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Evolution of a butterfly dispersal syndrome

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The existence of dispersal syndromes contrasting disperser from resident phenotypes within populations has been intensively documented across taxa. However, how such suites of phenotypic traits emerge and are maintained is largely unknown, although deciphering the processes shaping the evolution of dispersal phenotypes is a key in ecology and evolution. In this study, we created artificial populations of a butterfly, in which we controlled for individual phenotypes and measured experimentally the roles of selection and genetic constraints on the correlations between dispersal-related traits: flight performance and wing morphology. We demonstrate that (i) trait covariations are not due to genetic correlations, (ii) the effects of selection are sex-specific, and (iii) both divergent and stabilizing selection maintain specific flight performance phenotypes and wing morphologies. Interestingly, some trait combinations are also favoured, depending on sex and fitness components. Moreover, we provide evidence for the role of (dis)assortative mating in the evolution of these dispersal-related traits. Our results suggest that dispersal syndromes may have high evolutionary potential, but also that they may be easily disrupted under particular environmental conditions.

1. Introduction

Individuals are characterized by mosaics of traits that compose their phenotypes. It has long been demonstrated in nature that these traits are often correlated at the individual level (e.g. [1–3]). This implies that specialized phenotypes composed by suites of correlated morphological, physiological, behavioural and/or life-history traits assembled in syndromes are frequent [4]. Famous examples are plant defence syndromes [5], pollination syndromes [6], migratory syndromes [7], behavioural syndromes [4] and dispersal syndromes [8].

The observation that phenotypic syndromes are frequent across taxa and biological functions raises the question of their origin. Covariations between traits have been proposed to result either from environmental or genetic constraints (non-selected pleiotropic effects of genes), i.e. the constraint hypothesis, or from selection when a particular combination of traits work well together, i.e. the adaptive hypothesis [9–11]. Unravelling the genetic bases of trait covariation has important implications for its evolution. Indeed, genetically linked traits are obligatory co-evolving traits, while non-genetically linked traits can evolve independently. Therefore, deciphering the mechanisms responsible for the existence of trait correlations and the processes maintaining phenotypic architectures is a key to study the evolvability of many biological functions. Although not recent, the question of the origin of phenotypic syndromes still lacks empirical responses. This is mainly because it is often

64 difficult to determine in the same biological system the genetic and environmental part of variation on traits involved
65 in syndromes together with the investigation of the fitness
66 consequences of syndromes [12].

67
68 Dispersal, defined as movement potentially leading to
69 gene flow [13], is a key process in ecology and evolution,
70 from population and community regulation, to adaptation
71 and speciation [14]. Dispersal is crucial for multiple facets of
72 an individual's life because it limits competition with parents,
73 kin or conspecifics and allows the escape from unsuitable
74 environmental conditions (e.g. [15,16]). This means that it is
75 a key in organisms' response to global change [17]. According
76 to these important ecological and evolutionary roles, theory
77 predicts that dispersal can be either selected or counter-
78 selected (due to its inherent costs [18]) in particular environ-
79 mental and/or social conditions [19]. Thus, contrasted
80 dispersal-related phenotypes are susceptible to coexist
81 within a species, and contrasted selective pressures can gener-
82 ate individual heterogeneity in dispersal within populations.

83 The existence of dispersal syndromes, in which resident
84 and disperser individuals are characterized by suites of corre-
85 lated dispersal-related traits, have been intensively described
86 across many taxa, from unicellular organisms to animals and
87 plants (e.g. [20–22]). Indeed, distinct specialized phenotypes
88 can coexist and persist on the long-term not only at the inter-
89 specific level (e.g. [23]), but also at the intra-specific level
90 (e.g. [24,25]). Among the most emblematic examples are
91 heterotypic species for which individuals with distinct loco-
92 motory apparatus coexist within populations (e.g. plant
93 fruits with or without a flight apparatus (e.g. [26], or insects
94 with or without functional hind wings [27]).

