

RESEARCH ARTICLE

Intra- and inter-individual variation in flight direction in a migratory butterfly co-vary with individual mobility

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SUMMARY

Flight direction is a major component of an animal's migratory success. However, few studies have focused on variation in flight direction both between and within individuals, which is likely to be correlated with other traits implied in migration processes. We report patterns of intra- and inter-individual variation in flight direction in the large white butterfly *Pieris brassicae*. The presence of inter-individual variation in flight direction for individuals tested in the same conditions suggests that this trait is inherited in *P. brassicae* and we propose that a rapid loss of migratory skills may exist in the absence of selection for migration. The magnitude of intra-individual variation was negatively correlated to two surrogates of the potential for migration: mobility and wing length. Highly mobile and longed-winged individuals within the same family were found to fly in similar directions, whereas less mobile and short-winged individuals displayed divergent flight direction compared with the average direction of their kin. There was also a negative correlation between the variance to the mean flight direction of a family and its average mobility, but no correlation with wing length. We discuss these issues in terms of the evolution of traits potentially implied in both migration and dispersal in *P. brassicae*.

Key words: migration, *Pieris brassicae*, individual variation, orientation, mobility syndrome.

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INTRODUCTION

Migration is a process that allows animals to escape from deteriorating environmental conditions to exploit predictable favourable habitats (Liedvogel et al., 2011). This process involves a suite of adaptive traits, i.e. the migration syndrome, including morphological, physiological, sensory and behavioural characters (Åkesson and Hedenstrom, 2007). Among these traits, orientation (also called migratory direction or flight direction in flying species) is defined as an individual characteristic by which an animal moves in a given plane or compass direction (Baker, 1978) and is a major component of the migratory success of individuals (Holland et al., 2006). Indeed, Lepidoptera that migrate in the same regions showed common orientation (Chapman et al., 2010). To minimize migration time, natural selection should favour those traits that optimize the overall migration speed, including orientation, and help to achieve the so-called optimal migration model (Alerstam and Lindström, 1990; Hedenström, 2008). As a result, selection on orientation is key in the investigation of the genetic bases of migration (Berthold, 1991; Helbig, 1996; Liedvogel et al., 2011). This investigation is even more challenging for species with multi-generational migration patterns such as migrant butterflies [e.g. the large white *Pieris brassicae* (Spieth et al., 1998), the Monarch *Danaus plexippus* (Zhu et al., 2009) and the painted lady butterfly *Vanessa cardui* (Stefanescu et al., 2013)] especially when only a part of the population migrates [e.g. *P. brassicae* (Feltwell, 1982)].

Flying migratory species may exhibit great variation in flight direction (Helbig et al., 1994; Helbig, 1996; Thorup et al., 2007) despite rather high heritabilities of migratory traits in insects (Roff and Fairbairn, 2001) and birds (Pulido and Berthold, 2003). As a result, inter-individual variation in migration routes have been shown to be crucial to enable rapid evolution of migratory behaviours (Berthold, 1996). However, inter-individual variability is often neglected in both modelling and empirical studies of orientation systems (Thorup et al., 2007). Even less emphasis has been placed on intra-individual variation, although it might be an essential component of the individual's migratory success because a rectilinear trajectory may enable individuals to reach migratory sites more efficiently. For instance, intra-individual variation in flight direction was low in the butterfly *P. brassicae* and orientation was stable over lifetime and conditions (Spieth and Kaschuba-Holtgrave, 1996; Spieth et al., 1998). Inexperienced migrants of the Savannah sparrow *Passerculus sandwichensis* exhibited more intra- than inter-individual variation in flight direction, contrary to experienced migrants, suggesting that homogeneity in orientation plays a fundamental role in the migration behaviour (Moore, 1984). In addition, heterogeneity was frequent in flight direction in juvenile birds and interpreted as a possible bet-hedging strategy, given that the fitness of a parent's juvenile offspring has been observed to be very variable from year to year (Reilly and Reilly, 2009). How often such intra- and inter-individual variation is observed

and to what extent the long-term persistence of this variation is adaptive remains an open question.

The mechanistic components of orientation are relatively well documented in insects, especially in ants (Vowles, 1954) and in bees (Dyer and Could, 1983; Capaldi et al., 2000), but less attention has been devoted to butterflies, with the exception of *D. plexippus*, although this group is key in the study of both migration and dispersal patterns (Stevens et al., 2010). *Pieris brassicae* (Linnaeus 1758) is certainly one of the most intriguing migrant butterfly species. This species is well distributed in Europe (from north Scandinavia to the Iberia peninsula including the Balkans), in North Africa and Asia to the Himalayas (see Feltwell, 1982). It exhibits two to four generations per year from April to November, which are successively involved in the whole migration cycle of the species, and it hibernates with a pupal wintry diapause (Feltwell, 1982). In general, the spring generation flies northward and the autumn generation flies southward in Europe (Baker, 1968) as a result of the developmental mode (Spieth et al., 1998). However, *P. brassicae* shows important variation in flight direction which depends upon the geographic origin of individuals (Spieth and Kaschuba-Holtgrave, 1996; Spieth and Cordes, 2012) and a link between these flight directions and migratory directions has been suggested (Spieth and Kaschuba-Holtgrave, 1996). Interestingly, butterflies do not change their flight direction during the course of the day or with advancing age, and orientation skills are not essentially influenced by temperature or the sun's azimuth during the adult stage (Spieth et al., 1998). It has also been suggested that, within the species, only some of the individuals actually migrate (Feltwell, 1982), which raises the question of the existence of local adaptations in some individuals of the species.

As flight direction is a major component of the migratory syndrome, it is thus likely that it co-evolves with other migratory traits. This suite of traits implicated in the migratory syndrome is also likely to be involved in the dispersal syndrome, where dispersal is defined as any movement with potential consequences on gene flow (Ronce, 2007; Zera and Brisson, 2012), as they both concern traits related to movement ability. Migration of insects over several generations might even be considered as an extreme form of dispersal, as migration

entails colonization of empty habitats and gene flow. In general, dispersal and migration traits exhibit high heritability values but may also be dependent upon environmental factors (Clobert et al., 2009). The existence of behavioural syndromes, i.e. suites of correlated behaviours reflecting inter-individual consistency across both time and situation (Sih et al., 2004), has been described in several insects (Dingle, 2001; Dingle, 2006; Roff, 1992) including *P. brassicae*, which shows correlations between morphological, physiological and behavioural traits related to mobility (Ducatez et al., 2012b). To what extent selection on migratory traits can also influence the dispersal response (and the reverse) is an intriguing question of great importance in evolutionary ecology, but so far the relationship between mobility and flight direction has not been investigated in *P. brassicae*.

