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Significant effects of *Pgi* genotype and body reserves on lifespan in the Glanville fritillary butterfly

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Individuals with a particular variant of the gene phosphoglucose isomerase (*Pgi*) have been shown to have superior dispersal capacity and fecundity in the Glanville fritillary butterfly (*Melitaea cinxia*), raising questions about the mechanisms that maintain polymorphism in this gene in the field. Here, we investigate how variation in the *Pgi* genotype affects female and male life history under controlled conditions. The most striking effect is the longer lifespan of genotypes with high dispersal capacity, especially in non-reproducing females. Butterflies use body reserves for somatic maintenance and reproduction, but different resources (in thorax versus abdomen) are used under dissimilar conditions, with some interactions with the *Pgi* genotype. These results indicate life-history trade-offs that involve resource allocation and genotype×environment interactions, and these trade-offs are likely to contribute to the maintenance of *Pgi* polymorphism in the natural populations.

Keywords: allocation; histolysis; lifespan; *Melitaea cinxia*; phosphoglucose isomerase; reproduction

1. INTRODUCTION

A series of studies by Ward Watt and his associates on *Colias* butterflies have demonstrated significant differences in male mating success and female fecundity between different phosphoglucose isomerase (*Pgi*) allozyme genotypes (e.g. Watt *et al.* 1985; Watt 1992). More recently, working on the Glanville fritillary butterfly (*Melitaea cinxia*), we have found a comparable association between allozyme and DNA sequence genotypes in *Pgi* and variation in life-history traits such as egg clutch size (Saastamoinen 2007*a*; Saastamoinen & Hanski 2008) and in physiological traits such as flight metabolic rate (Haag *et al.* 2005). Individuals with the genotype *Pgi-f* have elevated flight metabolic rate (Haag *et al.* 2005), which is correlated with high dispersal rate in the field (Niitepõld *et al.* in press). Females with this genotype are able to be active in lower ambient temperatures and therefore initiate oviposition earlier in the day than other individuals, which explains, at least partly, the approximately 20 per cent larger clutches laid by *Pgi-f* than *Pgi-non-f* females (Saastamoinen 2007*a*; Saastamoinen & Hanski 2008). The genotypic effects on life-history traits are even reflected in population dynamics, as allelic composition of local populations in the well-studied metapopulation of the Glanville fritillary in Finland had a significant effect on their growth rate (Hanski & Saccheri 2006).

Although *Pgi-f* females appear to be superior over females with other genotypes, at least in terms of dispersal and clutch size, the frequency of the allozyme *f* allele in the large metapopulation in the Åland Islands in Finland is only approximately 0.25 (Haag *et al.* 2005; Hanski & Saccheri 2006). This raises the question: what maintains polymorphism in this gene in this metapopulation?

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Studies on other insects have often reported trade-offs between dispersal and reproduction (e.g. Zera & Denno 1997; Roff & Fairbarn 2007), which could maintain variation in these life-history traits and correlated traits. In the present case, however, there appears to be a positive rather than a negative association, as *Pgi-f* females have both higher dispersal capacity and greater clutch size than other females (Saastamoinen & Hanski 2008). On the other hand, a study conducted in a large outdoor population cage suggested that the more dispersive individuals may pay a cost in terms of reduced lifespan, as females from newly established isolated populations, in which females tend to be especially dispersive (Hanski *et al.* 2004, 2006) and the *Pgi-f* allele is more frequent (Haag *et al.* 2005), had a shorter lifespan than females from old populations (Hanski *et al.* 2006; Zheng *et al.* 2007). Taking into account that the more dispersive individuals have higher flight metabolic rate, a shorter lifespan might reflect increased oxidative damage due to free radicals produced in aerobic respiration. An inverse correlation between metabolic rate and longevity has been reported in both within-species and among-species comparisons (Harman 1956; Van Voorhies 2001)

Decreased lifespan poses a potentially important life-history cost, as survival rate is typically a major determinant of lifetime reproductive success in insects (Carroll & Quiring 1993; Heimpel *et al.* 1998; Boggs & Freeman 2005). In the Glanville fritillary butterfly, lifespan is the best explanatory variable of the cumulative number of eggs laid during lifetime (Saastamoinen 2007*b*). Increased lifespan may be particularly beneficial during years with adverse environmental conditions, such as low ambient temperatures or high precipitation, as opportunities for reproduction might then be especially limited for individuals with a short lifespan.

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Several factors influence lifespan in natural populations, and these factors are likely to interact with each other. Intrinsic factors such as hormones (juvenile growth hormone and insulin-like growth factor; e.g. Flatt & Kawecki 2007; Chistyakova 2008), oxidative damage due to metabolism and immune system decline (Van Voorhies 2001) have been shown to affect lifespan in numerous species. Additionally, adverse environmental conditions, for instance high ambient temperatures and deprivation of food resources, often decrease lifespan (e.g. Chi *et al.* 2004). The effect of resource depletion on lifespan is not, however, straightforward, as modest dietary restriction has been shown to increase lifespan in a number of organisms (e.g. Baldal *et al.* 2006; Pijpe *et al.* 2008; see also Lee *et al.* 2008). Some resources can also be renewed during the adult life of insects via feeding or mating (nuptial gifts; Wiklund *et al.* 1998; O'Brien *et al.* 2002; Voigt *et al.* 2008). Finally, studies on wing-dimorphic species, such as crickets, have demonstrated how resources can be reallocated within an adult individual, most notably by histolysis of the flight muscles following dispersal and reallocation of the resources for reproduction and maintenance (e.g. Zera & Denno 1997; Zera & Harshman 2001).

The aim of this study is to assess possible differences in lifespan among the *Pgi* genotypes in the Glanville fritillary under controlled conditions. We compared lifespan in butterflies that were allowed versus not allowed to reproduce, as possible genotypic effects on lifespan could interact with reproduction. To assess the generality of the results, we also included a third treatment in which the environmental conditions were very different from those in the other two treatments. Second, although flight muscle histolysis is not expected to occur in butterflies to the extent reported for many wing-dimorphic species, we measured the loss of thorax and abdomen dry weights in the course of adult life and analysed possible interactions with the *Pgi* genotype.

2. MATERIAL AND METHODS

(a) *Study system*

The Glanville fritillary butterfly (*M. cinxia*) occurs at its northern range margin in Finland, where it is found only in the Åland Islands in southwest Finland. In the Åland Islands, the butterfly has two host plant species, *Plantago lanceolata* and *Veronica spicata*, which grow on dry meadows (Nieminen *et al.* 2004). The habitat is highly fragmented and the butterfly has a classical metapopulation structure with a high rate of population turnover (extinctions and recolonizations; Hanski 1999). The butterfly has a univoltine life cycle with a flight period lasting from early June to mid-July. Females have the full number of oocytes in their ovarioles at eclosion (Boggs & Nieminen 2004). The eggs are matured in batches, and females may lay up to 10 clutches of usually 130–160 eggs in their lifetime (Saastamoinen 2007*a*). Larval survival is positively correlated with group size (Kuussaari *et al.* 2004).

(b) *Experimental set-up*

In spring 2007, 600 Glanville fritillary larvae were reared under common garden conditions in the laboratory (27 : 10°C; 12 : 12). These larvae represent 50 independent families and originated from a population cage experiment conducted in 2006 (Saastamoinen 2008). The larvae were the third generation in the laboratory. The original stock population was collected as post-diapause larvae from 60 different local populations across the Åland Islands in spring 2005 (Saastamoinen & Hanski 2008). In 2007, the larvae were reared in groups of 12 individuals with fresh leaves of *P. lanceolata*. Pupae were weighed (Mettler-Toledo XS 105 analytical balance, accuracy 0.01 mg) at the age of 1 day and maintained individually under common garden conditions to record the time of eclosion for each individual. After eclosion, butterflies were sexed and marked individually by writing a number on the underside of the hind wing.

