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Pgi genotype influences flight metabolism at the expanding range margin of the European map butterfly

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In species expanding their ranges in response to climate warming or for other reasons, individuals with superior dispersal capacity are often more likely than others to establish new populations beyond the current range boundary. Previous studies on butterflies have shown that molecular variation in the glycolytic enzyme phosphoglucose isomerase (*Pgi*) is associated with variation in flight metabolic rate, flight capacity under unfavourable conditions, and dispersal rate. We examined allelic variation in *Pgi* in the European map butterfly (*Araschnia levana*), which has rapidly expanded northwards in Finland. We show that the kinetically fastest *Pgi* allele, *Pgi-1*, is associated with superior flight metabolic rate and is more frequent at the eastern Finnish expansion front than in old established populations in Estonia. In eastern Finland, the *Pgi-1* allele is significantly more frequent in newly-established (age ca. 4 years) than in older populations (age 11 to 22 years), and the *Pgi-1* allele is more frequent in the spring than in the summer generation, possibly because the greater mobility of the summer generation butterflies selects for *Pgi-1*. Our results are consistent with the hypothesis that range expansion may select for increased dispersal capacity at the expansion front, which may in turn further elevate the rate of expansion.

Introduction

Many temperate and boreal animal and plant species are presently expanding their geographical ranges northwards in response to the ongoing climate warming (Parmesan 2006). Temperate butterflies have shown a particularly strong response (Parmesan *et al*. 1999, Hill *et al*. 2002), apparently because both the larvae and adult butterflies are sensitive to the thermal environment (Roy & Sparks 2000, Crozier 2004, Berger *et al*. 2008). For instance, of the 35 non-migratory European butterfly species with northern range limit in Great Britain, Sweden, Finland, or Estonia, 63% shifted their distributions northwards during the 20th century (Parmesan *et al*. 1999). Dispersal capacity largely determines whether a species is able to track the suitable climate (Warren *et al*. 2001, Pöyry *et al*. 2010). Poor dispersers and specialist species restricted to certain habitat types or using uncommon host plants have generally failed to expand their ranges,

most likely because the landscape is too fragmented for them to allow effective dispersal (Warren *et al*. 2001). In contrast, generalist species may expand their ranges surprisingly fast. One of the butterfly species that has successfully expanded in Finland is the European map butterfly (*Araschnia levana*), which moved northwards with the mean rate of 7.5 km per year in the period 1999–2004, with occasional annual jumps of up to 100 km (Mitikka *et al*. 2008).

In expanding species with other things being equal, individuals with the highest rate of dispersal are inevitably more likely than others to establish new populations beyond the current range boundary. Thereby the more dispersive individuals will contribute disproportionately to range expansion, and vice versa range expansion will favour traits that increase dispersal rate. For instance, Simmons and Thomas (2004) documented selection on morphological traits associated with flight in two wing-dimorphic species of bush crickets expanding in response to climate change in the United Kingdom. In Roesel's bush cricket (*Metrioptera roeselii*), only the long-winged (macropterous) individuals are able to fly, whereas the long-winged conehead (*Conocephalus discolor*) has long-winged and extra-long-winged individuals, both of which can fly, but only the extra-long-winged form is considered to disperse between habitat patches (Ando & Hartley 1982, Haes 1999, as cited in Simmons & Thomas 2004). Simmons and Thomas (2004) found that in both species the proportion of the macropterous form is higher at the expanding range margin than in more central populations. However, in a few years following the establishment of new populations the proportion of macropterous individuals decreased due to local selection involving a trade-off between reproduction and dispersal (Simmons & Thomas 2004). A trade-off between dispersal and fecundity is widespread in wing-dimorphic species, and is based on resource allocation to wings and wing muscles versus egg production (Roff 1986). On the other hand, whether wing-monomorphic species such as butterflies exhibit a comparable trade-off remains less clear (Hanski *et al*. 2006), and in these species high dispersal rate may involve other costs (Hanski *et al*. 2004).

Classic metapopulations with a high rate of population turnover provide a model system to study the biology of range expansion, because in such metapopulations new local populations are constantly being established even if there is no overall range expansion. In other words, classic metapopulations present excellent opportunities to study the process of establishment of new populations. Mark–recapture studies on the Glanville fritillary butterfly (*Melitaea cinxia*) in Finland have shown that the F1 offspring of females that established new populations in isolated habitat patches are more dispersive than the average individual in the metapopulation (Hanski *et al*. 2002, 2004). This result suggests that new populations in isolated habitat patches are established by particularly dispersive individuals and that the relevant trait or traits have high heritability. The latter inference is supported by an independent experimental study by Saastamoinen (2008), who compared the dispersal behaviour of females and their female offspring in a large outdoor population cage.

In the Glanville fritillary, there is no significant association between morphological traits and dispersal rate, but individuals in newly-established populations have, on average, higher flight metabolic rate than individuals in old populations (Haag *et al*. 2005), suggesting an association between flight metabolism and dispersal rate. A recent study using harmonic radar to track free-flying butterflies has indeed demonstrated such an association (Niitepõld *et al*. 2009). Furthermore, variation among individuals in flight metabolic rate is correlated with molecular variation in the phosphoglucose isomerase (*Pgi*) locus (Haag *et al*. 2005), and the *Pgi* allele that shows superior flight metabolism is disproportionally common in newly-established local populations (Hanski & Saccheri 2006). These results suggest that a certain *Pgi* genotype has superior flight capacity and dispersal rate, consistent with the classic studies by Ward Watt and his colleagues on *Pgi* and flight-related performance and fitness in *Colias* butterflies (Watt *et al*. 1983).

