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Environmentally induced dispersal-related life-history syndrome in the tropical butterfly, *Bicyclus anynana*

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Abstract

Dispersal is a key process for understanding the persistence of populations as well as the capacity of organisms to respond to environmental change. Therefore, understanding factors that may facilitate or constrain the evolution of dispersal is of crucial interest. Assessments of phenotypic variation in various behavioural, physiological and morphological traits related to insect dispersal and flight performance are common, yet very little is known about the genetic associations among these traits. We have used experiments on the butterfly *Bicyclus anynana* to estimate genetic variation and covariation in seven behavioural, physiological and morphological traits related to flight potential and hence dispersal. Our goal was to characterize the heritabilities and genetic correlations among these traits and thus to understand more about the evolution of dispersal-related life-history syndromes in butterflies. Using a version of the animal model, we showed that all of the traits varied between the sexes, and most were either positively or negatively (phenotypically and/or genetically) correlated with body size. Heritable variation was present in most traits, with the highest heritabilities estimated for body mass and thorax ratio. The variance in flight activity among multiple measurements for the same individual was high even after controlling for the prevailing environmental conditions, indicating the importance of behavioural switching and/or inherent randomness associated with this type of movement. A number of dispersal-related traits showed phenotypic correlations among one another, but only a few of these were associated with significant genetic correlations indicating that covariances between these traits in *Bicyclus anynana* are mainly environmentally induced.

Introduction

Dispersal is one of the key life-history traits that allows species and populations to occupy more of the available habitat and facilitates individuals in coping with both spatial and temporal heterogeneity. Due to its crucial role in the conservation of species experiencing habitat fragmentation, habitat loss and climate change, dispersal has gained much attention during the last decade (reviewed in Stevens *et al.*, 2010, 2011; Bonte *et al.*, 2012). In insects, dispersal propensity is relatively easy to assess in wing-dimorphic species, as in most cases only dispersive morphs produce wings and wing muscles (e.g. Roff & Fairbairn, 1991, 2007a). In wing-monomorphic insects, in which dispersal is a continuously varying trait, quantifying dispersal propensity is more difficult, and therefore, a number of proxies, such as flight endurance, thorax ratio and wing shape, are commonly used. These proxies, however, potentially measure different components of flight or mobility, some of which may or may not have shared functions. Even though the link between dispersal proxies and dispersal in the field is rarely demonstrated, examples do exist where dispersal-related proxies vary between dispersers and nondispersers (e.g. Hill *et al.*, 1999;

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Thomas *et al.*, 2001; Niitepõld *et al.*, 2009; Stevens *et al.*, 2010), highlighting their potential in studies of dispersal evolution.

Dispersal is often related to other behavioural, physiological and life-history traits (reviewed in Stevens *et al.*, 2011), and these covariations have been used to define the so-called dispersal syndromes. For example, the 'flight-oogenesis syndrome' is well documented in insects; here, dispersive individuals allocate resources into flight muscles with a cost of reduced reproduction and/or lifespan (e.g. in the speckled wood butterfly, Hughes *et al.*, 2003; in the sand cricket, Roff *et al.*, 1999). A contrasting syndrome is that of a 'colonizer syndrome', in which more dispersive individuals reproduce early, have high fecundity, and/or lifespan (e.g. in the Glanville fritillary butterfly; Saastamoinen, 2007; Saastamoinen *et al.*, 2009). Reasons for studying dispersal syndromes are manifold, including the links between dispersal syndromes and fitness consequences, the effective level of gene flow, colonization patterns and rates of spread (Ronce & Clobert, in press).

From an evolutionary perspective, knowledge about the genetic variation and covariation in different traits contributing to the phenotype is of fundamental importance. First, traits must be heritable for natural selection to result in evolutionary change. Second, genetic covariance among traits may either facilitate or constrain any evolutionary change (Futuyma, 2010; Kirkpatrick, 2010; Duputié *et al.*, 2012). Heritabilities of dispersal proxies have been estimated in both wing-dimorphic (e.g. Stirling *et al.*, 1999) and wing-monomorphic insects (e.g. Berwaerts *et al.*, 2008), including behavioural and physiological traits such as within-patch mobility (Saastamoinen, 2008), flight endurance (Gu & Danthanarayana, 1992) and take-off flight performance (Berwaerts *et al.*, 2008). However, estimates of genetic covariance among dispersal-related traits (between dispersal proxies and/or between dispersal proxies and other life-history traits; Stevens *et al.*, 2011) have been obtained mainly for wing-dimorphic insects such as the sand cricket (*Gryllus firmus*) (Zera, 2006; King *et al.*, 2011; Roff & Fairbairn, 2011), very little being known for wing-monomorphic insects.

Estimations of genetic variances and covariances for dispersal-related traits are hindered in part because many dispersal proxies are difficult to measure in an accurate and repeatable manner (e.g. Kruuk & Hadfield, 2007). Flight activity in most insects is highly dependent on prevailing environmental factors including population density (e.g. Albrectsen & Nachman, 2001), resource availability (biotic; Saastamoinen *et al.*, 2010) and ambient temperature (abiotic; e.g. Bonte *et al.*, 2008; Niitepõld *et al.*, 2009). These studies also highlight that dispersal may not be as fixed as has been assumed in most studies but may instead show high levels of phenotypic plasticity as is typical of life-history traits (e.g. Stjenholm *et al.*, 2005; Saastamoinen *et al.*, 2009, 2010). It is also noteworthy that the amount of genetic variation can depend on environmental conditions (G × E interaction), and hence, estimates of genetic variances and co-variances are context dependent (Hoffmann & Parsons, 1991).

