synthesizing population demography with quantitative genetics. Heritability is a measure of genetic determination of the trait, how much of the phenotypic variation in the trait of interest is explained by the additive genetic variance (Roff 1997). Depending on the amount and type of data available there are numerous methods that can be used to estimate heritabilities, including parent-offspring regression, full sib- and half sib-analysis, and maximum likelihood methods. Of these the latter is a more complex method that can be extended to examine several traits, dominance, and other complicating factors, but it requires a very large sample size (Roff 1997). In general, heritabilities of life history traits are assumed to be lower than heritabilities of e.g. morphological traits, because selection is expected to quickly reduce additive genetic variance of traits closely linked with fitness (Fischer 1930; Mousseau and Roff 1987). Price & Schuller (1991) alternatively suggested that heritability estimates of life history traits are often low because they are not only subject to the environmental variation in the life history trait but additionally to all the environmental variation in the underlying metric (physiological and morphological) traits. Consequently, any additional environmental variation affecting a life history trait enlarges only its total environmental component and, hence reduces its heritability (Price & Schuller 1991). Nevertheless, numerous studies have demonstrated that considerable genetic variation can also exist in life history traits in natural populations.

## 2. Aims of the thesis

This thesis had the following aims:

- Firstly, I wanted to assess the amount of variation in key life history traits, namely dispersal (mobility), clutch size, egg size, body mass, age at first reproduction, lifetime egg production, and lifespan, within the metapopulation of the Glanville fritillary butterfly (*Melitaea cinxia*) in the Åland Islands (**I, II & III**).
- The second aim was to understand which factors affect this variation and which traits are correlated with each other (**I, III, IV, V**). Comparisons of several life history traits were conducted between individuals originating from newly-established and old populations, as previous studies have shown that females from these two population types have dissimilar flight metabolic and dispersal rates (Haag *et al.* 2005; Hanski *et al.* 2002; 2004).
- Comparing individuals from the two population types allowed the study of the third aim of the thesis, the costs of being dispersive at the phenotypic level (**I, III & V**). I measured mobility directly in a population cage (**I**) and assessed its correlation with fecundity (**I, V**). Theoretical models were used to examine other possible costs of mobility (**I**).
- I examined genotypic differences among individuals in the gene *Pgi* (phosphoglucose isomerase) and the correlated variation in life history traits (**III, IV**).
- Finally, I estimated the heritability of mobility (**II**) and other life history traits under natural conditions.

In this thesis, I have been particularly interested in assessing the variation in phenotypes and the possible trade-offs between mobility and other life history traits in individuals living under natural conditions. All studies were conducted in a large population cage and with individuals collected from the wild either as post-diapause larvae or as newly-eclosed adults, except for the second generation of butterflies needed for the estimations of heritability (**III**).

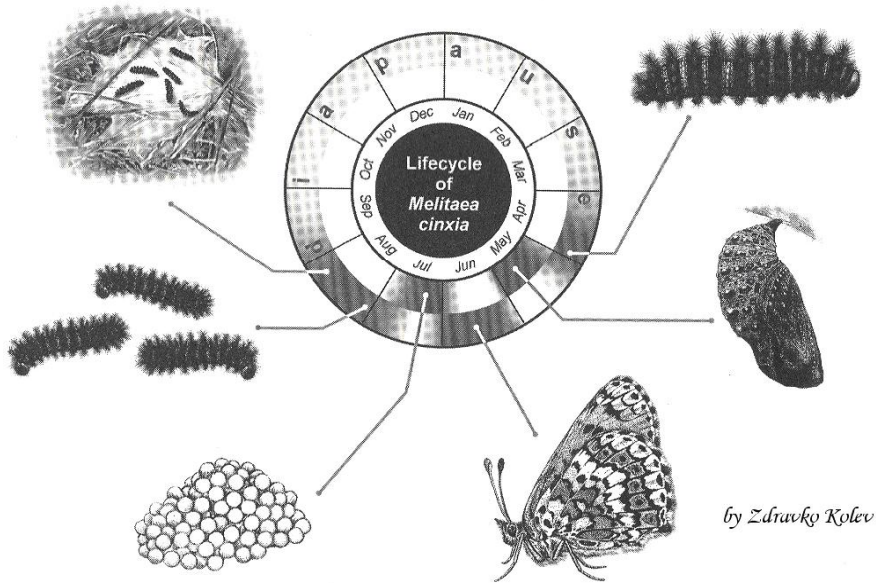
## 3. Study species and system

### *The Glanville fritillary butterfly*

The Glanville fritillary butterfly (*Melitaea cinxia*) is a member of the checkerspot tribe Melitaeini of the family Nymphalidae (Murphy *et al.* 2004). It is a medium-sized butterfly with a wide Palaearctic geographical range from western continental Europe to southern Siberia (Tolman and Lewington 1997). Even though the butterfly is common and widely distributed in much of southern and eastern Europe (Hanski and Kuussaari 1995), towards the north the distribution becomes increasingly fragmented. The butterfly has been and is still declining in numbers in many parts of central and western Europe. This is also the case in Finland, where the conservation status of the Glanville fritillary is “vulnerable” (Rassi *et al.* 2001). As is typical for checkerspots in general, the Glanville fritillary is a rather sedentary species, and therefore the species shows substantial intraspecific geographical variation in its ecology as populations respond both genetically and phenotypically to local conditions (Ehrlich and Hanski 2004). In most of Europe, including Finland, the butterfly has an obligate diapause and one generation per year (Fig. 1). Facultative diapause and from one to three generations per year are observed in parts of southern Europe and Asia (Kuussaari *et al.* 2004).