To further characterize the mechanisms responsible for the orientation trait in *P. brassicae*, the present study aimed to (i) quantify intra- and inter-individual variation in flight direction in both natural and breeding families of *P. brassicae* and (ii) study the relationships between flight direction and other mobility traits to further understand the behavioural syndrome described in *P. brassicae*.

MATERIALS AND METHODS

Breeding conditions

To assess the role of natural *versus* captive conditions in the evolution of flight direction, two types of eggs were collected and bred under similar laboratory conditions. The first was composed of four egg clutches originating from a captive breeding programme in Visan in South-East France (Vaucluse) after at least four captive generations. The second was composed of 16 egg clutches collected from the wild in South-West France (Ariège). Of these, 10 originated from Orgibet, two from Montégut-en-Couserans, three from Moulis and one from St-Girons (Table 1). Female *P. brassicae* usually mate only once and lay clutches of full-sibs (Feltwell, 1982) (N.L. and S.D., unpublished). The two types of clutches will be hereafter called 'breeding' and 'field' families. All individuals were the offspring of adults from diapaused pupae, emerged in June and July 2011 and were reared under similar conditions. Families were held separately in a climate chamber under photoperiod control (14 h:10 h light:dark period) and fixed temperatures (23±1°C in light periods, 18±1°C

Table 1. Number and origin of the butterflies used for flight direction tests

| Family code | N males | N females | Date of emergence | Origin | Type |
|-------------|---------|-----------|-------------------|-----------|----------|
| A | 4 | 4 | 26/06/11 | Visan | Breeding |
| B | 4 | 4 | 29/06/11 | Visan | Breeding |
| C | 4 | 4 | 25/06/11 | Visan | Breeding |
| D | 4 | 4 | 25/06/11 | Visan | Breeding |
| E | 10 | 9 | 10/07/11 | St-Girons | Field |
| F | 10 | 10 | 31/07/11 | Orgibet | Field |
| G | 10 | 10 | 29/07/11 | Orgibet | Field |
| H | 10 | 10 | 05/08/11 | Orgibet | Field |
| I | 10 | 10 | 26/07/11 | Orgibet | Field |
| J | 10 | 10 | 29/07/11 | Orgibet | Field |
| K | 10 | 10 | 28/07/11 | Orgibet | Field |
| L | 10 | 10 | 26/07/11 | Orgibet | Field |
| M | 10 | 10 | 26/07/11 | Orgibet | Field |
| N | 10 | 10 | 27/07/11 | Orgibet | Field |
| O | 10 | 10 | 28/07/11 | Orgibet | Field |
| P | 10 | 10 | 28/07/11 | Montégut | Field |
| Q | 10 | 10 | 29/07/11 | Montégut | Field |
| R | 9 | 3 | 27/06/11 | Moulis | Field |
| S | 10 | 7 | 24/06/11 | Moulis | Field |
| T | 10 | 10 | 27/06/11 | Moulis | Field |

'Breeding' families were bred in captivity for more than four generations. 'Field' families were collected in the wild.

in dark periods) inducing direct development. After hatching, larvae were bred in 40×20×10 cm boxes within the same climate chamber, and were fed *ad libitum* with fresh cabbage. During the first 24 h after adult emergence, each butterfly was individually marked and placed in a 1×1×1 m breeding cage with a water supply and nectariferous flowers. Males and females were separated regardless of their family and experienced similar densities in the laboratory. In total, 340 butterflies were used in the experiment (Table 1).

Flight direction

The assessment of flight direction was performed using a slightly modified version of an experimental design developed previously (Spieth and Kaschuba-Holtgrave, 1996). It consisted of a large outdoor cage (2×2×2 m), the sides of which were covered with gauze, with an octagonal base on which cardinal points were represented. The cage was fixed at exactly the same place in Moulis (Ariège, France) during all the experiments. Each butterfly was tested individually to prevent interactions with conspecifics inside the cage. Butterflies were all kept under similar laboratory conditions before the experiment. In order to slow down their metabolism and therefore to force them to acquire perceptual information before flying, individuals were cooled in a fridge for 30 s (5±1°C). They were then immediately placed in a 10×10×10 cm box covered by an opaque piece of tissue with an articulated arm that allowed the box to be opened on demand whilst the experimenter stayed outside the experimental cage. Thus, each butterfly was considered naive at the beginning of the experiment, i.e. facing a completely new environment. The direction of the body was randomized for each butterfly at the beginning of the experiment. Individuals were released in the centre of the cage and the first contact with the gauze was used as the measure of flight direction. Two subsets of individuals were tested. The first experiment, to quantify intra-individual variation in flight direction, was repeated 10 times on 10 individuals (1 male and 1 female from field family S and 1 male and 1 female from breeding families A, B, C and D). Replicates were performed at 1 h intervals over two consecutive days. Spieth and colleagues (Spieth and Kaschuba-Holtgrave, 1996; Spieth et al., 1998) showed that the main flight direction was constant over time and external conditions. To confirm this finding, this first set of individuals was also used to test whether the first record of flight direction was a good predictor of the mean individual flight direction over time. The first record of flight direction was the variable used in the second experiment where 340 individuals from the 20 families (175 females and 165 males) were used to quantify inter-individual variation in flight direction. Tests were conducted in June and July 2011 between 13:00 h and 18:00 h in sunny weather and temperatures greater than 25°C. Each butterfly was less than 3 days old when tested for the first time because dispersal events as well as migratory movements in females are performed during the first days of life in this species (Blunck, 1954; Trochet et al., 2013).

Mobility

In butterflies, flight capacity and wing length are likely to be fundamental in migratory behaviours. In addition, these traits have been described as being fundamental to dispersal capacity (Baguette et al., 2000; Berwaerts et al., 2002; Louy et al., 2007; Ockinger et al., 2010; Sekar, 2012; Stevens et al., 2012). We thus used a measure of these two traits on 70 butterflies (10 males and 9 females in total from breeding families A, C and D, and 30 males and 21 females in total from field families E, G, L, M, O, P and S) to test for their relationship with flight direction. Measurements of mobility were performed prior to flight direction tests.

Flight capacity

One day after emergence, butterflies were individually introduced into a 25×10×10 cm plastic chamber perforated at its base, and fixed to a rapid agitator (Vortex Genie 2, Scientific Industries, Bohemia, NY, USA). Experiments were performed at 25±1°C. Each butterfly was allowed to habituate to the chamber for 1 min. We then turned the vortex on, which strongly shook the chamber and impeded the perching of the butterfly on the chamber wall, and the butterfly's behaviour was observed for 1 min. During the test, the butterfly could either fly or lie uncomfortably at the bottom of the chamber, in a reduced area strongly shaken by the vortex. The time an individual spent flying during the test was calculated, with high values reflecting good mobility. This test has been described previously (Ducatez et al., 2012b) and is known as a good proxy of dispersal in experimental metapopulations (Legrand et al., 2012). Butterflies were never harmed by the test and previous studies showed that other tested butterflies flew and mated in experimental conditions (Ducatez et al., 2012b; Trochet et al., 2013).