The adult butterflies were assigned to one of the three treatments on the day of eclosion. For practical reasons, those males that were originally assigned to the treatment with reproduction but that did not mate within 48 hours were reassigned to the treatment without reproduction. Whenever possible, at least two males and two females from each of the 50 families were assigned into each treatment. In the first treatment, butterflies were allowed to reproduce and feed (1 : 5 honey : water solution, available continuously). These butterflies were kept in small cages (15×30 cm) with temperature and light conditions suitable for mating. Possible matings were observed every day between 11.00 and 15.00. Immediately after mating, the female was moved into another similar cage with a host plant (*V. spicata*). The number of egg clusters and eggs laid by each female was counted twice a week until the death of the butterfly. After mating, the male was moved into a larger cage (40×50 cm) with other males and honey water solution ad libitum.

In the second treatment, butterflies were not allowed to mate but they were allowed to feed as in treatment 1. Males and females were kept separately in large cages (40×50 cm), with the density of butterflies never exceeding 25 individuals per cage. In the third treatment, butterflies were transferred between warm and cold conditions such that they were kept at +7°C for 3 days followed by standard conditions (27 : 10°C; 12 : 12) for 24 hours. This cycle was continued until the death of the butterflies. In this treatment, butterflies were not allowed to mate or feed. This treatment was included to have a radically contrasting treatment to the other two treatments, and thereby to examine the generality of the results. In all treatments, the cages were checked three times per day for dead butterflies.

Wing samples were taken immediately after the death of the butterfly and stored in alcohol for *Pgi* genotyping (§2*c*). The rest of the body was frozen in −80°C for subsequent measurement of thorax and abdomen dry weights. The dry weights were measured (accuracy 0.01 mg) after drying in 60°C for 24 hours.

(c) Pgi *genotyping*

Genomic DNA was isolated from the hind-wing samples stored in −20°C using a Nucleo spin tissue extraction kit (Mackerey-Nagel, GmbH & Co. KG, Germany) with overnight incubation at 56°C. The SNP 111-AA (Orsini *et al.* 2008) at *Pgi* was genotyped by primer extension reactions (Sokolov 1990), in which the screening primers, designed with a 3′-end immediately adjacent to the SNP, undergo a single nucleotide extension by a fluorescent-labelled ddNTP that corresponds to the SNP allele. Each PCR reaction (20 µl) contained 20–30 ng genomic DNA, 1 µM each primer, 200 µM each dNTPs, 2.5 mM $MgCl_2$, 20 ng BSA and 0.2 U Taq DNA polymerase. An initial denaturing step (5 min at 95°C) was followed by 35 cycles of amplification with 1 min at 94°C, 1 min at the annealing temperature and

1.5 min at 72°C. A final extension step included incubation for 15 min at 72°C. PCR products were purified with Exo-SAP-IT (GE Healthcare, Europe GmbH, Germany) at a concentration of 1/10 µl PCR reaction. Primer extension reactions employed the SnuPE kit (GE Healthcare; Batley & Hayes 2003), following the manufacturer's instructions. The reactions were run on Megabace 1000 (GE Healthcare) and the genotypes called by SNP profiler (GE Healthcare). The calls were checked visually.

The *Pgi* genotype was characterized with the SNP 111-AA, which approximately corresponds to the PGI allozyme *f* used in previous studies (Haag *et al.* 2005; Hanski & Saccheri 2006). This and the other two SNPs are described in detail by Orsini *et al.* (2008). The genotypes *Pgi*-SNP 111-AC and *Pgi*-SNP 111-CC both correspond to *Pgi-f* individuals, including both *ff* homozygotes and *f** heterozygotes, while *Pgi*-SNP 111-AA correspond to *Pgi-non-f* individuals in the previous studies. Orsini *et al.* (2008) have shown with two large random population samples and laboratory crosses that there exists excess heterozygosity in the *Pgi*-SNP 111 in the Åland Islands, which is largely due to great deficiency of *Pgi*-SNP 111-CC homozygotes. In the present sample, there were only four individuals (three males and one female) with the *Pgi*-SNP 111-CC genotype (0.7% of the individuals). These four individuals were omitted from the analysis, and thus we compare the AC heterozygotes with the AA homozygotes. All our results remained qualitatively the same when the few CC individuals were combined with the AC heterozygotes (results not shown). Possible reasons for the deficiency of the CC homozygotes are discussed by Orsini *et al.* (2008), and they do not affect our results in this paper.

(d) *Statistical analysis*

Statistical analyses were performed with SAS v. 9.1 (SAS Institute 1999). Lifespan was analysed with generalized linear mixed models (GLMMs), with treatment and *Pgi* genotype as fixed factors and body mass (pupal weight) as a covariate. Thorax and abdomen dry weights were similarly analysed, with treatment and *Pgi* genotype as fixed factors and body mass, lifespan and either thorax or abdomen dry weight as covariates. In all analyses, family was included as a random factor. Individuals that experienced the low-temperature treatment during their adult life were excluded from initial models to focus on the condition reproduction versus not. We report results separately for models including also the low-temperature treatment if they were different from the initial models.

All explanatory variables were normally distributed and the dependent variables had similar variances. Starting with the full model including all second-order interactions, the model selection was carried out by backward elimination of non-significant factors. We excluded some marginally (0.05) significant interactions that would have yielded complex models with multiple marginally significant interactions. Separate models were calculated for each sex, as sex had a significant effect on all traits examined and would have complicated models for pooled data.

Additional linear models were fitted to females that were allowed to reproduce to analyse the effects of lifetime egg production or egg-laying rate on lifespan and thorax and abdomen dry weights. Egg-laying rate was calculated as the number of eggs laid per day during the period of time that the female laid eggs. Finally, we classified all individuals based on their sex and reproductive status (1, females that reproduced; 2, females that did not reproduce; 3, males that reproduced; 4, males that did not reproduce). This variable was used to analyse the sex-specific treatment effect on the loss of thorax dry weight.

3. RESULTS

(a) *Lifespan*

The average lifespan for females was 17 days ($n=253$), and significantly longer than the average lifespan for males, 14 days ($n=275$; t-test, $p<0.001$; table 1). Whether females reproduced or not made no significant difference to their lifespan (GLMM; $F_{1,141}=0.47$, $p=0.489$), and lifespan was not affected by body size ($F_{1,141}=0.0$, $p=0.977$). By contrast, there was a strong association between genotype and lifespan, as AC heterozygous females lived significantly longer than the AA homozygotes (18 and 14 days, respectively; $F_{1,141}=23.19$, $p<0.0001$; figure 1; table 1). None of the second-order interactions were significant, but it is noteworthy that, when analysed separately, the genotypic effect on lifespan was significant in non-reproducing females only (figure 2). The results were qualitatively similar when females experiencing the cold treatment were included in the model ($F_{2,197}=1.26$, $p=0.285$, $F_{1,197}=0.40$, $p=0.527$ and $F_{1,197}=29.76$, $p<0.0001$, respectively, for treatment, body size and *Pgi* genotype).