95 The long-standing debate on the origin of covariations
96 between phenotypic traits [9–11] has been recently synthe-
97 sized in the context of dispersal syndromes [8]. It has been
98 pointed out the necessity of distinguishing between the
99 roles played by proximal causes (i.e. genetic correlations,
100 environmentally induced trait covariations and effects of dis-
101 persal on the expression of other traits) and ultimate causes
102 (i.e. divergent selection on dispersal phenotypes, dispersal
103 plasticity and eco-evolutionary feedbacks) potentially
104 responsible for the emergence of dispersal syndromes. How-
105 ever, this task is not easy because it requires in the same
106 biological system the determination of the genetic and
107 environmental part of variation on dispersal traits involved
108 in syndromes, and the investigation of the fitness conse-
109 quences of dispersal syndromes [12]. This explains why
110 much more emphasis has been devoted to the study of the
111 mechanisms that maintain the integrity of dispersal poly-
112 morphisms rather than on those that generate variability.
113 Empirical and theoretical works indeed showed that disper-
114 sal symmetry [28] or asymmetry [29], dominance [30],
115 assortative mating [31] and balancing selection [32] can all
116 maintain dispersal polymorphisms. One of the most
117 famous examples refers to balancing selection acting in the
118 butterfly *Melitea cinxia* on the locus encoding the phospho-
119 glucose isomerase (PGI), a metabolic enzyme involved in
120 glycolysis [33]. Heterozygotes at this locus tend to have
121 higher fitness than homozygotes [34], and it has been
122 shown that this gene is involved in dispersal strategies
123 [35]. That being said, it remains generally unresolved if
124 and how selection acts to maintain not only values of a
125 specific dispersal-related trait, but also the combinations of
126 traits that determine dispersers and residents within

populations (see however [36,37]). This is mostly because
the proximal and ultimate causes of the emergence and the
maintenance of dispersal syndromes are poorly investigated
at the intra-specific level, although meta-analyses and phylo-
genetic studies have recently shed light on this topic at the
interspecific level [23,38–40]. Three main questions are at
the research front in the field of dispersal and echo back to
the more general debate on the evolution of correlated
traits raised above: (i) Do correlations between traits
involved in dispersal syndromes evolve as consequences of
a common genetic basis? (ii) Does correlational selection
operate to maintain covariations between dispersal-related
traits? (iii) By which mechanisms does selection operate to
maintain dispersal syndromes on the long-term?

In this study, we tackled these questions using an empiri-
cal approach by testing for the existence of selective and/or
genetic effects responsible for the emergence and mainten-
ance of a butterfly dispersal syndrome. We further tested
for the mechanistic role of assortative mating in the mainten-
ance of the variability of these traits. Non-random mating can
impact on phenotypes' distributions because of their influ-
ence on phenotypes' transmission. Indeed, disassortative
and assortative mating are mechanisms that may lead to or
evolve in response to stabilizing and disruptive selection,
respectively (see review in [41]). To do so, we used the
Large White butterfly *Pieris brassicae* as model species.
We focused our work on flight performance and wing
morphology because (i) significant correlation was consist-
ently measured between these two traits in two distinct
experiments involving unrelated *P. brassicae*'s populations
([42,43], see also in another butterfly species in [44]),
(ii) they are both commonly involved in butterflies' dispersal
syndromes [45,46].

Pieris brassicae is homotypic for its locomotory apparatus,
meaning that it is composed of a mixture of individuals
along a mobility and wing morphology gradient [47]. We
have recently shown that *P. brassicae*'s flight performance cov-
aries with wing morphology [42,43], exploratory behaviour
[42], orientation at emergence [48], dynamics of copulations
(unpublished) and dispersal [43]. Especially, we demonstrated
that disperser individuals in experimental metapopulations
had longer wings and higher flight performances than resident
individuals. Therefore, *P. brassicae* presents a dispersal syn-
drome involving morphological, physiological, behavioural
and life-history traits. It has also been shown that flight per-
formance increases with latitude using individuals sampled
along a south–north gradient in France. This means that mobi-
lity is likely to be selected at the species level [47]. Here, we first
determined the distributions of flight performance and wing
morphology in a natural population of *P. brassicae* to describe
the variation of these traits. Second, we tested on a breeding
pool of individuals whether specific flight performance
values and wing morphologies impacted several fitness com-
ponents under semi-natural conditions. We characterized
what kind of selection was at work on these traits: direct or cor-
relational; disruptive, stabilizing or directional. Third, based
on full pedigrees, we built the matrix of additive genetic var-
iances and covariances (G-matrix) between these traits to test
for the existence of genetic constraints at the basis of the
observed phenotypic covariations. Finally, we tested for a
potential role of (dis)assortative mating in the evolution of
the dispersal syndrome relating the patterns of dispersal-
related trait associations between sexual partners with fitness

127 components. We also tested for the effect of other phenotypic
128 traits commonly involved in butterflies' partner choice like
129 wing melanization or body morphology [49,50].
130
131

132 2. Material and methods

133 (a) Biological material and breeding conditions

134 To determine the natural distribution of flight performance and
135 wing morphology in *P. brassicae*, we collected eggs from a cabbage
136 field in Ariège (southwestern France) in summer 2010 and bred a
137 cohort of 87 individuals (from three different clutches) in common
138 garden conditions. Eggs and larvae were kept in a climate chamber
139 under controlled photoperiod (14 L : 10 D cycle) and temperature
140 ($23 \pm 1^\circ\text{C}$ during light and $18 \pm 1^\circ\text{C}$ during dark) conditions.
141 The larvae were fed ad libitum with fresh cabbage and kept in
142 $40 \times 20 \times 10$ cm boxes. Flight performance was measured on
143 adults following the protocol detailed below and wing length
144 was measured with a calliper.
145