Wing length

Immediately after the mobility test, butterflies were anaesthetized with nitric oxide in a 10×10×10 cm box (Inject+Matic *Sleeper* TAS, Geneva, Switzerland) and wing lengths were measured using a calliper by the same experimenter.

Statistical analyses

Data analyses were performed using R software version 2.12 (The R Foundation for Statistical Computing, Vienna, Austria) and the *circular* R-package (Agostinelli and Lund, 2011). The uniformity of the circular distribution of flight directions both within individuals (intra-individual variation) and within families (inter-individual variation) was tested using Rayleigh tests. Circular ANOVA were then used to compare the distributions of intra-individual (in total and between days) and intra-family flight directions. Spearman's correlation tests were used to assess the relationship between (i) individual mobility or wing length and consistency in flight direction, both in males and in females, and (ii) families' average mobility or families' average wing length and the variance in flight direction of these families. As the values in the mobility test exhibited a clearly bimodal distribution, individuals tested for inter-individual variation were classified into two subsets based on the median of the distribution: (i) individuals with high mobility (flying more than 35 s in the mobility test) and (ii) individuals with low mobility (flying for less than 20 s in the mobility test). Wilcoxon tests were performed to investigate the difference in the deviation from the mean flight direction of the family in high and low mobility groups. The same approach was used to test for the difference in inter-individual variation in flight direction between long-winged individuals and small-winged individuals. Groups were also defined according to the median of the distribution of wing lengths.

RESULTS

Intra-individual variation in flight direction

Among the 10 tested individuals, seven exhibited a preferential flight direction when released 10 times in the cage (all five females and two males with significant Rayleigh tests, Table 2; Fig. 1). The two individuals from a field family had a non-random distribution of flight directions, contrary to individuals in breeding families (five out of eight). Five individuals had a different mean flight direction between the first and second day of recording and all of these were from breeding families (Table 2). Females from breeding families flew in a range of angles between 0 and 90 deg (i.e. in northerly or

Table 2. Comparison of the first and subsequent flight directions

| Individual | Family | Sex | First value of flight direction | Mean flight direction over 10 assays | Mean flight direction without first assay | Upper limit | Lower limit | F-statistic (Rayleigh) | P-value | Comparison of day 1 and 2 (ANOVA) | P-value |
|------------|--------|-----|---------------------------------|--------------------------------------|---|-------------|-------------|------------------------|------------------|-----------------------------------|--------------|
| F1 | A | ♀ | 90 | 89.32 | 97.28 | 354.3 | 184.3 | 0.6992 | 0.005 | 0.003 | 0.954 |
| F2 | B | ♀ | 340 | 0.43 | 11.79 | 259.2 | 101.6 | 0.6663 | 0.008 | 16.59 | 0.004 |
| F3 | C | ♀ | 40 | 25.41 | 16.61 | 292.2 | 118.6 | 0.7086 | 0.004 | 11.95 | 0.009 |
| F4 | D | ♀ | 20 | 31.94 | 30.5 | 279.2 | 144.7 | 0.6038 | 0.022 | 7.049 | 0.003 |
| F5 | S | ♀ | 150 | 187.18 | 201.73 | 69.49 | 304.9 | 0.5775 | 0.031 | 2.092 | 0.186 |
| M1 | A | ♂ | 20 | 5.37 | 5.49 | 287.6 | 83.2 | 0.7866 | <0.001 | 4.7 | 0.062 |
| M2 | B | ♂ | 80 | 43.59 | 27.49 | 256.6 | 190.6 | 0.4243 | 0.167 | 0.191 | 0.674 |
| M3 | C | ♂ | 150 | 134.69 | 132.7 | 0.6 | 268.8 | 0.4904 | 0.088 | 6.274 | 0.037 |
| M4 | D | ♂ | 270 | 246.92 | 257.08 | 98.01 | 35.83 | 0.4151 | 0.181 | 9.938 | 0.014 |
| M5 | S | ♂ | 90 | 82.79 | 86.05 | 338.7 | 186.9 | 0.6504 | 0.011 | 0.186 | 0.678 |

Flight direction is given in degrees and was measured over 10 assays per individual. The first value was within the 95% confidence interval and close to the average flight direction for each tested butterfly.

Bold values indicate individuals with a significant non-random distribution of flight directions (Rayleigh uniformity test).

westerly directions) although they differed in their mean flight directions ($F=4.85$, d.f.=3, $P<0.01$, circular ANOVA). Males from breeding families exhibited very different mean flight directions ($F=14.13$, d.f.=3, $P<0.01$, circular ANOVA, Fig. 1). The first flight direction was generally very close to the average direction of the nine subsequent assays, as suggested by Table 2 (mean difference over all individuals between the first assay and the others, 22.59 deg), and was always within the 95% confidence interval around the individual mean flight direction. Furthermore, we found a positive correlation between the first assay and the individual average direction ($r=0.947$, $N=10$, $P=0.013$, Spearman's correlation). Altogether, these results provide good arguments to support the use of the first value as a proxy of flight direction in *P. brassicae*.

Inter-individual variation in flight direction

On average, individuals flew in a northward direction (mean 20.76 deg, $N=175$ and 165 for females and males, respectively, $P=0.024$, Rayleigh test). In addition, males and females displayed a similar average flight direction ($F=0.231$, d.f.=1, $P=0.632$, circular ANOVA). The flight direction of eight out of the 20 tested families, including the four breeding families, was different among siblings (Table 3).

In other words, in 12 out of the 16 field families, siblings flew in a similar direction. Additionally, field families exhibited different average flight direction ($F=50.58$, d.f.=3, $P<0.001$, circular ANOVA).

Relationship between flight direction and individual mobility

For the 70 individuals tested for a correlation between mobility traits and flight direction, we did not find a difference in mobility between males ($N=40$) and females ($N=30$, $P=0.283$, Wilcoxon test) even though females had larger wings than males ($P=0.013$, Wilcoxon test). Interestingly, among the 10 individuals tested for intra-individual variation, the standard deviation in flight direction was similar between males ($N=5$) and females ($N=5$, $P=0.31$, Wilcoxon test) as well as wing length ($P=0.222$, Wilcoxon test). However, males were slightly more mobile ($P=0.056$, Wilcoxon test).