Considering only females that reproduced, heavier females had a longer lifespan (table 2). Lifespan was negatively correlated with thorax dry weight at the end of life, and females with high egg-laying rate had a shorter lifespan, indicating a cost of reproduction (table 2). In this treatment, *Pgi* genotype had no effect, unlike in treatment 2 (no reproduction). We return to this point in §4*b*.

Males that mated lived significantly longer than those that did not mate (16 and 14 days, respectively; $F_{1,153}=9.52$, $p=0.002$), but as explained in §4, this result is likely to reflect variation in male quality rather than any benefit of mating. Body size did not have a significant effect on male lifespan ($F_{1,153}=0.09$, $p=0.771$), but as in females, AC heterozygous males had a longer lifespan than the AA homozygotes (17 and 14 days, respectively; $F_{1,153}=17.04$, $p<0.001$; figure 1). None of the second-order interactions were significant. Males in the cold treatment had a shorter lifespan than males that mated ($F_{2,217}=9.16$, $p=0.0002$, $F_{1,217}=0.64$, $p=0.425$ and $F_{1,217}=16.66$, $p<0.0001$, respectively, for treatment, body size and *Pgi* genotype), but there was no difference between males that did not mate and the ones in the cold treatment ($p=0.11$).

(b) *Thorax and abdomen dry weights at the end of life*

Thorax dry weight at the time of death was strongly and highly significantly affected by three factors in both females and males (table 3). Thorax weight was positively related to body mass at pupation and abdomen weight at death but negatively related to lifespan (table 3). Comparing the loss of thorax mass, corrected for initial body mass, among reproducing and non-reproducing females and males, it is evident that reproducing females lost significantly more of thorax dry weight with increasing lifespan than non-reproducing females and reproducing and non-reproducing males ($F_{1,325}=175.34$, $p<0.0001$,

Table 1. Comparison of the average (± s.d.) lifespan, body mass and thorax and abdomen weights at the end of life for the *Pgi*-SNP 111-AC and *Pgi*-SNP 111-AA females and males in the three different treatments. Sample sizes are given in parentheses.

	treatment 1 (reproduction)		treatment 2 (no reproduction)		treatment 3 (cold conditions)	
	females	males	females	males	females	males
lifespan						
Pgi-SNP 111-AC	18.3±6 (n=38)	18.2±6 (n=29)	18.3±5 (n=95)	15.3±6 (n=101)	19.1±5 (n=44)	13.0±3 (n=38)
Pgi-SNP 111-AA	16.2±7 (n=10)	14.8±5 (n=18)	13.8±4 (n=49)	12.3±4 (n=59)	16.9±6 (n=15)	12.6 (±3)(n=27)
body mass						
Pgi-SNP 111-AC	196±20 (n=38)	147±17 (n=29)	189±24 (n=95)	152±16 (n=101)	184±24 (n=44)	146±18 (n=40)
Pgi-SNP 111-AA	193±24 (n=10)	149±14 (n=18)	187±22 (n=49)	153±15 (n=59)	197±24 (n=15)	149 (±17)(n=25)
thorax weight						
Pgi-SNP 111-AC	6.3±1 (n=36)	5.3±1 (n=29)	6.4±1 (n=91)	5.7±1 (n=97)	7.4±1 (n=41)	5.9±1 (n=38)
Pgi-SNP 111-AA	6.7±2 (n=10)	5.5±1 (n=18)	7.1±1 (n=45)	5.7±1 (n=56)	7.7±1 (n=13)	6.0 (±1)(n=25)
abdomen weight						
Pgi-SNP 111-AC	9.2±4 (n=36)	3.8±1 (n=29)	12.1±3 (n=91)	4.8±2 (n=97)	9.5±3 (n=41)	3.7±1 (n=38)
Pgi-SNP 111-AA	12.2±4 (n=10)	4.9±2 (n=18)	13.1±5 (n=45)	4.4±2 (n=56)	10.5±2 (n=13)	3.7 (±1)(n=25)

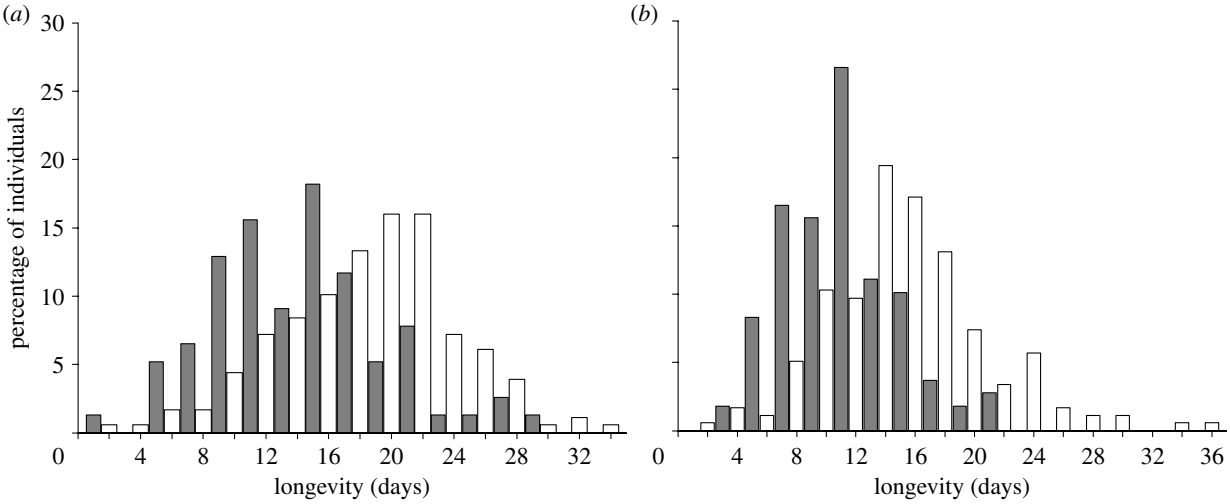

Figure 1. Frequency distribution of lifespan in (*a*) females and (*b*) males. White and grey bars are for *Pgi*-SNP 111-AC and *Pgi*-SNP 111-AA individuals, respectively. These results are for pooled data from treatments 1 and 2.

$F_{3,325}=2.56$, $p<0.05$ and $F_{3,325}=5.34$, $p<0.002$ for lifespan, sex-specific reproductive status and lifespan× sex-specific reproductive status, respectively; figure 3).

Focusing then on females that reproduced (treatment 1), thorax weight decreased with increased lifetime egg production (table 2; figure 4*a*). Conversely, lifetime egg production was best explained by thorax weight ($F_{1,16}=75.33$, $p<0.0001$), but a biologically more sensible model includes first lifespan, which explained lifetime egg production nearly as well as thorax weight and after which thorax weight made no significant additional contribution (table 2). It is noteworthy that no other variables, including body (pupal) mass ($F_{1,16}=0.05$, $p=0.828$), made a significant contribution to lifetime egg production after the effect of lifespan had been accounted for.

In contrast to the negative relationship between lifespan and thorax weight in the first two treatments, in the cold treatment (treatment 3), thorax dry weight at death was completely unrelated to lifespan (females: treatment× lifespan, $F_{2,183}=12.73$, $p<0.0001$, figure 3*a*; males: treatment×lifespan, $F_{2,204}=4.39$, $p=0.014$, figure 3*b*).

