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Ilkka Hanski: The legacy of a multifaceted ecologist

A candidate gene in an ecological model species: Phosphoglucose isomerase (*Pgi*) in the Glanville fritillary butterfly (*Melitaea cinxia*)

Kristjan Niitepõld & Marjo Saastamoinen*

*Metapopulation Research Centre, P.O. Box 65, FI-00014 University of Helsinki, Finland (*corresponding author's e-mail: marjo.saastamoinen@helsinki.fi)*

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The Glanville fritillary butterfly inhabits fragmented landscapes in the Åland Islands, Finland, where the long-term persistence of this classic metapopulation depends on frequent re-colonisation events. A number of studies have focused on understanding how dispersal propensity varies within this system, what the underlying mechanisms are, and how variation in dispersal influences metapopulation dynamics. In the mid-2000s the focus turned to a potential candidate gene, phosphoglucose isomerase (*Pgi*) that was soon found to have effects on phenotypic performance of individuals in regard to their dispersal potential but also other key life-history traits. Variation in *Pgi* has further been shown to affect population dynamics. Here we review 30 research papers in which associations of *Pgi* polymorphism with metabolic rate, mobility, fecundity, lifespan, and population growth rate in the Glanville fritillary are discussed. The studies highlight the role of environmental variation and landscape structure in maintaining genetic polymorphism in this study system.

Introduction

The genetic basis of life history traits, such as development time, age at maturation or lifespan is not clearly understood (Stapley *et al*. 2010, Klepsatel & Flatt 2011). Yet, understanding the genetics of how organisms adapt to changing environments is a fundamental topic in evolutionary ecology, and many life history traits are directly linked to fitness and hence crucial components of adaptation. Assessments of heritability indicate that life history traits often show a great amount of additive variance in natural populations (Houle 1992, Merilä & Sheldon 1999, Kruuk *et al*. 2000), but actually mapping fitness-related phenotypes to genotypes has so far mainly been done in a few model organisms, such as *Drosophila*, *Caenorhabditis elegans* and *Arabidopsis* (Partridge & Gems 2006, van Straalen & Roelofs 2006, Kenyon 2010, Richards *et al*. 2012). Even though genomic studies on life history adaptation in the wild are still relatively rare, there are some recent studies on *Arabidopsis thaliana* (Fournier-Level *et al*. 2011), the Soay sheep (Johnston *et al*. 2013) and the Atlantic salmon, *Salmo salar* (Barson *et al*. 2015) for example, that have identified candidate loci for local adaptation and key life

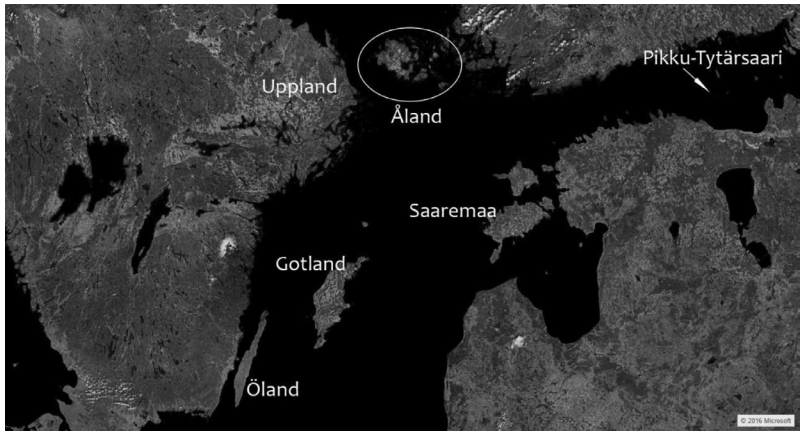

Fig. 1. Northern part of the Baltic Sea. Note the fragmented landscapes in the Åland Islands, the Uppland region on the Swedish mainland, and the fragmented landscapes in SW Finland where the Glanville fritillary persisted until the 1970s. The large islands of Saaremaa, Öland and Gotland sustain large, continuous populations of the Glanville fritillary. The island Pikku-Tytärsaari is completely isolated form other populations. The background image used according to Microsoft's terms and conditions of usage.

history traits in wild populations. Generally, due to their complexity, life-history traits are likely to be polygenic, but examples of one or a small number of genes having a large affect also exist (e.g. Barson *et al*. 2015).

The Glanville fritillary — an ecological model system

Demographic and microevolutionary dynamics of populations are often strongly affected by dispersal and gene flow among populations (Clobert *et al*. 2012). Dispersal is a crucial process in species living in fragmented landscapes and those characterized by metapopulation dynamics. The long-term persistence of metapopulations requires a sufficient amount of dispersal that leads to colonisation and re-colonisation of local populations, compensating for local extinctions (Hanski 1999). Due to its importance for metapopulations, dispersal has been studied extensively using the Glanville fritillary (*Melitaea cinxia*) butterfly in the Åland Islands (Fig. 1). The studies were initiated by Ilkka Hanski and colleagues in the early 1990s and the species has since become an ecological model system for studying how species respond to or cope with habitat fragmentation (Hanski 1999, 2011). The system consists of a network of 4000 habitat patches, characterized by the presence of one or both of the larval host-plant species *Plantago lanceolata* and *Veronica spicata* (Nieminen *et al*. 2004). The patches are surveyed twice a year by a group of research assistants, and we know that around 500 local populations are occupied by the butterfly in this large network in a given year (Ojanen *et al*. 2013). The Glanville fritillary in the Åland Islands has a classical metapopulation structure with a high rate of population turnover (extinctions and re-colonisations of local populations; Hanski 1999). Approximately, 100 local populations each year go extinct with a similar number of re-colonisations, and hence as a whole, the size of the metapopulation is relatively stable (Hanski 2011). Females lay clusters of 100–150 eggs (Saastamoinen 2007b) and one female can lay even a thousand eggs in her lifetime (Saastamoinen 2007a). Hence, a single mated female can easily establish a new population within this system.