146 To measure the effects of selection on dispersal phenotypes,
147 we used a second set of individuals issued from our laboratory
148 breeding. This breeding was established in august 2011 using
149 eggs originating from three locations in Ariège (southwestern
150 France, mean distance between locations: 10 km) and one place
151 in Vaucluse (Southeastern France). At least, six egg clutches
152 were used from each locality. In *P. brassicae*, fertilized females
153 usually lay few egg clutches composed of up to hundreds of
154 eggs for the first clutch, and of fewer eggs (often only a few
155 dozens) for the subsequent clutches. We only collected large
156 clutches, to minimize the possibility of collecting several egg
157 clutches from a single female. Therefore, our breeding was
158 likely established from at least 24 founding females. After two
159 generations (from August to October 2011) during which
160 adults were placed within a semi-natural common garden and
161 larvae bred under fully controlled conditions (the same as
162 above), we artificially induced a diapausing period to pupae
163 (from December 2011 to spring 2012, $20 \pm 1^\circ\text{C}$ during light
164 periods and $12 \pm 1^\circ\text{C}$ during dark periods). In spring 2012, we
165 stopped diapause and gradually led adults to emergence
166 throughout the experiment. These adults were maintained and
167 bred under common laboratory conditions until their release in
168 the mating cages (see below). We obtained a total of 210 butter-
169 flies on which we performed the following sequence of tests.
170 During the first 24 h after emergence, each butterfly was placed
171 in a $1 \times 1 \times 1$ m breeding cage with a water source and nectariferous
172 flowers. Males and females were separated to prevent
173 sexual interactions. On the day after emergence, butterflies
174 were individually tested for flight performance. On the same
175 day, they were weighed and scanned to obtain morphological
176 measurements (see below). Based on the distribution of flight
177 performance of these 210 butterflies, we selected 80 adults
178 (40 males and 40 females; thereafter called 'parental generation')
179 so as to maximize the variance in flight performance and mini-
180 mize the relatedness between individuals (individuals were
181 issued from 10 clutches from 10 distinct females). This parental
182 generation was then split into four replicates of 20 individ-
183 uals (10 males and 10 females in each replicate) ensuring the
184 continuum of flight performances needed in each artificial
185 populations. This density allows high survival rates in the
186 semi-natural cages used to follow mating behaviours [51] and
187 represents the maximal density allowing efficient observation
188 of matings and oviposition behaviour by experimenters (see
189 below). Larvae resulting from these artificial populations were
reared under the exact same conditions as their parents and the
resulting offspring were reared under common laboratory con-
ditions ($n = 214$). Tests on these adults were identical to those
performed on the parental generation.

(b) Measures of phenotypic traits

It has previously been demonstrated that both flight performance
and wing length are components of the *P. brassicae*'s dispersal
syndrome [43,51]. We have also demonstrated that wing surface
is related to flight performance [42]. These three variables were
thus used in this study.

We measured individual flight performance through a vali-
dated behavioural test monitoring flight performance in
stressful conditions [42,43,47,52], which consist in introducing
each butterfly into a shaken plastic chamber and measuring
their time in flight (see the electronic supplementary material,
for further information).

We then studied morphological traits using digital images.
Butterflies were anaesthetized with nitric oxide in a $10 \times 10 \times$
 10 cm box (Inject + Matic Sleeper TAS[®]). Each individual
was weighed using a scale with a precision of 0.1 mg (Precisa
80A-200 M) and subsequently fixed between two transparent
plastic sheets and placed within a slightly opened scanner
(Epson Perfection 2480 Photo, mode PROFESSIONAL). The
resulting images were then analysed using IMAGEJ [53] to
measure wing length and wing surface. One of our aims was
to test for the role of assortative mating in the evolution of
P. brassicae's dispersal syndrome. Several traits linked to body
and wing characteristics can influence butterfly mating [49,50];
see mating experiments below). Therefore, we also measured
total body length and percentage of melanized area on the
dorsal side of the left forewing [54] on the basis of images. For
each individual, we quantified melanized areas as those that
were below a threshold of grey intensity (i.e. 120 on a scale of
 $0 = \text{black}$ to $255 = \text{white}$). After the scanning procedure, butter-
flies were individually marked on both hindwings with a colour
pen allowing rapid identification during mating experiments.
We compared the melanized areas from the dorsal and ventral
sides of both the left and right forewings, as well as the lengths
of the two forewings, in a subsample of 20 butterflies to control
melanization and wing length measurements for each individual.
Each pair of measurements had a correlation coefficient greater
than or equal to 0.87. In addition, we performed 20 repetitions
of melanization and morphology measures for a single individual
to ensure that our established values were reliable (standard devi-
ation was 2.7 for the overall sample and 0.018 for the repeated
measurements of melanization; respectively, 1.57 and 0.0002 for
body length; 1.5 and 0.0004 for wing length).