The results on the Glanville fritillary and *Colias* butterflies prompted us to examine allelic variation at the *Pgi* locus in the European map butterfly in the context of its ongoing range expansion. The Glanville fritillary and the map butterfly belong to the same butterfly family

Nymphalidae, though they are not closely related, whereas *Colias* belong to the family Pieridae. Based on this, we could expect comparable *Pgi* genotypic effects in all three species as well as in many other butterflies. The map butterfly is widely distributed in central Europe and is currently expanding northwards. We compared populations from three regions, including two separate expansion fronts in Finland and populations in nearby Estonia, where the map butterfly has been present since the early 1900s (Keskula 1992) and is currently very common (T. Tammaru pers. comm.). Multiple independent populations were sampled in each region. The expansion in Finland occurs from the east and from the south (Fig. 1; details in Material and methods). To relate allelic variation in *Pgi* to dispersal-related individual performance, we measured the flight metabolic rate in a sample of butterflies. Finally, to test whether there are any morphological differences between butterflies at the expanding range margin and in more central populations, we applied the same body size measurements that have previously been taken on the speckled wood butterfly (*Pararge aegeria*) by Hill *et al*. (1999b) and Merckx and Van Dyck (2006) and which are supposed to be related to flight capacity.

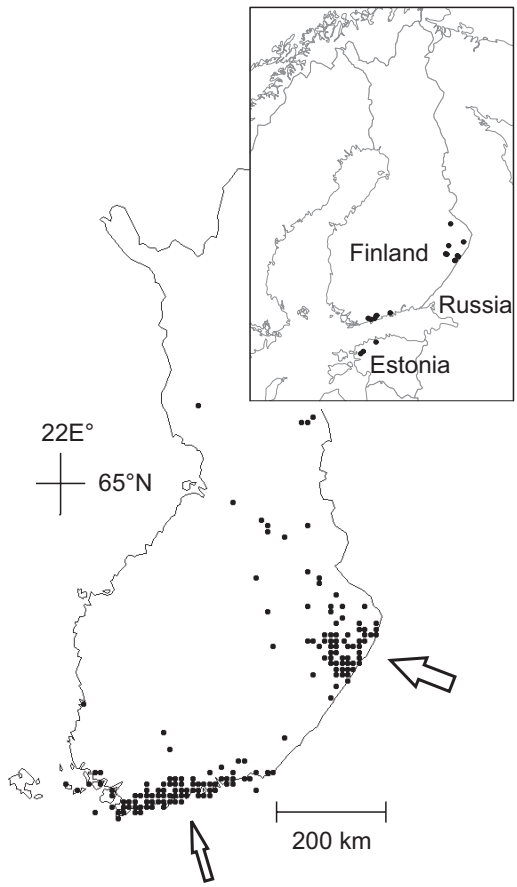

Fig. 1. Current distribution of the European map butterfly (*Araschnia levana*) in Finland (the larger map). The dots represent occupied 10 × 10 km grid cells, and the arrows the routes of expansion to Finland. The sites where samples were collected for allozyme work are shown in the smaller map (nine in eastern Finland, seven in southern Finland, and four in Estonia).

Material and methods

Study species and sampling

The European map butterfly is a palaearctic nymphalid butterfly with two phenotypically distinct generations per summer (Reinhardt 1972, Fric & Konvička 2002). The larval host plant is the common nettle (*Urtica dioica*), which is widely distributed across Europe, and hence larval resources and suitable habitat are not expected to prevent further range expansion. Females lay large clutches of eggs and the caterpillars develop gregariously (Ruf 2002). The most common habitats are moist semi-open meadows, pastures, forest openings, and river banks (Reinhardt 1972, Marttila *et al*. 1990).

The map butterfly has colonised Finland from two directions, from the east at the latitude of 62°N and from the south (Fig. 1). The expansion in eastern Finland started in 1983 and is a continuation of an earlier expansion in nearby areas in Russia. The map butterfly occurred in the St. Petersburg area already in the 19th century according to a literature reference by I. C. Sivers from 1863 (V. Gorbach pers. comm.). The species became more common in Russian Karelia close to eastern Finland before the 1990s, most likely due to changes in the habitats caused by extensive forestry from the 1960s onwards (V. Gorbach pers. comm.). In the first years of the 21st century the map butterfly was reported as far north as in the Arkhangelsk area (65°N, 35°E; Bolotov & Shutova 2006). Since 1983, the range margin in Finland has gradually

moved about 200 km westwards and northwards, excluding a few recent observations done up to 200 km west and north from the border of the more continuous range (Fig. 1).

The colonization of and expansion in southern Finland has occurred in a very different manner than in eastern Finland. The first observation on the south coast was made in 1992, but only very small numbers were observed until July 1999 (altogether 6 observations in 1992 to 1998 from several localities on the south coast). These few individuals probably originated from Estonia, but failed to establish permanent breeding populations. The situation changed in July 1999, when a very large influx of butterflies crossed the Gulf of Finland from Estonia in south-easterly air currents (Mikkola 2000, Saarinen & Marttila 2000), as several butterfly species occasionally do when conditions are favourable for long-range migration. These butterflies established permanent breeding populations, which have subsequently started to expand (Mitikka *et al*. 2008).

