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Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field

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Abstract. Dispersal is a key life-history trait, especially in species inhabiting fragmented landscapes. The process of dispersal is affected by a suite of morphological, physiological, and behavioral traits, all of which have a more or less complex genetic basis and are affected by the prevailing environmental conditions. To be able to identify genetic and phenotypic effects on dispersal, movements have to be recorded over relevant spatial and temporal scales. We used harmonic radar to track free-flying Glanville fritillary butterflies (*Melitaea cinxia*) released in the field and reconstructed their flight tracks for several hours. Flight track lengths for individual butterflies ranged from tens of meters to several kilometers. Butterflies were most mobile at midday and in intermediate temperatures. Flight metabolic rate (MR), measured prior to the tracking, explained variation in mobility at all scales studied. One-third of the variation in the distance moved in one hour could be attributed to variation in flight MR. Heterozygous individuals at a single nucleotide polymorphism in the phosphoglucose isomerase (*Pgi*) gene moved longer distances in the morning and at lower ambient temperatures than homozygous individuals. A similar genotype × temperature interaction was found to affect the metabolic rate. Our results establish connections from molecular variation in a single gene to flight physiology and movement behavior at the landscape level. These results indicate a fitness advantage to the heterozygous genotype in low temperatures and suggest a mechanism by which varying environmental conditions maintain genetic polymorphism in populations.

Key words: East Anglia, UK; flight ability; gene × environment interaction; Glanville fritillary butterfly; Melitaea cinxia; *metabolism; migration; mobility; reaction norm; respirometry; single nucleotide polymorphism (SNP); telemetry.*

INTRODUCTION

Dispersal is a key life-history trait especially in species living in fragmented landscapes (Hanski 1999, Ronce 2007). Dispersal is also a very complex and plastic trait, and exactly when, how fast, and how far an individual will disperse is affected by a suite of morphological, physiological, and behavioral traits (Zera and Denno 1997, Hughes et al. 2003, Phillips et al. 2006, Sinervo et al. 2006, Roff and Fairbairn 2007), all of which have a genetic basis and are affected by the environment (Cote et al. 2007, Urban et al. 2008). Dissecting dispersal into its components is nonetheless essential for predictive science (Nathan et al. 2008), which has important applications, for example, in predicting the consequences of global environmental changes, including habitat loss and fragmentation, climate change, and the spread and impact of invasive species.

Metapopulations with fast population turnover, in which many new populations are established annually by dispersing individuals (Hanski 1999), offer exceptional opportunities to investigate dispersal. Studies comparing individuals of the Glanville fritillary butterfly (*Melitaea cinxia*) originating from newly established vs. old local populations in the Åland Islands in southwest Finland have documented a higher dispersal tendency in the former in the field (Hanski et al. 2002, 2004). Individuals from new populations exhibited higher mobility also in a large outdoor population cage (Hanski et al. 2006) in which the movement rate was highly heritable (Saastamoinen 2008). Dispersal propensity is likely to be heritable also in the field, because the most parsimonious explanation for the higher mobility of butterflies in the newly established populations is that their mothers, who established the new population in the previous generation, were more dispersive than the average individual in the metapopulation.

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The difference in the dispersal rate of Glanville fritillaries from new vs. old populations is accompanied by differences in flight physiology, such as the [adenosine triphosphate]/[adenosine diphosphate] ([ATP]/[ADP]) ratio in flight muscles following active flight (which is thought to reflect the capacity to renew ATP) (Hanski et al. 2004) and flight metabolic rate (MR) as measured by CO_2 production (Haag et al. 2005). Furthermore, Haag et al. (2005) discovered a nonrandom allelic distribution in the phosphoglucose isomerase (*Pgi*) gene between the new and old populations. The frequency of one allele, which also was associated with high flight MR, was highest in isolated new populations (see also Hanski and Saccheri 2006). The locus *Pgi* encodes the glycolytic enzyme PGI, which has been extensively studied in *Colias* butterflies (Watt 1977, 1983, Watt et al. 2003). Studies on *Colias* have established that allozyme variation in PGI is correlated with individual performance and fitness via effects on flight.

The abovementioned studies on the Glanville fritillary have conclusively established that there exists small-scale spatial variation in phenotypes and genotypes that are most probably related to dispersal in a fragmented landscape, in which selection for increased dispersal is likely to vary in space and during the lifetime of a particular population (Hanski et al. 2004). What has been missing up until now are studies directly measuring dispersal rate in the field in the Glanville fritillary as well as in other butterfly species. Here we use harmonic radar to track movements of individual free-flying Glanville fritillaries. The harmonic radar is currently a unique method for obtaining detailed information about movement tracks of insects during prolonged periods of time in the natural environment. Previous studies have used harmonic radar to track, e.g., foraging movements of bumble bees (Riley et al. 1999) and honeybees (Riley et al. 2005) and flight patterns of butterflies (Cant et al. 2005).