Here, we used two laboratory-reared generations of the tropical butterfly *Bicyclus anynana* to assess the genotypic and phenotypic components of sex-specific flight activity under experimental conditions. We also measured commonly used proxies of dispersal, namely those related to wing shape (wing loading and aspect ratio) known to influence the aerodynamic aspects of flight (speed and manoeuvrability; Dudley, 2000) and to resource allocation to the thorax (i.e. thorax ratio), which has been used as a proxy of individual's dispersal capacity. A larger thorax can increase an individual's flight endurance and acceleration capacity (e.g. Dudley, 2000; Berwaerts & Van Dyck, 2004), the former relationship being shown also for *B. anynana* (Saastamoinen *et al.*, 2010). Finally, we measured three additional traits potentially correlated with dispersal, namely body size, resting metabolic rate (RMR) and fat percentage. RMR and fat percentage could potentially relate to flight, as maintenance costs (i.e. basal metabolic rate) of aerobically active tissues, such as the thorax, have been suggested (Steyermark *et al.*, 2005). Fat, on the other hand, may be used to fuel flight (Dudley & Srygley, 2008). A priori expectations of the correlations are difficult to make due to inconsistent results on other Lepidoptera (Stevens *et al.*, 2010, 2011).

We estimated the heritabilities for the above-mentioned traits and examined the correlations among them, both at the phenotypic and genotypic levels. To do so, we used an animal model that accounted for differences caused by sex, age and generation, as well as variation in prevailing environmental conditions and in the measurement used for flight activity. We show evidence for a dispersal-related life-history syndrome in *B. anynana*, which, in this study, is mainly environmentally rather than genetically induced.

Materials and methods

Insect rearing

Bicyclus anynana occurs from South Africa to Ethiopia, and adults feed on fallen fruit. The butterfly shows seasonal polyphenism in both life history and morphology, as the wet-season forms are smaller, reproduce faster and have shorter lifespan compared with the dry-season form (e.g. Brakefield & Reitsma, 1991; Pijpe *et al.*, 2007; Brakefield & Kesbeke, 1997). Although little is known about dispersal of *B. anynana* in the field, variation in flight ability (Saastamoinen *et al.*, 2010) and dispersal-related wing morphology (Frankino *et al.*, 2005) has been observed. Butterflies of the wet-season form are more active than those of the dry

season in the field (Brakefield & Reitsma, 1991); butterflies spend much of the cool dry season in a rather quiescent state before reproducing with the next rains (see; Brakefield & Larsen, 1984). The individuals used in the present experiment originated from a laboratory colony at the University of Bayreuth, Germany. The original laboratory population was founded more than 100 generations ago from about 80 gravid females collected in Malawi (Van't Hof *et al.*, 2005).

For both generations, 400–500 larvae were reared in mesh-covered sleeves (12 × 25 × 50 cm), with no more than 40 individuals per sleeve to avoid crowding effects. The larvae in the first generations originated from eggs collected from the wild-type population on a single day. The wild-type laboratory population consists of several hundred butterflies, and therefore, the eggs used here came from multiple families. All larvae were reared in a climate room under wet-season conditions (27 °C, photoperiod of L : D 12 : 12 h, RH 70%) on fresh maize (*Zea mayz*) *ad libitum*. On the day after eclosion, individuals were individually marked on their ventral hind wing. Males and females were then kept separately in cylindrical hanging cages (Ø 25 cm, height 60 cm). Adults were kept in the climate rooms at 27 °C (photoperiod of L : D 12 : 12 h, RH 70%) with moist banana *ad libitum* during their entire lifetime. At the age of ca. 4 days, the butterflies were assessed for flight activity and resting metabolic rate. Following these measurements, the butterflies were mated, and females were allowed to oviposit. Butterflies were killed 3 days after the assessment of their flight activity (regardless of whether the butterfly had mated) and preserved at −25 °C for subsequent morphological measurements.

Butterflies were mated in cylindrical hanging cages (Ø 25 cm, height 60 cm) between 3 pm and 6 pm within 3 days of assessment of their flight activity. All matings were recorded, and the mating pairs were removed from the cage to prevent multiple pairing. In the first generation, mated females were then placed individually in plastic pots (6 cm of diameter) with a cutting of a natural host plant (*Oplismenus africanus*) for oviposition. Newly hatched larvae of each family were transferred to a net sleeve. In the second generation, mated females were placed in hanging cages with continuous access to host plants for oviposition. Although we did not raise the offspring of the second generation, we allowed the butterflies to mate and to oviposit to ensure that the butterflies were in a similar reproductive state during the assessments of the morphological traits.

Traits measured

Flight activity

Flight activity of individuals was assessed in three identical and parallel outdoor poly-tunnels (polyethylene-covered commercial greenhouses; L × W × H = 20 m × 6 m × 3 m) within 2-week periods in May and July 2008 for the first and second generations, respectively. The interior of the poly-tunnel was bare, and the ground was covered with woodchips mimicking dry leaves. The average ambient temperature inside the poly-tunnels during the assessments was 27.2 °C (ranging from 24.0 to 33.3 °C). Flight activity was assessed only if the ambient temperature reached 24 °C, as females in particular were unlikely to fly at lower temperatures. One end of the polytunnels was situated nearby some trees making few metres of these ends more shaded compared with the other end of the tunnel, which was sunnier. Prereproductive butterflies (average age 5 days ± 1.9 (SD)) were placed on the ground at the shady end of each tunnel at 11 am, and the time of arrival to the other end of the tunnel was recorded, after which butterflies were captured and kept in shady conditions. At the end of the trial (3 h), those butterflies that had not moved through the tunnel were captured, and their location was noted down to measure how far (metres from the release point) the butterflies had moved. As a main flight-activity measure, we used the speed of the observed displacement (m/h). As alternative measures, we used the displacement distance during the three-hour trial (i.e. 20 m for all those individuals who reached the goal end of the tunnel) and a binary variable (0/1) indicating whether the individual reached the goal end during the experiment. We note that all these measures are influenced by chance events in the butterflies' behaviour, so that, for example, a highly mobile individual may have obtained a low score if it turned back and forth and ended close to the release end of the tunnel. As the butterflies were not tracked continuously during the trials, information, for example, about the length of their track was unfortunately not available. However, most butterflies that flew actively were attracted to the sunnier end of the tunnel and hence reached the goal end of the tunnel (M. Saastamoinen, personal observation). To increase the accuracy of the butterfly-specific flight-activity estimates and to quantify the measurement variance, each individual was measured twice, weather permitting, on consecutive days.