In June–July 2005, we collected first-generation adult butterflies and their offspring (caterpillars) from multiple populations in three regions, eastern Finland (nine distinct populations within an area of 12 000 km^2), southern Finland (seven populations within 4000 km^2), and Estonia (four populations within 2500 km^2; Fig. 1). In each population, the larval samples consisted of individuals from different families (larval groups) and thus most likely represent the offspring of different females. We kept the adult butterflies collected from the field alive until frozen in –80 °C in the laboratory. We reared the caterpillars in captivity under common garden conditions (L:D 15:9 h, ca. 25 °C) to second-generation butterflies.

Unfortunately, we do not know the exact ages of the local populations from which the samples were collected. Given the known time courses of the two expansions, the populations in eastern Finland are likely to have been < 22 years old, and the populations in southern Finland were about 6 years old. The ages of the study populations were estimated based on the first year of observation within the respective 10 by 10 km grid cell, recorded by volunteer amateur and professional lepidopterists in the national butterfly recording scheme (NAFI) since 1992 (Saarinen *et al*. 2003). Older observations concerning the early years of the expansion in eastern Finland were obtained from the records collected by the Finnish Lepidopterist Society and from individual lepidopterists. Based on these records, we classified five of the nine populations in eastern Finland as being from 11 to 22 years old, while four populations were considered to be about four years old. These estimates are however not accurate, because the individual local populations from which we obtained the samples could have been more recent than the regional presence of the species in the respective 10 by 10 km grid cell. In southern Finland, there is little variation in the ages of the local populations, all being about 6 years old based on the observations in the nearby areas.

The relevant source area for the expansion in southern Finland is Estonia. The source area for the eastern Finnish expansion is Russian Karelia, from where we do not have samples. However, given the proximity of the St. Petersburg region to Estonia, without any barriers to dispersal between them, it seems reasonable to consider the Estonian populations as representing the source areas also for the eastern Finnish populations. We make this assumption while comparing the *Pgi* allele frequencies between the regions.

Morphological measurements

We measured the wing length and the dry weights of the thorax and the abdomen in a sample of second-generation females, collected from the field as caterpillars (above) and reared under common garden conditions in the laboratory (L:D 15:9 h, ca. 25 °C). To avoid pseudoreplication we performed the analysis with population means, including only one individual per family. The sample sizes were 8, 18, and 13 individuals representing 6, 6, and 3 populations from eastern and southern Finland and Estonia, respectively (Table 1).

We detached the wings, mounted them under a cover glass and photographed them with a digital camera (Nikon coolpix 995, Nikon corporation, Tokyo, Japan) using a ruler for scale. There were more high-quality samples of left than right

wings and hence we used left wings only. For the measurements we used the image analysis program ImageJ 1.37v by Wayne Rasband (http://rsb.info.nih.gov/ij/). The fore-wing length was measured from the junction of the two main veins at the base to the outer margin of the wing at the end of the second vein down from the apex. The accuracy of the wing measurement was 0.01 mm. We dissected the thorax and the abdomen, dried them at 60 °C for 24 hours and weighed them with a Mettler AE163 balance (accuracy 0.1 mg).

Allozyme typing of samples

We typed samples of wild-caught adult butterflies and butterflies reared from caterpillars collected in the field for phosphoglucose isomerase (*Pgi*), aspartate aminotransferase (*Aat*), and phosphoglucomutase (*Pgm*). The sample sizes are given in Table 1. The latter two loci were selected as reference loci to compare with *Pgi* and to control for any historical demographic effects on molecular variation, which could be expected to be the same in all loci. Unfortunately, the 2 or 3 alleles present in *Pgm* could not be scored reliably and hence we had to exclude this locus. Scoring of *Aat* was successful, but there was almost no variation, as only two alleles were present and the minor allele was detected in only four individuals. *Pgm* and *Aat* did not therefore provide any useful information and we analyse the results for *Pgi* only.

We performed the genotyping using cellulose acetate electrophoresis (Hebert & Beaton 1993). For *Pgi*, we homogenized the head of the butterfly in 100 μl of water. The gels were run in Tris Glycine buffer at 200 V for 15 min and *Pgi* was scored on the gels right after staining.

Flight metabolic rate

All butterflies used in the measurement of flight metabolic rate were second-generation butterflies reared from caterpillars under common garden conditions. The caterpillars originated from eight populations in eastern Finland, four populations in southern Finland, and two populations in Estonia (Table 1). Following their eclosion in early August 2005, we marked the butterflies individually and released them into a large outdoor population cage ($32 \times 26 \times 3$ m) at the Tvärminne Zoological Station (for the cage *see* Hanski *et al*. 2006), allowing the butterflies to fly under natural conditions. We caught the butterflies for the experiment from the cage between 2 and 9 August after they had spent 2 to 7 days in the cage, and allowed them to adjust to the room temperature for several hours before the measurement. We measured altogether 40 individuals (19 females and 21 males) that were all in good condition. These butterflies mostly originated from eastern Finland (Table 1), because during the time when the metabolic rates were measured mostly butterflies from eastern Finland were available.

Flight metabolic rate was measured as CO_2 output using the standard respirometry technique described in Lighton (1991) and applying the procedure described by Haag *et al*. (2005). Butterflies were stimulated to fly as continuously as possible in a transparent 1-litre jar with constant flow of CO_2-free air through the chamber. The average temperature in the jar, measured at the time of peak CO_2 emission (maximum metabolic rate), was 32.8 ± 1.1 °C (SD). The measurement was continued for 15 min, followed by the measurement of the baseline CO_2 production while the butterfly was at rest (the jar covered with a dark cloth). Following the measurement we

Table 1. Sample sizes and the numbers of populations and families sampled per region (eastern Finland, southern Finland and Estonia) for the *Pgi* allozymes, measurement of flight metabolic rate, and morphometric measurements.