Our aim was to study in detail the process of dispersal of individual butterflies in the field. We have investigated whether flight metabolism, measured as CO_2 output in a flow-through respirometer, and the *Pgi* genotype are associated with dispersal rate in the field and how these variables interact with one another and with the prevailing environmental conditions in affecting dispersal rate. The study was carried out in East Anglia, UK, where the species has occurred previously but does not occur currently. To rule out the possibility of establishing a population in the study area we released only unmated females. Females rather than males were released, because the former are considered to be the more dispersive sex in this (Kuussaari et al. 1996) and related species (Singer and Hanski 2004) and because only females are capable of establishing new populations. To ensure the generality of the results, we used individuals from several source populations in the Åland Islands, Estonia, and China, all reared under the same common garden conditions. Results describing detailed movement patterns in relation to environmental conditions and the population of origin are reported elsewhere (Ovaskainen et al. 2008).

MATERIALS AND METHODS

Rearing and handling of butterflies

Female butterflies used in this study originated from three regions: the Åland Islands in southwest Finland (individuals from old and new local populations), the island of Saaremaa in West Estonia, and the Tian Shan mountains in West China. All larvae were reared in common garden conditions from winter diapause onwards. A detailed description of the origin and maintenance of larvae is presented by Ovaskainen et al. (2008).

Butterflies were weighed to the nearest milligram and marked with a felt tip pen on the day of eclosion by writing a running number on the underside of the right hind wing. Small pieces of both hind wings (~2 × 2 mm) were sampled for DNA and preserved in ethanol. Following marking the butterflies were placed in a mesh cage and kept in shade. On the following day the cage was moved outdoors in sunny weather or, in the absence of sunshine, under a light source indoors, and the butterflies were allowed to pump out excess meconium and fly in the cage for ~1 h. This improves their flight ability and allows reliable measurements of flight MR (K. Niitepõld, *personal observation*). After the flight practice, butterflies were provided with water.

Pgi *genotyping*

All individuals were genotyped for the single nucleotide polymorphism (SNP) site AA111 at *Pgi* as described by Saastamoinen and Hanski (2008) and Orsini et al. (2009). This SNP in the coding region of *Pgi* roughly distinguishes between the *f* allozyme and other allozymes as used in the previous studies by Haag et al. (2005) and Hanski and Saccheri (2006). Genotype frequencies in relation to population of origin are presented in Appendix D.

Flight metabolic rate

The flight MR of 73 females was measured on their second full day following eclosion using open-flow respirometry. Measurements were done in a field cabin with limited control of measurement temperature (variation from 30.8° to 35.1°C), which was included as an explanatory variable in the analyses. Individuals were forced to fly for a 10-min period as continuously as possible by gently shaking and tapping the respirometer that was kept under a UV light source. Some individuals fatigued before the end of the experiment, while others flew until the end. For further details about the measurements see Appendix A.

The highest rate of CO_2 production and the total volume of CO_2 produced during the 10-min flight experiment were calculated for each individual. The results were qualitatively the same; here we use the total volume in the analyses. In all analyses explaining mobility, we used as an explanatory variable the residual from a linear regression of the total volume of CO_2 against pupal mass, measurement temperature, and ambient air temperature during the measurement. When flight MR was entered as an explanatory variable along with the *Pgi* genotype, we used residual metabolic rate that accounted for the interactions between *Pgi* and measurement temperature. These residuals thus contained variation in flight metabolism that could not be ascribed to the interaction between temperature and *Pgi*.

Measurement of dispersal with harmonic radar

On the third day following eclosion a 7-mg transponder was attached to the thorax of the butterfly (see Ovaskainen et al. [2008] for further details). The mass of the transponder corresponds to 6% of the average body mass of the butterflies in this study. The mass corresponds to 30–50% of the mass of a typical egg clutch laid by a female (K. Niitepõld, *unpublished data*) and is significantly less than a mean honey water load ingested by an individual (17.6 ± 8.7 mg [mean ± SD]; K. Niitepõld, *unpublished data*). We did not observe any effect of the transponder on butterfly behavior prior to or during the field trial. In other butterflies added loads up to 15% of the body mass have not influenced field-measured survival or flight speed (Srygley and Kingsolver 2000).

We released females carrying a transponder and followed their movements with a harmonic radar within an area of 570 × 860 m of flat heathland at East Wretham in Norfolk, UK. The heath, with its low and sparse vegetation and low density of the natural larval host plant *Plantago lanceolata*, resembles the dry grasslands inhabited by the butterfly in northern Europe and Asia (see Appendix F). In the Åland Islands local populations tend to be small and low densities of conspecifics have been documented to increase dispersal (Kuussaari et al. 1996).

