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Combination of morphometric and isotopic tools for studying spring migration dynamics in *Episyrphus balteatus*

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Abstract. Migration and population dynamics are important in organisms that provide ecosystem services as they determine the occurrence of individuals in a given place at a given time, which, in turn, determines the efficiency of the service provided. To design appropriate landscape management strategies to improve the efficiency of biological control, it is essential to know the origin of beneficial insects that colonize agricultural fields. However, studying migration dynamics in insects is complicated by their small size and short life span which largely prevent the use of conventional techniques based on capture-mark-recapture, or remote sensing.

Stable isotopes and morphological characters are intrinsic markers that can be used to infer the geographical origin of many organisms including insects. In this study, we first determined whether the hydrogen isotopic ratio and the wing morphometrics are appropriate markers to study the spring migration dynamics of a major aphidophagous hoverfly species (*Episyrphus balteatus*). To this end, we assessed the magnitude of variation of these indicators in seven populations along a north-south gradient in Western Europe. Second, we used the two markers in *E. balteatus* individuals collected in a French agricultural landscape over the course of the spring to assess the proportion of immigrants from southern regions and the period of immigration.

Our results revealed uncertainties associated with the use of the hydrogen isotopic ratio and wing morphometrics, but showed that the spring population of *E. balteatus* in Western France very likely comprises mainly local individuals, with the probable arrival of some immigrants from nearby regions located further south. The low proportion of immigrants in the spring population has important implications for the biological control provided by *E. balteatus* in terms of landscape management strategies.

Key words: biological control; deuterium; *Episyrphus balteatus*; Europe; hoverfly; migration; stable isotopes; wing morphometrics.

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INTRODUCTION

Arable lands are highly disturbed environ-

ments because of crop rotation and farming practices (Bianchi et al. 2006). These frequent disturbances lead to the repeated colonization of

cultivated fields by pests and by their natural enemies (Wissinger 1997). Field colonization can result from arrivals from adjacent fields or field margins, or from large-scale migrations (Vialatte et al. 2007). To be efficient, pest management programs require a sound understanding of field colonization processes. The origin of immigrants and the relative contribution of local versus large-scale immigration are particularly important to determine the appropriate scale and the locations to target when designing management actions. For example, local landscape management measures would not be really efficient if the population is mostly composed by large-scale immigrant. In insects, their small size and short life span complicate the study of their migration behavior at large scale and generally prevent the use of conventional techniques based on capture-mark-recapture, or on remote sensing methods (e.g., GPS or radio tracking). As an alternative, the use of intrinsic markers such as molecular markers, stable isotopes or morphometric markers may help obtain information on the geographical origin of insects without requiring prior marking (Hobson 1999), and their use is increasingly widespread in ecology.

Stable isotopes are widely used powerful tools to infer the trophic position or geographical origin of a broad range of organisms including insects (Hobson et al. 1999, Malausa et al. 2005, Hobson et al. 2012b, Sullivan et al. 2012). Hydrogen has three stable isotopes, including ^1H and ^2H (also called deuterium). Hydrogen isotopic ratio (δD) is calculated from the relative proportions of ^1H and ^2H in a sample. Hydrogen isotopic ratio in precipitation reveal continent-wide patterns that are reflected in plants and ultimately in the tissues of higher trophic level consumers (Hobson 1999). Hence, δD ratio in metabolically inert tissues of insects such as wing chitin can be used to infer the geographical region in which the larvae developed (Hobson et al. 1999). The main difficulty with this method is its low spatial resolving power. Consequently, it is suitable for efficiently tracking movements only at large scales, e.g., at a continental scale (Farmer et al. 2008). The δD ratio has been successfully used to determine migration patterns in various insect species and in various parts of the world, including the monarch butterfly *Danaus plexippus* (L.) in North America

(Wassenaar and Hobson 1998), the dragonfly *Pantala flavescens* (Fabricius) in the Indian Sub-continent (Hobson et al. 2012a), and the red admiral butterfly *Vanessa atalanta* (L.) in Europe (Brattström et al. 2010). Ouin et al. (2011) investigated the possibility of using δD ratio to study the migration of the aphidophagous hoverfly *Episyrphus balteatus* (De Geer) in Europe. They concluded that it could be a valuable tool as long as inter-individual variability in a given geographic area and inter-annual variability of δD in precipitation are taken into account.