The initial studies on dispersal in the Glanville fritillary included the more traditional mark–release–recapture studies that assess the actual dispersal event, as well as those that inferred successful dispersal events from the long-term survey data. Based on the autumn survey data, we know which previously empty habitat patches have been re-established in a given year (Ojanen *et al*. 2013). Together these

THE MANY NAMES OF *M. CINXIA Pgi* ALLELES

The nomenclature associated with *Pgi* is almost as diverse as the locus itself. We refer to the enzyme and allozyme locus as PGI, whereas the genetic locus is called *Pgi*. In the Glanville fritillary, the two most common allozyme alleles are known as d and f. The single nucleotide polymorphism (SNP) (Pgi):c.331A>C is being used to divide between the *Pgi-f* or *Pgi*-non-*f* individuals. The same SNP is also called *Pgi* AA111, in some studies, according to the amino acid sequence. There are three possible genotypes at this SNP: AA, AC, and CC, of which AA determines the non-*f* genotype. In this review, we only refer to the SNP genotypes if there are results that differ between AC and CC individuals, otherwise we pool individuals as *Pgi-f* and *Pgi*-non-*f* individuals (i.e. AA *vs.* AC & CC).

Fig. 2. The many names of *M. cinxia Pgi* alleles.

data provide information such that most colonisations occur within 2.5 km of the nearest possible source population and that the longest distances moved are 4–5 km, though such long-distance movements are rare (Hanski *et al*. 1994, Kuussaari *et al*. 1996, van Nouhuys & Hanski 2002). These studies also focused on factors that affect the decision to disperse, such as patch size, density, host-plant and nectar availability (Kuussaari *et al*. 1996). Since the late 1990s, most of the studies related to dispersal have utilised the knowledge of the population size, age and connectivity. Such information allows comparing individuals collected from newly established more isolated patches against individuals collected from well-connected previously occupied populations. This way the assessment is not made on the colonizer itself but on the offspring of the colonizer, i.e. after the successful colonising event (establishment of the population).

Phoshoglucose isomerase (*Pgi*) — the gene that encodes a glycolytic enzyme

Phosphoglucose isomerase (PGI, also known as glucose-6-phosphate isomerase, GPI) is a dimeric enzyme that catalyses the second step in glycolysis (Fig. 2). As a central metabolic enzyme, it is present in all living organisms and it is often polymorphic. PGI became a model system for detecting signs of natural selection in the wild due to the groundbreaking work by Ward Watt who has studied PGI in North American *Colias* (Pieridae) butterflies since the late 1970s (Fig. 3). Watt and colleagues have established that the polymorphic PGI enzymes differ in terms of kinetics and thermal stability, and that this molecular variation is connected to flight performance, mating success, fitness and allele frequencies in wild *Colias* butterflies (Watt 1977, Watt *et al*. 1985, Watt 1992, Kohane & Watt 1999, Watt *et al*. 2003). *Colias* and *Melitaea* represent distantly related clades and differ in terms of egg-laying strategies, diapause, and habitat requirements, but flight plays an important role in the life of both butterflies. The work on PGI in *Colias* butterflies served as an inspiration for Ilkka Hanski to start exploring the role of molecular variation in explaining the dynamics of dispersal in fragmented landscapes. The modelling work by Heino and Hanski (2001) showed that the dispersal rate should first decrease, then increase as an evolutionary response to habitat fragmentation. It was then experimentally shown that female Glanville fritillaries from newly established populations had a higher emigration rate than females from old populations (Hanski *et al*. 2002), which confirmed predictions based on the hypothesis that individuals colonising new habitat patches should be more mobile than the average individual in a metapopulation. Further work showed that patterns in a physiological proxy of flight capacity, the ratio between ATP and ADP after forced flight, followed the same predictions of high flight capacity in isolated, newly established populations (Hanski *et al*. 2004). This work formed the basis for the hypothesis of the glycolytic enzyme PGI also playing a role in the dispersal of the Glanville fritillary.

PGI was found to be a polymorphic enzyme in the Glanville fritillary already in the allozyme study by Saccheri *et al*. (1998) where PGI was among the seven allozyme loci and one microsatellite locus that were used to assess heterozygosity in the Åland metapopulation. Saccheri *et al*. (1998) detected a total of seven PGI alleles of which the two most common alleles, d and f, had a combined frequency of 0.72. When PGI was