Butterflies were released in groups of five on average, one from each of several fixed release points distributed around the study area. On each day, butterflies were released in two sessions, at approximately 11:00 and at 14:00. Each individual was tracked for the rest of the day. At the end of the day, butterflies still present at the heath were captured and the transponders were removed for later use. Butterflies were tracked on nine consecutive days in early June 2006. We released altogether 68 unmated females, of which the metabolic rate was measured for 55 butterflies. Three individuals did not move spontaneously during the radar experiment, and they were removed from the analyses.

Weather data were collected by recording wind speed at 2.7 m above the ground level with cup anemometers and ambient temperature at 1.5 m above the ground level. For the analyses, the weather data were averaged for periods of 5 min or 1 h. The 5-min means for wind speed varied from 0.3 to 3.9 m/s and ambient temperatures from 13.5° to 28.5°C.

Original radar data were filtered and flight tracks were reconstructed using algorithms described by Ovaskainen et al. (2008). To quantify dispersal-related flight activity we used the maximum distance moved in any 1-h period, measured along the flight track. This measure of dispersal distance was considered preferable to the total length of an individual's flight track or the net displacement distance, because the total tracking time was not the same for all individuals due to differences in release times. Furthermore, sooner or later many individuals flew beyond radar coverage (see examples of flight tracks and distances moved in Appendix E). For five individuals that disappeared within one hour, we calculated a linear correction by dividing the distance moved with the time observed in minutes and multiplying by 60. The 1-h movement distance was $\log_{10}$ transformed to normalize the distribution.

We divided the full-length flight tracks into periods of 5 min. Because environmental conditions often changed substantially during the tracking period of an individual, analyzing the results in 5-min periods allowed us to disentangle the factors affecting activity and distance moved. An individual was classified as active during a 5-min period if it moved a minimum distance of 5 m, which was greater than the typical radar error (Ovaskainen et al. 2008). The distances moved in 5 min were $\log_{10}$ transformed.

Statistical analyses

All statistical analyses were performed with SAS 9.1 using PROC GLIMMIX (SAS Institute, Cary, North Carolina, USA). We used logistic regressions to model the probability of becoming active in a 5-min period following a 5-min period of inactivity and vice versa. The activity state during a 5-min period was considered a Markov process, and the probability of becoming active (or inactive) was modeled with individual (nested within population or genotype) as a random factor. The distance flown in 5 min was modeled as a repeated-measures mixed model. We accounted for temporal autocorrelation in the data by using the covariance structure $\text{cov}(y_{t1}, y_{t2}) = \sigma^2\rho^{|t1-t2|}$, where t is time. This takes into account unequal temporal spacing of observations.

The initial models contained all main effects, two-factor interactions, and squared terms, and we used stepwise backward elimination to remove nonsignificant ($P > 0.05$) terms from the model. Nonsignificant main effects were kept in the model if the factor was involved in a significant interaction or the squared term was significant. In models with quadratic terms we used Type I sum of squares, as recommended for polynomial models containing higher-order terms (Littell et al. 1996). Factors were entered in the models in the following order: population type or genotype, pupal mass, flight MR, time, temperature, wind speed, two-factor interactions between individual traits and environmental variables, and finally the squared terms of

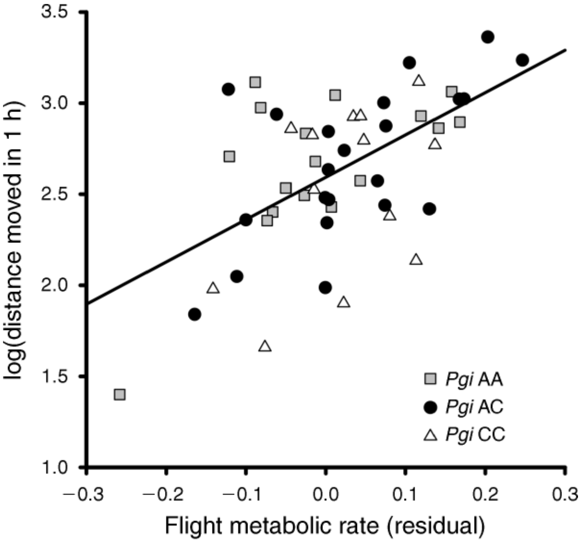

FIG. 1. Maximum distance moved in 1 h vs. flight metabolic rate (flight MR). The flight MR was corrected for pupal mass, ambient temperature during the measurement, and the measurement temperature. The regression is significant ($t_{51} = 4.84$, $P < 0.0001$) and explains 32% of the variation in the distance moved. The distance ranged from 25 to 2300 m. No significant differences were found between the phosphoglucose isomerase (*Pgi*) genotypes in this measure.

environmental variables. Individual and day were included as random factors in all repeated-measures models (see Appendix B).

We constructed several models of varying complexity to assess the robustness of the results. In the first model type we included the environmental factors, population, body mass, flight MR, and all two-factor interactions. In the second model we replaced flight MR and population with *Pgi* genotype. In the third model we included both flight MR and *Pgi* genotype. The results of this final model are reported in Table 2, and the results of the first two models are presented in Appendix C.