Hence, six phenotypic variables were measured, hereafter
called *flight performance*, *wing length*, *wing surface*, *body length*,
body mass and *wing melanization*.

(c) Mating experiments and fitness measurements

In April and May 2012, 10 males and 10 females were released
into each of four replicated $10 \times 10 \times 2$ m outdoor cages, which
are part of the Metatron experimental platform [51]. Butterflies
were released at the same hour for the four replicates, and the
replicates were all run on sunny days. To stimulate reproduction
and provide opportunities for egg laying, a fresh cabbage was
placed at the centre of each cage. For each replicate, we recreated
artificial populations for which the variance in flight perform-
ance was maximized, by releasing individuals selected on the
basis of their scores on the flight performance test. Individuals
spent on average 2 days in the laboratory (range = 1–6 days,
but only four individuals were kept more than 4 days). There
was no difference in the distribution of variables within each
replicate based on sex, with the exception of melanization and
wing length in males (ANOVA, both p -values = 0.011). Butter-
flies were monitored daily from 9.00 to 18.00. Every 30 min, we
identified each butterfly in the cages and checked for mating
and egg laying. Mating and egg-laying durations were indeed
never shorter than 30 min (authors' personal observation).

Using this procedure, egg clutches were unambiguously attributed to their corresponding parents and were reared under the conditions described in the *Biological material and breeding conditions* paragraph.

Four different components of fitness were estimated during this experiment:

- (1) *lifespan* was measured as the time individuals lived after their release in the experimental mating cages;
- (2) *mating success* scored if an individual mated or not;
- (3) *egg production* scored if a mated individual produced eggs or not; and
- (4) *offspring production* scored if a mated individual produced imagoes or not.

(d) Analyses of selection on syndrome traits

We first estimated the correlations among pairs of traits for each individual and for each sex using Spearman's correlations to assess whether flight performance and wing morphology were effectively correlated in our sample. Then, we placed our study in the general framework of the measurement of selection on correlated characters developed by Lande & Arnold [10]. Briefly, multiple regressions with fitness components as the response variable and correlated phenotypic traits as explanatory variables are used to detect the presence of selection and estimate its intensity [10]. Significant linear terms indicate the presence of linear selection (i.e. directional selection), significant quadratic terms indicate the presence of nonlinear selection (i.e. stabilizing or disruptive selection), and significant cross product terms indicate the presence of selection on combinations of traits (i.e. correlational selection). A limitation of this general framework is that the strength and significance of nonlinear selection may be underestimated, especially in the presence of correlative selection [55,56]. To overcome these limitations, we used Partial Least-Squares regressions (PLS) to analyse the data. PLS regression is a robust modelling method for data analysis, especially when the effect of a great number of correlated explanatory variables is investigated from a restricted number of observations [57]. This means that PLS regression is specifically pertinent in the context of selection analyses for traits assembled in syndromes because a complete model including numerous correlated traits and their interactions can be fitted without the need to summarize variables with Principal Component Analyses (in order to limit multi-collinearity which may drastically bias the coefficient estimations, [58]). Moreover, it allows reliable estimations of the significance and intensity of selective effects using very small sample sizes, like one of these studies. The basic principles of PLS regressions and the detailed procedure used in this study can be found in the electronic supplementary material.

(e) Heritability and genetic correlations

To assess genetic covariances and correlations between the six phenotypic traits, we estimated the G matrix using multivariate animal models [59]. Animal models have proved very useful to estimate heritabilities and genetic correlations compared with parent-offspring regressions when sample sizes are limited like in our study [60]. Additive genetic effect was added as a random effect, and sex was included as a fixed effect as sexual differences in morphology are known in *P. brassicae* [42,47]. Phenotypic traits were standardized to a mean of 0 and a variance of 1 to avoid that our conclusions be based on traits with larger means [61,62]. We thus used the following model:

Phenotypic trait = Sex + animal;

with *wing length*, *wing surface*, *flight performance*, *melanization*, *body mass* and *body length* being the standardized 'phenotypic traits' to explain and 'animal' the individual effect linked to the

pedigree to assess additive genetic variance. The procedure used to run Animal Models is detailed in the electronic supplementary material.