Considering the abdomen dry weight at the end of life in females, the major difference in the results on thorax weight was the lack of the effect of pupal weight but strong effect of treatment: females that reproduced had significantly lighter abdomens (9.9 mg) at death than non-reproducing females (12.4 mg; table 1). Unexpectedly, females in the cold treatment without food lost more abdomen weight than females in the other two treatments, including treatment 1 in which females reproduced ($F_{2,185}=41.82$, $p<0.0001$), and hence they had the very lightest abdomens (9.7 mg; $p<0.02$ and <0.001, respectively, compared with mated and unmated females under warm conditions; table 1; figure 5*a*). In reproducing

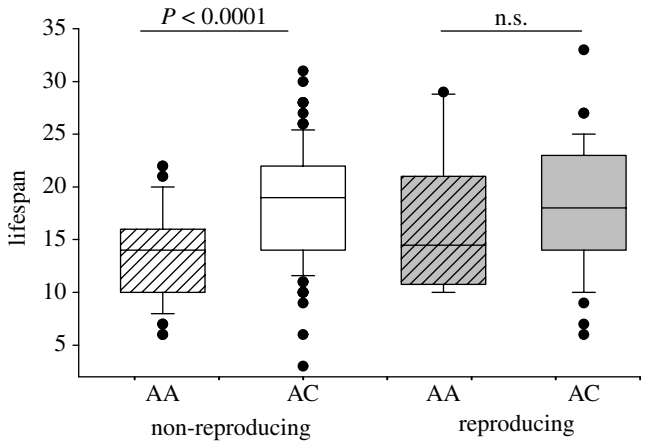

Figure 2. Lifespan in *Pgi*-SNP 111-AA (dashed boxes) and *Pgi*-SNP 111-AC (empty boxes) females in treatments without (white boxes) and with (grey boxes) reproduction.

Table 2. Factors affecting female lifespan, lifetime egg production and thorax and abdomen dry weights at the time of death in females that reproduced (treatment 1).

dependent variable explanatory factor	*F*	*p*-value	effect direction
lifespan[a]			
body mass (pupal weight)	14.40	0.002	+
thorax dry weight	109.68	<0.0001	−
egg-laying rate	7.82	0.0143	−
lifetime egg production[b]			
lifespan	32.80	<0.0001	+
thorax dry weight[c]			
body mass (pupal weight)	19.22	0.0007	+
lifespan	37.90	<0.0001	−
abdomen dry weight	4.67	0.0499	+
lifetime egg production	14.54	0.0022	−
abdomen dry weight[d]			
Pgi genotype	1.14	0.3064	
thorax dry weight	16.54	0.0016	+
egg-laying rate	6.27	0.0277	−
Pgi genotype × egg-laying rate	10.53	0.0070	
thorax dry weight × egg-laying rate	8.30	0.0138	

[a] d.f. = 1,14.
[b] d.f. = 1,16.
[c] d.f. = 1,13.
[d] d.f. = 1,12.

females (treatment 1), abdomen dry weight was positively correlated with thorax weight (table 2) and negatively correlated with egg-laying rate (number of eggs/number of reproductive days). The results were qualitatively similar when lifetime egg production was used instead of the egg-laying rate. Interestingly, abdomen mass decreased with increased egg-laying rate only in AC heterozygous females, suggesting a life-history trade-off involving resource allocation. Interaction between thorax mass and egg-laying rate suggested that the relationship between abdomen and thorax mass was stronger in females with lower egg-laying rate (table 2; figure 4*b*).

In males, abdomen dry weight was not related to body mass at pupation ($F_{1,147}=1.87$, $p=0.174$), but it was strongly correlated with thorax weight and decreased with lifespan, though not nearly as strongly as in females (table 3). Similarly to females, males in the cold treatment without food had much lighter (3.7 mg) abdomens than males in the other treatments ($F_{2,207}=9.54$, $p=0.001$; figure 5*b*).

4. DISCUSSION

(a) Pgi *genotype and lifespan*

We found a striking association between *Pgi* genotype and lifespan in the Glanville fritillary butterfly, the AC heterozygotes at SNP 111 having a significantly longer lifespan than the AA homozygotes in both sexes. This finding extends the apparent superiority of the AC heterozygotes, corresponding to the *Pgi-f* allozyme genotype in the Glanville fritillary, as previous studies have shown that *Pgi-f* individuals have higher flight metabolic rate (Haag *et al.* 2005), higher dispersal rate (Niitepõld *et al.* in press), greater activity in low ambient temperatures (Saastamoinen & Hanski 2008) and greater egg clutch size (Saastamoinen 2007*a*).

The effect of *Pgi* genotype on lifespan was consistent across the three treatments. However, the difference in lifespan between the genotypes was smaller among reproducing (treatment 1) than non-reproducing females (treatment 2), which suggests reduced genotypic effect under conditions of greater activity. In support of this, in a previous field experiment, we found that 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). In that experiment, butterflies were studied in a large population cage (30×30 m) in the field, in which they could fly freely under natural conditions.

The pioneering studies by Watt (1977, 1983) on *Colias* butterflies reported an increasing frequency of heterozygous PGI allozyme genotypes in natural populations with advancing flight season, which was interpreted as indicating higher survival of the PGI heterozygotes. Furthermore, heterozygous individuals, which possess the kinetically superior form of the PGI enzyme, did better especially under low and moderate temperatures, whereas, under warm conditions, the performance difference between the genotypes was reversed and the thermally more stable genotypes did better (Watt 1977, 1983; Watt *et al.* 1983). Whether the longer lifespan of AC heterozygotes than AA homozygotes in the Glanville fritillary would disappear under higher temperatures than tested in the present experiment remains an open question. Kallioniemi (2008) found an interaction between *Pgi* genotype and temperature in affecting larval growth that is consistent with Watt's hypothesis, as the AC heterozygotes did worse in high temperatures than the AA homozygotes.

(b) *Reproduction and lifespan*

The present study was designed to examine possible differences in the relationship between the rate of reproduction and lifespan among the *Pgi* genotypes. A negative relationship is commonly reported in both comparisons between different species (Roff 2002; Jervis *et al.* 2007)

Table 3. Factors affecting thorax and abdomen weights in males and females.

	females[a,b]			males[c,d]		
dependent variable: explanatory factor	*F*	*p*-value	effect direction	*F*	*p*-value	effect direction
thorax weight						
body mass	102.40	<0.0001	+	143.11	<0.0001	+
lifespan	138.02	<0.0001	−	59.67	<0.0001	−
abdomen weight	43.67	<0.0001	+	31.80	<0.0001	+
abdomen weight						
treatment	25.93	<0.0001				
lifespan	14.08	0.0003	−	5.59	0.0194	−
thorax weight	46.64	<0.0001	+	41.97	<0.0001	+
lifespan×thorax weight	10.41	0.0016				

[a] 1,134 d.f. for thorax models.
[b] 1,133 d.f. for abdomen weight models.
[c] 1,147 d.f. for thorax models.
[d] 1,148 d.f. for abdomen weight models.