The three genotypes in the SNP AA111 show a significant deviation from the Hardy-Weinberg (HW) equilibrium in the Finnish population, possibly due to overdominance at *Pgi* and/or to linkage with deleterious alleles (Orsini et al. 2009). In contrast, the Chinese population shows no deviation from the HW equilibrium, suggesting a different functional role of this mutation or lack of linkage with deleterious alleles (L. Orsini, *personal communication*). In the small sample of Chinese butterflies in this study, all individuals happened to be CC homozygotes in SNP AA111. Because the functional significance of different mutations in *Pgi* may be different in the European and Chinese populations, we excluded the 11 Chinese butterflies from the models involving the *Pgi* genotype as an explanatory variable. Seven individuals in the remaining sample were CC (Appendix D) and they were also excluded, thus leaving the contrast between the AC heterozygotes and the common AA homozygotes.

RESULTS

Individual butterflies were successfully tracked for 2.7 h on average ($n = 52$, SD = 1.6, minimum = 0.4 h, maximum = 6.3 h). Ten percent of butterflies were not observed by the radar after 1 h, 38% were lost in 2 h, and 77% in 4 h. Twenty-seven percent of the individuals were recorded to leave the area covered by the radar, whereas a larger fraction settled in the area and were hidden in the vegetation. Our first measure of dispersal rate is the maximum distance moved in 1 h, selecting for each individual the 1-h period during which it moved the longest distance, measured along the actual flight track. This distance varied from 25 to 2300 m. Flight MR was the only factor with a significant effect on the distance moved in 1 h, but this effect was very strong, explaining 32% of variation in the distance moved (Fig. 1).

Flight MR was measured over a range of temperatures. Examining the influence of measurement temperature on flight MR revealed a significant interaction with *Pgi* genotype (Table 1): CO_2 production increased with increasing measurement temperature in AA homozygotes but decreased with temperature in AC heterozygotes (Fig. 2). Flight metabolic rate also increased with the outside temperature during the measurement (Table 1), apparently because the ambient temperature influenced the activity of butterflies in the cage in which they were kept prior to the measurement. There was no statistical correlation between the measurement temperature and the ambient temperature. The measurement temperature followed its own dynamics and only occasionally was it affected by the high temperature in

TABLE 1. Factors influencing flight metabolic rate.

	Model A: all genotypes			Model B: excluding CC homozygotes		
Variable	*F*	df	*P*	*F*	df	*P*
Pupal mass	8.94	1, 65	0.0039	5.78	1, 49	0.0201
MT	1.40	1, 65	0.2418	1.43	1, 49	0.2382
AT	7.55	1, 65	0.0078	4.04	1, 49	0.0499
Pgi	3.83	2, 65	0.0268	8.74	1, 49	0.0048
MT × *Pgi*	3.80	2, 65	0.0276	8.67	1, 49	0.0049

Notes: Model A contains phosphoglucose isomerase (*Pgi*) genotypes AA, AC, and CC in the single nucleotide polymorphism (SNP) AA111. Model B excludes *Pgi* CC homozygotes. Key to variables: MT, measurement temperature; AT, ambient temperature.

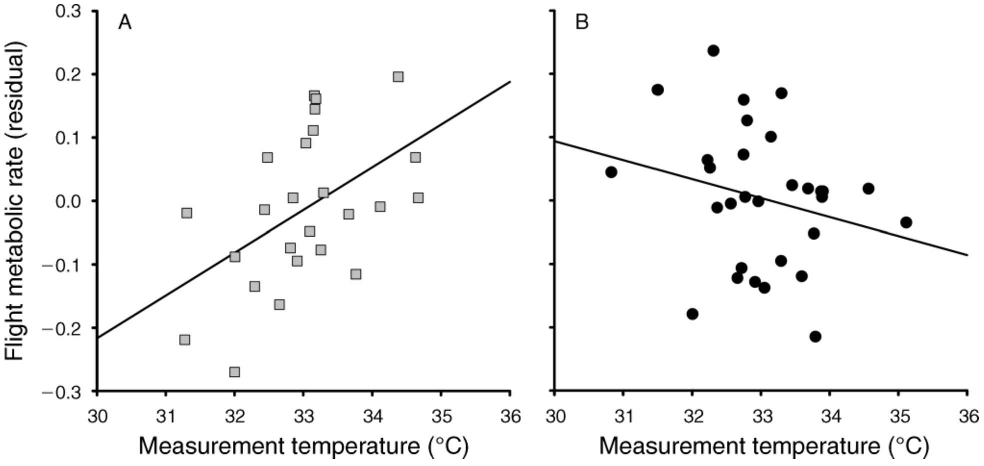

FIG. 2. Flight metabolic rate (flight MR) vs. measurement temperature: the phosphoglucose isomerase (*Pgi*) gene (single nucleotide polymorphism [SNP] AA111) (A) AA homozygotes and (B) AC heterozygotes differ in their reaction to temperature. Metabolic rate was corrected for pupal mass and ambient temperature during the measurement. For statistics see Table 1.

the cabin in which the measurements were performed. As expected, there was also a significant positive correlation between flight MR and the size of an individual, measured as pupal mass.