The standard deviation in flight direction calculated over the 10 replicates performed for 10 individuals was strongly related to mobility and wing length ($r=-0.93$, $N=10$, $P<0.001$ for wing length and $r=-0.90$, $N=10$, $P<0.001$ for mobility, Spearman's correlation). These correlations persisted when considering the sexes separately for mobility ($r=-0.9$, $N=5$, $P=0.083$ in both males and females, Spearman's correlation). The correlation also persisted for wing

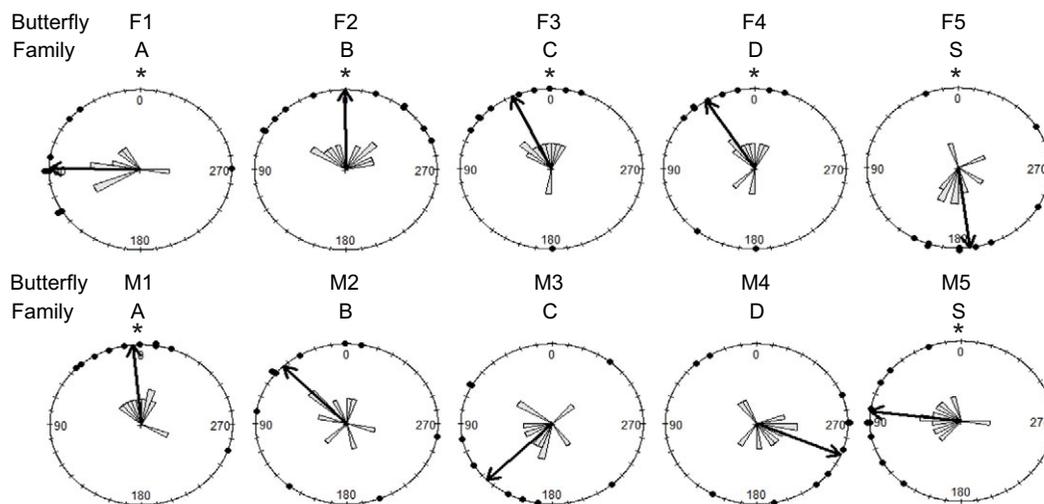


Fig. 1. Flight directions in degrees (0 deg represents North) of 10 butterflies (five females: F1 to F5 and five males: M1 to M5). F1 and M1, F2 and M2, F3 and M3, F4 and M4 originated from 'breeding' families A, B, C and D, respectively. F5 and M5 originated from 'field' family S. Each dot represents the first contact with the gauze over the 10 replicates of the experiment. The arrows represent the average individual flight direction. Asterisks indicate individuals with a significantly non-random distribution of flight directions.

Table 3. Mean flight direction for the 20 families and significance of the Rayleigh uniformity test

| Family | Type | N males | N females | Mean flight direction (deg) | F-statistic (Rayleigh) | P-value |
|--------|----------|---------|-----------|-----------------------------|------------------------|------------------|
| A | Breeding | 4 | 4 | 46.16 | 0.209 | 0.717 |
| B | Breeding | 4 | 4 | 353.93 | 0.292 | 0.519 |
| C | Breeding | 4 | 4 | 95.65 | 0.330 | 0.432 |
| D | Breeding | 4 | 4 | 352.91 | 0.227 | 0.675 |
| E | Field | 10 | 9 | 103.13 | 0.574 | 0.001 |
| F | Field | 10 | 10 | 151.97 | 0.722 | <0.001 |
| G | Field | 10 | 10 | 87.64 | 0.564 | 0.001 |
| H | Field | 10 | 10 | 0.74 | 0.741 | <0.001 |
| I | Field | 10 | 10 | 17.64 | 0.523 | 0.003 |
| J | Field | 10 | 10 | 315.67 | 0.702 | <0.001 |
| K | Field | 10 | 10 | 184.89 | 0.581 | <0.001 |
| L | Field | 10 | 10 | 233.19 | 0.232 | 0.345 |
| M | Field | 10 | 10 | 45.18 | 0.539 | 0.002 |
| N | Field | 10 | 10 | 272.75 | 0.586 | <0.001 |
| O | Field | 10 | 10 | 58.67 | 0.644 | 0.001 |
| P | Field | 10 | 10 | 313.24 | 0.481 | 0.008 |
| Q | Field | 10 | 10 | 218.36 | 0.572 | <0.001 |
| R | Field | 9 | 3 | 32.77 | 0.265 | 0.439 |
| S | Field | 10 | 7 | 14.34 | 0.141 | 0.718 |
| T | Field | 10 | 10 | 324.97 | 0.333 | 0.108 |

Bold values indicate families with a significantly non-random distribution of flight directions.

length in females ($r=-0.9$, $N=5$, $P=0.083$, Spearman's correlation) but not in males ($r=-0.7$, $N=5$, $P=0.23$, Spearman's correlation). Thus, butterflies exhibiting very consistent directions throughout the assays were long-winged and highly mobile individuals (Fig. 2). Importantly, wing length and mobility were themselves highly correlated in these 10 individuals ($r=0.91$, $N=10$, $P<0.001$, Spearman's correlation), although this correlation was not found in the overall sample ($r=0.13$, $N=70$, $P=0.251$, Spearman's correlation). This is probably due to the over-representation of good flyers (i.e. mobile individuals) in the overall sample (mobility 33.1 ± 7.9 and 40.5 ± 20.4 s and wing length 23.9 ± 0.98 and 24.4 ± 1.7 mm in the subsample and the overall sample, respectively). Also, the subset

composed of highly mobile individuals showed a difference to the mean flight direction of their families that was marginally different from that of the less mobile group ($N_{\text{high}}=53$, $N_{\text{low}}=17$, $P=0.058$, Wilcoxon test, Fig. 3A). In other words, more mobile individuals tended to fly in directions similar to that of their siblings. In contrast, the same analysis showed that the two subsets of individuals with longer or smaller wings exhibited the same deviation to the mean flight direction of their family ($N_{\text{high}}=N_{\text{low}}=35$, $P=0.978$, Wilcoxon test). There was also a negative correlation between the variance in flight direction of the family and the average mobility ($r=-0.684$, $N=10$, $P=0.035$, Spearman's correlation, Fig. 3B). That is, families composed of individuals with high mobility also displayed a higher consistency in flight direction. The same analysis revealed no correlation between the variance in flight direction and the average wing length ($r=0.305$, $N=10$, $P=0.271$, Spearman's correlation).