(f) Detection of assortative mating

To assess the presence of non-random mating, we built six models corresponding to the six phenotypic traits in which males' trait values were expressed as a function of females' trait values using Generalized Linear Mixed Models (GLMM) with the lmerTest R package [63]. *P*-values were calculated by means of the Satterthwaite method with the replicate (cage) as a random variable. To rule out the possibility that the observed association of phenotypes between sexual partners could have been generated by purely stochastic processes, mate resemblance was compared to a theoretical distribution of trait association between randomly chosen partners. We generated 1000 sets of 33 pairs randomly chosen among the males and females that reproduced within each replicate of the experiment. For each replicate, we performed Spearman's correlations between trait values of paired males and females and compared the observed ρ to the distribution of the simulated ρ . Assortative mating was detected if 95% of the simulated values were inferior to the observed value. Similarly, if 95% of the simulated values were superior to the observed ρ , disassortative mating was detected. All traits were standardized prior to analyses by subtracting the mean and dividing this value by the standard deviation of their respective distributions. For (dis)assorted traits, we tested for the role of selection in the maintenance of these non-random associations between sexual partners. To do so, we built normalized similarity indexes consisting in the differences between males' and females' trait values. We checked for multi-collinearity between indexes and then used Ridge regressions, because they allow coping with strong collinearity between explanatory variables [64,65], provided that models are simple. Two models were built with *egg production* and *offspring production* as responses variables and similarity indexes as explanatory variables.

All statistical analyses were performed using the software R v. 3.1 [66].

3. Results

(a) Trait distributions and correlations between traits

We assessed the frequency distribution of flight performance and wing length in a cohort of 87 individuals from a natural population. The flight performance distribution was not normal ($W = 0.84$, $p < 0.001$, Shapiro test) but rather (albeit not perfectly) bimodal (electronic supplementary material, figure S1a), which means that most individuals could be allocated to one of two opposite classes (low or high flight performance). By contrast, wing length followed a normal distribution ($W = 0.99$, $p = 0.46$, Shapiro test; electronic supplementary material, figure S1b). The shapes of distributions were similar for both sexes.

To create our artificial populations, we selected 80 individuals among a pool of 210 butterflies of our breeding, for which we ensured that the distribution of flight performance and wing length were similar to those of the natural population, i.e. bimodal and normal (see the electronic supplementary material, figure S2). Correlations between the six measured phenotypic traits are shown in the electronic supplementary material, table S1 for each sex. Melanization was the only trait uncorrelated to any other trait. Among significant correlations, some were found in both sexes and others were

sex-specific. Wing length and wing surface were positively correlated in both sexes, as were body length and body mass. Three pairs of traits (exclusively morphological traits: wing surface and body length, wing surface and body mass, wing length and body length) showed significant positive correlations in females. In males, two pairs of traits linked to dispersal showed significant negative correlations. Flight performance was indeed correlated to wing length and wing surface. A similar sex-specific correlation between flight performance and wing length was also observed in the natural population (Spearman's $\rho = 0.126$, $p = 0.39$ for females and Spearman's $\rho = 0.477$, $p = 0.001$ for males).

(b) Selection analyses on dispersal traits

To assess individual fitness, we recorded lifespan, mating success and fecundity by collecting eggs and raising offspring until adult emergence. All PLS were significant and explained between 41.1 and 100% of variance in fitness components, with male dispersal-related traits explaining a higher part of variation in the four fitness components than female dispersal-related traits (electronic supplementary material, table S2). However, among the four fitness components, only three were significantly affected by dispersal-related traits: lifespan, mating success and offspring production (table 1). These analyses revealed that both linear and nonlinear selections are acting on dispersal-related traits. Indeed, intermediate male flight performance favoured lifespan, while extreme flight performance favoured male mating success (figure 1) and offspring production. Extreme flight performance favoured female lifespan, while only low flight performance favoured female offspring production. Wing length had a significant quadratic effect on male mating success, with intermediate males having a higher probability to mate compared with males with extreme wing lengths. Male offspring production increased for two trait combinations: high flight performance/small wings and low flight performance/long wings. Finally, the age at release had a strong significant effect on lifespan, with the oldest butterflies being those that survived the longest. Wing surface had no significant effect on fitness.

(c) Heritability and genetic correlations

The flight performance and morphology of adult offspring were compared with parental values to assess their heritability using animal models with sex as a covariate. Only wing surface was not heritable, whereas the five other phenotypic traits were significantly heritable: heritability rates were high for wing melanization and wing length, intermediate for flight performance and body mass, and low for body length (electronic supplementary material, table S3a). Sex was retained in the selection of model terms for three traits: females were more melanized than males and also displayed higher body mass and body length compared with males. With regards to genetic covariances and correlations, only the positive genetic correlation between wing length and wing surface was significant (see the electronic supplementary material, table S3b).