We next analyzed the movement data in 5-min segments. The probability of becoming active in a 5-min period following inactivity was highest at intermediate ambient temperatures, peaking at 21.8°C, and in the early afternoon, as indicated by the significant quadratic terms in Table 2. Flight MR had a highly significant positive effect, but the *Pgi* genotype did not enter the model.

Flight MR was the only factor explaining the probability of being inactive in a 5-min period following activity in the previous 5-min period. Individuals with a high flight MR were less likely to stop flying than individuals with a low MR ($F_{1,349} = 4.37$; $P = 0.037$). The *Pgi* genotype had no significant effect.

The distance moved in 5 min was longest at intermediate temperatures, and it increased toward the afternoon (Table 2). Distance moved tended to increase with flight MR ($P = 0.07$), consistent with the result in Fig. 1. The main effect of the *Pgi* genotype was not significant, but there was a significant temperature × genotype interaction, such that AC heterozygotes moved longer distances in low temperatures than AA homozygotes (Fig. 3A). In addition to this effect, there was a significant time × genotype interaction, indicating that AC heterozygotes moved more than AA homozygotes in the early hours of the day. Finally, there was a significant interaction between the flight MR and *Pgi* genotype: AC heterozygotes showed a significant positive correlation between flight MR and distance moved, whereas in AA homozygotes there was a nonsignificant negative correlation (Fig. 3B). Body mass was used as a covariate, but it had no effect on movement in any of the models.

DISCUSSION

Conventional methods of recording insect movements in the field, such as mark–recapture studies (Hanski et al. 2004) and tracking of individuals on foot (Schtickzelle et al. 2007, Kőrösi et al. 2008), are often useful but they are not appropriate for all purposes. Mark–recapture studies provide valuable information about

TABLE 2. Models explaining activity and distance flown with phosphoglucose isomerase (*Pgi*) genotype, flight metabolic rate (flight MR), and environmental variation. Results are presented for factors influencing (A) the probability of being active in a 5-min period following a 5-min period of inactivity and (B) the distance moved in a 5-min period.

	A) Probability of activity in 5-min period			B) Distance moved in 5 min		
Variable	df	*F*	*P*	df	*F*	*P*
Pgi	NS	NS	NS	1, 32	0.94	0.3388
Flight MR	1, 954	13.24	0.0003	1, 284	3.26	0.0722
Time	1, 954	2.98	0.0845	1, 284	7.59	0.0062
Temperature	1, 954	4.82	0.0284	1, 284	0.01	0.9093
Flight MR × *Pgi*	NS	NS	NS	1, 284	9.33	0.0025
Time × *Pgi*	NS	NS	NS	1, 284	5.41	0.0207
Temperature × *Pgi*	NS	NS	NS	1, 284	5.82	0.0165
Time^2	1, 954	6.76	0.0094	NS	NS	NS
Temperature^2	1, 954	4.26	0.0392	1, 284	6.74	0.0099

Note: NS indicates a nonsignificant term left out of the final model.

the outcome of movements at large spatial scales, for instance on the shape of the dispersal kernel (Fric and Konvicka 2007) and on dispersal across different habitat types (Ovaskainen 2004). On the other hand, mark–recapture studies cannot uncover the actual movement tracks and transit times. Following butterflies visually on foot and marking turning points with physical markers or with a GPS can be very time consuming due to the unpredictable behavior of butterflies. There is also the danger that a person tracking a butterfly may unintentionally influence its behavior.

Tracking of freely moving insects over "field scales" with harmonic radar yields records of movement trajectories with a high degree of accuracy. High spatial and temporal resolution of movement tracks is necessary when the purpose is to disentangle the causes of variation among individuals in movement behavior and dispersal. Previous studies on the Glanville fritillary have estimated a mean lifetime displacement distance of ~1 km from the natal population (Ovaskainen 2004). This makes an interesting comparison with the full-day movement paths recorded in this study, which were often surprisingly long (see Appendix E).

As a robust measure of mobility in the field we used the maximum distance moved in 1 h. The distance varied up to almost 100-fold between individuals and is sure to have biological significance. To take full advantage of the spatial and temporal resolution of the harmonic radar data we split all flight tracks into segments of 5 min. As a general measure of activity we modeled the probability of an individual becoming active if it was resting in the previous 5-min period, and vice versa. To add further detail to the understanding of flight behavior, we analyzed the distances moved in active 5-min periods. These latter measures provide high-resolution data for examining genotypic, phenotypic, and environmental effects on movement behavior.