PGI POLYMORPHISM IN OTHER INVERTEBRATES

Butterflies: Pioneering work on PGI and associations with fitness-related traits in the wild has been conducted by Ward Watt and colleagues in *Colias* butterflies (Picture A) (reviewed in Watt *et al*. 2003, Wheat & Hill 2014). The work on *Colias* is the only one that has demonstrated a connection between biochemical functional differences among PGI genotypes, variation in individual performance under different thermal conditions, fitness, and PGI frequencies in the wild.
In *Araschnia levana* (Picture B), variation in PGI correlates with flight metabolic rate, and the allele related to high flight metabolic rate is most common at the leading edge of an expansion front at the northern range limit, in newly-established populations, and in the more mobile spring generation (Mitikka & Hanski 2010).
In *Lycaena tityrus*, variation in PGI is associated with thermal tolerance and cold adaptation, and the genotypes differ in development time, growth rate, pupal mass and adult chill-coma recovery time, but not in flight performance (Karl *et al*. 2010). Consistently, high and low altitude populations also differ in the PGI allele frequency (Karl *et al*. 2009).
In *Polyommatus coridon*, genotypic variation in PGI is not associated with thermal adaptation (Ashoff *et al*. 2014) and in *Maniola jurtina* variation in PGI is not associated with dispersal, and allozyme frequencies do not vary along altitude (Goulson 1993).
In *Danaus plexippus,* variation in PGI influences the ability to fly at low temperatures (Hughes & Zalucki 1993).
Beetles: Strong directional selection on PGI alleles differing in thermal stability and kinetic efficiency in natural populations of the montane beetle C*hrysomela aeneicollis* (Picture C). PGI polymorphism interacts with the expression of heat shock proteins (HSP), with impacts on individual performance and fitness (Dahlhoff & Rank 2000, Dahlhoff *et al*. 2008).
No association between thermal tolerance and variation in PGI was found in soil-inhabiting larvae of two beetle species, *Costelytra zealandica* and *C. brunneum* (Lefort *et al*. 2014).
Australian stick insects: Evidence of positive selection on *Pgi* (Dunning *et al.* 2013).
Bumblebees: No intraspecific amino acid variation in *Pgi* and only moderate levels of interspecific divergence (five species; Ellis *et al*. 2013).
Damselflies (*Ischnura elegans*)**:** PGI genotypes differ in larval development time and body mass, but not in larval growth rates nor adult life span between northern and southern populations. The effect of PGI further dependent on developmental resources (De Block & Stoks 2012).
Parasitoid wasps (family *Encyrtidae*): PGI affects soldier development time and the outcome of competition. Homozygous broods bearing the developmentally disadvantageous allele produce more adult females than broods bearing alternate common alleles (Bowker *et al*. 2014).
Marine copepods: Potential habitat-specific selection on PGI in *Tigriopus californicus* (Schoville *et al*. 2012).
Barnacles (*Semibalanus balanoides*)**:** Allele frequency differences in PGI between high *vs*. low intertidal habitats that differ in thermal conditions (Véliz *et al.* 2006).
***Drosophila melanogaster*:** Experimental work that manipulates enzyme concentrations, including PGI, in central metabolism and quantifies their impact on flight performance (Eanes *et al.* 2006). No effect of PGI was found.

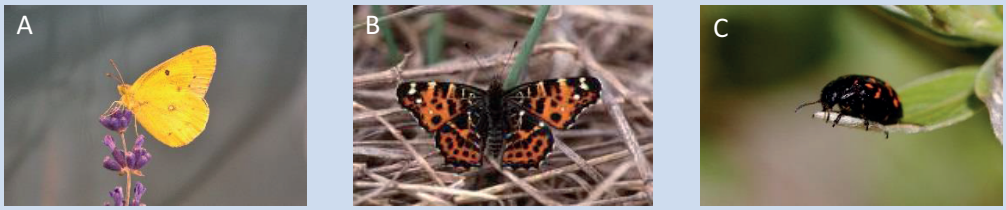

Fig. 3. *Pgi* polymorphism in other invertebrates.

selected as a dispersal-related candidate locus in the study by Haag *et al*. (2005), the combined frequency of the d and f alleles was found to be a strikingly similar 0.76. In their study, Haag *et al*. (2005) found associations between the f allele and two important phenotypic traits, flight metabolic rate and fecundity. The coding region of the *Pgi* locus was sequenced by Orsini *et al*. (2009) who identified three single nucleotide polymorphisms (SNPs) that could be used to determine the most common allozyme alleles. In subsequent studies, most of the focus was directed at one particular SNP, pgi:c.331A>C (also known as *Pgi* AA111, *see* Fig. 2) that segregates between the *Pgi-f* and non-*f* genotypes. At the DNA level, there are three possible genotypes, AA, AC, and CC, of which CC is sometimes found to be disproportionally rare. The SNPs do not exactly match those of the sequenced *Pgi* gene in *Colias eurytheme*, but it is noteworthy that in both species the nonsynonymous SNPs are located in a loop region in the protein which is likely to be functionally important (Wheat *et al*. 2006, Storz & Wheat 2010). Unlike in *C. eurytheme*, studies on enzyme function in *M. cinxia* have not been successful. To this date, the exact mechanisms of why *Pgi* is repeatedly found to correlate with phenotypic performance in the Glanville fritillary butterfly, are not fully known. Wheat and Hill (2014) identify two possible (non-exclusive) hypotheses, the so-called 'flux hypothesis' and 'the signalling and moonlighting hypothesis'. In short, according to the flux hypothesis (Watt 1986, 2003), natural selection acts on metabolic enzymes to ensure that each step of the metabolic pathway

is sufficient for the maximum flux rate that is required under physiologically demanding situations, such as rapid flight. The signalling and moonlighting hypothesis (Marden 2013) highlights the other, often surprising, roles of metabolic enzymes as signalling molecules that can affect for example oxygen transport in insects or nerve growth in the case of mammals.

Here our aim is to review over 30 papers that have assessed how variation in *Pgi* affects a number of key life history traits in the Glanville fritillary butterfly, and how these associations play a role in the eco- evolutionary dynamics of the butterfly metapopulation in the Åland Islands. We start by describing the associations observed on variation at the phenotypic level, first by focusing on flight metabolic rate and development, which we consider to be so-called primary associations. This is followed by describing associations between some other life-history traits (secondary associations), which are more likely to occur at least to some extent due to the influence of *Pgi* on the primary associations. Finally, we describe how the genetic polymorphism in *Pgi* is coupled with the ecological and evolutionary dynamics at the population level. We finish this review by highlighting the unknowns and the potential for future studies.