(d) Mating preference

Thirty-three of the 40 released females mated (each mating only once), while 26 of the 40 males mated (seven of them

Table 1. Sex-specific magnitude of the significant effect of dispersal traits on fitness. This table details the regression coefficients associated for each significant variable with their 95% confidence intervals and p -values. These regression coefficients were doubled for quadratic variables [67] and adjusted following [68] to obtain estimators of selection coefficients [10]. For male offspring production, it was impossible to estimate selection coefficients likely due to a Q2 limited sample size and/or a very high predictive power of our model, which led PLS regression to compute large coefficient values.

fitness component	significant variables	regression coefficient	p -value	selection coefficient	selection type
male survival	flight performance ²	-0.052 [-0.111;0.007]	0.047	-0.104	stabilizing selection
	age at release	0.207 [0.105;0.264]	0.0002	0.207	directional selection
female survival	flight performance ²	0.099 [-0.003;0.017]	0.03	0.198	disruptive selection
	age at release	0.233 [0.122;0.198]	0.011	0.233	directional selection
male mating success	flight performance ²	3.22 [0.841;214.99]	0.01	0.341 [¶]	disruptive selection
	with length ²	-2.008 [-155.371; -0.892]	0.002	-0.213 [¶]	stabilizing selection
male offspring production	flight performance × wing length	-149.02 [-216.185;0.04]	0.031	n.a.	correlational selection
	flight performance ²	57.56 [-1.154;88.009]	0.05	n.a.	disruptive selection
female offspring production	flight performance	-4.73 [-112.597;2.052]	0.05	-0.189 [¶]	directional selection

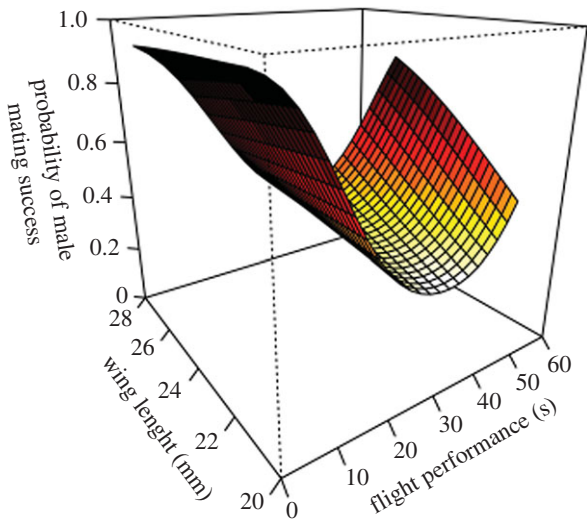


Figure 1. Fitness landscape of male mating success. The relationship was built on the basis of PLS result for the mean wing surface and age at release within the male population, and for weighted means of each replicate/family combinations. In this figure, we only represented the complex fitness landscape presenting two significant variables with estimable selection coefficient. (Online version in colour.)

mated twice). Correlations between traits of mating partners are shown in the electronic supplementary material, figure S3. Males and females of the same pair had significantly divergent body lengths ($\rho = -0.464$, $p = 0.004$), wing lengths ($\rho = -0.368$, $p = 0.021$) and wing surfaces ($\rho = -0.319$, $p = 0.031$), reflecting disassortative mating for these traits. By contrast, males and females of the same pair had significantly similar flight performances ($\rho = 0.575$, $p < 0.001$), reflecting assortative mating for flight performance. No correlation between mating partners' phenotypes was observed for body mass and wing melanization. Ridge regressions showed that only disassortative mating on the basis of wing length significantly affected a fitness component (offspring production was favoured when sexual partners had different wing lengths, t -value = 3.396; $p = 0.0007$).

4. Discussion

Wing length and flight performance are key phenotypic traits in butterflies because they are involved in many biological functions: e.g. in mate location, male–male competition, food search, thermoregulation ability or escape behaviour. Recent meta-analyses showed that they are also key traits in butterflies' dispersal ability [45,46]. This was experimentally validated in *P. brassicae* that exhibits a dispersal syndrome in which dispersers have longer wings and a higher flight performance than residents [43]. Such correlations between phenotypic traits can be the consequence of constraints, either genetic or environmental (i.e. without any selection effects on phenotypes), or can result from divergent and/or correlational selection (i.e. phenotypic syndromes are adaptive) [9–11]. Here, we measured four fitness components of butterflies with known phenotypes issued from our breeding and placed under semi-natural conditions to decipher the mechanisms by which a dispersal syndrome can emerge and evolve. We used a complete methodology including animal models and PLS regressions. Notably, PLS appeared to be a powerful tool to study selection on correlated traits

analogous to the Lande and Arnold method [10] but with the advantage of robustness to small sample sizes and to provide an unbiased coefficient estimation despite the presence of multi-collinearity among explanatory variables (which is highly valuable in the context of phenotypic syndromes). We showed that complex and sex-specific selective effects, and not heritable genetic constraints, can shape the evolution of *P. brassicae*'s trait covariations. We hereafter mostly discuss these results in the context of evolution of dispersal syndromes, but also point out their importance for other aspects of butterflies' ecology when relevant.