During each trial, 42 ± 4 individuals (sexes separately) were tested together in each tunnel. Two trials were omitted and repeated the next day, as <30% of butterflies reached the goal end of the tunnel, and thus, the environmental conditions were considered suboptimal. In total, 405 (178 females and 227 males) and 557 individuals (276 females and 281 males) were tested in the first and second generation, respectively. Some of the butterflies were lost or killed by spiders during the assessment, and hence, of all butterflies, two measurements of flight activity were successfully obtained for 72% (144 females and 147 males) and 71% (197 females and 200 males) in the first and second generations, respectively.

Body mass
Adults were weighed (Sartorius AC 210P with accuracy 0.1 mg) 1 day after their eclosion.

Thorax ratio
Dry weights of thorax and abdomen (after 24 h at + 60 °C; Sartorius RC 210D) were measured to the nearest 0.01 mg. Allocation to flight muscles was measured by thorax ratio, defined as dry thorax mass/(dry thorax mass + dry abdomen mass).

Fat percentage
After assessing the dry mass, thoraces and abdomens were submerged in ethyl-acetate for 4 days, dried at 60 °C for 24 h and weighed again (Zwaan *et al.*, 2001). The absolute fat content was defined as the difference between initial dry weight and weight after fat extraction. Fat percentage was calculated as a ratio of the absolute fat content to the total dry mass.

Wing loading and aspect ratio
Following freezing down, forewings of 300 and 409 butterflies in the first and second generations, respectively, were photographed (Leica DC200; 10 × 1.25 magnification) to assess wing loading (adult weight/forewing area) and aspect ratio (wing span^2/forewing area). Wing span and the area of ventral right forewing (or left one if the right one was too worn to prevent accurate measurement) were analysed with Image-Pro 6.2 software (Media Cybernetics Inc., Rockville, MD, USA).

Resting metabolic rate
Resting metabolic rate (RMR) was measured with a Li-Cor LI-6251 CO_2 analyzer in a Sable systems respirometer set-up with a push-through flow of 100 ml min^{-1} for 156 females and 113 males in the second generation only. Individuals were measured in the morning a day after their second flight-activity assessment (i.e. before they were mated). RMRs of the individual butterflies were measured in small cylindrical class containers, which were kept in a temperature-controlled climate chamber (27 °C). Butterflies were measured in the dark, so they would stay inactive, and data of two repeated measurements were used (Pijpe *et al.*, 2007) and then analysed using Expedata software (Sable systems, Las Vegas, NV, USA).

Data analysis

Phenotypic correlations
Sex-specific Pearson correlation coefficients were computed for uncorrected phenotypic traits pooled over both generations as well as for phenotypic traits corrected for differences between generations. For the flight activity, we used the average of the two measurements (log-transformed values for the speed of displacement). The speed of the displacement correlated very highly with the two other measures of flight activity (Table 1). As we considered the speed of the displacement to combine information about both the time and the distance moved with the polytunnel, we mainly focused on this trait, and the genetic analyses were solely performed with this measure.

Table 1 Phenotypic correlation among the three measures of fight activity obtained from the polytunnel separately for females and males (above and below diagonal, respectively). The flight-activity measures used are the average values from the two separate runs.

	Speed of displacement (m/h)†	Distance travelled in 3 h	Success in reaching the goal end
Speed of displacement (m/h)†	–	0.85 ***	0.88***
Distance travelled in 3 h	0.81***	–	0.83***
Success in reaching the goal end	0.82***	0.84***	–

†Measure used in the analyses.
***$P < 0.0001$.

Genetic variances and covariances
The data were analysed using a version of the animal model (Lynch & Walsh, 1998) to decompose the variation in the measured trait values into fixed effects, additive genetic effects and environmental effects. For flight activity, we additionally included a random effect modelling variation in experimental conditions under which this trait was measured. For flight activity, we further included a random effect modelling inherent higher level of variation associated with the measurement of this trait: if the same individual would be measured twice under identical conditions, the measured values would not be expected to be identical due to variation in the internal state of the individual and chance events associated, for example, with turning back before the goal end of the tunnel. All data for both generations and all traits were analysed in a single multivariate model. We treated female and male flight activity as separate and a priori independent traits. Thus, all individuals (males and females) were assumed to have an additive genetic value both for the female flight activity and male activity traits, although only one of the two trait values was expressed (the one corresponding to the sex of the focal individual) and thus could be measured for each individual. Traits with positive values (i.e. female and male flight activity, body mass, wing loading, aspect ratio and resting metabolic rate) were log transformed, and those between zero and one (i.e. thorax ratio and fat percentage) were logit transformed to allow the use of a multivariate normal model.

We denote by $\mathbf{y}$ the vector containing all the data, that is, the measurements of all traits for all individuals,

including repeated measurements for flight activity. In addition to a measure of female flight activity being missing for all males and *vice versa*, also some other trait values were missing for some of the individuals. Thus, the number of measurements in **y** varies among the individuals. We modelled **y** using the multivariate normal animal model, $\boldsymbol{y}\sim\mathrm{MN}(\boldsymbol{\mu}, \mathbf{V})$, where the vector $\boldsymbol{\mu}$ includes the fixed effects and the variance–covariance matrix **V** includes the random effects.