Analysis	Eastern Finland			Southern Finland			Estonia		
	Pop	Fam	Ind	Pop	Fam	Ind	Pop	Fam	Ind
Pgi genotypes	9	37	141	7	49	60	5	27	33
Flight metabolic rate	8	17	29	4	6	7	2	3	4
Morphometric measures	6	8	8	6	18	18	3	13	13

froze the butterflies in –80 °C for subsequent *Pgi* genotyping.

We calculated the peak flight metabolic rate and the integrated CO_2 emission during the 15 min measurement period as explained by Lighton (1991) and Haag *et al*. (2005). As a measure of flight capacity, we used the residual of the peak flight metabolic rate regressed against the integrated metabolic rate. This measure reflects the maximum flight capacity of the butterfly in relation to its longer term metabolism, as reflected by the integrated CO_2 emission, which is affected by body size and physiological condition.

Statistical analyses

We used one-way ANOVA (SAS) to analyze differences among the regions (southern and eastern Finland and Estonia) in thorax and abdomen weights and in wing length as well as in the ratios of these measures. In the case of the ratio of wing length to the sum of the thorax and abdomen weights, the variances were unequal and a non-parametric Kruskall-Wallis test was used instead of the parametric ANOVA. We calculated the effect size for the ANOVA for thorax weight to obtain the smallest significant (α = 0.05) difference that could have been detected with our material. The effect size is a measure of the difference between the null and alternative hypotheses (Buchner *et al*. 1997, Thomas 1997), and we used it to compare our results with the results of a comparable previous study. The effect sizes were calculated from the sample means and standard deviations with the program G*Power 3.0.5, (http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/), using the method for one-way ANOVA according to Faul *et al*. (2007). Allometric relationships involving the wing length were analysed with ANCOVA, in which we used region as a factor and thorax weight or the sum of the thorax and abdomen weights as covariates.

We analysed the *Pgi* genotype frequencies among the three regions for deviations from the Hardy-Weinberg equilibrium using the exact HW test in Genepop 4.0 (Rousset 2008). The exact *G* test in Genepop 4.0 was used to compare genotype frequencies in the two generations and among the regions. The nine populations from eastern Finland were classified into two classes, old (presumed age from 11 to 22 years; 5 populations) and new (age about 4 years; 4 populations; *see* section "Study species and sampling"), and the effect of population age on *Pgi* genotype frequencies was tested with the exact test in Genepop 4.0.

As an alternative approach, we modelled allelic variation among the regions using a mixed model with a logistic link function and a binomial response distribution (presence or absence of the focal allele, two per individual; GLIMMIX macro, SAS). In this model, we included population as a random factor nested within region (fixed factor). The estimate of the random factor was very small. The results of this analysis were similar to the ones obtained with the analysis of genotype frequencies and are hence not reported.

Variation in metabolic rates among the *Pgi* genotypes was tested with one-way ANOVA. Variation in the respective mass-specific rates was tested by analysing residuals from the metabolic rate *vs*. body size regressions. We used ANCOVA to study whether genotype and sex explained the peak flight metabolic rate in relation to total CO_2 emitted during the 15 min measurement. Differences between particular genotypes were examined with Tukey's HSD. Finally, we contrasted the genotypes with and without the *Pgi-1* allele, as Haag *et al*. (2005) did for the putatively analogous genotype in the Glanville fritillary butterfly (*Pgi-1* is the kinetically fastest *Pgi* allele in the map butterfly). Integrated total CO_2 emitted during the 15 min flight period, sex, presence of the *Pgi-1* allele, and the interaction between sex and *Pgi-1* allele were used as explanatory variables in ANCOVA to explain the peak flight metabolic rate.

Results

Morphological measurements

There were no statistically significant differences among the females from the three regions in thorax and abdomen dry weights, wing length,

or in their ratios (Table 2, *see* Table 1 for sample sizes and composition). We repeated the calculations for the total material including multiple individuals per family, but the results were the same (not shown).

There was a significant difference among the regions in the allometric increase of wing length with body size, measured as the sum of the thorax and abdomen weights. The main effects of region and body mass as well as their interaction were significant ($F_2 = 7.72$, $P = 0.01$, $F_1 = 44.86$, $P < 0.0001$, and $F_1 = 8.25$, $P = 0.009$, respectively; Fig. 2). The increase in wing length with body size was steepest in eastern Finland and least steep in southern Finland. Considering small butterflies, wings were shortest in eastern Finland and longest in Estonia, whereas in the case of large butterflies wings were shortest in southern Finland.