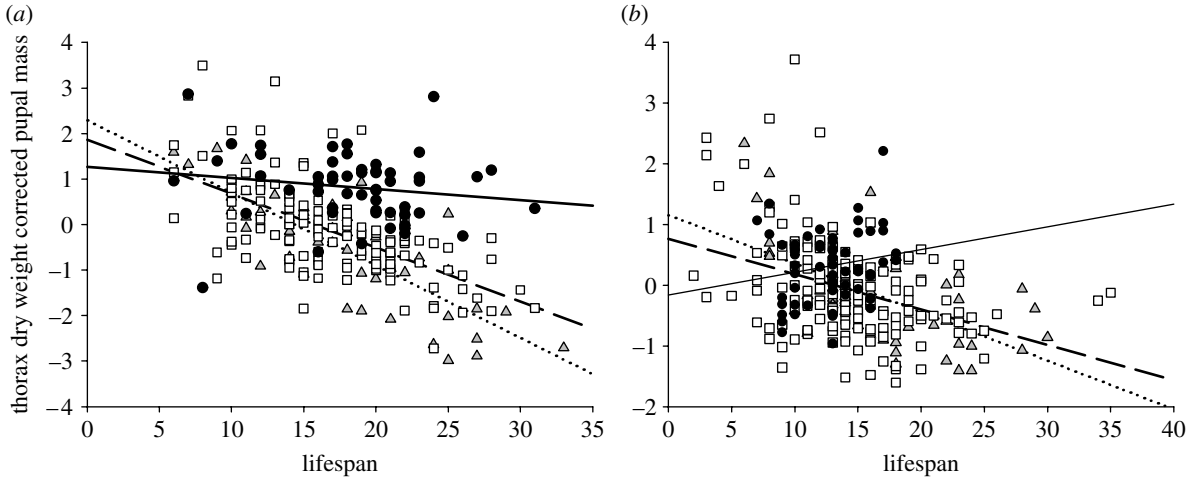

Figure 3. Thorax dry weight corrected for body (pupal) mass in relation to lifespan in (*a*) females and (*b*) males. Dotted, dashed and solid lines are for individuals in warm-temperature treatment with (triangles) and without (squares) reproduction and in low-temperature treatment with no reproduction (circles), respectively (for statistics see table 2).

and studies on single species (e.g. Van Voorhies 1992; Fischer 2007; Lewis & Wedell 2007). These results are usually explained by allocation of limited resources to reproduction and somatic maintenance (the *Y*-model; Van Noordwijk & De Jong 1986). On the other hand, a positive relationship between reproduction and lifespan is also common and is explained by variation in resource availability and fitness among individuals. For instance, Wiklund *et al.* (1998) demonstrated a positive relationship between the rate of reproduction and lifespan in butterflies when females received resources from males by mating multiple times.

In the Glanville fritillary females, there is a positive association between lifespan and lifetime egg production (see also Saastamoinen 2007*b*). This relationship most probably reflects fitness variation among individuals, but there is also a simpler reason, as females that live longer, for whatever reason, have more time for reproduction. By contrast, using the rate of egg-laying as a measure of reproduction, which eliminates the latter factor, yielded the opposite relationship: females that reproduced at a faster rate had a reduced lifespan, suggesting a trade-off. In this respect, the different *Pgi* genotypes showed no difference. Our results on the loss of thorax and abdomen weights (discussed in §4*c*) shed further light on the possible mechanisms of this cost of reproduction.

Males that reproduced in our experiment lived significantly longer than the non-reproducing males, but this most probably reflects differences in male quality rather than any benefit of mating itself. While running the experiment, we increased the rate of matings by often changing males that did not mate within 48 hours, and many of the unmated males, apparently of lower quality in general, were assigned to the treatment without reproduction (treatment 2). In nature, a trade-off between reproduction and survival is often thought to occur due to courtship, as searching for mates and mating may increase the risk of predation (Roff 2002), but these external causes of mortality played no role in the present laboratory experiment. On the other hand, we may conclude from our results that physiological or molecular costs of reproduction, such as energy diverted into reproduction reducing an individual's immunocompetence and therefore increasing the risk of death due to disease (Roff 2002), did not have

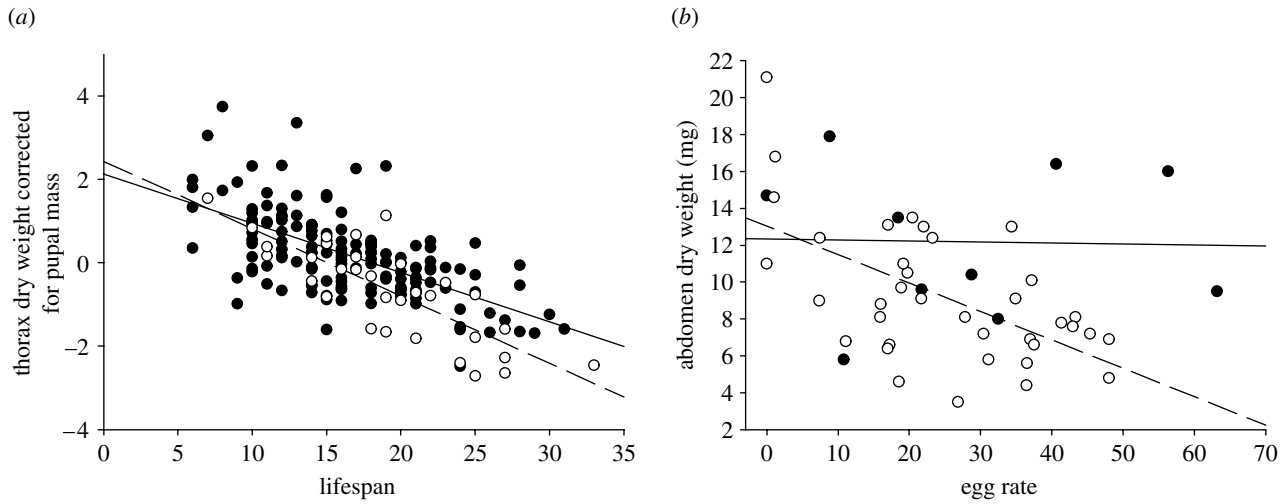

Figure 4. (*a*) Relationship between thorax dry weight (corrected for variation in body mass) and lifespan in females with lifetime egg production less than 150 eggs (filled circles and solid line) and those with lifetime egg production greater than 150 eggs (open circles and dashed line). (*b*) Relationship between abdomen dry weight and egg-laying rate in *Pgi*-SNP 111-AC (open circles and dashed line) and *Pgi*-SNP 111-AA (filled circles and solid line) females.

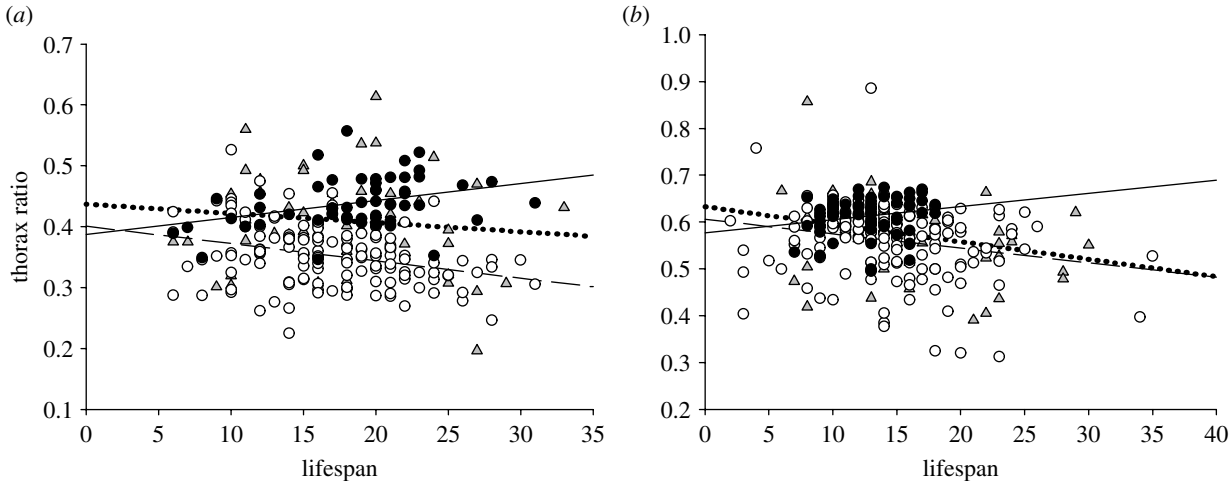

Figure 5. Thorax ratio (thorax weight/(thorax + abdomen weight)) in relation to lifespan in (*a*) females and (*b*) males. Dotted, dashed and solid lines are for individuals in warm-temperature treatment with (triangles) and without (open circles) reproduction and in low-temperature treatment with no reproduction (filled circles), respectively (for statistics see table 2).

a substantial effect on longevity in males. By contrast, in females, higher rate of reproduction appears to come with a cost of reduced lifespan, as mentioned above.