In Finland, the Glanville fritillary is present only in the Åland Islands, an archipelago in the northern Baltic south-west from the mainland Finland (Fig. 2). In the Åland Islands, the adult flight season lasts from early June to mid July. Females eclose with the full complement of oocytes in their ovarioles but egg maturation is influenced by the availability of adult food resource, nectar (personal observation). A large fraction (85-90%) of females mates only once. Females lay eggs in clutches of 130-160 eggs on average, and the lifetime egg production can be more than 1 000 eggs. Clutch size is an important fitness measure as larval survival in all but the last larval instar is positively correlated with group size (Kuussaari *et al.* 2004). During winter, the larvae diapause in a densely span “winter-web” in a family group (Kuussaari *et al.* 2004). In the

**Figure 1.** Life-cycle of the Glanville fritillary butterfly (*Melitaea cinxia*) in the Åland Islands (Murphy *et al.* 2004).



Åland Islands, the larvae have two host plant species, *Plantago lanceolata* and *Veronica spicata* (Kuussaari *et al.* 1998). Both host plant species contain iridoid glycosides, which are sequestered by the larvae and make both the larvae and the adults distasteful to predators (Harvey *et al.* 2005; Suomi *et al.* 2003). There does not seem to be any important natural enemies, apart from parasitoids (Van Nouhuys and Hanski 2004), attacking the Glanville fritillary and checkerspots in general (Ehrllich and Hanski 2004).

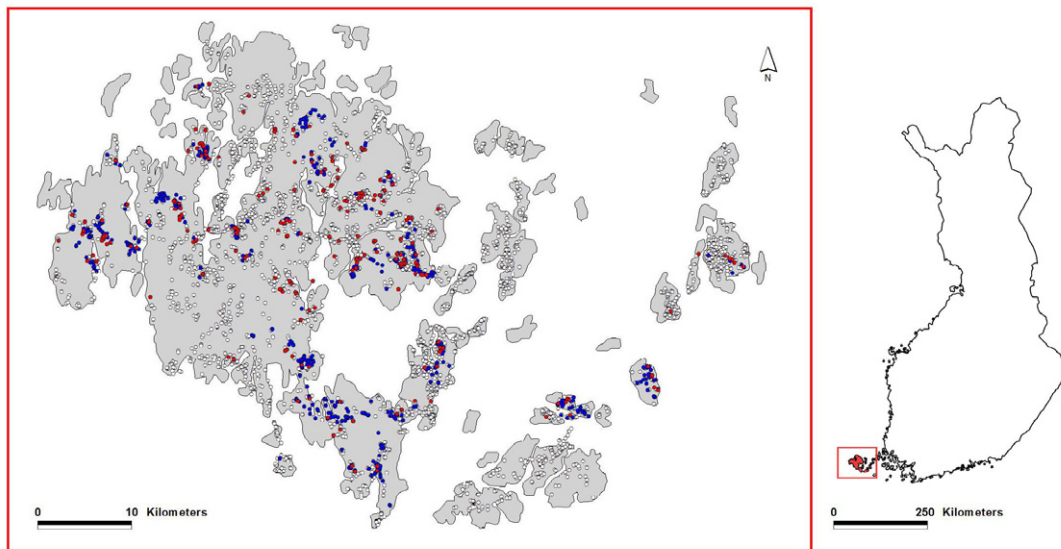
### *The Åland metapopulation*

The Glanville fritillary occurs in a highly fragmented landscape and has a classic metapopulation structure in the Åland Islands. The metapopulation dynamics have been studied since 1991 (Hanski 1999). Knowledge about the occupancy of the habitat patches in the entire patch network is gathered bi-annually by surveying all patches with the help of a large group of students (Hanski 1999). Of the roughly 4,000 habitat patches in the entire study area some 500-700 are occupied in any given year (Hanski 1999). The metapopulation has a high rate of population turnover (extinctions and

recolonisations; Nieminen *et al.* 2004), and of the 500-700 local populations approximately 100 are newly-established at a habitat patch that was unoccupied in the year before. These populations are hereafter referred to as newly-established populations. The individuals originating from such newly-established populations are the offspring of females that established the new population in the previous year. Local populations that had existed for > 5 years are referred to as old populations.

Females establishing new populations are unlikely to be a random sample from the entire metapopulation, and in fact Hanski *et al.* (2004) showed that F1 females sampled from newly-established isolated populations had higher dispersal rate than females originating from old populations. This conclusion was based on a theoretical model, mark-release-recapture data and a physiological study in which the [ATP]/[ADP] ratio in the flight muscles of butterflies was measured after constant flight in an experiment (Hanski *et al.* 2004). The latter result suggested a difference in metabolic performance between females from newly-established and old populations. Consistent results were obtained by Haag *et al.* (2005), who showed that females from newly-established populations have higher flight

**Figure 2.** Map and location of the Åland Islands in the northern Baltic Sea. The other map shows the newly-established and old populations in 2004, represented by red and blue symbols, respectively.



metabolic rate than old-population females, which is likely to be related to the difference in their dispersal rate.

### *The population cage*

The study material used in this thesis consists of butterflies sampled either as larvae (years 2003 and 2005) or as adults (2004) from 40 to 60 local populations located across the Åland Islands. In all years the local populations included both newly-established and old populations with varying connectivity to other existing populations. The larvae were reared in the laboratory under common garden conditions.

After eclosion adult butterflies were weighed and individually numbered under the wind wing, and released into a large population cage (32 x 26 x 3 m) constructed upon a natural habitat (dry meadow) in the field (Fig. 3). The cage is covered with a mesh preventing the butterflies from escaping but allowing natural environmental conditions (rain, sunlight and wind) inside the cage. Potted host plants were provided for oviposition in the central part of the cage, which was relatively bare of vegetation compared with the edges. The edges of the cage had taller vegetation and greater abundance of flowering plants that provided nectar for adult butterflies.