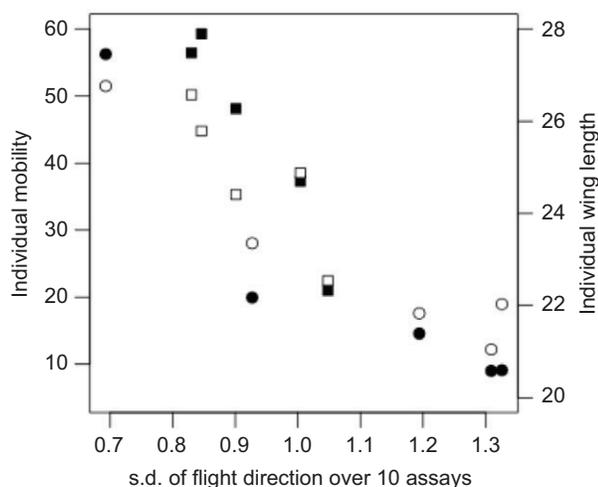


Fig. 2. Standard deviation of the distribution of flight direction (in deg) in the 10 individuals (males, circles; females, squares) used to test the intra-individual variation in flight direction (individuals released 10 times in the orientation cage). The standard deviation of the resulting distribution is negatively correlated to mobility (filled symbols, $r=-0.9$, $P<0.001$, Spearman's correlation) and to wing length (open symbols, $r=-0.93$, $P<0.001$, Spearman's correlation).

DISCUSSION

Repeatability and mode of inheritance of flight direction

This study highlights the consistency in flight direction in *P. brassicae*. Indeed, we have shown that intra-individual variation in flight direction was low when individuals were repetitively subjected to the same experiment. Our estimations were made over 2 days, while *P. brassicae* individuals live for around 10 days, and half of the individuals presented different mean flight directions between the 2 days of the test. In contrast, Spieth and Kaschuba-Holtgrave (Spieth and Kaschuba-Holtgrave, 1996) showed that flight direction in *P. brassicae* did not vary with advancing age of individuals. The difference we observed between the 2 days of the test may thus be due either to undetermined environmental factors that varied between the 2 days of the test or to poor statistical support due to a limited sample size (only five assays performed during a period of 2 days). The latter hypothesis is also supported by the observation that individuals showed different flight directions between days 1 and 2 while exhibiting a preferred flight direction over the 10 assays. Our results thus contrast with the results of a study in birds showing that flight direction is likely to vary over a lifetime (Moore, 1984). Birds are likely to migrate several times over their lifetime, so they will benefit from their previous experience and adjust their flight

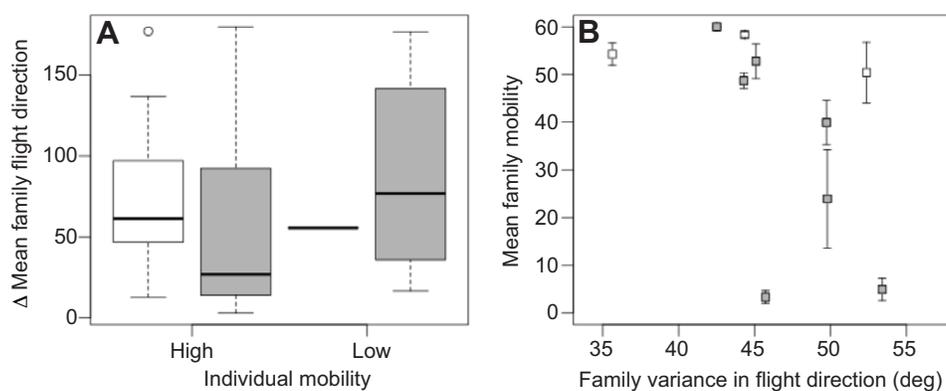


Fig. 3. (A) Individual deviation from the mean family flight direction of highly and poorly mobile groups in breeding ($N_{\text{high}}=18$, $N_{\text{low}}=1$) and field ($N_{\text{high}}=35$, $N_{\text{low}}=16$) families. (B) Negative correlation between the variance in flight direction of a family (in degrees) and its average mobility ($r=-0.684$, $N=10$, $P=0.035$). Error bars represent s.e.m. In A and B, breeding families are represented by open symbols and field families by filled symbols.

direction accordingly. According to Newton (Newton, 2007), age differences in migration patterns can be the result of at least two factors: the timing of annual cycle events, such as moult, and body size or dominance, which depends on age class. In contrast, migration occurs only once in insect species and is probably determined by genetic or epigenetic factors.

Interestingly, although individuals showed a general tendency to orientate in northern directions, inter-individual variation in flight direction was found both between and within families, which may have several non-exclusive origins. Flight direction may be inherited in *P. brassicae* (and may possibly be due to parental effects), which would be consistent with the studies of Spieth and colleagues (Spieth and Kaschuba-Holtgrave, 1996; Spieth and Cordes, 2012). In the sister species *Pieris rapae*, Baker also concluded that migratory direction was a selected and inherited trait that was independent of the mother's orientation and was determined solely by the male parent (Baker, 1968). Alternatively, variation can reflect different responses to environmental cues not measured in our experiment (daytime, wind, larvae density, etc.). Nevertheless, tests were performed under very similar conditions (temperature, sunshine, position of the experimental cage), individuals were bred under similar conditions, and orientation was highly repeatable in this species [see our results and those of Spieth and colleagues (Spieth and Kaschuba-Holtgrave, 1996; Spieth et al., 1998)]. This gives more credit to the inheritance hypothesis than to the environmental hypothesis. Interestingly, both innate and environmentally induced orientation have been described in migrating birds (Pasinelli et al., 2004; Ogonowski and Conway, 2009) and flight direction depends greatly on the flight direction of the mother. Other experiments are now needed to enable conclusions to be drawn on the mode of inheritance of flight direction in *P. brassicae*, especially as inherited traits related to migration and dispersal have been suggested to have both a genetic (Spieth and Cordes, 2012) and a non-genetic basis (Ducatez et al., 2012a) in this species.

In this work, we compared results between field and breeding families of butterflies in the same experiment. Although caution must be taken with regard to our conclusions because of the very limited sample size, in terms of both the number of breeding families ($N=4$) compared with field families ($N=16$) and the number of individuals in breeding families ($N=8$) compared with the number of individuals in field families ($N=12-20$), this experiment is particularly informative because the comparison between two sets of individuals that either originated from a natural environment or were bred in captivity for several generations has been poorly used in migratory birds (Helbig, 1996) or in insects (but see Nesbit et al., 2009). In our design, butterflies kept under breeding conditions for a minimum of four generations did not show consistency in their flight direction, unlike the majority of field families, which may

suggest a loss of migratory skills in captive butterflies in a very small temporal scale (only a few generations). This would be congruent with the rapid change of migratory behaviour described in the blackcap *Sylvia atricapilla* (Berthold et al., 1992). Results are different in reared butterflies of *V. cardui* that exhibited a significant bias in flight direction, whereas field butterflies flew in different directions (Nesbit et al., 2009). However, this species uses a sun compass to orientate contrary to *P. brassicae*. An alternative hypothesis would support the existence of different adaptations between breeding and field families because of the different geography of their sampling locations (breeding families are from the Vaucluse and field families are from Ariège, about 400 km to the west). Geographical location is a major factor influencing flight direction in *P. brassicae* because individuals adapt their flight direction to the geographical barriers they face (Spieth and Cordes, 2012). Nonetheless, variation was observed between families from Ariège, suggesting that the mechanisms underlying flight direction in this species are more complex than simply being geography dependent. Further experimental studies with sufficient sample size are now required to definitely conclude on the existence of a rapid shift in flight direction in *P. brassicae* and to characterize the mechanisms underlying such variation.