(c) *Thorax and abdomen weights at the end of life*

Histolysis (degeneration) of flight muscles and reallocation of the respective resources for reproduction and, possibly, somatic maintenance has been conclusively demonstrated for wing-dimorphic water striders (e.g. Kaitala & Huldén 1990) and crickets (e.g. Zera & Denno 1997; Zera & Harshman 2001). In crickets, flight muscle histolysis occurs concurrently with ovarian development, leading to a very strong negative correlation between flight and reproduction (Zera & Denno 1997). Far less is known about possible resource reallocation in species, in which flight capacity is a continuously varying trait (wing-monomorphic species) and flight ability cannot be lost entirely without severe cost to fitness.

Stjenholm *et al.* (2005) have reported that the loss of thorax and abdomen mass with age is a common phenomenon among nectar-feeding temperate-zone butterflies, and suggested that thorax resources may be reallocated for reproduction. Our results support this suggestion, as the decline in thorax mass was significantly greater in reproducing females than in non-reproducing females and males. Karlsson (1998) demonstrated that in the green-veined white butterfly (*Pieris napi*), resources from the thorax were, in fact, incorporated into eggs. Nonetheless, in butterflies in which flight ability is absolutely necessary during the entire life, reallocation of resources from flight muscles cannot be as complete as in wing-dimorphic species. Stjenholm *et al.* (2005) pointed out that as the primary function of flight muscles is to provide power for flight, flight ability and thorax weight (mass of flight muscles) do not need to be strongly correlated if abdomen weight also decreases with age, in which case the relative thorax weight would remain unchanged. In the present experiment, the relative thorax mass decreased, though only slightly, in reproducing and non-reproducing females in the warm treatments (treatments 1 and 2), whereas it increased with lifespan in the food-limited low-temperature treatment. To what extent

flight ability decreases with age due to reduced thorax mass, and whether such effects might differ among the *Pgi* genotypes, remain unanswered questions. The answers may depend on the environmental conditions.

A recent study on the alternatively spliced forms of the flight muscle protein troponin-t (Tnt) in the Glanville fritillary points to a possible mechanism that may allow butterflies to retain adequate flight capacity in spite of the loss of flight muscle mass (Marden *et al.* 2008). The relative frequency of the large Tnt isoform, which is expected to promote force and power output (Marden *et al.* 2001), increased with decreasing ratio of thorax mass at the end of life to initial body mass. We suggest that there may be a non-random distribution of the Tnt isoforms in flight muscles and, consequently, enrichment of certain splice forms due to the selective loss of muscle cells. Thereby, the relative abundance of large, contraction-enhancing isoforms would increase with age and potentially reduce the impact of muscle mass loss on flight capacity.

In the Glanville fritillary, abdomen weight decreased in both sexes with increasing lifespan, and, in general, there was a clear positive correlation between abdomen and thorax dry weights at the time of death. However, in females, there was a striking difference in abdomen weight loss between the treatments. Females in the food-limited low-temperature treatment (treatment 3) lost significantly more weight in their abdomen than in their thorax, whereas non-reproducing females in the warm-temperature treatment (treatment 2) lost thorax weight but much less abdomen weight. We suggest that these results indicate the mobilization of dissimilar resources under different conditions. As developing eggs in females and sperm in males are stored in the abdomen, it is likely that resources in the abdomen are not reallocated unless absolutely necessary, because this would greatly affect future reproductive performance. The low temperature combined with food deprivation in treatment 3 may have represented such a situation, in which individuals had no choice but to start using the fat-rich reserves in their abdomens. Boggs & Ross (1993) have shown that in the butterfly *Speyeria mormonia*, lifespan was conserved under adult resource stress by reallocating resources away from reproduction via resorbing the oocytes. Similarly, numerous studies on *Drosophila* have shown the importance of lipid reserves on lifespan and/or starvation resistance, whereas the protein content of the body seems to be of less importance (Harshman *et al.* 1999; Baldal *et al.* 2006). It is particularly noteworthy that the biggest difference in the lifespan between the two sexes was in the low-temperature treatment. Males have much smaller abdomens than females (table 1), and hence males would run out of resources much faster in the abdomen, as soluble sugars, glycogen, lipids and proteins have often been shown to increase with body weight (e.g. Blanckenhorn *et al.* 2007; Pijpe *et al.* 2008). As a matter of fact, female lifespan was longer in the low-temperature treatment than in the warm-temperature treatment without reproduction (treatment 2), which may be due to the resources in the abdomen mobilized only in the former treatment. Nonetheless, in the present study, unlike in many studies on other insects, body size (pupal weight) had no significant effect on lifespan except in reproducing females.

The difference in the performance of the two sexes in the low-temperature treatment may also reflect dissimilar adaptations to the thermal environment, although with the present material we cannot discriminate between the effects of low temperature and food deprivation. In the Glanville fritillary, females generally live longer than males and are likely to tolerate unfavourable and stressful conditions better than males. Artificial selection experiments have often demonstrated that lines selected for increased longevity have increased stress resistance, and thus these two sets of traits have been suggested to share the same genetic regulatory mechanisms (e.g. *Drosophila*: Baldal *et al.* 2006; *Bicyclus anynana*: Pijpe *et al.* 2008). As males are smaller than females, they may be able to be active under lower temperature than females (which could be an adaptation), and therefore they would lose energy-generating resources at a faster rate than females. A previous study on the Glanville fritillary showed that males can fly with lower body temperature and be active under lower ambient temperatures in the field than females (Saastamoinen & Hanski 2008).

Finally, females that were allowed to reproduce in the warm-temperature treatment (treatment 1) lost both thorax and abdomen weights. In their case, reduced abdomen weight was apparently related to egg production, as abdomen weight at the end of life was significantly and more strongly correlated with lifetime egg production than with lifespan (table 2). The loss of thorax weight in reproducing females was highly significantly affected by both lifespan and lifetime egg production, hence apparently resources in the thorax were mobilized for both somatic maintenance and reproduction. O'Brien *et al.* (2002) demonstrated how the primary constraint on egg production in a nectar-feeding hawkmoth is the availability of essential amino acids that originate entirely from the larval diet. However, they also suggested that these essential amino acids may, in theory, be obtained by the breakdown and turnover of structural proteins formed during metamorphosis, such as proteins in flight muscles (O'Brien *et al.* 2002). In the present experiment, the loss of thorax mass was most evident in reproducing females, suggesting that these females may indeed have reallocated limited essential amino acids from the thorax to egg production.