**Figure 3.** The population cage in the Åland Islands.



The cage was divided into 8 x 8 grid cells, which were surveyed systematically every second hour between 9:00 and 17:00 hours to obtain data about the location and mobility (see below) of the butterflies. During the surveys mating pairs were carefully recorded. Additional searches were conducted during the warmer hours of the day to minimise the number of matings missed. Based on the number of females that laid eggs but had not been observed to mate, 81% of the matings were observed directly. Potted host plants (*Plantago lanceolata* and *Veronica spicata*) located in a group in the central part of the cage were monitored continuously to record all the ovipositions. The first oviposition indicated the age at first reproduction. Every evening host plants were searched for possible additional egg clusters. In these studies, only 7% of the ovipositions were missed by not observing the actual oviposition. Following oviposition the clutch of eggs was removed, together with the leaf on which the eggs had been laid. To determine the clutch size the eggs were counted at the age of 3-5 days with a small paint brush. For each female eggs in all her clutches were summed up to obtain a measure of lifetime egg production. In 2005 and 2006 some clutches were weighed to determine the average egg weight.

As a measure of individual mobility in the cage I used the residual from the regression of the number of grid cells in which the butterfly had been observed against the number of observations during the surveys in the cage. Mobility was calculated separately for different age groups: 0-3 days, 4-9 days and > 9 days of age, as the reproductive state of an individual may affect movements.

#### *Phosphoglucose isomerase (PGI) and its genotyping*

Phosphoglucose isomerase (PGI) is a glycolytic enzyme (Box 2) and hence plays a role in providing fuel for energy-demanding processes such as flight. Studies on *Colias* butterflies have demonstrated that allelic variation in PGI is correlated with variation in fitness. In *Colias*, genotypes (allozymes) differ in their flight performance (Watt *et al.* 1983). Both female fecundity and male mating success are directly

influenced by flight performance and thereby are correlated with the PGI genotype (Watt 1992). Female *Colias* with a particular PGI genotype (3/4 heterozygotes) are able to fly at lower ambient temperatures and may thus have more time for oviposition than females with other genotypes (Watt 1992).

Previous studies on the Glanville fritillary have identified seven different allozyme genotypes at PGI (Haag *et al.* 2005; Saccheri *et al.* 1998). Studies conducted nine years apart showed that the frequencies of these alleles have remained stable over time (Haag *et al.* 2005; Saccheri *et al.* 1998). Among the alleles, PGI-*d* and PGI-*f* are present in high frequencies, 0.49 and 0.29, respectively (Haag *et al.* 2005; Saccheri *et al.* 1998). Haag *et al.* (2005) showed that the frequency of PGI-*f* is higher in newly-established populations, in which females tend to be more dispersive than females in older populations (Hanski *et al.* 2004). More recently, Wheat *et al.* (in review) have shown that underlying the variation at the allozyme level there is extensive variation at the DNA sequence level at *Pgi*. Orsini *et al.* (in prep.) have identified a set of three single nucleotide polymorphisms (SNP) that suffice to discriminate among the numerically dominant allozymes, including the PGI-*f* allele. In this thesis, the *Pgi* genotypes have been identified using two different methods. In chapter III, when the SNPs were not yet available, PGI genotypes were identified using allozymes. In chapter IV, we used the SNPs to identify the respective genotypes (Box 3).

## 4. Results and discussion

#### *Variation in mobility between individuals from newly-established and old populations (I, II, V)*

Previous studies by Hanski and others (2002, 2004) had demonstrated a difference in dispersal rate between females originating from newly-established and old populations. Can the same difference be observed in the population cage? Results of the first chapter (I) showed that females from newly-established isolated populations were more mobile than females from old populations. Even though, mobility measured in the cage (see

## Box 2. Glycolysis and phosphoglucose isomerase (PGI)

Glycolysis is a metabolic pathway by which glucose and fructose, the principal components of flower nectar, are transformed into two molecules of pyruvic acid. Glycolysis serves three principal functions. High-energy molecules, ATP and NADH are generated in glycolysis and the downstream pathways, which glycolysis feeds into (e.g. aerobic respiration). ATP provides the energy for many cellular functions as well as high energy needed for flight. Additionally, a variety of six- and three-carbon intermediate compounds are produced, which may be removed at various steps in the process for other cellular functions.

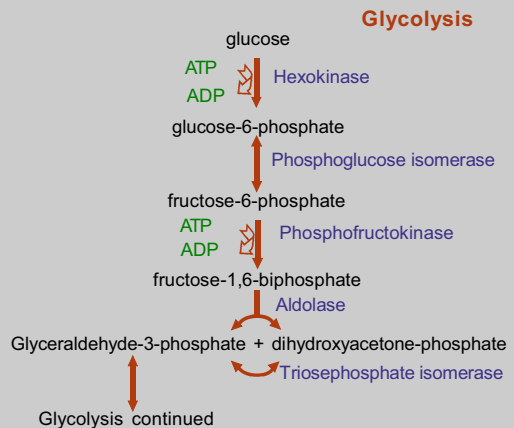
The second step of glycolysis is the conversion of glucose-6-phosphate to a fructose-6-phosphate, which is catalyzed by the enzyme *phosphoglucose isomerase* (PGI). Comprehensive studies on *Colias* butterflies have demonstrated that *Pgi* genotypes (determined as allozymes) differ in their metabolic capacity, which leads to predictable differences in flight performance and affect both female and male reproductive success (Watt et al. 1983, Watt 1992). Recent studies on the Glanville fritillary butterfly have shown that flight metabolic rate and female fecundity correlate with *Pgi* genotype (Haag et al. 2005).