Migration routes and local adaptation in *P. brassicae*

Our results show that the mean flight direction was skewed towards North. All butterflies emerged in June and July 2011, meaning that they were from the first generations of the year. The northern preference of individuals is thus in accordance with the migratory routes described in *P. brassicae*, with spring and summer generations migrating northward and autumn generations migrating southward in Europe (Feltwell, 1982; Spieth and Cordes, 2012). Nonetheless, it is interesting to note that families with uniform distributions in flight direction sampled in the same site during the same period were not all orientated in the same direction (see families J and K in Table 3). This means that migratory patterns may be different in the same geographical region. Several mechanisms can explain this pattern. First, different migration sites may exist in *P. brassicae* that are genetically determined and have not been described yet. Second, individuals may experiment with different routes to cross the barriers encountered during their migration in northern directions (our field families were all sampled in the French Pyrenees). This hypothesis is congruent with the results of Spieth and Cordes (Spieth and Cordes, 2012), which showed that *P. brassicae* butterflies change their flight direction during migration according to the geographic barriers they face. Third, in the case of maternal transmission of flight direction, mothers may modulate the mean flight direction of their progeny either to avoid kin competition when they lay several clutches in the same

location or to avoid intra-specific competition when several mothers lay egg clutches at the same place. Finally, different flight directions between families in the same location may account for non-migrating strategies, i.e. resident strategies, corresponding to local adaptations. Feltwell (Feltwell, 1982) (and references therein) suggested that only some of *P. brassicae* individuals are migrants. More generally, insects are often facultative migrants that respond to changes in habitat availability, quality or level of crowding, reflecting bet-hedging strategies (Holland et al., 2006). This statement makes sense when considering the variation in flight direction in *P. brassicae* between individuals of the same families. Indeed, partitioning the progeny into migrant and non-migrant individuals represents a good strategy to limit the negative impact of rapid changes in environmental conditions or to adapt to local favourable conditions. It is noteworthy that local adaptation in *P. brassicae* has been observed in traits related to migration and mobility (Spieth and Cordes, 2012; Ducatez et al., 2013).

Relationship with flight performance

The existence of behavioural syndromes has been extensively documented in recent years and appears central to the evolution of organisms (Sih et al., 2004; Sih et al., 2012). These syndromes rely on a suite of phenotypic traits that co-evolve to determine the behavioural types of individuals. Such syndromes have been extensively studied in the context of migration because migration implies selection on morphological characters for long distance movements (Van Noordwijk et al., 2006) resulting in different characteristics between migrant and resident individuals (Sandberg and Moore, 1996; Löhmus et al., 2003; Marchetti and Zehindjiev, 2009). For example, Leisler and Winkler showed that migrant birds are generally smaller and have shorter tails and longer wings than non-migrant individuals (Leisler and Winkler, 2003). Interestingly, *P. brassicae* is a facultative migrant and exhibits a syndrome related to mobility (Ducatez et al., 2012b), which could be related to its migratory behaviour. Our results demonstrate that flight direction is correlated to some of these mobility traits, both in males and in females. Although females were larger winged, we did not find any difference in mobility or standard deviation in flight direction between males and females, which suggests that this correlation is a general pattern in the species, and not the result of a divergence in dispersal traits between sexes. In particular, we found a correlation between intra-individual variation in flight direction and mobility for both sexes and between intra-individual variation in flight direction and wing length for females (the absence of correlations for males probably results from the small sample size used to perform the rank test, $N=5$). It is noteworthy that mobility and wing length were strongly correlated in the subset of individuals used to test repeatability in flight direction. Although the origin of this correlation remains unknown, this means that good performers, with longer wings, exhibit lower variation in their flight direction than bad performers, with smaller wings. Further, the results show that good performers were also those whose flight direction was the closest to the mean flight direction of their families of origin (Fig. 3A), a relationship that was not found using long-winged and small-winged groups of individuals. In other words, highly mobile siblings have a tendency to orientate in the same direction. The observed negative correlation between the variance in flight direction of a family and its average mobility (non-significant result when using average wing length) is also congruent with the hypothesis of a migratory syndrome in *P. brassicae* involving flight direction and mobility. Altogether, our results are consistent with those of a previous study (Bäckman and Alerstam, 2003), which showed that fast flying bird species had more accurate heading directions (i.e.

less intra-individual variation). Future research should focus on the evolutionary mechanisms underlying such interactions between traits as it is difficult to know which traits effectively co-evolved into a migratory syndrome and which correlations are simple by-products of selection acting independently on traits responsible for migration (Pulido and Berthold, 2003; Pulido, 2007; Teplitsky et al., 2011).

The dispersal behaviour also implies a suite of correlated traits that may be the same as those described in migratory syndromes. This implies that dispersers on the one hand and residents on the other hand may harbour the same characteristics. For example, in *P. brassicae*, dispersers have been shown to exhibit longer wings and higher mobility than residents (Legrand et al., 2012). Dispersers and residents may also use different types of movements. Dispersal has been described as a mixture of fast directed movements and routine movements (e.g. foraging, mating, etc.) in several butterfly species (Van Dyck and Baguette, 2005; Hovestadt et al., 2011). Consequently, it has been hypothesized that residents may restrict their movements to the routine type, thus leading to a potential uncoupled evolution of routine and dispersal movements. Such patterns may form the basis of a divergent evolution of flight direction in both dispersers and residents, as the two types of movement require different capacities. To prevent the return to previously visited areas, dispersers should exhibit rectilinear trajectories, implying little intra-individual variation, whereas residents should benefit more from a more accurate habitat exploration, and thus should be able to shift easily between different directions. These differences in types of movement between dispersers and residents would lead exactly to the patterns of intra- and inter-individual in flight direction we observed in this study. In particular, the intra-family variation in flight direction (putative presence of residents and dispersers in the same family) can be viewed as a way to maximize the survival of the progeny because some siblings will optimally use the local resources while others will search for new reproductive sites (bet-hedging strategy, see above). Furthermore, the difference in intra-individual variation in flight direction between females and males in our study – all females exhibited a significantly non-random distribution in flight direction in contrast to males (two out of five only) – would explain the tendency of females to disperse more in *P. brassicae* (D.L., A.T., O.C. and M.B., unpublished).