We denote by $I(\mathrm{i})$ the individual ($I(\mathrm{i}) = 1,\ldots,n$) and by $T(i)$ the trait ($T(i) = 1,\ldots,8$) for measurement i. We write the animal model as

$$y_i = \mu_i + a_i + e_i + r_i + m_i,$$

where a_i and e_i denote the random effects for additive genetic and environmental effects, respectively. The components r_i and m_i were included for flight activity only, and they model variation in experimental conditions and inherent variation associated with this trait. These four variance components are assumed to be independent of each other. The additive and environmental effects are assumed to follow the covariance structures

$$\mathrm{Cov}(a_i, a_j) = 2\theta_{I(i)I(j)}G_{T(i)T(j)},$$

$$\mathrm{Cov}(e_i, e_j) = \delta_{I(i)I(j)}E_{T(i)T(j)}$$

where **G** and **E** denote the 8 × 8 variance–covariance matrices to be estimated. The coancestry coefficient $\theta_{I(i)I(j)}$ between individuals $I(i)$ and $I(j)$ is determined through the breeding design including parents and their offspring, full-sibs, and nonrelated individuals, and $\delta_{I(i)I(\mathrm{j})}$ is Kronecker's delta which is one if $I(i)$ and $I(j)$ are the same individual and otherwise zero. As male and female flight activity was never measured for the same individual, the covariance between these traits in **E** is immaterial and does not affect the likelihood of the data. This is not the case for **G**, as the data allow one to assess, for example, the correlation between flight activity of a male and its female offspring.

For the experimental effects and the measurement errors associated with flight activity, we assume the covariance structures

$$\mathrm{Cov}(r_i, r_j) = \delta_{Z(i)Z(j)}R_{T(i)T(j)},$$

$$\mathrm{Cov}(m_i, m_j) = \delta_{ij}M_{T(i)T(j)},$$

Here, **R** and **M** are 2 × 2 variance–covariance matrices, and the Kronecker's delta $\delta_{z(i)z(\mathrm{j})}$ indicates whether the two measurements of flight activity were made in the same experiment Z or in different experiments. We note that the off-diagonal elements of the matrices **R** and **M** do not influence the likelihood of the data and are thus irrelevant. Regarding the matrix **R**, this is the case because flight-activity experiments were made for the two sexes separately.

Heritability for each trait was measured as the ratio $h^2 = \frac{G_{ii}}{G_{ii}+E_{ii}}$. As for female and male flight activities, the measure h^2 excludes variance due to experimental and measurement errors, and it measures the heritability of inherent flight activity under standardized conditions and based on average performance of the individual. To assess the roles of the experimental and measurement variances, we also computed the ratio $\lambda^2 = \frac{G_{ii}}{G_{ii}+E_{ii}+R_{ii}+M_{ii}}$, which measures how repeatable flight-activity measurements are due to genetic additive effects.

The fixed effects were modelled as a function of the following covariates: generation (except for RMR, which was assessed only in the second generation), sex (except for flight activity which was treated as a separate trait for males and females), the age of the individual at the time of the respective measurement, measured as number of days after eclosion (included only for those traits with variation among the individuals in the time of the measurement, that is, flight activity, thorax ratio, fat percentage and resting metabolic rate) and an indicator variable describing whether the individual had mated (included only for those traits with variation among the individuals in their mating status, that is, thorax ratio and fat percentage). Thus, the covariates included varied between the traits as follows. For flight activity (female or male), we included generation and age. For wing loading, aspect ratio and body mass, we included generation and sex. For thorax ratio and fat percentage, we included generation, sex, age, sex*age, mated and sex*mated. For resting metabolic rate, we included sex, age and sex*age.

We fitted the model to the data using the Bayesian approach that has become increasingly popular in quantitative genetics largely due to its flexibility in fitting hierarchical models (Sorensen & Gianola, 2002; Beaumont & Rannala, 2004; O'Hara *et al.*, 2008). The parameters of the model are the variance–covariance matrices, **G**, **E**, **R** and **M**, and the vector of regression coefficients $\boldsymbol{\beta}$ associated with the overall mean and the fixed effects. For the variance–covariance matrices, we assumed a Wishart prior with expectation set to the identity matrix and degrees of freedom set to one plus the dimension of the matrix. For the regression coefficients, we assumed a flat prior in the real scale. We applied a Monte Carlo Markov Chain (MCMC) scheme in which we first updated $\boldsymbol{\beta}$ by sampling it directly from its full conditional distribution and then updated each of the variance–covariance matrices using the Metropolis–Hastings algorithm (Gelman *et al.*, 2004) with a Wishart proposal that was centred at the current value and degrees of freedom adjusted manually to lead to a acceptance ratio in the range (0.3, 0.5). We run the MCMC for 200 000 iteration rounds and used a thinned sample of size 1000 in the analyses. The algorithm was programmed with Mathematica 6.0 (Wolfram Research, Inc., 2007,

Champaign, IL, USA), the source code being found in the Supporting Information (Appendix S1).

Some individuals did not move at all during the flight assessment trials, and to assess whether this biased our results for flight activity, we additionally estimated the variances and covariances with a model in which these cases were omitted.