### References:

Haag, C. R., M. Saastamoinen, J. H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proceedings of the Royal Society of London Series B Biological Sciences* 272:2449-2456.

Watt, W. B et al. 1983. Adaptation at specific loci. III. Field behaviour and survivorship differences among *Colias* Pgi genotypes are predictable from in vitro biochemistry. *Genetics* 103:725- 739.

Watt, W. B. 1992. Eggs, enzymes, and evolution: Natural genetic variants change insect fecundity. *Proceedings of the National Academy of Sciences, USA* 89:10608-10612.



above) is not the same as dispersal among habitat patches in the field, these two types of movements are likely to be correlated. This is because more active or mobile individuals are more prone to come to contact with habitat boundaries, and therefore more likely to leave the habitat patch than less mobile individuals (but see Van Dyck and Baguette 2005). The difference in mobility between females originating from newly-established and old populations was only apparent in their early life (< 4 days; Fig. 4). This makes sense as after 4 days female butterflies initiate oviposition and their activity may thereafter be more directed towards the centre of the cage where the host plants were located. There

was no difference in the mobility of males originating from newly-established versus old populations. This latter result is consistent with previous results (Hanski *et al.* 2004), and may, at least partly, be explained by mobility and dispersal serving a different function in the two sexes (see below).

Studies conducted in 2004 and 2005, however, found no difference in mobility between females from newly-established and old populations. This is likely to be due to various other factors apart from population age affecting the measure of mobility in the population cage. Firstly, in 2004 individuals were collected from the wild as adults and hence many of the



### Box 3. Methods of genotyping different *Pgi* types in the Glanville fritillary butterfly

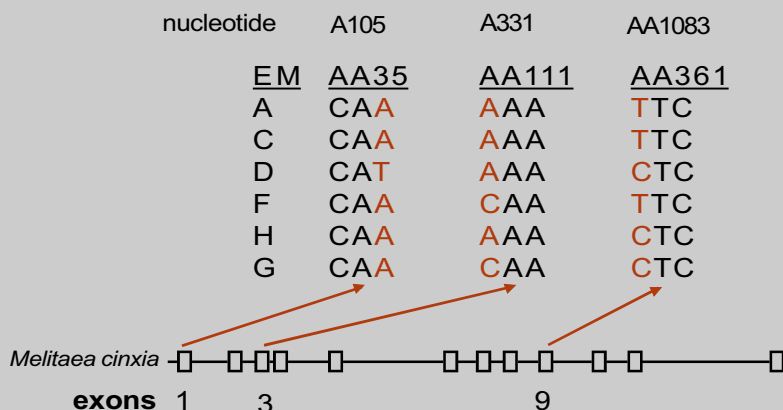
In the Glanville fritillary, the *Pgi* genotypes have been identified based on the electrophoretic mobility of the different allozyme isoforms (Haag *et al.* 2005). Recently, Wheat *et al.* (in review) have shown that underlying the variation at the allozyme level there is extensive variation at the DNA sequence level. Orsini *et al.* (in prep.) have identified a set of SNPs that suffice to discriminate among the numerically dominant allozyme isomorphs, principally the PGI-*f* and PGI-*d* allele.

#### Allozymes (III):

Allozymes were analysed through electrophoresis, in which tissue homogenates were loaded on a gel and the different allozyme electromorphs were separated on the basis of their electrophoretic mobility arising from charge changing amino acid variation among the isomorphs (allozymes). In the Glanville fritillary seven different allozyme electromorphs have been identified in the Åland Islands, of which PGI-*f* and PGI-*d* are the most frequent (Saccheri *et al.* 1998, Haag *et al.* 2005).

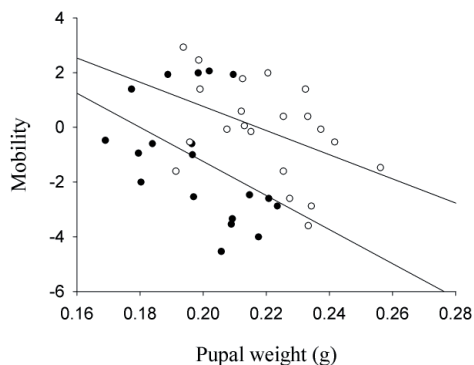
#### SNP (IV):

SNP are single nucleotide polymorphisms in DNA sequences. SNPs of interest for *Pgi* genotyping in the Glanville fritillary fall within the coding region of the *Pgi* gene, and are responsible for the charge changing amino acid variation underlying the allozyme isomorphs (or in linkage). Orsini *et al.* (in prep.) have identified a set of three SNPs that are sufficient to distinguish between all of the allozyme electromorphs (EM) found in the Åland Islands (see table below). A combination of two SNPs, AA111F and AA361R, however, suffice to distinguish the PGI-*f* allozyme electromorph, as can be seen in the table below (Orsini *et al.* in prep.). Individuals with bases AC or CC in AA111F and with bases CT or TT in A361R correspond to PGI-*f*. These two SNPs are strongly linked and hence the results would be qualitatively the same for AA111F only, in which case AC and CC individuals were contrasted with AA individuals.



#### References:

- Haag, C. R., M. Saastamoinen, J. H. Marden, and I. Hanski. 2005. A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proceedings of the Royal Society of London Series B Biological Sciences* 272:2449-2456.
- 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.



**Figure 4.** Early-life mobility against body mass in females originating from newly-established and old populations (open symbols with dashed line and closed symbols with solid line, respectively) in 2003 (chapter I).

butterflies were probably already older than three days (it was the early-life mobility where the difference was observed in 2003). Secondly, in 2005, the samples were not obtained from the most isolated newly-established populations, which in previous studies have been shown to contain the most dispersive females. Finally, the measure of mobility obtained in the cage must be greatly influenced by prevailing weather conditions, which vary among the years.