CONCLUSIONS

To conclude, our results demonstrate that flight direction, a fundamental trait involved in both migration and dispersal behaviour, is probably inherited and highly variable in *P. brassicae* populations. This study also adds new elements to the mobility syndrome described in *P. brassicae*. We have shown that highly mobile individuals, which have been described as being more prone to exploration and bolder than less mobile individuals, also have a tendency to keep the same flight direction and to orientate in the same flight direction. Clearly, *P. brassicae* populations contain individuals with distinct phenotypes corresponding to different migration and/or dispersal strategies, opening the possibility of the co-existence of local adaptations and bet-hedging strategies in this butterfly.

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AUTHOR CONTRIBUTIONS

D.L. and M.B. designed the experiment. N.L., D.L. and O.C. conceived the experimental setting and performed the experiment. N.L., D.L. and M.B. wrote the manuscript. N.L., S.D. and A.T. performed the statistical analyses. All authors participated in the interpretation of the results, and read and approved the manuscript.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

- Agostinelli, C. and Lund, U. (2011). R package 'circular'. Circular statistics (version 0.4-3). <https://r-forge.r-project.org/projects/circular/>.
- Åkesson, S. and Hedenström, A. (2007). How migrants get there: migratory performance and orientation. *Bioscience* **57**, 123-133.
- Alerstam, T. and Lindström, A. (1990). Optimal bird migration: the relative importance of time, energy and safety. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 331-351. Berlin: Springer.
- Bäckman, J. and Alerstam, T. (2003). Orientation scatter of free-flying nocturnal passerine migrants: components and causes. *Anim. Behav.* **65**, 987-996.
- Baguette, M., Petit, S. and Queva, F. (2000). Population spatial structure and migration of three butterfly species within the same habitat network: consequences for conservation. *J. Appl. Ecol.* **37**, 100-108.
- Baker, R. R. (1968). A possible method of evolution of the migratory habit in butterflies. *Philos. Trans. R. Soc. B* **253**, 309-341.
- Baker, R. R. (1978). *The Evolutionary Ecology of Animal Migration*. London: Hodder and Stoughton.
- Berthold, P. (1991). Genetic control of migratory behaviour in birds. *Trends Ecol. Evol.* **6**, 254-257.
- Berthold, P. (1996). *Control of Bird Migration*. London: Chapman & Hall.
- Berthold, P., Helbig, A. J., Mohr, G. and Querner, U. (1992). Rapid microevolution of migratory behaviour in a wild bird species. *Nature* **360**, 668-670.
- Berwaerts, K., Van Dyck, H. and Aerts, P. (2002). Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Funct. Ecol.* **16**, 484-491.
- Blunck, H. (1954). Beobachtungen fiber Wanderflüge von *Pieris brassicae*. *Beitr. Entomol.* **4**, 485-528.
- Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M. et al. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature* **403**, 537-540.
- Chapman, J. W., Nesbit, R. L., Burgin, L. E., Reynolds, D. R., Smith, A. D., Middleton, D. R. and Hill, J. K. (2010). Flight orientation behaviors promote optimal migration trajectories in high-flying insects. *Science* **327**, 682-685.
- Clobert, J., Le Galliard, J.-F., Cote, J., Meylan, S. and Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecol. Lett.* **12**, 197-209.
- Dingle, H. (2001). The evolution of migratory syndromes in insects. In *Insect Movement: Mechanisms And Consequences. Proceedings of the Royal Entomological Society's 20th Symposium* (ed. I. Woivod, D. R. Reynolds and C. D. Thomas), pp. 159-181. Wallingford, UK: CABI.
- Dingle, H. (2006). Animal migration: is there a common migratory syndrome? *J. Ornithol.* **147**, 212-220.
- Ducatez, S., Baguette, M., Stevens, V. M., Legrand, D. and Fréville, H. (2012a). Complex interactions between paternal and maternal effects: parental experience and age at reproduction affect fecundity and offspring performance in a butterfly. *Evolution* **66**, 3558-3569.
- Ducatez, S., Legrand, D., Chaput-Bardy, A., Stevens, V. M., Fréville, H. and Baguette, M. (2012b). Inter-individual variation in movement: is there a mobility syndrome in the large white butterfly *Pieris brassicae*? *Ecol. Entomol.* **37**, 377-385.
- Ducatez, S., Baguette, M., Trochet, A., Chaput-Bardy, A., Legrand, D., Stevens, V. and Fréville, H. (2013). Flight endurance and heating rate vary with both latitude and habitat connectivity in a butterfly species. *Oikos* **122**, 601-611.
- Dyer, F. C. and Could, J. L. (1983). Honey bee navigation: the honey bee's ability to find its way depends on a hierarchy of sophisticated orientation mechanisms. *Am. Sci.* **71**, 587-597.
- Feltwell, J. (1982). *Large White Butterfly: The Biology, Biochemistry and Physiology of Pieris Brassicae (Linnaeus)*. The Hague: W. Junk Publishers.
- Hedenström, A. (2008). Adaptations to migration in birds: behavioural strategies, morphology and scaling effects. *Philos. Trans. R. Soc. B* **363**, 287-299.
- Helbig, A. (1996). Genetic basis, mode of inheritance and evolutionary changes of migratory directions in palaeartic warblers (Aves: Sylviidae). *J. Exp. Biol.* **199**, 49-55.
- Helbig, A., Berthold, P., Mohr, G. and Querner, U. (1994). Inheritance of a novel migratory direction in Central-European blackcaps. *Naturwissenschaften* **81**, 184-186.
- Holland, R. A., Wikelski, M. and Wilcove, D. S. (2006). How and why do insects migrate? *Science* **313**, 794-796.
- Hovestadt, T., Binzenhöfer, B., Nowicki, P. and Settele, J. (2011). Do all inter-patch movements represent dispersal? A mixed kernel study of butterfly mobility in fragmented landscapes. *J. Anim. Ecol.* **80**, 1070-1077.
- Legrand, D., Guillaume, O., Baguette, M., Cote, J., Trochet, A., Calvez, O., Zajitschek, S., Zajitschek, F., Lecomte, J., Bénard, J. et al. (2012). The Metatron: an experimental system to study dispersal and metaecosystems for terrestrial organisms. *Nat. Methods* **9**, 828-833.