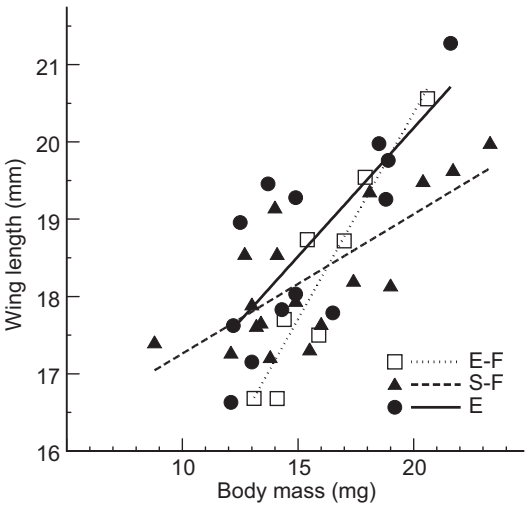

Fig. 2. Allometric relationship between wing length and body mass (thorax plus abdomen dry weights) in the three regions (E-F = eastern Finland, S-F = southern Finland, and E = Estonia). The main effects of region and body mass, as well as their interaction, are significant (*see* the text).

Allelic variation in *Pgi*

We identified two common *Pgi* alleles, denoted by *Pgi-1* and *Pgi-2*, of which the former is the kinetically faster one. A third rare allele, *Pgi-3*, was present in three individuals in the same family in southern Finland and as a single copy in two populations in eastern Finland. The genotype frequencies were in the Hardy-Weinberg equilibrium in all three regions ($P > 0.7$ in all cases).

There was a significant difference in the genotype frequencies between the two generations ($\chi_2^2 = 18.06$, $P = 0.0001$), reflected in the higher frequency of the *Pgi-1* allele in the first than the second generation in eastern and southern Finland (Table 3). In Estonia, there was no such difference, but the sample size for the first generation was very small (Table 3).

We compared the genotype frequencies in the three regions both in the pooled material and in the material for the second generation, for which the sample size was > 27 in all regions. In the pooled material, there was a highly significant difference in the genotype frequencies between the eastern and southern Finland ($\chi_2^2 = 21.44$, $P = 0.00002$) and between eastern Finland and Estonia ($\chi_2^2 = 16.81$, $P = 0.0002$), but not between southern Finland and Estonia ($\chi_2^2 = 0.33$, $P = 0.85$). The results were qualitatively the same for

Table 2. Measurements (mean ± SE) of the thorax and abdomen dry weights (mg), wing length (mm) and their ratios, and the ratio of the wing length to the sum of thorax and abdomen weights for females from the three study regions, eastern Finland, southern Finland and Estonia. Samples included one individual per family and the tests (ANOVA and Kruskall-Wallis) were done on population means (for sample sizes *see* Table 1). The last two lines give the test statistics and the *P* values.

Region	Thorax	Abdomen	Wing length	Thorax/abdomen	Wing length/(thorax + abd.)
E Finland	5.06 ± 0.32	11.49 ± 0.71	18.57 ± 0.54	0.44 ± 0.02	1.13 ± 0.04
S Finland	5.06 ± 0.29	11.06 ± 1.17	18.37 ± 0.24	0.50 ± 0.05	1.18 ± 0.09
Estonia	5.28 ± 0.50	10.60 ± 0.85	18.80 ± 0.42	0.55 ± 0.06	1.19 ± 0.07
Test value	$F_2 = 0.09$	$F_2 = 0.16$	$F_2 = 0.20$	$F_2 = 1.34$	$\chi^2_2 = 0.73$
P	0.91	0.85	0.82	0.30	0.70

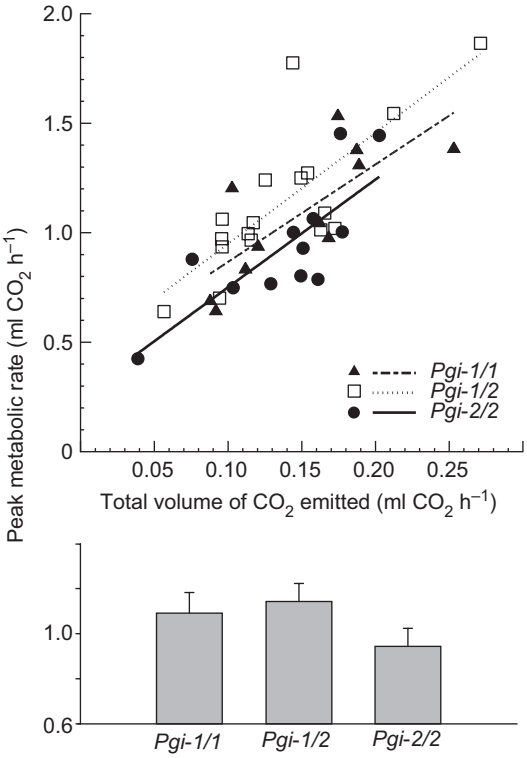

Fig. 3. Upper panel: Peak flight metabolic rate plotted against the total volume of CO_2 emitted during 15 min of flight in the three *Pgi* genotypes, *Pgi-1/1* homozygotes (triangles), *Pgi-1/2* heterozygotes (open squares), and *Pgi-2/2* homozygotes (black dots). Lower panel: Means and standard errors of peak flight metabolic rates for the genotypes. For statistics *see* Table 4 and the text.

the second generation samples (eastern and southern Finland: $\chi_2^2 = 9.21$, $P = 0.01$; eastern Finland and Estonia: $\chi_2^2 = 6.82$, $P = 0.03$; and southern Finland and Estonia: ($\chi_2^2 = 0.00$, $P = 1.0$).

We compared the *Pgi* genotype frequencies among the newly-established (age ca. 4 years) and older populations (age 11 to 22 years) among in eastern Finland. In the first-generation butterflies, there was a significant difference ($\chi_2^2 = 7.10$, $P = 0.03$) in the expected direction, the *Pgi-1* allele being more frequent in the new (0.59) than in the old populations (0.42). There was no difference in the second-generation butterflies, but here the sample size was very small (Table 3).