#### *Heritability of dispersal and other life history traits (II)*

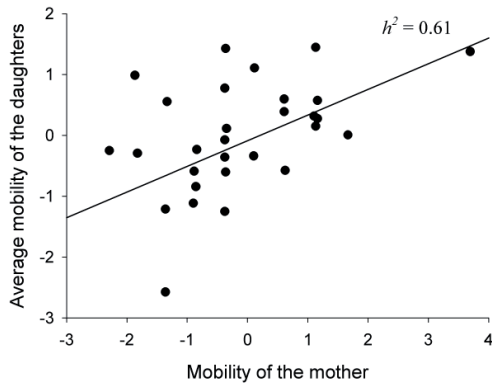
Variation in dispersal rate between females from newly-established and old populations does not alone suffice to demonstrate the evolutionary importance of such variation. For a trait to respond to selection and to evolve it needs to be heritable from parents to offspring. The aim of the second chapter was to assess whether mobility and related life history traits are heritable under the field conditions in the population cage.

Results supported the previous inferences from indirect data that dispersal rate is indeed heritable in the Glanville fritillary, but only from the mother to her female offspring (Fig. 5). In the field, differences in dispersal capacity between individuals from newly-established and old populations are, consistently, only evident in females (Hanski *et al.* 2002, 2004). Furthermore, it

was only the early-life mobility that was significantly heritable from mother to daughters. Similarly, previous studies conducted in the population cage showed that differences in mobility between population types were only evident in the pre-reproductive stage. This suggests that mobility in older butterflies is primarily affected by environmental variation.

Reasons for mobility being heritable only from the mother to her female offspring can only be speculated. Rather than being sex-linked, I suggest that mobility is dissimilarly expressed in the two sexes. The function of early-life mobility in males is likely to be primarily mate searching as most females only mate once and do so soon after eclosion. The measure of mobility used in the cage may not distinguish well differences in male movement behaviour, namely differences in mate searching strategies (Van Dyck and Matthysen 1998). In females, on the other hand, flight is necessary for searching for nectar and oviposition sites within habitat patches as well as for dispersal to other patches, and general differences in mobility among individuals may be easier to distinguish than in males. The most likely explanation for the maintenance of variation in dispersal rate in females is the spatially varying selection pressures in the metapopulation, for which there is now direct empirical evidence (Hanski and Saccheri 2006).

I estimated heritabilities of other life history traits apart from dispersal, and many of these traits were highly heritable. Body mass was moderately heritable ( $h^2 = 0.36$ ), but greater resemblance between mother rather than a father and her offspring suggests that maternal effects may be inflating this estimate (see below). Heritability estimate for body mass-corrected egg weight was very high, close to one. Additionally, mothers that reproduced early in life had offspring that also started reproducing early. In contrast, clutch size and lifetime egg production, traits most closely linked with female fitness, showed low heritability as predicted by life history theory (Fischer 1930; Mousseau and Roff 1987; Roff 2002). Apparently, additive genetic variance in these traits has been reduced by natural selection and environmental factors cause most of the variation to be observed. In general, heritability estimates for mobility, age at first reproduction,



**Figure 5.** Mother–daughter regression for early-life mobility (< 4 days) in 29 females and their daughters (chapter II).

body size and egg size were surprisingly high. Spatial population structure and spatially varying selection pressure are likely to maintain genetic variation in these life history traits, especially those that are correlated with dispersal.

A potential problem in estimating heritabilities with parent-offspring regression is that it is impossible to account for maternal effects or the effect of the common environment affecting the traits of both the mothers and the offspring (Roff 1997). This may bias and inflate the heritability estimates. It is not however that obvious how maternal effects could affect the heritability estimates of mobility, especially as mobility was significantly heritable only from mothers to daughters. Maternal effects themselves could possibly be sex-specific as has been shown to be the case in recent studies on birds (Badyaev 2005; Badyaev *et al.* 2005; 2006). Whether the estimation of heritability of mobility between mother and her female offspring in chapter II is inflated by maternal effects only expressed in females therefore remains an unanswered but intriguing possibility.

Heritability estimates are greatly affected by environmental variation, and hence under different environmental conditions the estimates may be different. Estimates of heritability for dispersal have been previously obtained for other species of Lepidoptera (e.g. the codling moth, the light brown apple moth, and the African armyworm moth), and the value of  $h^2 = 0.61$

obtained in chapter II is consistent with the values estimated in these other studies ( $h^2 \sim 0.5$ ; Dingle 1991). The present results are, however, among the first to demonstrate significant heritability for dispersal in an insect population under field conditions.

#### *Female traits affecting clutch size and lifetime egg production (III, IV, V)*

A good measure of female fitness is the total number of eggs laid in its lifetime. In the Glanville fritillary, clutch size is additionally an important fitness component, as larval survival in all but one larval instar and especially over winter is highly positively dependent on group size (Kuussaari *et al.* 2004). Finally, a trait generally assumed to be closely related to fitness is egg size, as larger eggs produce larger newly-hatched larvae (Fischer *et al.* 2002; Fox and Czesak 2000). In two chapters in this thesis I assessed which factors, considering both the traits of the female as well as environmental variables, explain best variation in clutch size (III, IV), egg size and lifetime egg production (V).

Numerous female traits affected clutch size in the Glanville fritillary. Clutch size decreased with the number of eggs laid previously, which correlates positively with female age. As the study was conducted in two consecutive years it allowed also the assessment of the generality of the results. Factors affecting clutch size varied to some extent between the years, as both the interval between ovipositions and female body weight affected clutch size in one year but not in the other. This implies that prevailing environmental conditions may have a big influence on clutch size. For example, in some years larger females, as suggested by Gotthard *et al.* (2007), may not be able to lay all their eggs due to generally unfavourable environmental conditions. Alternatively, poor environmental conditions may prevent smaller butterflies from compensating shortage of resources obtained during larval stage (body size) by increased adult feeding (O'Brien *et al.* 2004), which may limit fecundity.