Results

Table 2 summarizes average values for the different traits measured, and Table 3 shows the effects of the fixed factors. The sexes differed in all of the traits measured. Thus, males had a higher flight activity in the polytunnels than females, in which a relatively high proportion failed to move across the tunnel during one of the two flight trials (Fig. 1). The results related to the flight activity were nevertheless qualitatively same in the model where the cases in which an individual did not move during the flight trial were omitted (Appendix S2 in Table 1). Males were also smaller, allocated more resources to their thorax (i.e. higher thorax ratio) and had a higher fat percentage than females. In addition, males had a higher aspect ratio, lower wing loading and lower resting metabolic rate than females. There were also differences between the two generations, as individuals from the first generation had higher wing loading and fat percentage than individuals from the second generation. Differences between the generations may be explained in part by the experimental set-up (see Discussion). Additionally, older individuals had a lower fat percentage and a higher thorax ratio than younger individuals. Mated females had a higher fat percentage and higher thorax ratio than unmated females (Table 3).

Heritability estimates

The estimated levels of heritability for the traits are shown in Fig. 2. Body mass exhibited the highest heritability estimate ($h^2 = 0.54$), and thorax ratio and wing loading had moderate heritabilities, whereas the heritability estimates for aspect ratio, fat percentage and RMR were rather low. The data were not informative (posteriors very similar to the priors) on the heritabilities for female or male flight activity (heritability estimates based on only those cases where individuals moved during the flight trial are provided in Appendix S2 in Table 2). This is because the repeatability of the flight ability measurement was low, two assessments of flight activity for the same individual showing a significantly positive but low correlation (Pearson correlation coefficient = 0.10, $P < 0.01$). In other words, the amount of inherent variation in measuring flight activity was high (Table 4) for both sexes, and furthermore, for males, the environmental conditions within the poly-tunnel explained a high proportion of the total phenotypic variance (Table 4).

Phenotypic correlations

Table 5 summarizes the phenotypic correlations for the different traits separately for males and females. Generally, heavier individuals had higher wing loading and a smaller thorax ratio. Similarly, in both sexes, individuals with higher allocation to thorax (i.e. higher thorax ratio) had a lower resting metabolic rate and lower fat percentage. In males, flight activity correlated positively with aspect ratio, wing loading and body mass. However, the latter two only correlated with flight activity in the first generation. Several other sex-specific phenotypic correlations were also observed, and some of the correlations were stronger in one generation than in the other one (Table 5). Phenotypic correlation among the average flight-activity measures obtained from the poly-tunnel (i.e. speed of displacement, distance moved and the success of reaching the goal end of the tunnel), and the six life-history traits separately for females and males are provided in the Appendix S3 (Table 1).

Table 2 Average values (± SE) for the dispersal-related traits given separately for males and females and for the two generations. Sample sizes are presented in parenthesis. Values are based on the raw data (not corrected for other fixed effects than sex and generation).

	1st generation		2nd generation	
Variable	Females	Males	Females	Males
Adult weight (mg)	70.8 ± 0.8 (*n* = 178)	48.8 ± 0.5 (*n* = 227)	73.8 ± 0.6 (*n* = 276)	45.2 ± 0.3 (*n* = 281)
Flight activity (m/h)	46.5 ± 5.9 (*n* = 144)	65.0 ± 7.1 (*n* = 147)	63.5 ± 10.5 (*n* = 197)	94.9 ± 13.9 (*n* = 200)
Fat percentage (%)	24.9 ± 0.7 (*n* = 161)	31.5 ± 0.7 (*n* = 171)	19.7 ± 0.4 (*n* = 220)	29.9 ± 0.6 (*n* = 216)
Fat absolute (mg)	5.3 ± 0.2 (*n* = 161)	3.1 ± 0.1 (*n* = 171)	5.1 ± 0.2 (*n* = 220)	3.2 ± 0.1 (*n* = 217)
Thorax ratio	0.29 ± 0.006 (*n* = 162)	0.48 ± 0.007 (*n* = 172)	0.27 ± 0.003 (*n* = 221)	0.49 ± 0.005 (*n* = 218)
Thorax weight (mg; absolute)	5.7 ± 0.08 (*n* = 162)	4.3 ± 0.06 (*n* = 179)	6.7 ± 0.05 (*n* = 221)	4.8 ± 0.05 (*n* = 219)
Wing loading	0.35 ± 0.002 (*n* = 147)	0.31 ± 0.003 (*n* = 153)	0.33 ± 0.002 (*n* = 209)	0.27 ± 0.002 (*n* = 200)
Aspect ratio	1.94 ± 0.006 (*n* = 147)	2.01 ± 0.007 (*n* = 153)	1.94 ± 0.006 (*n* = 209)	2.04 ± 0.006 (*n* = 199)
Resting metabolic rate	–	–	0.06 ± 0.001 (*n* = 156)	0.05 ± 0.001 (*n* = 113)

Table 3 Estimated effect of the covariates and fixed factors on dispersal-related traits. The explanatory variables for which a positive (or negative) effect was detected with at least 95% posterior probability are shown in bold. Sex codes the effect of the individual being a male (female normalized to zero), and generation codes the effect of belonging to the second generation (first generation normalized to zero).

Trait	Median	2.5% Quantile	97.5% Quantile
Male flight activity			
Generation	−0.39	−1.36	0.66
Age	0.32	−0.64	1.17
Female flight activity			
Generation	−0.72	−1.37	0.03
Age	0.12	−0.57	0.87
Thorax ratio			
Generation	0.03	−0.03	0.09
Sex	**1.01**	**0.95**	**1.06**
Age	**0.35**	**0.25**	**0.46**
Sex*age	−0.13	−0.27	0.02
Mated	**0.10**	**0.03**	**0.17**
Sex*mated	**−0.21**	**−0.31**	**−0.12**
Wing loading			
Generation	**−0.09**	**−0.10**	**−0.07**
Sex	**−0.18**	**−0.19**	**−0.17**
Aspect ratio			
Generation	0.01	0.00	0.01
Sex	**0.04**	**0.04**	**0.05**
Resting metabolic rate			
Sex	**−0.26**	**−0.34**	**−0.18**
Age	−0.05	−0.18	0.08
Sex*age	−0.11	−0.28	0.10
Fat percentage			
Generation	**−0.26**	**−0.34**	**−0.19**
Sex	**0.43**	**0.36**	**0.51**
Age	**−0.18**	**−0.34**	**−0.03**
Sex*age	0.09	−0.12	0.30
Mated	**0.10**	**0.00**	**0.19**
Sex*mated	0.09	−0.05	0.22
Body mass			
Generation	0.00	−0.02	0.02
Sex	**−0.45**	**−0.47**	**−0.43**