In the present study, we have demonstrated that movement rate depends, as expected, on environmental factors such as the time of the day and ambient temperature, but also on an individual's flight MR and *Pgi* genotype. Furthermore, there are significant interactions between these factors. The interactions between *Pgi* genotype and time and between *Pgi* genotype and temperature add to the existing body of literature about the role of PGI as a temperature-sensitive enzyme that affects individual performance. The interaction between *Pgi* and flight MR represents a new finding. Below, we discuss in turn the influence of abiotic factors, flight MR, and *Pgi* genotype on movement rate, with emphasis on the likely biological mechanisms involved in the interactions of the explanatory variables.

Environmental effects and constraints on dispersal

The probability of becoming active, in the sense of moving within a 5-min period that was preceded by a 5-min period of inactivity, was highest around the solar noon and hence at the time of maximal solar radiation. No direct measurements of solar radiation or cloud cover were made but it is likely that the general diel pattern of solar radiation remained the same between days despite variation in the ambient temperature. In relatively low temperatures the activity of ectothermic insects is generally likely to follow the intensity of short-waved radiation (Watt 1968). On the other hand, though the probability of becoming active decreased in the afternoon, the distance flown in 5-min periods increased with time. A possible explanation is that the higher afternoon ambient temperatures decreased convective heat loss during flight, enabling longer flight bouts. The motivation to fly may also have been different; for instance, flight in the morning may have been more oriented toward foraging.

Temperature had a clear nonlinear effect on both activity and the distance flown in 5 min: the longest distances were flown at intermediate temperatures, slightly above 20°C (Fig. 3A). These findings represent typical reaction norms to temperature, which in most biological systems peak between the extremes (Knies et al. 2006). It is clear that initially, starting from low temperatures, an increase in ambient temperature increases the activity of ectothermic insects. The decrease at high temperatures was less expected as the highest ambient air temperature measured in this experiment was 28.5°C. At the ground level, however, temperature must have reached much higher values and was potentially high enough to reduce activity. Basking butterflies are known to reach thoracic temperatures above 40°C when exposed to direct sunlight (Watt 1968, Heinrich 1986). We observed that on the warmest days butterflies stopped basking and instead positioned themselves on the ground with wings closed to reduce the generation of heat.

Flight MR and dispersal

Apart from temperature, the variable that most consistently explained variation in dispersal rate was flight MR. Flight MR was the only factor that had a significant effect in all the models in which it was included as a candidate variable, and it was the only significant factor explaining the aggregated measure of distance moved in 1 h.

Flight MR was measured on the day before the radar trial. In the measurements, an individual was pushed to its maximum performance by forcing it to fly in a 1-L respirometry chamber. Whenever the individual stopped flying and landed, the chamber was shaken to force the butterfly to fly again. Due to the real-time nature of the measurements, a decrease in activity or ability could be detected almost immediately as a decline in the CO_2 curve. The purpose of these measurements was not to simulate natural flight but to measure an individual's maximum flight metabolic capacity.

Our results show that individuals with a high flight MR are more mobile in the field than individuals with a low flight MR. From an energy budget point of view it

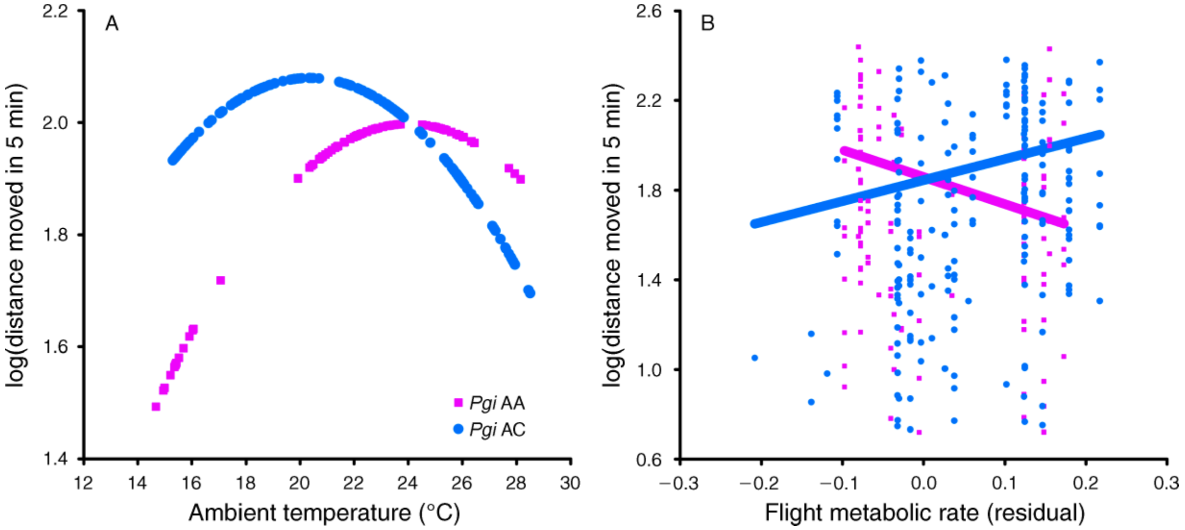

FIG. 3. (A) Distance moved in 5-min intervals for the phosphoglucose isomerase (*Pgi*) gene AA (pink squares) and AC (blue circles) individuals, showing the model-predicted values against measured ambient air temperature (see model 2B in Appendix C). (B) Distance moved in 5-min intervals as predicted by model B in Table 2, showing both the predicted values (lines) and the observed values (squares and circles) against flight metabolic rate (flight MR).