Several female traits also affected the lifetime egg production under the semi-natural conditions in the large outdoor population cage

(V). As expected, lifespan had the greatest effect on lifetime egg production. Other factors included eclosion time (date) and number of matings. The effect of the number of matings on lifetime egg production was not straightforward, however, as it seemed that females that mated multiple times had higher fecundity already prior to their second mating. It is also possible that due to their increased fecundity they were running out of resources and therefore needed to mate for the second time.

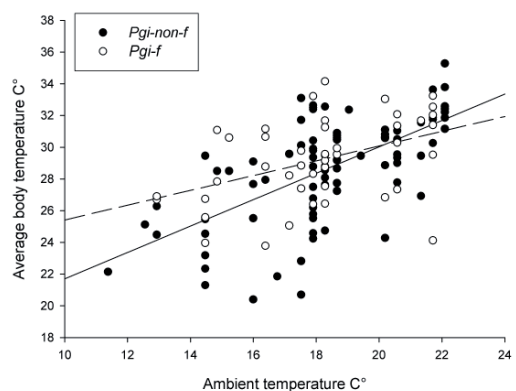
#### *Effect of environmental variation (III, IV, V)*

Results of chapter III, which demonstrated that many female traits influence clutch size in the Glanville fritillary, also suggested that females that initiated oviposition earlier in the day, when the environmental conditions were often most favourable (high temperature), laid larger clutches than females that laid eggs later on in the day. Furthermore, allelic variation in the phosphoglucose isomerase enzyme (PGI) was correlated with timing of egg-laying, such that females with certain genotypes (*PGI-f*) initiated oviposition earlier in the day than other females, which may potentially explain the larger clutch size in *PGI-f* females.

These results led us to conduct an experiment (IV) in which the effects of the time of oviposition were studied in more detail. Results of this study together with previous results strongly suggest that genotypic differences correlate with traits related to flight performance and reproduction. Individuals with the *Pgi-f* allele appear to have superior flight performance compared with individuals without this allele, as *Pgi-f* individuals were able to attain or maintain higher body temperature at low ambient temperatures in the morning, potentially allowing them to fly under sub-optimal conditions (Fig. 6). This may greatly enhance dispersal, as dispersive individuals may often encounter unfavourable weather conditions. The ability to fly under sub-optimal conditions may even be a trait under selection due to the metapopulation structure of the Glanville fritillary in the Åland Islands. The present results also demonstrate the fitness advantage of being able to fly under cool morning hours as females are thereby able to initiate

oviposition during the favourable environmental conditions when clutches tend to be larger. The results of these two studies (III, IV) illustrate how a range of phenotypic and genotypic traits combine with environmental factors to determine clutch size and thereby influence individual fitness and potentially affect population dynamics. Furthermore, in contrast to the general assumption that most life history traits are influenced by many genes of small effects (Stearns 1992), even single genes can have strong influence on life history traits (see also De Luca *et al.* 2003; Haag *et al.* 2005; Mackay 2001). Finally, the results highlight the importance of conducting life history studies under natural environmental conditions.

Females had higher average body temperature at flight than males, consistent with other studies on Lepidoptera (Gilchrist 1990; Pivnick and McNeil 1986). The likely reason is sexual size difference, as in the Glanville fritillary and most other butterflies females are larger than males. Smaller butterflies tend to heat up faster than heavier ones but they also cool off faster, allowing females to maintain elevated body temperature at flight for longer (Heinrich 1993). In the European skipper (*Thymelicus lineola*), the ability of males to fly at lower body temperature has been attributed to lower wing loading (body mass/wing area) in males, which



**Figure 6.** The relationship between the average body temperature at flight and ambient temperature. Open symbols with dashed line represent individuals with the *Pgi-f* allele and closed symbols with solid line represent individuals without the *Pgi-f* allele (chapter IV).

allows them to fly with lower wing beat frequency, which in turn is temperature-dependent (Pivnick and McNeil 1986). Differences between the sexes may also arise due to dissimilar selection pressures, as males need to invest more on behaviours and physiological processes that enable them to compete for mating success (Pivnick and McNeil 1986). Females, on the other hand, invest primarily in egg production (abdomen size), as female reproductive success is first of all limited by the number of eggs produced (Clutton-Brock and Parker 1992; Clutton-Brock and Vincent 1991). However, selection for increased flight ability under sub-optimal weather conditions is likely to affect also females, as flight under sub-optimal conditions may often be required for nectar and host plant searching and for oviposition.

*The costs of being dispersive – no apparent trade-off between dispersal and reproduction (I, II, III, V).*

The main aim of chapters **I** and **V** was to investigate whether the generally assumed trade-off between dispersal and reproduction is also apparent in the Glanville fritillary, which could potentially explain variation in dispersal rate at the metapopulation level. Results of these studies were in conflict with those for many wing-dimorphic species, as the present results strongly suggested that there is no general trade-off between dispersal and reproduction in the Glanville fritillary. This conclusion is based on the following results.