Influence of *Pgi* on life-history variation

Flight metabolic rate and developmental time (primary associations)

In the seminal work by Haag *et al*. (2005), PGI genotype was found to be associated with variation in flight metabolic rate (FMR) in field-collected female Glanville fritillaries. Females with the PGI-f allele exhibited higher mass-independent peak metabolic rate and a larger total volume of CO_2 emitted during the 15 min measurement than females without the f allele. High flight metabolic rate reflects metabolic capacity and hence flight performance as insect flight requires extremely high energy flux rates (Suarez 2000). The association between the *Pgi-f* genotype and high flight metabolic rate was corroborated in laboratory-reared females by Niitepõld (2010) who used low (26 °C) and moderate (31 °C) measurement temperatures. Notably, at higher temperatures (33–35 °C), the flight performance of *Pgi-f* females was found to decrease with temperature, not increase as it did in females without the *f* allele (Niitepõld *et al*. 2009, Niitepõld 2010). The work by Wong *et al*. (2016) provided further insight into the different thermal dynamics of *Pgi* genotypes as low temperatures experienced during the first days after emergence reduced flight performance later in life. However, the *Pgi-f* genotype suffered less from the low-temperature treatment than the non-*f* genotypes, again suggesting that *Pgi-f* outperforms other genotypes in low temperatures.

The first studies on *Pgi* and flight metabolic rate were performed on females only, but the studies by Mattila (2015) and Wong *et al*. (2016) also included males and found that *Pgi-f* genotypes had higher flight metabolic rate than the non-*f* genotypes. Curiously in both studies no significant effects of *Pgi* on FMR were observed in females (Mattila 2015, Wong *et al*. 2016). Similarly, Mattila and Hanski (2014) found no associations between *Pgi* and flight metabolic rate (though there was a non-significant trend in females), yet the heritability of flight metabolic rate was high. On the contrary, resting metabolic rate was not found to be heritable. This is in accordance with other studies, as all associations between *Pgi* genotype and metabolic rate have been found on flight metabolic rate, whereas variation in *Pgi* does not associate with resting metabolic rate in any study.

At the gene expression level, *Pgi* has been linked to between-population differences in flight performance. Individuals from the small and completely isolated island Pikku-Tytärsaari in the eastern Baltic Sea show high levels of inbreeding, reduced heterozygosity and low flight metabolic rate compared to the Åland mainland population, and a four-fold difference in basal expression of *Pgi* (Kvist *et al*. 2015). Focusing on the Åland metapopulation, Wheat *et al*. (2011) specifically assessed whether expression phenotypes become assorted among the newly established and old populations. They assessed how gene expression and flight metabolic phenotypes vary between allelic forms of *Pgi* and another polymorphic metabolic gene

(*Sdhd*). Firstly, the results confirmed the previous observation of phenotypic differences in flight and reproductive performance between females from new and old populations, as females from new populations had higher expression of genes involved in egg provisioning as well as maintenance of flight muscle proteins. Regarding *Pgi*, the results showed that the gene expression pattern also varied to some extent between the two allelic forms. Specifically, the *Pgi-f* genotype, which is associated with higher fecundity and flight metabolic rate had higher expression of genes involved in the final step of oogenesis as well as oxidoreductase and ribosomal complex genes (Wheat *et al*. 2011).

How does the link between *Pgi* and flight metabolic relate to actual dispersal in the field? A direct connection between individual mobility and *Pgi* genotype was shown by Niitepõld *et al*. (2009) who used a harmonic radar to follow individual, free-flying Glanville fritillary females in the field. The study detected a strong positive relationship between flight metabolic rate and the distance moved in the field, and showed that flight activity followed ambient temperature in a non-linear fashion, peaking at approximately 20–24 °C. Under low to moderate ambient temperatures, individuals with the *Pgi-f* allele covered longer distances than individuals without the *f* allele. A second study examined the association of *Pgi* genotype on the frequency of between-patch movements and movement distance in wild-caught males and females using mark-recapture methods. Here, females with the *Pgi-f* genotype showed a trend towards higher dispersal, whereas males with the *Pgi-f* genotype were less dispersive (Niitepõld *et al*. 2011). The result likely reflects the different roles of flight in the two sexes, as females with high flight capacity can establish new populations, whereas in males the main purpose of flight is to locate females. Life history studies in the Glanville fritillary have benefited from the use of large population enclosures ($32 \times 26 \times 3$ m) that cover a semi-natural habitat patch. However, in these studies *Pgi* has not been found to correlate with mobility (Klemme & Hanski 2009, de Jong *et al*. 2014; A. Duplouy unpubl. data), possibly because of the scale of the studies and the crudeness of the mobility measure. In a more indirect approach, the link between *Pgi* and dispersal has been shown by the higher occurrence of the *Pgi-f* allele in isolated, newly established populations (Haag *et al*. 2005, Hanski & Saccheri 2006, Niitepõld *et al*. 2009) (discussed further below). Such a pattern is expected if dispersal is a non-random process and the genotypes of the colonising individuals are reflected in the genotypes of their offspring.