Flight metabolic rate

The peak flight metabolic rate and the total CO_2 emitted during the 15 min experiment did not vary significantly among the *Pgi* genotypes ($F_{2,37} = 1.48$, $P = 0.24$, and $F_{2,37} = 0.22$, $P = 0.80$, respectively). The metabolic rates were only weakly and not significantly related to body size (peak flight metabolic rate; $F_{1,19} = 2.68$ $P = 0.12$, $F_{1,17} = 0.41$, $P = 0.53$, total CO_2 emitted; $F_{1,19} = 3.19$ $P = 0.09$, $F_{1,17} = 0.01$, $P = 0.92$ for males and females, respectively), and mass-specific metabolic rates did not vary significantly among the genotypes (peak flight metabolic rate; $F_{1,18} = 0.26$ $P = 0.78$, $F_{1,16} = 1.30$, $P = 0.3$, total CO_2 emitted; $F_{1,18} = 0.22$, $P = 0.8$, $F_{1,16} = 0.19$, $P = 0.82$, for males and females, respectively). The peak flight metabolic rate in relation to total CO_2 emitted during the 15 min measurement period was marginally significantly explained by genotype and sex ($F_{2,35} = 3.22$, $P = 0.05$, $F_{1,35} = 4.19$, $P = 0.05$, respectively; ANCOVA, Fig. 3). Tukey's HSD for genotype showed a significant (0.05) difference between the *Pgi-1/2* heterozygotes and the *Pgi-2/2* homozygotes (Fig. 3). Contrasting the *Pgi-1/2* heterozygotes with pooled data for the two homozygotes gave a significant difference ($F_{1,36} = 4.75$, $P = 0.04$).

Working on the Glanville fritillary butterfly,

Table 3. Frequencies of the two common *Pgi* alleles and the corresponding *Pgi* genotypes in the two generations and three regions. *n* is the number of individuals. *See* the text for analyses.

Region	*n*	*Pgi-1*	*Pgi-2*	*Pgi-1/1*	*Pgi-1/2*	*Pgi-2/2*
Eastern Finland (gen 1)	104	0.47	0.53	0.22	0.49	0.29
Eastern Finland (gen 2)	37	0.38	0.62	0.14	0.49	0.38
Southern Finland (gen 1)	11	0.27	0.73	0.00	0.55	0.45
Southern Finland (gen 2)	49	0.20	0.80	0.04	0.33	0.63
Estonia (gen 1)	6	0.17	0.83	0.00	0.33	0.67
Estonia (gen 2)	27	0.20	0.80	0.04	0.33	0.63

Haag *et al*. (2005) contrasted individuals with and without the dominant kinetically fast *Pgi* allele, *Pgi-f*. In our case, the effect of the kinetically fast *Pgi-1* on peak flight metabolic rate was significant ($P = 0.02$), and the interaction between *Pgi-1* and sex was nearly significant ($P = 0.08$; the entire model: $F_{4,35} = 19.69$, $P = 0.001$, Table 4). Analysing the sexes separately showed that the effect of *Pgi-1* on peak flight metabolic rate was significant in females ($F_{2,16} = 6.11$, $P = 0.03$) but not in males ($F_{2,18} = 0.45$, $P = 0.5$), paralleling the results for the Glanville fritillary (Haag *et al*. 2005).

Discussion

A wide range of animal and plant species are currently expanding their geographical ranges northwards in response to climate warming (Parmesan 2006). European butterflies are amongst the best-studied taxa, and in their case, excluding species whose spreading through landscapes is restricted by fragmented habitat, range expansion is the rule rather than the exception (Pollard *et al*. 1995, Hill *et al*. 2001, Warren *et al*. 2001). The European map butterfly is not much restricted by host plant availability (the common nettle) nor by habitat selection, as it occurs in various kinds of semi-open habitats (Reinhardt 1972, Marttila *et al*. 1990) including forest edges (V. Mitikka unpubl. data), which are very common in the fragmented Finnish landscapes. The map butterfly is expanding in Finland (Fig. 1) and elsewhere in northern Europe (Öckinger *et al*. 2006), which is consistent with the hypothesis about climate warming as the causal mechanism. On the other hand, the map butterfly has expanded westwards in the Netherlands and France (Radigue 1994, Parmesan 2001) and southwards in Spain (C. Stefanescu pers. comm.), which is less obviously related to climate change and would warrant further study.

Though range expansions are rampant, and though one could expect widespread selection for enhanced dispersal at range boundaries, as explained in the Introduction, only a few studies have examined such selection, usually by comparing populations from the expanding range margin with those from the more central parts of the range. Perhaps the most convincing example is the study of the wing-dimorphic Roesel's bush cricket (*Metriopera roeselii*) by Simmons and Thomas (2004) referred to in the Introduction. The genetic basis of wing length determination is not known in this particular case, but it is nonetheless likely that the observed difference in the frequency of the wing morphs in newly-established populations at the range boundary *versus* old populations in more central parts of the geographical range is due to selection for long-winged individuals at expanding range boundary. Other studies of wing-dimorphic insects have demonstrated a simple genetic mechanism of wing-length determination in many (Aukema 1990) though not in all cases (Roff & Shannon 1993).