- Firstly, lifetime egg production and individual mobility as measured in the cage were not negatively correlated.
- Secondly, more mobile females actually initiated reproduction earlier, not later, than the more sedentary females (in terms of mating in 2003 and in terms of oviposition in 2004 and 2005). In chapter **III** a genetic correlation, even though not statistically significant, between mobility and age at first reproduction similarly indicated that more mobile females start reproducing at younger age than more sedentary females.
- Thirdly, previous studies have shown that females from newly-established

populations are generally more dispersive in the field than old-population females (based on mark-release-recapture studies; Hanski *et al.* 2002; 2004) and the former have higher flight metabolic rate (Haag *et al.* 2005), which is correlated with dispersal rate (Niitepõld *et al.* in prep.). Yet in the studies conducted in three consecutive years there was no difference in the lifetime egg production of butterflies representing the two population types. In 2003, the results even indicated that females from newly-established populations may have higher lifetime fecundity than females from old populations as the cumulative number of clutches laid increased faster with age in new population than in old-population females (**I**). The difference was significant in females older than 13 days. The same pattern was also observed in cumulative egg production, though in this case it was not statistically significant.

- Most importantly, there was a consistent and significant (in 2 out of 3 years) interaction between mobility and population age in affecting reproduction: in new populations, lifetime fecundity was positively correlated with mobility, while in old populations there was a significant interaction between lifespan and mobility, such that long-lived more sedentary females had the highest lifetime egg production. This latter result suggests dissimilar selection on mobility and related life history traits between newly-established and old populations, which may maintain variation in dispersal rate in the metapopulation as a whole.

Positive correlation between dispersal and reproduction in generally dispersive species or populations has been explained by the selective advantage of such correlation due to increased success of individuals colonizing new habitats (Dingle *et al.* 1988; Lavie and Ritte 1978). Such associations between high dispersal rate, high fecundity and short lifespan have been referred to as the “coloniser-syndrome” (Baker and Stebbins 1965) or “fugitive species” (Hutchinson 1951) and assumed to be characteristic for species living in highly unstable habitats (Ronce and

Olivieri 2004). On the other hand, for individuals living in more permanent populations high dispersal rate is not essential and in such cases selection may favour individuals with other fecundity-related life history traits, for instance increased longevity, as indicated by the present results for females in old populations (**I**, **V**). These results are reminiscent of some previous results comparing dispersive versus sedentary species/populations (Lavie and Ritte 1978; Wu 1981).

Even though no support for a general trade-off between mobility and reproduction was found in this thesis (**I**, **III** or **V**), mobility or dispersal capacity is surely not cost-free in the Glanville fritillary. The study conducted in the population cage in 2003 indicated that females from newly-established populations have shorter maximal lifespan than females from older populations (see also Zheng *et al.* 2007). As females from newly-established populations have been shown to be more dispersive than females from older populations this suggests a cost of dispersal in terms of longevity, possibly related to the higher flight metabolic rate of the more dispersive females (Haag *et al.* 2005). A well-known hypothesis posits that individuals with higher metabolic rate consume more oxygen and therefore produce harmful free-radical by-products at a higher rate, which leads to a higher rate of damage to critical cellular targets and hence higher rate of cellular aging (Adelman *et al.* 1988).

Secondly, in fragmented landscapes, an ecological trade-off exists between being more dispersive and therefore spending more time in the landscape matrix versus having more time for reproduction in the habitat (**I**). In chapter **I**, we showed with a dispersal model that for this reason the lifetime egg production is 4% smaller, on average, in the more dispersive butterflies. Furthermore, mortality in the matrix may be higher than in the habitat patches and therefore the cost of being dispersive may be even greater (this was not included in the model). Hanski and Saccheri (2006) recently demonstrated that the allelic composition of the glycolytic enzyme phosphoglucose isomerase, which is known to affect flight metabolic rate and some life history traits in the Glanville fritillary (chapters **III**, **IV**; see also Haag *et al.* 2005), affects the growth of local populations. Importantly, however, the effect

is context dependent, so that the strength and the sign of the molecular effect on population growth depend on the size and the connectivity of the habitat patches (Hanski and Saccheri 2006). Thus, though in small populations (small habitat patches) the more dispersive females had higher lifetime reproductive output than the more sedentary females, the cost of dispersal becomes apparent in large populations, where the more dispersive types perform worse than the sedentary types, possibly due to their shorter lifespan and shorter residence time (Hanski and Saccheri 2006).

Finally, it is important to keep in mind that correlation studies cannot unambiguously demonstrate whether or not traits interact functionally (Zera and Harshman 2001; Roff and Fairbairn 2007), and therefore some internal (functional) trade-offs between dispersal and reproduction are possible in spite of the results in this thesis. Physiological studies should be conducted to validate the present results. As a matter of fact, a previous study on the Glanville fritillary demonstrated reduced potential fecundity (number of oocytes at eclosion) in females from newly-established populations compared with females from old populations (Hanski *et al.* 2004), though the results in this thesis show no comparable difference in realized fecundity (lifetime egg production). Furthermore, positive correlations between traits that may be assumed to correlate negatively can be found under conditions when resource availability is not restricted (e.g. Leroi *et al.* 1994; Tessier *et al.* 2000). As concluded by Reznick *et al.* (2000), “super phenotypes” are real, but it may be that they can only be “super” under particular conditions. Results of the present thesis suggest that even though functional trade-offs are likely to exist between dispersal ability and reproduction, they are not often apparent at the phenotypic level under the environmental conditions in which natural populations occur.