- Leisler, B. and Winkler, H. (2003). Morphological consequences of migration in passerines. In *Avian Migration* (ed. P. Berthold, E. Gwinner and E. Sonnenschein), pp. 175-186. Berlin: Springer-Verlag.
- Liedvogel, M., Åkesson, S. and Bensch, S. (2011). The genetics of migration on the move. *Trends Ecol. Evol.* **26**, 561-569.
- Löhmus, M., Sandberg, R., Holberton, R. L. and Moore, F. (2003). Corticosterone levels in relation to migratory readiness in red-eyed vireos (*Vireo olivaceus*). *Behav. Ecol. Sociobiol.* **54**, 233-239.
- Louy, D., Habel, J. C., Schmitt, T., Assmann, T., Meyer, M. and Muller, P. (2007). Strongly diverging population genetic patterns of three skipper species: the role of habitat fragmentation and dispersal ability. *Conserv. Genet.* **8**, 671-681.
- Marchetti, C. and Zehndtjiev, P. (2009). Migratory orientation of sedge warblers (*Acrocephalus schoenobaenus*) in relation to eating and exploratory behaviour. *Behav. Processes* **82**, 293-300.
- Moore, F. (1984). Age-dependent variability in the migratory orientation of the savannah sparrow (*Passerculus sandwichensis*). *Auk* **101**, 875-880.
- Nesbit, R. L., Hill, J. K., Woivod, I. P., Sivell, D., Bensusan, K. J. and Chapman, J. W. (2009). Seasonally adaptive migratory headings mediated by a sun compass in the painted lady butterfly, *Vanessa cardui*. *Anim. Behav.* **78**, 1119-1125.
- Newton, I. (2007). *The Migration Ecology of Birds*. Amsterdam: Elsevier.
- Ockinger, E., Schweiger, O., Crist, T. O., Debinski, D. M., Krauss, J., Kuussaari, M., Petersen, J. D., Pöyry, J., Settele, J., Summerville, K. S. et al. (2010). Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. *Ecol. Lett.* **13**, 969-979.
- Ogonowski, M. S. and Conway, C. J. (2009). Migratory decisions in birds: extent of genetic versus environmental control. *Oecologia* **161**, 199-207.
- Pasinelli, G., Schiegg, K. and Walters, J. R. (2004). Genetic and environmental influences on natal dispersal distance in a resident bird species. *Am. Nat.* **164**, 660-669.
- Pulido, F. (2007). The genetics and evolution of avian migration. *Bioscience* **57**, 165-174.
- Pulido, F. and Berthold, P. (2003). Quantitative genetic analysis of migratory behavior. In *Avian Migration* (ed. P. Berthold, E. Gwinner and E. Sonnenschein), pp. 53-77. Berlin: Springer-Verlag.
- Reilly, J. R. and Reilly, R. J. (2009). Bet-hedging and the orientation of juvenile passerines in fall migration. *J. Anim. Ecol.* **78**, 990-1001.
- Roff, D. A. (1992). *Evolution of Life Histories: Theory and Analysis*. New York: Chapman & Hall.
- Roff, D. A. and Fairbairn, D. J. (2001). The genetic basis of dispersal and migration and its consequences for the evolution of correlated traits. In *Dispersal* (ed. J. Clobert), pp. 191-202. Oxford: Oxford University Press.
- Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu. Rev. Ecol. Syst.* **38**, 231-253.
- Sandberg, R. and Moore, F. (1996). Migratory orientation of red-eyed vireos, *Vireo olivaceus*, in relation to energetic condition and ecological context. *Behav. Ecol. Sociobiol.* **39**, 1-10.
- Sekar, S. (2012). A meta-analysis of the traits affecting dispersal ability in butterflies: can wingspan be used as a proxy? *J. Anim. Ecol.* **81**, 174-184.
- Sih, A., Bell, A. and Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol. Evol.* **19**, 372-378.
- Sih, A., Cote, J., Evans, M., Fogarty, S. and Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecol. Lett.* **15**, 278-289.
- Spieth, H. R. and Cordes, R. (2012). Geographic comparison of seasonal migration events of the large white butterfly, *Pieris brassicae*. *Ecol. Entomol.* **37**, 439-445.
- Spieth, H. R. and Kaschuba-Holtgrave, A. (1996). A new experimental approach to investigate migration in *Pieris brassicae* L. *Ecol. Entomol.* **21**, 289-294.
- Spieth, H. R., Cordes, R. G. and Dorka, M. (1998). Flight directions in the migratory butterfly *Pieris brassicae*: results from semi-natural experiments. *Ethology* **104**, 339-352.
- Stefanescu, C., Páramo, F., Åkesson, S., Alarcón, M., Ávila, A., Brereton, T., Carnicer, J., Cassar, L. F., Fox, R., Heliölä, J., et al. (2013). Multi-generational long-distance migration of insects: studying the painted lady butterfly in the Western Palaearctic. *Ecography* **36**, 474-486.
- Stevens, V. M., Turlure, C. and Baguette, M. (2010). A meta-analysis of dispersal in butterflies. *Philos. Trans. R. Soc. B* **365**, 625-642.
- Stevens, V. M., Trochet, A., Van Dyck, H., Clobert, J. and Baguette, M. (2012). How is dispersal integrated in life histories: a quantitative analysis using butterflies. *Ecol. Lett.* **15**, 74-86.
- Teplitsky, C., Mouawad, N. G., Balbontin, J., De Lope, F. and Møller, A. P. (2011). Quantitative genetics of migration syndromes: a study of two barn swallow populations. *J. Evol. Biol.* **24**, 2025-2039.
- Thorup, K., Rabøl, J. and Erni, B. (2007). Estimating variation among individuals in migration direction. *J. Avian Biol.* **38**, 182-189.
- Trochet, A., Legrand, D., Larranaga, N., Ducatez, S., Calvez, O., Cote, J., Clobert, J. and Baguette, M. (2013). Population sex ratio, dispersal and colonisation in experimental butterfly metapopulations. *J. Anim. Ecol.* doi: 10.1111/1365-2656.12082.
- Van Dyck, H. and Baguette, M. (2005). Dispersal behaviour in fragmented landscapes: routine or special movements? *Basic Appl. Ecol.* **6**, 535-545.
- Van Noordwijk, A., Pulido, F., Helm, B., Coppack, T., Delingat, J., Dingle, H., Hedenström, A., Van der Jeugd, H., Marchetti, C., Nilsson, A. et al. (2006). A framework for the study of genetic variation in migratory behaviour. *J. Ornithol.* **147**, 221-233.
- Vowles, D. M. (1954). The orientation of ants I. The substitution of stimuli. *J. Exp. Biol.* **31**, 341-355.
- Zera, A. J. and Brisson, J. A. (2012). Quantitative, physiological, and molecular genetics of dispersal/migration. In *Dispersal Ecology and Evolution* (ed. J. Clobert, M. Baguette and T. G. Benton). Oxford: Oxford University Press.
- Zhu, H., Gegeer, R. J., Casselman, A., Kanginakudru, S. and Reppert, S. M. (2009). Defining behavioral and molecular differences between summer and migratory monarch butterflies. *BMC Biol.* **7**, 14.