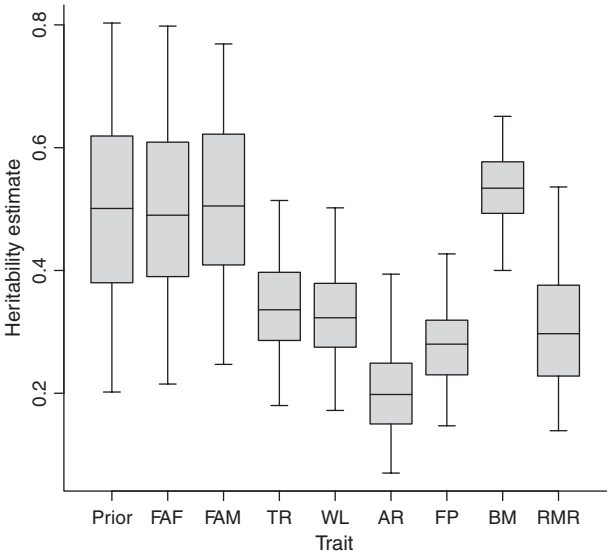

Fig. 2 Estimated heritabilities for flight activity in females (FAF) and in males (FAM), thorax ratio (TR), wing loading (WL), aspect ratio (AR), fat percentage (FP), body mass (BM) and resting metabolic rate (RMR). The lines show the median estimates, the boxes the 50% quartiles and the error bars the 95% quantiles. The heritability estimate induced by the prior distribution (common for all traits) is shown for comparison.

Genetic and environmental correlations

Genetic and environmental correlations among the traits are summarized in Table 6. Strong evidence for genetic correlations was found only for two pairs of traits: body mass and wing loading showed a positive genetic correlation, whereas thorax ratio showed a negative genetic correlation with fat percentage. Additionally, there was weaker evidence for a negative genetic correlation between thorax ratio and body mass. Body mass and wing loading were positively correlated, and thorax ratio and fat percentage were negatively correlated also for the environmental effects. A number of other trait pairs showed environmental correlations: aspect ratio was positively correlated with male flight activity, resting metabolic rate was positively correlated with body mass, fat percentage and wing loading, and resting metabolic rate was negatively correlated with thorax ratio (Table 6, see also Appendix S2 in Table 3).

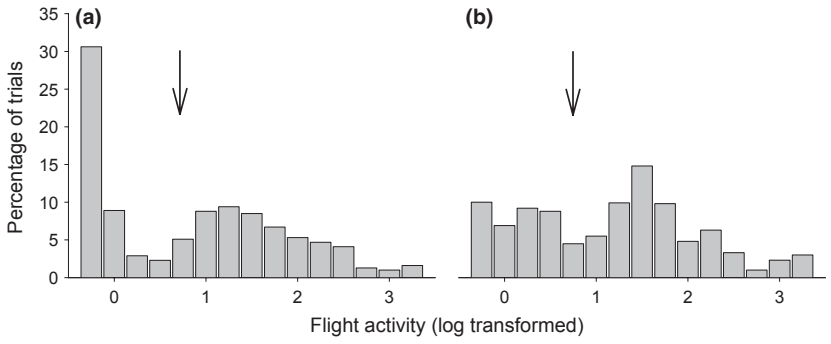

Fig. 1 Frequency distribution of the log-transformed flight activity trials in (a) females and (b) males. The arrows separate those individuals that reached the goal end of the polytunnel at the end of the trial from those individuals that did not.

Table 4 Relative variances in flight activity due to additive genetic, environmental and experimental effects and due to inherent variation associated with measuring this trait. Values represent the 2.5%, 50% and 97.5% quantiles of the posterior distribution.

	Females			Males		
	2.5%	50%	97.5%	2.5%	50%	97.5%
Additive genetic	0.04	0.09	0.15	0.02	0.05	0.09
Environmental	0.03	0.09	0.17	0.02	0.04	0.08
Experimental	0.03	0.07	0.14	0.17	0.27	0.41
Inherent variation	0.66	0.76	0.83	0.51	0.63	0.73

Discussion

Studies on many organisms have shown that dispersive individuals can differ from nondispersive individuals in a number of dispersal-related proxies but also in many behavioural, physiological and life-history traits. Such covariation among traits defines the so-called dispersal syndromes (Ronce & Clobert, in press). Although any assessment of the evolution of dispersal inevitably requires an understanding of how dispersal-related traits covary, very few studies have actually estimated whether such traits are genetically or environmentally correlated. This study aimed to fill this gap by asking to what extent dispersal syndromes (covariance with dispersal proxies and the other life-history traits) or covariances between the dispersal-related proxies in a species of tropical butterfly are genetically or environmentally induced.