Pgi has also been found to be related to larval growth and survival. Interestingly, while in adult butterflies there is a consistent pattern of higher performance of the *Pgi-f* genotype under cooler conditions that would seem typical to the Åland Islands, patterns seen in the studies on larvae appear reversed. The larval period is critical for acquiring resources used for building the adult body plan and storing resources for later use (fecundity and maintenance), and the larvae are known to be sensitive to environmental stress, such as poor weather conditions (Hanski & Meyke 2005, Ojanen *et al*. 2013). Kallioniemi and Hanski (2011) reared larvae under three different thermal conditions and found that survival during the final instar decreased with decreasing temperature. In the lowest temperature, there was a significant effect of *Pgi* genotype as the non-*f* genotype had the highest survival. In addition, individuals with the non-*f* genotype had higher pupal mass than the *Pgi-f* genotype. In a similar vein, A. Duplouy (unpubl. data) examined post-diapause larvae and found that larvae of the non-*f* genotype were heavier than *Pgi-f* larvae. The latter result is likely a general one, as the experimental individuals represented several geographic locations around the Baltic: the fragmented landscapes of Åland and Uppland (Sweden), and continuous populations from Saaremaa (Estonia), Gotland and Öland (Sweden). During larval development, *Pgi* has also been found to be associated with plasticity in the number of larval instars. In *M. cinxia*, larvae, especially those that are initially smaller after diapause, can compensate for poor conditions by moulting one extra time before pupation. Saastamoinen *et al*. (2013) found that the prevalence of this extra instar was higher in larvae that originated from newly established populations, during cool conditions, and in female larvae that need to acquire a heavier body mass than males.

In addition, the likelihood of adding an instar was associated with polymorphisms in three loci, one of them being *Pgi*. Here, the *f* allele which has been associated with poor larval growth was found to be associated with higher occurrence of the extra instar in female larvae, suggesting a developmental mechanism to compensate for sub-optimal conditions. At the gene expression level, however, *Pgi* did not show significant variation in the global gene expression analysis of over 8000 genes by Kvist *et al*. (2013). The material included last-instar larvae reared under three different thermal conditions.

Other life-history traits (secondary associations)

Flight is critical for life history traits such as mating success and fecundity. Several studies on the Glanville fritillary butterfly have assessed how individuals from newly established and old populations differ in key fitness traits and what the role of *Pgi* is in these associations. Haag *et al*. (2005) showed that based on the allozyme genotype, individuals with the PGI-f genotypes had significantly higher body mass-adjusted average clutch sizes than individuals without this allele, and that 29% of the total variance in clutch size was explained by the presence of PGI-f. The association between *Pgi* polymorphism and clutch size has been observed in a number of later studies as well. Interestingly, the approximately 20% higher clutch size obtained by the females with the PGI-f genotype (Saastamoinen 2007b, Saastamoinen & Hanski 2008) is especially apparent under cooler thermal conditions. Saastamoinen (2007b) showed that under semi-natural conditions females with the PGI-f genotype initiated oviposition earlier in the day, which allowed them to take advantage of the most favourable environmental conditions for oviposition and lay larger clutches compared with females with the PGI-d genotype. A subsequent study by Saastamoinen and Hanski (2008) showed using experimental and observational data that experiencing low temperatures in the morning delayed the initiation of oviposition and decreased clutch size. With the use of a thermal image camera it was further showed that the polymorphism at *Pgi* was associated with thoracic temperature at low ambient temperatures. Females that are able to fly at lower ambient temperatures generally initiate oviposition earlier in the afternoon and lay larger clutches. The results hence highlighted how variation in physiological and molecular capacity to sustain active flight at low ambient temperatures has significant fitness-related consequences. The association between *Pgi* and clutch size was also shown in a study by de Jong *et al*. (2014). However, in this study the result was non-significant after applying the Bonferroni adjustment for multiple testing (corrected for 10 genes) but nevertheless the direction was the same as in the previous studies. In a comparison between genotypes from the fragmented populations in Åland and Uppland, and continuous populations from Saaremaa, Gotland and Öland, *Pgi* AC females were found to lay larger clutches than *Pgi* AA and CC females (A. Duplouy unpubl. data). Interestingly the difference in clutch size between *Pgi* genotypes does not seem evident under laboratory conditions, potentially due to the 'too optimal' thermal conditions for *Pgi*-non-*f* females (Saastamoinen *et al*. 2009). The fecundity differences have also been confirmed to some extent at the gene expression level, as Wheat *et al*. (2011) showed that the *Pgi-f* genotypes have higher expression of genes involved in the final step of oogenesis. Though females with the *Pgi-f* genotype seem to have higher clutch size this does not seem to translate to the lifetime egg production (Klemme & Hanski 2009, de Jong *et al*. 2014), even though at least in some case lifetime egg production seems to show a heritable component (Klemme & Hanski 2009). Klemme and Hanski (2009) found that SNP variation in *Pgi* explained variation in egg hatching rate. Interestingly, the proportion of eggs that hatched was significantly higher in *Pgi*-non-*f* females than *Pgi-f* females but this effect seems largely due to the CC homozygotes having extremely low hatching success (38%). Hence, if the comparison is made only between AA and AC females the difference is relatively minor (68% & and 61%, respectively for AA and AC females). Yet, the difference in hatching rate potentially explains at least partially why no differences between *Pgi* genotypes were observed