may seem counterintuitive that individuals with the highest energy consumption rates would be moving the longest distances, thus resulting in high total energy usage. One could hypothesize that individuals with the least cost of flight would fly the longest distances. One way to reduce the energy cost of flight would be to fly at a lower speed. This may, however, bear a cost in terms of increased predation risk, supported by the finding that slow-flying tropical butterfly species are typically unpalatable, whereas edible species have high flight speeds (Srygley and Dudley 1993). The ability to move over long distances across unsuitable habitats and to avoid predation requires speed and maneuverability. As flight is the most energy-consuming mode of animal locomotion and as flying insects are thought to have metabolic enzymes working at flux rates close to their theoretical maxima (Suarez 2000), the only plausible way to improve flight ability is to increase energy consumption. High flight ability is expected to yield fitness benefits as activities such as foraging, egg laying, and dispersal all require flight. Besides, flight ability is needed when time is a limiting factor, which is the case with short-lived butterflies. It thus seems that the most successful dispersers among the heavy egg-carrying females are equipped with a fast but energetically expensive "sports car engine" rather than a slow and cheap "city car engine."

The present results suggest that flight MR is a good predictor of mobility and dispersal. So far, wing dimorphism is the best trait predicting dispersal in insects (Zera and Denno 1997), as short-winged or wingless individuals are not able to disperse over long distances. Long-winged individuals have the potential to disperse, yet not all of them do so. In some studies on wing-monomorphic insect species, wing length (Kuussaari et al. 1996) or more subtle wing morphometric measures (Breuker et al. 2007) have correlated with dispersal rate or distance, but other studies have found no effect of wing length on flight speed during migration (Dudley and Srygley 1994), colonization success (Charrette et al. 2006), or emigration rate (Hanski et al. 2002). One much-used indicator of flight performance is the thorax mass : body mass ratio, which has been found to explain take-off ability (Marden 1987, Berwaerts et al. 2002), flight speed during migration (Dudley and Srygley 1994), and escape flight (Almbro and Kullberg 2008) and which has varied in a predictable manner between core and marginal populations in an expanding butterfly species (Hughes et al. 2003). Thorax size and metabolic rate are likely to correlate as both greatly depend on flight muscle mass. However, flight MR measurements do not require dissecting individuals and represent the direct energy cost to an individual.

Pgi *genotype and dispersal*

The glycolytic enzyme PGI has been studied in a large number of taxa, often as a marker locus (e.g., Altermatt et al. 2007) but also at the functional level (Dahlhoff and Rank 2000, Watt et al. 2003, Wheat et al. 2006). We found a significant interaction between *Pgi* genotype and ambient temperature in explaining the distance flown in 5-min periods. The AC heterozygotes moved longer distances than AA homozygotes in low to moderate ambient temperatures. The average distance flown by heterozygotes peaked at 20.3°C, whereas in the AA homozygotes the mean distance moved was longest at 23.7°C. At the very highest temperatures AA homozygotes moved longer distances than AC heterozygotes. Additionally, there was a significant interaction between time and *Pgi* genotype, as AC individuals moved longer distances than AA individuals in the morning when solar radiation (the main source of body heat) was low.

Consistent with the results on radar-measured mobility, there was a similar *Pgi* genotype × temperature interaction in the measurements of flight MR. Within the range of measurement temperatures, from 30.8° to 35.1°C, AA homozygotes showed a linear increase in flight MR with temperature, whereas AC heterozygotes showed a negative trend. The regression lines crossed at ~33°C, whereas the radar-measured flight distances of the two genotypes were equal at an ambient air temperature of ~24°C. The apparent mismatch is due to the very different temperatures in the two measures. In the case of the metabolic rate, the measurement temperature should agree closely with thoracic temperature, as the source of heat to the butterflies was heated ambient air. In the field experiment, butterflies gained their heat from solar radiation. The thoracic temperature of Glanville fritillaries caught in flight in the field typically exceed the corresponding ambient air temperature by ~10°C (Saastamoinen and Hanski 2008). It is thus highly likely that the same biological reasons caused the observed interactions in both experiments.