## 5. Conclusions

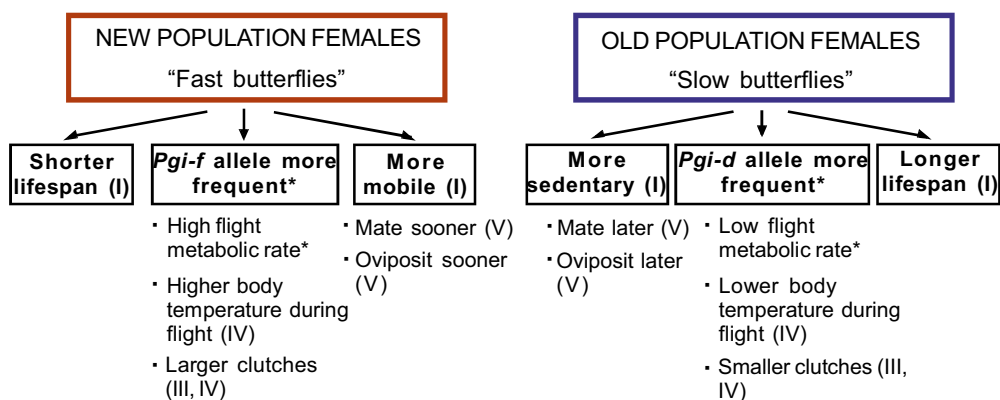
Most studies of life history evolution are based on the assumption that species exist as undisturbed and spatially homogeneous

populations (Ronce and Olivieri 2004). In the real world, however, this is often not the case as populations are spatially structured, and therefore the characteristics of metapopulations should be taken into account while studying factors affecting life history evolution. The spatial context has been considered to some extent in the theoretical study of life history evolution during the last few years, but corresponding empirical experiments and research are still rare (Olivieri and Gouyon 1997; Ronce and Olivieri 2004). This is, at least to some extent, due to the practical difficulties in studying metapopulation-level processes in life history evolution empirically (but see Thomas *et al.* 1998; Hill *et al.* 1999a,b). In this thesis, I have tried to narrow this gap between theoretical and empirical studies on life history evolution by using the well-known Glanville fritillary metapopulation as a model system. An attempt was also made to integrate the consequences of genetic variation on life history ecology.

Results of this thesis together with those obtained by others (Haag *et al.* 2005; Hanski *et al.* 2002; 2004) show that in the Glanville fritillary, empirical evidence broadly supports the theoretical expectations for metapopulations, as individuals originating from different kinds of populations within the larger metapopulation vary in their dispersal ability. Females from the newly-

established isolated populations are more dispersive than females from old well-connected populations. As dispersal ability correlates with some other life history traits, it appears that the metapopulation includes females with two types of “strategies”, though in reality these are likely to be extremes of a continuum. Females from newly-established populations move more, reproduce early and live for shorter time. Equivalent lifetime egg production is achieved by females from old populations, but these females, who move less, seem to compensate for the slower reproduction by longer lifespan (Fig. 7). Interestingly, these differences are correlated with variation in one gene, *Pgi*, encoding an enzyme that is catalyzing glycolysis. *Pgi* is significantly correlated with flight metabolic performance (Haag *et al.* 2005), which in turn is correlated with dispersal rate (Niitepõld *et al.* in prep). Other studies have shown the significance of this enzyme at the metapopulation level (Haag *et al.* 2005, Hanski and Saccheri 2006). It now seems evident that *Pgi*, or some gene(s) closely linked with it, plays an important role in the life history as well, as individuals with a certain genotype are able to be active and lay eggs under more unfavourable environmental conditions than others.

Further studies on the effect of *Pgi* on other life history traits, such as developmental rate and



\* 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.

**Figure 7.** Variation in life history traits between females originating from newly-established and old populations in the Åland Islands.

lifespan, are likely to reveal new interesting results. It will be important to obtain more mechanistic knowledge about the underlying molecular mechanisms of the effects of *Pgi*. However, work on *Pgi* and its association with life history traits has already demonstrated the importance of integrative approaches in studies of life history evolution. Comparable integrative approaches have recently been implemented in many areas of biology including evolutionary ecology (e.g. Brakefield and Roskam 2006). In this thesis, I have only started the demanding project of understanding variation in life history traits from genes all the way to the level of metapopulations, but this work has much potential for more research.

The present results support the idea that the generality of the assumptions of life history theory should be validated by experiments conducted with wild individuals under natural environmental conditions. It seems apparent that in the Glanville fritillary more mobile females do not pay a cost in terms of reduced reproductive output under natural conditions, as females rarely live long enough to lay the full potential number of eggs they have at birth.

Even though dispersal plays an important role in the metapopulation context and is therefore one of the most important life history traits in species living in fragmented habitats, it is also important to consider interactions with other traits. It seems evident that other life history traits such as lifespan and age at first reproduction may be affected by the spatial structure of populations and even independently of dispersal. In this thesis, I have only touched upon this issue but it is apparent that there is a lot of variation also in these other life history traits at the metapopulation-level and that one of the main mechanisms maintaining this variation may indeed be spatial heterogeneity. Interactions between dispersal and other life history traits are also apparent. For example, more dispersive individuals initiated reproduction (mating and/or oviposition) sooner than more sedentary individuals, and most likely as a direct result of being more mobile.

To attain a more mechanistic understanding of the patterns described in this thesis more experimental techniques should be applied in

future under controlled environmental conditions. In the present studies variation in the environmental conditions was either natural or only the ambient temperature was manipulated. Another important environmental factor that should be investigated is the amount of resources, both larval and adult resources, and their effects on life histories, including trade-offs between dispersal and reproduction. For example, more dispersive individuals may be able to maintain equal reproductive output with more sedentary females due to increased resource acquisition (Reznick *et al.* 2000; but see Huberty and Denno 2006). If the amount of resources available would be experimentally manipulated very different patterns to those obtained in the present thesis could arise (see review in Reznick *et al.* 2000). Finally, as estimates of heritabilities from parent-offspring regressions may be inflated by maternal effects comparing estimates obtained by other methods, such as direct selection experiments, could be useful and informative. With such experiments one could also assess the genetic basis of the possible trade-offs and developmental constraints (e.g. Fischer *et al.* 2006; Frankino *et al.* 2005).

## 6. Acknowledgements

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