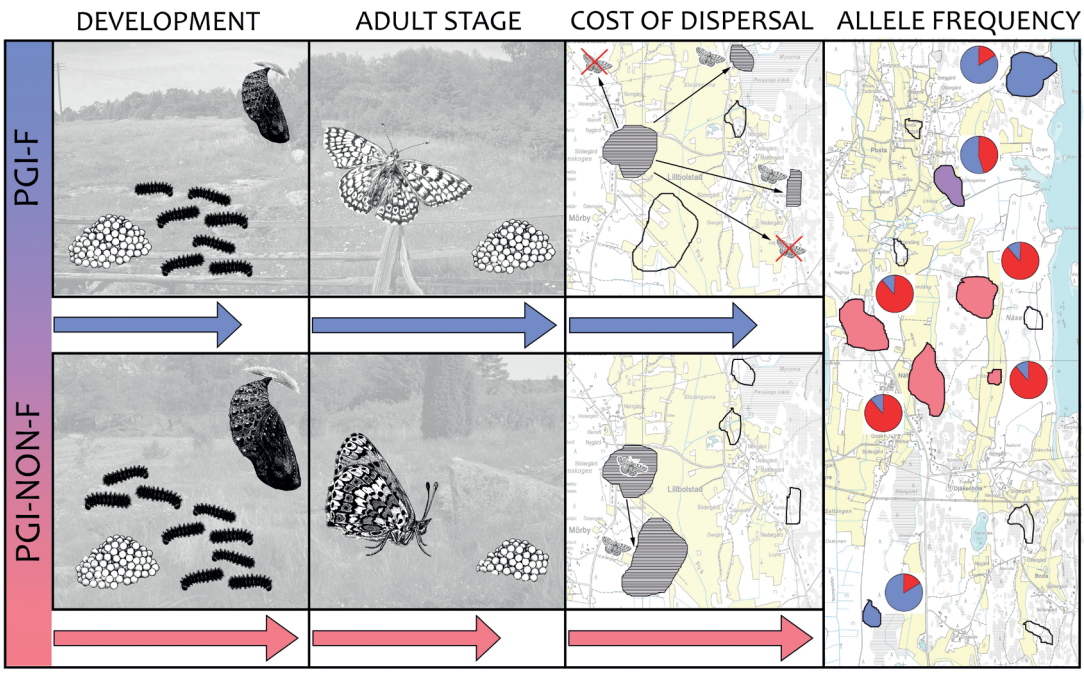

Fig. 4. Allelic variation at the *Pgi* locus (*Pgi-f vs. Pgi*-non-*f*) influences development and adult life-history traits. *Pgi* also has consequences on dispersal propensity which leads to variation in allele frequencies at *Pgi* among the newly established and old populations within the Glanville fritillary metapopulation in the Åland islands. **Developmental stage**: The hatching success of clutches from *Pgi-f* females is reduced compared to those from *Pgi*-non-*f* females. The larval survival is also lower in *Pgi-f* individuals and pupal mass is reduced. The differences are potentially influenced by maternal effects and amplified under poor thermal and resource conditions during development. **Adult stage**: Females with the *Pgi-f* genotype are more dispersive than females with the *Pgi*-non-*f* genotype, especially under cooler thermal conditions. *Pgi-f* females also have increased clutch size but there is no difference in lifetime egg production **Dispersal costs**: Even though there is no direct trade-off between dispersal and reproductive performance, the higher tendency to disperse away from a habitat patch is likely to entail risks and consequently reduce survival of *Pgi-f* genotypes, especially when population connectivity is low. **Allele frequency**: The *Pgi-f* genotype is more frequent in newly established isolated populations, whereas *Pgi*-non-*f* genotypes are more common in well-connected old populations. The length of the arrows under the columns represents the survival of each genotype within each stage (blue and red respectively for the *Pgi-f* and *Pgi*-non-*f* individuals).

in lifetime egg production even if clutch size seemed to differ (Fig. 4).

Lifespan is another trait that shows considerable variation that has been attributed to molecular variation at the *Pgi* locus. Klemme and Hanski (2009) found that under semi-natural field conditions *Pgi-f* females lived longer than *Pgi*-non-*f* females. Consistent results have also been observed under more controlled environmental conditions (Niitepõld & Hanski 2013). Notably, the differences in lifespan are sometimes evident only in females (Klemme & Hanski 2009). Saastamoinen *et al*. (2009) specifically assessed how variation in the *Pgi* genotype affects female and male adult resource allocation and life history under controlled conditions. Consistent with the studies described above, *Pgi* genotype significantly affected lifespan, with 29% longer lifespan in *Pgi-f* genotypes compared with that of non-*f* genotypes. Even though the effect of *Pgi* genotype on lifespan was consistent across the three treatments, it was especially evident in non-reproducing females. This study further assessed how butterflies use their body reserves for somatic maintenance and reproduction, and how this may depend on environmental conditions and interact with the *Pgi* genotype. The results showed that there is a highly significant difference between the *Pgi* genotypes in how the rate of reproduction influ-

ences the weight loss of the abdomen, which was much greater in the *Pgi-f* females. This result was hypothesized to indicate a trade-off between fecundity and body maintenance based on the following: if non-*f* females are able to reproduce with a smaller reduction of resources in their abdomen, these resources can be used for somatic maintenance, which could prolong lifespan under some conditions, for instance under mild food stress. This idea was further supported by the fact that although the *Pgi-f* individuals had a consistently longer lifespan than the non-*f* individuals, the difference was small and non-significant when assessed in reproducing females alone. Unfortunately, differences in allocation patterns between reproduction and body maintenance under variable environmental conditions have not been tested in other studies. Finally, it is noteworthy that in the study by Saastamoinen *et al*. (2009), the increased lifespan in individuals with the *Pgi-f* genotype was also apparent in males, which has not been found it other studies and may again reflect differences between assessments under controlled laboratory conditions and those conducted under more natural environmental settings. The increased lifespan in *Pgi-f* individuals compared with non-*f* genotypes can be attributed to several factors that generally may influence lifespan in natural populations. Potential, intrinsic factors include hormones (juvenile growth hormone and insulin-like growth factor; *see* e.g. Flatt & Kawecki 2007, Chistyakova 2008), oxidative damage due to side-effects of energy metabolism, and immune system decline (Van Voorhies 2001). We do not know whether these factors differ between the genotypes. Furthermore, adverse environmental conditions, such as ambient temperatures and deprivation of food resources are likely to be important. It is noteworthy here that in a previous field experiment, females that originated from newly established populations, in which the *Pgi-f* genotype is most frequent, had a shorter lifespan than females from older populations (Hanski *et al*. 2006, Zheng *et al*. 2007).