The relevance of wing length for dispersal is self-evident in the case of wing-dimorphic species, because usually only the long-winged individuals are able to fly and hence disperse any longer distances. The situation is less obvious in butterflies and other wing-monomorphic species. Several studies on expanding butterflies have suggested that morphological differences in relative wing area, aspect ratio, total dry mass, relative thorax mass or thorax shape between marginal and core populations are indicative of selection for dispersal at the range margin (Hill *et al*. 1999a, 1999b, Hughes *et al*. 2003). However, these studies have not demonstrated that the differences in the morphological traits actually make a difference to dispersal rate in the field. Alternatively, morphological differences could reflect latitudinal or some more complex spatial variation in morphology, possibly related to other life-history traits than dispersal.

In the present study, there were no significant differences in any morphological measurements

Table 4. Linear regression model (ANCOVA) of the peak flight metabolic rate (ml CO_2 h^{-1}) explained by the integrated total CO_2 emission (ml CO_2 h^{-1}) during 15 min of flight, and by sex and the *Pgi-1* allele. Sample size was 19 females and 21 males.

Factor	df	Type III SS	*F*	*P*
Total CO_2	1	2.06	60.89	< 0.0001
Sex	1	0.07	2.00	0.1664
Pgi-1	1	0.22	6.46	0.0156
Sex × *Pgi-1*	1	0.11	3.18	0.0833

between the expanding populations in eastern and southern Finland and the old populations in Estonia. We calculated an effect size of 0.12 mg for the comparison of the thorax weight between the three regions in this study, which is the smallest significant (0.05) difference that we could have detected with our material. Hughes *et al*. (2003) found a significant difference in thorax weight between the core and the marginal populations of *Pararge aegeria* in England, for which result we calculated an effect size of ca. 0.65 mg based on their results. Therefore, given our sample size, we should have been able to detect a much smaller difference than found by Hughes *et al*. (2003).

The results on allometric scaling of wing length with body mass (Fig. 2) showed significant differences among the three regions, but these differences are hard to interpret as indicating more dispersive butterflies in eastern Finland. If anything, the butterflies in the expanding populations in eastern Finland appeared to have smaller wing loading ratios than butterflies in the stable populations in Estonia (Fig. 2), which is not expected to make the former more powerful fliers. It is worth emphasizing that our measurements were taken on F1 individuals reared under common garden conditions, reducing the possibility that some latitudinally varying environmental factors would have influenced their development and thus obscured the genotypic effect on the measurements.

Results on the well-studied Glanville fritillary butterfly in the Åland Islands in south-western Finland cast further doubt on any general association between morphological traits and flight capacity in butterflies. Glanville fritillary females from newly-established populations are known to be more dispersive than females from old populations based on direct measurement of dispersal rate in the field (Hanski *et al*. 2002, 2004), but there are no corresponding differences in any morphological traits (Hanski *et al*. 2002).

Insect flight is energetically exceptionally demanding (Suarez 2000). Enzymes of the central metabolic pathway, including phosphoglucose isomerase (*Pgi*) catalysing the inter-conversion of glucose-*6*-phosphate and fructose-*6*-phosphate, are essential in the production of energy (ATP) for flight muscles. Pioneering studies on *Colias* butterflies have suggested that different isoforms of *Pgi* exhibit differences in kinetics and thermal stability that may lead to dissimilar flight performance (Watt *et al*. 1996). More recent studies on the Glanville fritillary have demonstrated more directly that individuals with different *Pgi* genotypes indeed differ in their flight metabolic rate (Haag *et al*. 2005, Hanski & Saccheri 2006) which is related to dispersal rate in the field (Niitepõld *et al*. 2009). Furthermore, there is an interaction between *Pgi* genotype and ambient air temperature in affecting movement activity (Saastamoinen & Hanski 2008, Niitepõld *et al*. 2009), supporting the hypothesis that there are kinetic differences between the different *Pgi* isoforms. Saastamoinen and Hanski (2008) recorded with a thermal image camera the body temperatures of butterflies exposed to low ambient temperatures in the field. They found that females with the *Pgi-f* allele, which is associated with high flight metabolic rate and is analogous to the kinetically fast *Pgi-1* allele in this study, were able to initiate flight in lower temperatures than females without the *f* allele.

It is noteworthy that *Colias* butterflies and the Glanville fritillary are not closely related, as they shared a common ancestor around 80 million years ago (Braby *et al*. 2006). The two species show dissimilar patterns of molecular variation in the coding region of the *Pgi* gene with likely functional significance (Wheat *et al*. 2010), yet there is a striking association between molecular variation in *Pgi* and performance variation among individuals in both species. This result implies functional convergence and makes it less likely that the molecular effects would be due to some other locus linked with *Pgi*. At the same time, this result suggests that comparable *Pgi* effects are likely to be present in many other butterflies and probably also in many other taxa. Given the results of this study and the previous studies on *Colias* and the Glanville fritillary, it is tempting to suggest that the high frequency of the *Pgi-1* allele in the eastern Finnish populations of the map butterfly is due to association between the *Pgi-1/1* or *Pgi-1/2* genotypes, or both, and high dispersal rate. According to this hypothesis, repeated establishment of new populations at the gradually expanding range boundary in eastern

Finland has favoured the *Pgi-1* allele. Within eastern Finland, the populations that were classified as new (about 4 years old) had indeed higher frequency of the *Pgi-1* allele on average than the populations classified as old (11 to 22 years old). The difference was statistically significant only in the first-generation butterflies, but the sample size for second-generation butterflies was very small. It is also noteworthy that the estimates of the ages of the local populations are inaccurate, as they are based on limited records of the butterfly within large (10 by 10 km) areas in the neighbourhood of the focal populations (*see* Material and methods).