Many of the traits showed phenotypic correlations among one another (Table 5 and Appendix S3 in Table 1), but the quantitative genetic analyses indicated that in most cases, this was likely due to an environmental correlation, strong statistical evidence for genetic correlation being found only for two trait pairs. A positive genetic (as well as environmental) correlation was found between body mass and wing loading, implying that larger individuals may exhibit increased flight speed compared with smaller individuals (Dudley, 2000). This is consistent with a highly positive genetic correlation found in the codling moth (*Cydia pomonella*) for body size and flight distance (Schumacher *et al.*, 1997) and in the British Papilionoidea, where migratory butterflies are larger than nonmigratory ones (Roff, 1991). In contrast, a negative genetic (as well as environmental) correlation was found between thorax ratio and fat percentage presumably indicating the commonly assumed allocation trade-off between flight and fecundity and/or body maintenance (e.g. Zera & Harshman, 2001; Roff & Fairbarn, 2007b). Fat reserves are used throughout the adult life for maintenance costs as well as egg production in females and nutritional donations at mating in males (Chown & Nicolson, 2004). Alternatively, the negative correlation between thorax

Table 5 Phenotypic correlations among the dispersal-related traits given separately for females and males (above and below diagonal, respectively). Values without brackets are based on the raw data, and those within brackets are corrected for generation differences.

	Flight activity	Thorax ratio	Wing loading	Aspect ratio	Resting metabolic rate	Fat percentage	Body mass
Flight activity	–	0.094 (0.067)	0.081 (0.014)	0.029 (0.114)*	−0.046 (–)	−0.001 (−0.083)	−0.054 (−0.070)
Thorax ratio	−0.044 (−0.036)	–	0.071 (0.056)	0.043 (0.045)	−0.282*** (–)	−0.269*** (−0.336)***	−0.115* −0.086
Wing loading	0.137* (0.014)	−0.045 (0.017)	–	0.064 (0.063)	0.111 (–)	−0.184** (−0.290)***	0.731*** (0.802)***
Aspect ratio	0.181** (0.108*)	−0.043 (−0.057)	0.012 (0.141*)	–	0.001 (–)	0.015 (0.019)	−0.098 (−0.100)
Resting metabolic rate	0.162 (–)	−0.187 (–)	0.282** (–)	0.144 (–)	–	0.275** (–)	0.166 (–)
Fat percentage	0.050 (0.071)	−0.779*** (−0.779***)	−0.009 (−0.065)	−0.024 (0.045)	0.150 (–)	–	−0.270*** (−0.242)***
Body mass	0.173** (0.065)	−0.144** (−0.089)	0.738*** (0.750***)	−0.070 (−0.045)	0.317** (–)	0.206*** (0.113*)	–

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Table 6 Median estimates of genetic and environmental correlations (above and below diagonal, respectively) among the dispersal-related traits (2.5% and 97.5% quantiles are presented in parenthesis). The cases where the correlation is positive (or negative) for the entire 95% interquantile range are shown in bold, whereas for the grey entries, this is the case for the interquartile range (from 25% quantile to 75% quantile).

	Male Flight activity	Female Flight activity	Thorax ratio	Wing loading	Aspect ratio	Resting metabolic rate	Fat percentage	Body mass
Male Flight activity	–	−0.011 (−0.57; 0.56)	0.076 (−0.50; 0.52)	0.032 (−0.45; 0.60)	0.245 (−0.42; 0.79)	−0.128 (−0.65; 0.47)	−0.161 (−0.62; 0.44)	0.146 (−0.30; 0.63)
Female Flight activity	−0.087 (−0.55; 0.55)	–	−0.020 (−0.51; 0.52)	0.082 (−0.54; 0.57)	0.099 (−0.61; 0.68)	0.074 (−0.58; 0.62)	0.007 (−0.47; 0.50)	−0.016 (−0.48; 0.44)
Thorax ratio	0.011 (−0.55; 0.33)	0.058 (−0.30; 0.50)	–	−0.103 (−0.51; 0.31)	0.360 (−0.16; 0.72)	−0.054 (−0.61; 0.48)	**−0.651 (−0.85; −0.33)**	**−0.274 (−0.56; 0.01)**
Wing loading	0.100 (−0.31; 0.57)	0.036 (−0.36; 0.46)	0.025 (−0.14; 0.19)	–	0.007 (−0.48; 0.56)	0.218 (−0.40; 0.71)	−0.162 (−0.55; 0.23)	**0.807 (0.67; 0.91)**
Aspect ratio	**0.518 (0.16; 0.79)**	0.099 (−0.25; 0.49)	**−0.117 (−0.28; 0.03)**	**0.091 (−0.07; 0.25)**	–	0.204 (−0.51: 0.72)	0.081 (−0.41; 0.56)	−0.119 (−0.53; 0.27)
Resting metabolic rate	0.171 (−0.33; 0.58)	−0.077 (−0.55; 0.36)	**−0.367 (−0.59; −0.14)**	**0.213 (0.01; 0.42)**	0.054 (−0.13; 0.25)	–	−0.039 (−0.52; 0.48)	0.213 (−0.35; 0.65)
Fat percentage	0.135 (−0.33; 0.57)	−0.106 (−0.50; 0.25)	**−0.572 (−0.67; −0.46)**	**−0.118 (−0.26; 0.03)**	0.029 (−0.10; 0.16)	**0.420 (0.21; 0.62)**	–	−0.072 (−0.40; 0.26)
Body mass	0.115 (−0.32; 0.51)	−0.087 (−0.59; 0.35)	−0.022 (−0.19; 0.14)	**0.804 (0.73; 0.86)**	−0.087 (−0.25; 0.08)	**0.295 (0.02; 0.54)**	−0.019 (−0.17; 0.14)	–

ratio and fat percentage could result from more fat reserves being used to fuel flight in individuals with higher allocation to thorax, as has been found in some migratory butterflies (i.e. higher potential dispersal capacity; Dudley & Srygley, 2008). On the basis of our results, it seems that environmental conditions play a major role in structuring the covariance structure between the dispersal-related life-history traits in *B. anynana*, so that some environmental conditions 'produce' large individuals in terms of body mass and wing loading, with high fat percentage and increased RMR, whereas other types of environmental conditions produce the opposite types of individuals. A negative environmental correlation between thorax ratio and RMR may be explained by expensive relative maintenance (basal) costs of a large mass of aerobically active tissues (thorax ratio) resulting in lower RMR in those individuals with a relatively heavy thorax (Steyermark *et al.*, 2005).