(a) Association between phenotypic traits

Our results revealed complex associations between some of these traits. The positive correlations between body length and mass, and between wing length and surface observed both in males and females are well known as universal scaling relationships (e.g. [69]). The other, sex-dependent associations are more intriguing. First, we showed that body length was positively related to wing length and wing surface in females only, while body length and wing morphology were not genetically correlated. In insects, female fecundity scales positively with body size (e.g. [70] for insects, [71] for butterflies). We suggest that egg load is such a strong constraint on flight that it generates an obligatory positive scaling relationship between female body size and wing size.

We also report that two pairs of traits involved in *P. brassicae*'s dispersal syndrome were correlated in males: flight performance was negatively related to wing length and wing surface. We determined that wing length and wing surface were highly genetically correlated, i.e. they share a common genetic basis likely responsible for the covariation we observed at the phenotypic level. However, only wing length was significantly heritable. Therefore, wing length, and not wing surface, is probably one of the main drivers of wing morphology evolution in our model species. As well, we did not detect any impact of wing surface on fitness components. Therefore, we will hereafter only discuss the role of wing length and flight performance on dispersal syndrome evolution. Wing length and flight performance are two traits under selection in flying insects (e.g. [72,73]), which is expected given their role in many insect biological functions. We confirm that selection influences the evolution of these two traits in *P. brassicae*. Interestingly, we found that the effects of selection were sex-specific and differed between fitness components.

(b) Sex-specific selection

Sex-specific selection, where fitness landscapes are different between males and females, and sexually antagonistic selection, where the signs of the covariance between a trait and fitness differ between males and females, have been extensively documented (see reviews in [74]) and can occur in the context of dispersal. Indeed, differences in dispersal rates between males and females, i.e. sex-biased dispersal, have been described in many taxa including butterflies (e.g. [75,76]). In *P. brassicae*, females tend to disperse more than males [43]. However, we did not evidence selective effects that favoured dispersers' trait values specifically in females as compared to males: high (and low) flight performance favoured female lifespan, but also male mating success

and offspring production. Furthermore, females with longer wings had no benefit compared to other, while long-winged males with low flight performance were favoured. Therefore, the dispersal bias toward females in *P. brassicae* does not seem to be linked to female-specific phenotypic attributes, which is in accordance with the absence of significant interactive effect between sex and phenotypic traits on dispersal decisions in this species [43]. We suggest that phenotypic adaptations to dispersal are the same in the two sexes, although dispersal rates are different, and that other sex-specific ecological processes linked to males' and females' lifestyles also influence the evolution of wing length and flight performance (for example intra- and inter-sex interactions, or resource use strategy). This hypothesis is in accordance with the observations that correlations between flight performance and wing length is highly consistent in males ([42,43], this study), while the same correlation is less frequently observed in females [43]. The existence of sex-specific syndromes has rarely been investigated except in the context of plant morphology ([6], see however [77]); here, we provide an example of a sex-specific syndrome in the context of animal dispersal.

(c) Flight performance, wing length and fitness components

We measured the effect of flight performance and wing length on four fitness components. Among them, egg production was unaffected by phenotypic traits. The study of the three other fitness components (female lifespan, male mating success and male offspring production) revealed that disruptive selection generally drives the evolution of flight performance. This means that both low and high performers (i.e. potentially residents and dispersers) have a selective advantage compared with intermediate phenotypes, which may be responsible for the long-term maintenance of dispersal polymorphisms in this species. Strong disruptive selection can lead to strong divergence between populations, and even speciation [78]. This process could thus result in the isolation of high and low performers in *P. brassicae*. However, a stabilizing effect on flight performance was also detected on male lifespan, which should limit the distribution of the flight performance trait towards extreme values. At broader spatial and temporal scales, it is also likely that fluctuating environmental conditions favouring either low or high performers (balancing selection) prevent strong divergence between extreme flight performance phenotypes within *P. brassicae*. Besides, probability of offspring production was lower for females with high flight performance, which would suggest the existence of an oogenesis-flight syndrome in *P. brassicae*, i.e. a negative correlation between reproduction (here offspring production) and flight performance [79]. All in all, our results suggest that the effects of selection on this dispersal-related trait could promote the existence of dispersers and residents without provoking a strong divergence between them.