One further detail in the allele frequencies is worth noting in this context. Both in eastern and southern Finland the frequency of *Pgi-1* was significantly higher in the first than in the second generation. The second-generation map butterflies are much more dispersive than the first-generation butterflies (Fric & Konvička 2002). If *Pgi-1* is associated with mobility, the observed difference in the frequency of *Pgi-1* between the two generations could be generated by selection favouring more dispersive butterflies in the second generation and less dispersive butterflies in the first generation.

We measured the flight metabolic rate in a sample of map butterflies in an attempt to directly test the hypothesis that there are differences in flight metabolism between the *Pgi* genotypes comparable to the differences reported for the Glanville fritillary (Haag *et al*. 2005, Niitepõld *et al*. 2009). We found a marginally significant difference in the right direction in the scaling of the peak flight metabolic rate with total CO_2 produced in the 15 min experiment. We examined this scaling on the assumption that the total CO_2 produced during the experiment would control for any variation in individual condition, while the peak flight metabolic rate would reflect the maximal flight capacity of the butterfly. The difference in mass-specific peak flight metabolic rate was in the same direction but not significant. These results remain inconclusive and further study is needed, but it is noteworthy that both in the Glanville fritillary and in the map butterfly it is the kinetically fast allele (*Pgi-f* and *Pgi-1*, respectively) that is associated with high peak flight metabolic rate.

The frequency of the *Pgi-1* allele is significantly higher in eastern Finland than in Estonia, but at the other expanding front in southern Finland the frequency of *Pgi-1* is only a little higher than in Estonia (Table 3). This result does not appear to support the hypothesis about range expansion leading to selection on *Pgi-1*. However, one should recall that there is a fundamental difference between the expansion processes in eastern and southern Finland. In eastern Finland and presumably also in Russian Karelia from where the Finnish expansion has originated, the range boundary has been expanding gradually and continuously for at least 30 years (Mitikka *et al*. 2008). The range expansion is the result of individual butterflies having dispersed through the heterogeneous landscape at the average rate of 7.5 km per year (Mitikka *et al*. 2008). In this process, phenotypic and genotypic differences among the butterflies can be expected to accumulate to generate the contrast between the marginal and central populations in dispersal rate. In contrast, the individuals of the map butterfly that established the populations in southern Finland took advantage of the air current during a singular migration event in July 1999 (*see* Material and methods). It is plausible that the large numbers of butterflies that crossed the Gulf of Finland represented a random sample of butterflies in the source areas in Estonia. In any case the butterflies did not fly on their own to Finland. Our hypothesis predicts that since the original colonization in 1999, *Pgi* allele frequency differences start accumulating during the subsequent expansion. This is a testable hypothesis but requires careful sampling of local populations with dissimilar known ages.

The significantly higher frequency of *Pgi-1* in eastern Finland than in Estonia is consistent with the hypothesis that selection on dispersers at the range margin has favoured the *Pgi* genotype or genotypes associated with high dispersal rate. There are three partly related alternative hypotheses that remain to be tested. First, it is possible that *Pgi-1* is additionally favoured in eastern Finland by the generally lower ambient temperatures than in southern Finland and Estonia. The comparable kinetically fast allele in the Glanville fritillary performs especially well in low ambient temperatures (Saastamoinen & Hanski 2008,

Niitepõld *et al*. 2009). Second, the deviating frequency of *Pgi-1* may be due to a genetic bottleneck through which the eastern Finnish population may have gone. Unfortunately, we could not genotype in this study other loci to examine the bottleneck hypothesis more closely. And third, the Estonian population may not represent well the allele frequencies in the source area for the eastern Finnish expansion. To test this hypothesis requires extensive sampling in eastern Europe.

Since 1999, the rate of northwards expansion has been slower in southern than in eastern Finland. In eastern Finland, the rate of expansion has been positively correlated with the degree-days during the growing season and with July-August temperatures in the period from 1983 to 2004, while there has been no such correlation in southern Finland (Mitikka *et al*. 2008). These differences call for further comparative studies, but the faster rate of expansion in the generally cooler climate in eastern Finland is suggestive of intrinsic differences in dispersal rate, possibly due to 30-yr selection at the continuously advancing range margin. The second generation, which is more dispersive than the first generation (Fric & Konvička 2002), has been observed regularly in Finland since 1999. The butterflies that arrived to the south coast of Finland from Estonia in summer 1999 were second generation individuals. Also in eastern Finland the second generation has occurred regularly since 1999, possibly due to higher summer temperatures from 1999 onwards.

Our results showed only limited molecular variation in *Pgi* in the map butterfly, much less than in the Glanville fritillary (Haag *et al*. 2005) and *Colias* butterflies (Watt 1983, Wheat *et al*. 2006). Generally, *Pgi* has been found to be highly polymorphic in many kinds of animals and plants (Zera 1987, Zamer & Hoffmann 1989, Patarnello & Battaglia 1992, Katz & Harrison 1997, Filatov & Charlesworth 1999, Dahlhoff & Rank 2000). Furthermore, there was similarly little variation in the two enzymes that were genotyped in this study for the map butterfly, *Aat* and *Pgm*. Why there is so little enzyme polymorphism in the European map butterfly remains unknown, but one possibility is historical bottlenecks at the European scale. It would be helpful to genotype samples from different parts of the geographical range of the species.

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