Geometric morphometrics (Bookstein 1991) is another method that can be used in insect species for differentiating local from migrant individuals and for inferring the geographical origin of these individuals. This method is based on the principle that local conditions can shape the development of animals (Bateson et al. 2004), and that different environmental pressures can lead to morphological differences (Alibert et al. 2001, Francoy et al. 2011). Morphology could thus reveal the local conditions that prevailed during the development of the organism, and the geographical origin associated to these local conditions. Variation in wing morphology could also reveal some life history traits independent of geographical origin. Migration is notably known to shape wing development and to affect wing morphology in different insect taxa including dragonflies (Dockx 2007), butterflies (Johansson et al. 2009) and whiteflies (Byrne 1999). Land-mark-based geometric morphometrics offer better discrimination capabilities than classical morphometrics (Rohlf and Marcus 1993, Bookstein 1996, Villemant et al. 2007) as they make it possible to study the shape in addition to the size of organisms. Geometric morphometrics and in particular wing geometric morphometrics have already been used in insect taxa to characterize and identify populations from different geographical origin (Alibert et al. 2001, Camara et al. 2006, Francoy et al. 2011, Gaspé et al. 2012). However, geometrical morphometrics have rarely been used to assess issues related to migration in insect species (but see Francuski et al. 2013).

The hoverfly species *E. balteatus* (Diptera: Syrphidae), which is distributed worldwide, is one of the most important aphid predators in Europe (Ankersmit et al. 1986, Tenhumberg and Poehling 1995). In Western Europe, *E. balteatus* is

Table 1. Sampling sites, sampling period and size of *Episyrphus balteatus* samples collected at seven European locations in 2011 and used for geometric morphometrics and deuterium analyses. GPS coordinates of the sampling sites are as follows: Ha (Hanover, Germany), 52°22' N, 9°42' E; Ge (Gembloux, Belgium), 50°33' N, 4°42' E; Pf (Pleine-Fougères, France), 48°32' N, -1°33' E; Ch (Chizé, France), 46°8' N, -0°23' E; To (Toulouse, France), 43°16' N, 0°51' E; Bo (Bologna, Italy), 44°29' N, 11°20' E; Al (Alicante, Spain), 38°20' N, -0°29' E.

| Sampling site | Morphometry | | | Deuterium analysis | |
|---------------|-----------------|--------------------|----------------------|--------------------|-------------|
| | Sampling period | Sample size, males | Sample size, females | Sampling period | Sample size |
| Ha | 6/14 to 7/07 | 27 | 27 | 6/14 to 6/30 | 10 |
| Ge | 6/01 to 6/15 | 15 | 18 | 6/01 to 6/15 | 10 |
| Pf | 6/30 to 7/13 | 25 | 30 | 6/30 to 7/13 | 10 |
| Ch | 6/23 to 7/13 | 7 | 13 | 6/31 to 7/13 | 10 |
| To | 5/18 to 6/30 | 32 | 32 | 5/18 to 6/01 | 10 |
| Bo | 5/21 to 6/19 | 3 | 7 | 5/21 to 6/19 | 10 |
| Al | 5/05 to 5/20 | 0 | 0 | 5/05 to 5/20 | 2 |