During the past few years, few studies have concentrated on assessing in more detail whether *Pgi* genotype is associated with thermal stress tolerance. These studies were motivated by those indicating the superiority of the *Pgi-f* genotype in regard to dispersal and flight metabolic rate under low ambient temperatures. Luo *et al*. (2014) examined whether Finnish and Chinese populations of the Glanville fritillary butterfly differ in cold or heat tolerance, and whether *Pgi* genotype influenced these traits in the Finnish population. Their results showed that variation in *Pgi* was associated with heat knock-down time (at +53 °C), so that individuals with *Pgi* AA and AC were more heat tolerant than *Pgi* CC, but maybe somewhat unexpectedly, there was no significant difference between *Pgi* AA and AC (the generally used comparison). Furthermore, there was an indication that *Pgi* influenced cold tolerance (–5 °C for 2 h) so that *Pgi* AA individuals had the longest chill-coma recovery time. Similarly, M. de Jong and M. Saastamoinen (unpubl. data) tested for genetic associations between chill-coma recovery and a set of candidate genes, including *Pgi*. However, no significant association was found between variation in *Pgi* and cold tolerance, whereas polymorphism in the gene *flightin*, which has a known function in insect flight and locomotion, was associated with chill-coma recovery.

Pgi and association with population dymanics

Above we have demonstrated how variation in *Pgi* is associated with a number of key life history traits in the Glanville fritillary butterfly. Even though this in itself is interesting and has made a significant contribution to examples in which allelic variation in a single gene is connected to variation in life-history in wild populations, the story does not end here. Maybe even more influential is the fact that the molecular variation is reflected in population dynamics, most likely through the connective link of dispersal. The first study in which the allelic variation in PGI was assessed among local populations is surprisingly the well-known study by Saccheri *et al*. (1998), which was one of the first demonstrations of inbreeding increasing the extinction rates of local populations in natural systems. Mistakenly, however, in this study polymorphism in PGI was assumed neutral. Since then, in a number of studies it has been shown how the

allelic composition in *Pgi* varies among the local populations in the well-studied metapopulation of the Glanville fritillary in relation to population age and population connectivity. In the study in which the first association between PGI and flight metabolic rate was demonstrated with a relatively small sample size, the frequency of the allele associated with high flight capacity was found to be highest in the newly established isolated populations (Haag *et al*. 2005). There is systematic variation in the PGI allelic composition among local populations, which is related to the spatio-temporal dynamics of these populations, as newly established isolated populations are colonised by females that are more dispersive and hence more likely to carry the specific allele of the PGI that relates to high flight capacity (Hanski & Saccheri 2006). A recent study also indicated that the allelic frequency of *Pgi* is reflected on an even larger scale when comparisons are made between Glanville fritillary populations living in fragmented (Åland, Upland) *versus* continuous (Saaremaa & Öland) landscapes (A. Duplouy unpubl. data). More specifically this study found 15 SNPs in eight genes with significant allele frequency differences between the fragmented and continuous landscapes, and *Pgi* was identified as one of these outlier loci (A. Duplouy unpubl. data). In a study by Fountain *et al*. (2016), comparisons were made among now extinct populations in SW Finland (based on museum samples), fragmented populations (Åland and Uppland) and continuous populations (Saaremaa and Öland). The now extinct population in the Finnish mainland had significantly reduced genetic diversity before extinction and allele frequencies that gradually diverged from those in contemporary Åland populations over 80 years. Even though the allele frequencies of outlier loci in SW Finland resembled those in fragmented landscapes as opposed to continuous landscapes in the Baltic region, *Pgi* was not identified as one of the outlier loci (Fountain *et al*. 2016). However, when looking at gene expression, the variation in differentially expressed genes observed between new and old populations in the Åland Islands do not appear when comparisons are made between individuals originating from fragmented and continuous landscapes at a larger scale. Somervuo *et al*. (2014) used RNA-seq data, and showed that even though a large number of genes (1841) were differentially expressed between the landscape types, *Pgi* was not among them. Nevertheless, in general the study confirmed that genes that were more highly expressed in butterflies from newly established than old local populations within a fragmented landscape in Åland were also more highly expressed, at the landscape level, in fragmented than continuous landscapes.

Differential performance of individual genotypes, leading to differential fitness, can even influence population dynamics, as was demonstrated by the examples that show how allelic variation in PGI affects the growth rate of the local populations (Hanski & Saccheri 2006). In this analysis of hundreds of local populations in the large metapopulation, the strength and the sign of the molecular effect on population growth rate was sensitive to the ecological context, namely the area and spatial connectivity of the local population, which in turn affects genotype-specific gene flow and dispersal in the local population. The effects found for PGI were not present between population growth rate and allelic variation at six other loci genotyped in the same material. The data also demonstrate how spatial configuration of habitat and spatial dynamics of populations contribute to maintenance of PGI polymorphism in this species.