Seasonal polyphenism is an adaptation that is induced entirely by alternating environmental conditions, namely by ambient temperature during the final larval instar and early pupal stage (Oostra *et al.*, 2011). The covariance among the life-history traits observed when rearing all individuals under wet-season conditions actually mirrors the differences between the two seasonal forms, as the dry-season form is, in general, larger, has delayed reproduction, higher metabolic rate and increased lifespan (Brakefield & Zwaan, 2011). Furthermore, individuals eclosing early within cohorts of each generation have been found to tend to be more wet-season like in wing pattern than those eclosing late, even if all individuals are raised under identical conditions from eggs laid over a single day (Zijlstra *et al.*, 2003). These observations and our results indicate phenotypic variation in development time and related morphological and life-history trajectories, even within seasons.

We expected most of the traits to be sex specific, as males and females have very different life-history strategies associated with flight and dispersal (Van Dyck & Wiklund, 2002; Gibbs & Breuker, 2006). Indeed, the sexes of *B. anynana* differed in all of the traits measured. Females, in general, were larger and apparently maximize their fitness by allocating resources to their abdomen at the expense of reduced allocation to flight muscles (Tammaru *et al.*, 1996). Males had narrower but larger wings relative to their mass (higher aspect ratio and lower wing loading), indicating higher allocation to flight ability in general and manoeuvrability in particular, which may be beneficial when males compete for access to females. Lower wing loading may also allow males to be active at lower ambient temperatures, as it reduces wing-beat frequency and lowers the energetic cost of flight (Wikman, 2009 and references therein). Even though *B. anynana* is a tropical butterfly, it generally inhabits shady wooded habitats, and hence, adaptations to increased activity in lower ambient temperatures may be important for males as they search for

females. Males also showed a higher fat percentage and lower resting metabolic rate than females, consistent with previous studies on this species (e.g. Oostra *et al.*, 2011). Concerning the phenotypic correlations, we found that flight activity was positively correlated with wing loading, aspect ratio and body size mainly in males. In line with our finding, Berwaerts & Van Dyck (2004) found that in the speckled wood butterfly *(Pararge aegeria*), the relative thorax size and aspect ratio explained variation in acceleration capacity in males, which again may relate to competition for females. In addition, some of the measured traits also differed between the two generations and/or were affected by the reproductive state of the individuals, implying that dispersal and flight may be highly state and condition dependent (Bonte *et al.*, 2008; Saastamoinen *et al.*, 2010) and that individuals may use up or re-allocate resources, namely from the abdomen but also from the thorax, as they age (Stjenholm *et al.*, 2005; Dudley & Srygley, 2008; Saastamoinen *et al.*, 2009).

Experimental variation and the inherent variation associated with the measurement of flight activity explained a high proportion of the observed phenotypic variance in this trait both for female and male flight activity, hindering the estimation of heritabilities for these traits. This was partially expected, as flight activity was measured under experimental conditions and for logistic reasons only in two trials per individual. The stronger effect of environmental conditions on male compared with female flight activity (Table 4) may potentially be explained, at least in part, on thermoregulatory aspects, as smaller insects in general both heat up and cool down faster than larger ones (Kingsolver, 1983). In the wet season, *B. anynana* males are also in general more active than females, spending much time searching for mates.

Heritability estimates as well as genetic covariance between traits are known to be dependent on the environmental conditions (Hoffmann & Merilä, 1999; Charmantier & Garant, 2005), and therefore, the presented results could be different under other environmental settings, for example, under more stressful developmental conditions. In particular, these data suggest high dependency of flight activity on prevailing environmental conditions. Thus, in future research, it would be interesting to study phenotypic plasticity of this trait by measuring its reaction norm against, for example, ambient temperature, and by searching for patterns of genetic covariance between this reaction norm and other life-history traits. As the presented results are dependent on our estimate of flight ability, other measures of mobility and dispersal may produce dissimilar patterns and should be tested. It was evident that, especially in females, many individuals did not move at all during one of the two trials. Even though the results were qualitatively same when such cases were not included in the model, they do imply that there may be variation in the motivation to move, dispersal component which should be studied in more detail in the future. We also note that studies with field-collected individuals could show very different covariance structure, as laboratory populations can undergo adaptation to the captive environment, which can reduce both phenotypic and genetic variance in a number of life-history traits (e.g. Lewis & Thomas, 2001).

The focus of this study was mainly on morphological and physiological traits linked with dispersal; however, published examples of 'dispersal syndromes' have indicated that dispersal often covaries also with fitness-related life-history traits such as age at first reproduction, lifetime fecundity or lifespan (e.g. Saastamoinen, 2007), which kinds of traits were not assessed here. In summary, our results show that at least within the wet-season morph in *B. anynana*, many of the measured traits are phenotypically but not genetically correlated with each another. It thus seems evident that environmental conditions have a considerable influence on traits assumed to be related to dispersal, and hence, general conclusions about evolution of dispersal solely based on these morphological proxies should be made cautiously. Our results highlight the importance of condition dependence in phenotypic variance and show that at least in *B. anynana* many dispersal-related traits are genetically not strongly constrained.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Data files.

Appendix S2 Results for the genetic variances and covariances based on the model in which cases where individuals did not move during the trial were omitted.

Appendix S3 Phenotypic correlations among the three flight activity measures and the six life history traits.

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