Our previous studies have shown a difference in clutch size between the *Pgi* genotypes (Saastamoinen 2007*a*; Saastamoinen & Hanski 2008), which was not observed here. This result supports the hypothesis that the ability of AC heterozygous (*Pgi-f*) females to be active earlier in the day in the field than the AA homozygotes (*Pgi-non-f*) is the cause of the difference in clutch size, as discussed by Saastamoinen & Hanski (2008). In the present study, there was a highly significant difference between the genotypes in the effect of the rate of reproduction on abdomen weight loss, which was much greater in the AC heterozygotes. This result points to a trade-off between fecundity and maintenance; if AA homozygous females are able to reproduce with a smaller reduction of resources in the abdomen, the latter can be used for somatic maintenance, which could prolong lifespan under some conditions, for instance under mild food stress. In fact, although the AC heterozygotes had a consistently longer lifespan in the present experiment than the AA homozygotes, the difference was small and non-significant in reproducing females.

5. CONCLUSION

Survival is clearly a complex phenomenon, which is affected by individual's genotype, environment and their interaction, as well as by changing life-history traits in ageing individuals. As shown by the present results, resource acquisition, both the use of new resources and the reallocation of existing resources, will greatly affect survival and thereby lifespan. A particular complication is that individuals living under dissimilar environmental conditions may use resources from different reserves. Thus, in the present experiment, under low temperature and food deprivation, resources from the abdomen were used more than resources from the thorax, whereas under warm conditions the reverse was the case.

In general, high metabolic rate that is correlated with high oxidative damage appears to speed up ageing and thereby reduce lifespan (Van Voorhies 2001 and references therein). However, in the Glanville fritillary, individuals with the AC (*Pgi-f*) genotype that is associated with high flight metabolic rate had higher, not lower, lifespan than AA individuals under the present experimental conditions (see also Melvin *et al.* 2007). One important consideration here is that the metabolic rate that has been measured is the flight metabolic rate and not the basal metabolic rate. The cost of high flight metabolic rate may become expressed only under conditions in which butterflies fly much, as suggested by some field experiments (Hanski *et al.* 2006; Zheng *et al.* 2007).

Our results add to the growing body of evidence suggesting superiority of the *Pgi*-SNP 111-AC genotype in the Glanville fritillary (Haag *et al.* 2005; Hanski *et al.* 2006; Saastamoinen 2007*a*; Saastamoinen & Hanski 2008). This study failed to demonstrate any simple and strong life-history trade-offs between longevity, dispersal and fecundity, but our results suggest the presence of more complex trade-offs involving resource allocation and genotype×environment interactions. First, both thorax mass and abdomen mass at the end of life were smaller in AC females than in AA females, suggesting that the former are using resources at a faster rate and may become more resource limited under some conditions. Second, there was a greater loss of abdomen weight with increasing egg-laying rate in AC females than in AA females. Third, the longer lifespan of the AC genotype was smallest or entirely non-existent in reproducing females. In summary, it appears that the mechanisms maintaining variation at *Pgi* are complex and involve mechanisms at the molecular (Orsini *et al.* 2008), life-history (present study) and even population level (Hanski & Saccheri 2006).

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REFERENCES

Baldal, E. A., Brakefield, P. M. & Zwaan, B. J. 2006 Multitrait evolution in lines of *Drosophila melanogaster* selected for increased starvation resistance: the role of metabolic rate and implications for the evolution of longevity. *Evolution* **60**, 1435–1444. (doi:10.1554/05-693.1)

Batley, J. & Hayes, P. K. 2003 Development if high throughput single nucleotide polymorphism genotyping for the analysis of *Nodularia* (cyanobacteria) population genetics. *J. Phycol.* **39**, 248–252. (doi:10.1046/j.1529-8817.2003.02073.x)

Blanckenhorn, W. U., Fanti, J. & Constanze, R. 2007 Size-dependent energy reserves, energy utilization and longevity in the yellow dung fly. *Physiol. Entomol.* **32**, 372–381. (doi:10.1111/j.1365-3032.2007.00589.x)

Boggs, C. L. & Freeman, K. D. 2005 Larval food limitation in butterflies: effects of adult resource allocation and fitness. *Oecologia* **144**, 353–361. (doi:10.1007/s00442-005-0076-6)

Boggs, C. L. & Nieminen, M. 2004 Checkerspot reproductive biology. In *On the wings of checkerspot: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 92–111. Oxford, UK: Oxford University Press.

Boggs, C. L. & Ross, C. L. 1993 The effect of adult food limitation on the life-history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology* **74**, 433–441. (doi:10.2307/1939305)

Carroll, A. L. & Quiring, D. T. 1993 Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera Canadensis*. *Oecologia* **93**, 233–241. (doi:10.1007/BF00317676)

Chi, Y. C., Sakamaki, Y., Tsuda, K. & Kusigemati, K. 2004 Effect of temperature on oviposition and adult longevity of the legume pod borer, *Maraca vitrata* (Fabricius) (Lepidoptera: Crambidae). *Jpn J. Appl. Entomol. Zool.* **49**, 29–32. (doi:10.1303/jjaez.2005.29)

Chistyakova, O. V. 2008 Signalling pathway of insulin and insulin-like growth factor 1 (IGF-1) as a potential regulator of lifespan. *J. Evol. Biochem. Physiol.* **44**, 1–11.

Fischer, K. 2007 Control of reproduction and a survival cost to mating in female *Bicyclus anynana* butterflies. *Ecol. Entomol.* **32**, 674–681. (doi:10.1111/j.1365-2311.2007.00922.x)

Flatt, T. & Kawecki, T. J. 2007 Juvenile hormone as a regulator of the trade-off between reproduction and life span in *Drosophila melanogaster*. *Evolution* **61**, 1980–1991. (doi:10.1111/j.1558-5646.2007.00151.x)

Haag, C. R., Saastamoinen, M., Marden, J. H. & Hanski, I. 2005 A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proc. R. Soc. B* **272**, 2449–2456. (doi:10.1098/rspb.2005.3235)

Hanski, I. 1999 *Metapopulation ecology*. Oxford, UK: Oxford University Press.

Hanski, I. & Saccheri, I. 2006 Molecular-level variation affects population growth in a butterfly metapopulation. *PLoS Biol.* **4**, 0719–0726. (doi:10.1371/journal.pbio.0040129)

Hanski, I., Erälahti, C., Kankare, M., Ovaskainen, O. & Siren, H. 2004 Variation in migration propensity among individuals maintained by the landscape structure. *Ecol. Lett.* **7**, 958–966. (doi:10.1111/j.1461-0248.2004.00654.x)

Hanski, I., Saastamoinen, M. & Ovaskainen, O. 2006 Dispersal-related life history trade-offs in a butterfly metapopulation. *J. Anim. Ecol.* **75**, 91–100. (doi:10.1111/j.1365-2656.2005.01024.x)

Harman, D. 1956 Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300.

Harshman, L. G., Moore, K. M., Sty, M. A. & Magwire, M. M. 1999 Stress resistance and longevity in selected lines of *Drosophila melanogaster*. *Neurobiol. Aging* **20**, 521–529. (doi:10.1016/S0197-4580(99)00091-3)

Heimpel, G. E., Mangel, M. & Rosenheim, J. A. 1998 Effects of time limitation and egg limitation on lifetime reproductive success of a parasitoid in the field. *Am. Nat.* **152**, 273–289. (doi:10.1086/286167)

Jervis, M. A., Boggs, C. L. & Ferns, P. N. 2007 Egg maturation strategy and survival trade-offs in holometabolous insects: a comparative approach. *Biol. J. Linn. Soc.* **90**, 293–302. (doi:10.1111/j.1095-8312.2007.00721.x)

Kaitala, A. & Huldén, L. 1990 Significance of spring migration and flexibility in flight-muscle histolysis in waterstriders (Heteroptera Gerridae). *Ecol. Entomol.* **15**, 409–418. (doi:10.1111/j.1365-2311.1990.tb00824.x)

Kallioniemi, E. 2008 Survival and growth of Glanville fritillary larvae: interaction between the effects of temperature and *Pgi* genotype. MSc thesis, University of Helsinki, Helsinki, Finland.