The strong coupling between demographic and microevolutionary dynamics at the *Pgi* gene in the Åland metapopulation of the Glanville fritillary butterfly described above in terms of metapopulation size and population persistence has also been assessed with a few additional models. Zheng *et al*. (2009) used an individual-based evolutionary sexual model of dispersal for species occupying a metapopulation in a habitat patch network. The model was parameterised based on empirical data, so that dispersal rate, fecundity and survival were assumed to be affected by variation in the *Pgi* locus (A *vs*. C; individuals with the C allele being more mobile). The model was then tested with independent empirical data sets, and confirmed that at the level of local populations, the frequency of the C allele is highest in newly established isolated populations and lowest in old isolated populations. Second, at the level of sub-networks that

differ in patch number and connectivity, the frequency of the C allele increases with decreasing network size and hence with decreasing average metapopulation size. The frequency of C is highest in landscapes where local extinction risk is high and where there are abundant opportunities to establish new populations. The results hence highlight that the strength of the coupling of the ecological and evolutionary dynamics depends on the spatial scale and is asymmetric, as demographic dynamics have a greater immediate impact on genetic dynamics than vice versa. In the other example, an eco-evolutionary model for dispersal was constructed, in which a stochastic patch occupancy metapopulation model was combined with a model for changes in the frequency of fast-dispersing individuals in local populations (Hanski & Mononen 2011). The outcome of the model was tested with data on allelic variation in *Pgi*. Population-specific measures of immigration and extinction rates, and the frequency of *Pgi-f* individuals among the immigrants explained 40% of the spatial variation in the *Pgi* allele frequency among the local populations. This model highlights the importance of founder events and gene flow in the evolution of dispersal in the Glanville fritillary system and clarifies the consequences of habitat loss and fragmentation on the evolution of dispersal (*see* also Hanski 2011).

In his last study on the impact of allelic variation in *Pgi* and population dynamics, Hanski focused on local extinctions. In this study the long-term data set, more than 20 years of ecological data on the occurrence of the Glanville fritillary butterfly in the over 4000 habitat patches in the Åland Islands were used. Hanski *et al*. (2017) built a metapopulation model to predict the distribution of the butterfly in this system. The results showed how the majority of the networks into which the meadows were clustered exist below the extinction threshold for long-term persistence. However, among the networks that were above the threshold (26%), the spatial configuration and habitat quality rather than the pooled habitat area predicted metapopulation size and persistence. Interestingly, the allelic variation in *Pgi* explained 30% of variation in metapopulation size in addition to the landscape structure parameters, again highlighting how molecular variation at *Pgi* can crucially influence population dynamics in this system.

Conclusion

The work on *Pgi* in the Glanville fritillary has discovered multiple associations between molecular polymorphism, phenotypic traits and population dynamics. We have classified these effects as primary effects that we believe to be functionally important phenotypic traits, and secondary effects that follow from the primary effects. The first primary effect is related to developmental success as the genotypes have been shown to differ in egg hatching rate, larval growth rate and pupal mass. Here, the most common *Pgi* genotype, *Pgi*-non-*f*, performs better than the *Pgi-f* genotype (Fig. 4). The primary effect recorded in adults, flight performance, however, shows an opposite pattern, where the *Pgi-f* genotype is associated with high flight metabolic rate and superior flight capacity under cool to intermediate temperatures, which represent typical conditions in the Åland Islands. Flight capacity is positively related to mobility in the field in females and forms the mechanistic basis for many fitness traits. In the case of these secondary effects, *Pgi* has been shown to be associated with higher egg production under field conditions. At the population level, the *Pgi-f* genotype is most common in isolated, newly established populations, reflecting the genotypes of the founding individuals, and *Pgi* is connected to population growth rate alongside with habitat area and configuration.

Despite the well-documented patterns, we still lack a mechanistic understanding of the biochemical basis of these effects, and so far, the causal role of *Pgi* as a driver of these processes has not been proven. Many of the aspects are in concordance with the enzyme flux hypothesis, but in the absence of successful measurements and manipulations of enzyme function, this remains uncertain. It seems however highly likely, that the 'filtering effect' of fragmented landscapes imposes strong selection on flight capacity, as only the individuals best suited for long-distance flight will regularly establish new populations. *Pgi* genotype may therefore serve as a marker of a successful combination of

alleles at multiple loci that allows butterflies to reach and colonise new habitat patches. The work by Wheat *et al*. (2011) showed that *Pgi* may epistatically interact with other genes, such as succinate dehydrogenase (*Sdhd*), as an allelic combination at these two loci was associated with maximal metabolic endurance. The Glanville fritillary was among the first butterflies whose full genome was sequenced (Ahola *et al*. 2014), which combined with gene expression studies provides a promising opportunity for the detection of other loci that are connected to flight performance and fitness. Future work should incorporate both genetic and life history studies to understand the multi-stage process of dispersal and factors contributing to the behavioural decision to emigrate, as well as the consequences of dispersal at the individual and population level. Open questions include: how do conditions experienced at the larval stage affect later life history stages, and how does flight affect fecundity and lifespan? While much of the work on adult Glanville fritillaries has suggested that the *Pgi-f* genotype exhibits superior performance over the non-*f* genotype, it should not be forgotten that the frequency of the allozyme f allele in the large metapopulation in the Åland Islands is only approximately 0.25 while the frequency of the d allele is over 0.50 (Haag *et al*. 2005, Hanski & Saccheri 2006). This raises the question: what maintains polymorphism in this gene in the metapopulation? The work on the Glanville fritillary has provided three general factors contributing to the surprising success of the non-*f* genotype: differences in larval performance, costs of dispersal, and low survival of the *Pgi* CC homozygotes. The latter phenomenon is still under scrutiny and we are still developing an understanding of the dynamics of the C allele. Some studies have also failed to find any associations between *Pgi* and life history traits. We have gained new insight into effects such as the role of conditions experienced prior to phenotypic measurements (Wong *et al*. 2016) and how these could affect the measurements. As demonstrated in the case of the interaction between the *Pgi* and *Sdhd* loci (above), it is clear that *Pgi* does not operate in a vacuum, but instead is connected to a number of factors at different organizational levels. Nevertheless, *Pgi* in the Glanville fritillary with its associations between molecular variation and individual performance and fitness components, is still perhaps the best documented genetic polymorphism affecting interacting ecological and evolutionary dynamics (Hanski & Saccheri 2006, Zheng *et al*. 2009).

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