The genotype × temperature interactions on flight MR and dispersal rate strongly suggest that AC heterozygotes can reach higher levels of activity than AA homozygotes at low ambient and body temperatures, but they suffer from high temperatures. A similar result has been previously reported for *Colias* butterflies for different measures of activity. Thus female *Colias* with a particular heterozygous PGI genotype are able to fly at lower ambient temperatures than the homozygotes (Watt 1992). In mountain-living willow beetles, allozyme variation at PGI shows a clinal pattern resulting from differences in kinetic properties and thermal stability of the common allozyme alleles (Dahlhoff and Rank 2000). The temperature-sensitive alleles are also associated with a higher expression of heat shock proteins that protect enzymes from stress-induced damage (Dahlhoff and Rank 2000). The PGI allozyme variation has additionally been linked to variation in running speed in the larvae and adults of the willow beetle when exposed to extreme temperatures (Rank et al. 2007), again highlighting the connection between *Pgi* and mobility. Another related result on the Glanville fritillary is due to Saastamoinen and Hanski (2008), who found that AC heterozygotes had higher thoracic temperatures in flight than AA homozygotes at low ambient temperatures in the morning. There are several possible reasons for this result (Saastamoinen and Hanski 2008), but the pattern is consistent: superior performance of certain genotypes at low temperatures.

What could allow the heterozygotes to perform better at low ambient temperatures? Ectothermic butterflies can actively control their body temperature and thereby activity by altering their body position in relation to the sun (Watt 1968, Heinrich 1986). Differences in basking behavior could thus explain variation in activity in the radar experiment. On the other hand, in the flight MR measurements the body temperature was not controlled by the butterfly, but nonetheless at equally low body temperatures heterozygotes had higher flight MR. A likely mechanism for differences in activity in low temperatures is, rather than sole basking behavior, dissimilar temperature dependence in the kinetic performance of the different forms of the PGI enzyme, as suggested by Watt (1977) for *Colias*. Different forms of the PGI enzyme differ in their kinetic properties and heat tolerance: homozygotes have narrow thermal ranges whereas the heterozygous form can function well at low temperatures and it also remains relatively stable at higher temperatures (Watt 1983).

Differences in body temperature due to basking can also contribute to our results, as the ability to move in low temperatures allows faster heating when individuals can reach faster favorable locations and positions for basking. Finally, the rate of cooling of small flying butterflies may be slower in individuals with a high flight MR due to internal heat production.

In addition to the main effects of *Pgi* genotype and flight MR on the distance flown in 5 min, there was a significant interaction between these two factors. The AA homozygotes with high flight MR moved less than homozygotes with low flight MR. This interaction was not significant in the aggregated measure of distance moved in one hour (Fig. 1), though a nonsignificant effect in the same direction was present. Apparently, the effect of the *Pgi* genotype on dispersal is not through the effect on flight MR only, though this effect is also present (Haag et al. 2005), but the movement behaviors of different genotypes differ in some other ways as well. This is comparable to what has previously been found for the two sexes, because only females originating from new populations, in which the frequency of the AC heterozygotes is highest (Haag et al. 2005, Hanski and Saccheri 2006), are particularly dispersive. Both AC males and females may have equal flight capacities, but this may be exhibited in males as territorial behavior rather than long-distance dispersal (K. Niitepõld, A. Mattila, P. J. Harrison, and I. Hanski, *unpublished data*).

The *Pgi* allelic composition varies in a predictable manner among natural populations of the Glanville fritillary in relation to their age and isolation from other populations in the large metapopulation in the Åland Islands (Haag et al. 2005, Hanski and Saccheri 2006). The present results support the hypothesis that variation among the populations is due to dissimilar dispersal capacities of different *Pgi* genotypes. From the ecological point of view, it is particularly noteworthy that the genotypic differences become amplified at low ambient temperatures (see also Saastamoinen and Hanski 2008). This is relevant because the weather conditions in northern temperate regions are often marginal for butterfly activity, and hence much of the dispersal must occur under challenging environmental conditions. In such situations, the ability to fly and disperse in low ambient temperatures can be very advantageous. Other fitness advantages of flight capacity under suboptimal conditions include high rate of mating (Watt et al. 1985) and, in females, the capacity to produce large eggs clutches (Watt 1992, Saastamoinen and Hanski 2008). Therefore, even a modest genotype × environment interaction can lead to major fitness consequences.

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APPENDIX A

Respirometry details (*Ecological Archives* E090-155-A1).

APPENDIX B

Statistical analyses: random factors in mixed models (*Ecological Archives* E090-155-A2).

APPENDIX C

Factors explaining mobility (*Ecological Archives* E090-155-A3).

APPENDIX D

The *Pgi* genotype frequencies with respect to the population of origin (*Ecological Archives* E090-155-A4).

APPENDIX E

Study area with flight tracks (*Ecological Archives* E090-155-A5).

APPENDIX F

Photographs of study area and typical habitat (*Ecological Archives* E090-155-A6).