Karlsson, B. 1998 Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. *Ecology* **79**, 2931–2940. (doi:10.2307/176527)

Kuussaari, M., van Nouhuys, S., Hellmann, J. & Singer, M. 2004 Larval biology. In *On the wings of checkerspot: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 138–160. Oxford, UK: Oxford University Press.

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Soran, N. & Raubenheimer, D. 2008 Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc. Natl Acad. Sci. USA* **105**, 2498–2503. (doi:10.1073/pnas.0710787105)

Lewis, Z. & Wedell, N. 2007 Effect of adult feeding on male mating behaviour in the butterfly, *Bicyclus anynana* (Lepidoptera: Nymhalidae). *J. Insect Behav.* **20**, 201–213. (doi:10.1007/s10905-007-9075-2)

Marden, J. H., Fitzhugh, G. H., Girgenrath, M., Wolf, M. R. & Girgenrath, S. 2001 Alternative splicing, muscle contraction and intraspecific variation: associations between troponin T transcripts, calcium sensitivity, and the force and power output of dragonfly flight muscles during oscillatory contraction. *J. Exp. Biol.* **204**, 805–814.

Marden, J. H., Fescemyer, H. W., Saastamoinen, M., MacFarland, S. P., Vera, J. C., Frilander, M. J. & Hanski, I. 2008 Weight and nutrition affect pre-mRNA splicing of a muscle gene associated with performance, energetics and life history. *J. Exp. Biol.* **211**, 3653–3660. (doi:10.1242/jeb.023903)

Melvin, R. G., Van Voorhies, W. A. & Ballard, J. W. O. 2007 Working harder to stay alive: metabolic rate increases with age in *Drosophila simulans* but does not correlate with lifespan. *J. Insect Physiol.* **53**, 1300–1306. (doi:10.1016/j.jinsphys.2007.07.006)

Nieminen, M., Siljander, M. & Hanski, I. 2004 Structure and dynamics of *Melitaea cinxia* metapopulations. In *On the wings of checkerspot: a model system for population biology* (eds P. R. Ehrlich & I. Hanski), pp. 63–91. Oxford, UK: Oxford University Press.

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

O'Brien, D. M., Fogel, M. L. & Boggs, C. L. 2002 Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proc. Natl Acad. Sci. USA* **99**, 4413–4418. (doi:10.1073/pnas.072346699)

Orsini, L., Wheat, C. W., Haag, C. R., Kvist, J., FRilander, M. J. & Hanski, I. 2008 Fitness differences associates with *Pgi* SNP genotypes in the Glanville fritillary butterfly (*Melitaea cinxia*). *J. Evol. Biol.* **22**, 367–375. (doi:10.1111/j.1420-9101.2008.01653.x)

Pijpe, J., Brakefield, P. M. & Zwaan, B. J. 2008 Increased lifespan in a polyphenic butterfly artificially selected for starvation resistance. *Am. Nat.* **171**, 81–90. (doi:10.1086/524200)

Roff, D. A. 2002 *Life history evolution*. Sunderland, MA: Sinauer Associates.

Roff, D. A. & Fairbarn, D. J. 2007 The evolution of trade-offs: where are we? *J. Evol. Biol.* **20**, 433–447. (doi:10.1111/j.1420-9101.2006.01255.x)

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

Saastamoinen, M. 2007*b* Mobility and lifetime fecundity in new versus old populations of the Glanville fritillary butterfly. *Oecologia* **153**, 569–578. (doi:10.1007/s00442-007-0772-5)

Saastamoinen, M. 2008 Heritability of dispersal rate and other life history traits in the Glanville fritillary butterfly. *Heredity* **100**, 39–46. (doi:10.1038/sj.hdy.6801056)

Saastamoinen, M. & Hanski, I. 2008 Genotypic effects on flight activity and oviposition in the Glanville fritillary butterfly. *Am. Nat.* **171**, 701–712. (doi:10.1086/587531)

SAS Institute 1999 *SAS/STAT software user's guide, release 8.00*. Cary, NY: SAS Institute, Inc.

Sokolov, B. P. 1990 Primer extension technique for the detection of single nucleotides in genomic DNA. *Nucleic Acids Res.* **18**, 3671. (doi:10.1093/nar/18.12.3671)

Stjenholm, F., Karlsson, B. & Boggs, C. L. 2005 Age-related changes in thoracic mass: possible reallocation of resources to reproduction in butterflies. *Biol. J. Linn. Soc.* **86**, 363–380. (doi:10.1111/j.1095-8312.2005.00542.x)

Van Noordwijk, A. J. & De Jong, G. 1986 Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137–142. (doi:10.1086/284547)

Van Voorhies, W. A. 1992 Production of sperm reduces nematode life-span. *Nature* **360**, 456–458. (doi:10.1038/360456a0)

Van Voorhies, W. A. 2001 Metabolism and lifespan. *Exp. Gerontol.* **36**, 55–64. (doi:10.1016/S0531-5565(00)00208-4)

Voigt, C. C., Kretzschmar, A. S., Speakman, J. R. & Lehmann, G. U. C. 2008 Female bushcrickets fuel their metabolism with male nuptial gifts. *Biol. Lett.* **4**, 476–478. (doi:10.1098/rsbl.2008.0282)

Watt, W. B. 1977 Adaptations at special loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: biochemical and population aspect. *Genetica* **87**, 177–194.

Watt, W. B. 1983 Adaptation at specific loci. II. Demographic and biochemical elements in the maintenance of the *Colias* PGI polymorphism. *Genetics* **103**, 691–724.

Watt, W. B. 1992 Eggs, enzymes, and evolution: natural genetic variants change insect fecundity. *Proc. Natl Acad. Sci. USA* **89**, 10 608–10 612. (doi:10.1073/pnas.89.22.10608)

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

Watt, W. B., Carter, P. A. & Blower, S. M. 1985 Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of *Colias* butterflies. *Genetics* **109**, 157–175.

Wiklund, C., Kaitala, A. & Wedell, N. 1998 Decoupling of reproductive rates and parental expenditure in a polyandrous butterfly. *Behav. Ecol.* **9**, 20–25. (doi:10.1093/beheco/9.1.20)

Zera, A. J. & Denno, R. F. 1997 Physiology and ecology of dispersal polymorphism in insect. *Ann. Rev. Entomol.* **42**, 207–230. (doi:10.1146/annurev.ento.42.1.207)

Zera, A. J. & Harshman, L. G. 2001 The physiology of life history trade-offs in animals. *Ann. Rev. Ecol. Syst.* **32**, 95–126. (doi:10.1146/annurev.ecolsys.32.081501.114006)

Zheng, C., Ovaskainen, O., Saastamoinen, M. & Hanski, I. 2007 Age-dependent survival analyzed with Bayesian models of mark–recapture data. *Ecology* **88**, 1970–1976. (doi:10.1890/06-1246.1)