We have evidenced a positive effect of the age of release on lifespan in both sexes, meaning that individuals that spent a longer time under laboratory conditions survived longer within semi-natural cages than those that spent less time. Butterflies living their first days of life under optimal laboratory conditions may endure reduced physiological costs than individuals living their first days under more

costly semi-natural conditions (e.g. variation in weather conditions, predation risk), explaining this result.

We also detected significant correlational selection favouring short-winged males with high flight performance and long-winged males with low flight performance. This result shows that particular combinations of these two traits can be the target of selection, and thus influence the emergence and maintenance of dispersal syndromes. Interestingly, wing length and flight performance were genetically uncorrelated, i.e. the emergence of *P. brassicae*'s dispersal syndrome is probably not due to genetic constraints. A corollary is that this syndrome is susceptible to be disrupted rapidly if selective pressures on these traits vary intensively. This might explain why a positive correlation between wing length and flight performance was reported in our previous studies [42,43] and in our natural sample, while we found a negative correlation in our experimental sample. We also suspect that such differences across individual pools with distinct histories reflect a high evolutionary potential of *P. brassicae*'s syndrome. This hypothesis would deserve further experimental investigations, which could be more generally performed in the general context of phenotypic syndromes.

By contrast, we found a disruptive effect of correlational selection on wing length (short-winged males with high flight performance and long-winged males with low flight performance were favoured) that is opposed to the stabilizing effect we evidenced measuring male mating success. The evolution of trait correlations can thus be complex and driven by several, and sometimes discordant, selective effects.

(d) (Dis)assortative mating on dispersal-related traits

In this study, we have also tested for the mechanistic role of (dis)assortative mating in the maintenance of dispersal polymorphisms. We found significant assortative mating on the basis of flight performance, and significant disassortative mating on the basis of wing length, wing surface and body length. In butterflies, several studies showed the key role of wing colour patterns in the choice of sexual partners (e.g. [50,80]). Our study highlights that wing morphology and flight performance may also be cues for non-random associations between sexual partners. To what extent these traits may serve as signals for mate choice or drive non-random mating patterns without choice (for example, if flight performance influences the probability to encounter partners) is still an open question that surely deserves further investigation. Assortative mating on the basis of flight performance has the potential to accelerate the spread of resident and disperser phenotypes in populations of *P. brassicae* through the fitness benefits we have demonstrated above. Interestingly, we also found that a direct fitness reward to disassortment was found for wing length, because the dissimilarity in wing lengths between sexual partners was associated with a higher number of offspring, which should favour intermediate wing lengths. Clearly, the existence of adaptive disperser and resident phenotypes in populations tends to increase the frequency of short and long-winged individuals. But other selective pressures and/or morphological constraints also skew the distribution of wing length towards normality in *P. brassicae*, as we highlighted with the detection of disassortative mating on wing length,

which may explain the high frequency of individuals with intermediate wing length observed in our natural population.

5. Conclusion

Determining the potential causes of the emergence and long-term maintenance of phenotypic syndromes is a fundamental, but challenging question in evolutionary ecology. In a butterfly, we have determined that the correlations between phenotypic traits involved in a dispersal syndrome can arise independently of genetic constraints, and that they may be adaptive. Furthermore, nonlinear and linear selection can act independently and concomitantly to shape the evolution of traits involved in syndromes, by favouring specific trait values or specific combinations of traits. Determining the processes that shape the evolution of dispersal syndromes can be challenging because we showed that the effect of selection can be sex-specific and divergent between fitness components. Interestingly, such observations suggest a high evolutionary potential of dispersal syndromes, meaning that correlations between phenotypic traits can emerge and may be rapidly disrupted in response to specific environmental conditions. Such observations may have important

implications for species' abilities to respond to changing environments, and should therefore be the focus of future experiments. Finally, we strongly suggest that future work on fitness landscapes should integrate several fitness proxies and appropriate methodology to study correlated traits, in order to correctly understand and predict the evolution of phenotypic syndromes.

Data accessibility. The data supporting this article are available in the Dryad Digital Repository, with identifier <http://dx.doi.org/10.5061/dryad.s73t1>.

Authors' contributions. D.L., N.L. and M.B. conceived the study. D.L., N.L. and O.C. performed the experiment. All authors participated to the statistical analyses at the exception of PLS regressions performed by R.B. and animal models performed by S.D. D.L. wrote the first draft of the manuscript, all authors contributed substantially to revisions.

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