a partial migratory species: part of the population remains in its summer habitat and overwinters at immature or adult stage (Dušek and Láška 1974, Hart et al. 1997, Sarthou et al. 2005), while the other part of the population moves southward in search of milder climatic conditions (Aubert and Goeldlin 1981). Therefore, in the spring, the population of *E. balteatus* in agricultural fields may partly comprise the individuals which overwintered locally and partly immigrants from regions further south where spring arrives earlier. Many authors report observations of north-south migratory flights by hoverflies in the fall (Lack and Lack 1951, Aubert and Goeldlin 1981), but there are no reports of large scale south-north flights in the spring. The lack of information on spring migration, and the short life span of *E. balteatus* suggests that this species has a one-way migration pattern (i.e., a succession of uni-directional movements through a series of breeding areas over several generations that results in a multi-generation round trip; Hobson and Norris 2008), with a large scale rapid flight to the southern overwintering sites in the fall, and a gradual return northward in spring and summer over several generations. Although most aphidophagous hoverfly species are migratory and provide ecosystem services that are important for agriculture, issues related to migration have rarely been addressed in these species. Lack of genetic differentiation between *E. balteatus* overwintering strategies (Raymond et al. 2013a) as between geographical origins (Hondelmann et al. 2005, Raymond et al. 2013b) leads to consider alternative approaches to understand migration patterns of this species.

The main objective of this study was to evaluate the magnitude of variation in the wing morphology and in the δD ratio in *E. balteatus* across Europe to check whether isotopic and/or morphometric markers could be used to study migration dynamics in this species. We then used the two markers to (1) validate the hypothesis of a one way migration pattern, (2) determine the proportion of immigrants in an *E. balteatus* spring population in Western France and (3) identify the period of immigration in this region. Assessing the proportion of immigrants and local overwintering individuals should provide information about the relative contribution of these two overwintering strategies to the biological control of aphids and help define appropriate landscape management strategies for better control of aphid populations.

MATERIAL AND METHODS

Insect sampling

Episyrphus balteatus specimens were collected at seven sites located across Western Europe to establish a gradient of δD ratio in hoverfly tissues and to evaluate the variations in wing morphology on a north-south axis in Europe. Specimen were caught in agricultural landscapes using Malaise traps or sweep nets in 2011 during the spring-summer period (from May to July; the sampling period for each site is listed in Table 1). To limit the risk of catching immigrants, we collected individuals during the period of maximum abundance of *E. balteatus* in each region, avoiding the beginning of spring and the end of summer, which are the most probable migratory

Table 2. Sampling sites, sampling period and size of *Episyrphus balteatus* samples collected in Pleine-Fougères during spring 2011 and used for geometric morphometrics and deuterium analyses.

| Sampling site | Sampling period | Sample size | | |
|---------------|-----------------|--------------------|----------------------|--------------------|
| | | Morphometry, males | Morphometry, females | Deuterium analysis |
| Pf | 5/11 to 5/20 | 0 | 11 | 13 |
| Pf | 5/21 to 5/26 | 6 | 4 | 10 |
| Pf | 5/27 to 6/03 | 8 | 8 | 16 |
| Pf | 6/04 to 6/10 | 8 | 8 | 16 |
| Pf | 6/11 to 6/17 | 8 | 8 | 16 |
| Pf | 6/18 to 6/24 | 8 | 8 | 16 |
| Pf | 6/25 to 7/01 | 9 | 6 | 15 |
| Pf | 7/02 to 7/13 | 18 | 27 | 10 |

periods (Aubert and Goeldlin 1981). The number of individuals collected at each sampling site ranged from 2 to 55 (Table 1).

In the aim of evaluating variations in wing morphology in relation with the overwintering strategy (i.e., migration, local overwintering at the adult stage or local overwintering at the nymphal stage) we used a sampling protocol that allowed us to catch unambiguously individuals using each of the three strategies. The sampling protocol is described in Raymond et al. (2013a), but in the present study, we used only specimens trapped in “Vallées et Coteaux de Gascogne” (43°17' N, 0°54' E) for the local overwintering strategies and from Boucharo pass (42°42'13" N, -0°3'52" E) for the migratory individuals. The sample sizes for the three overwintering strategies were: $N = 55$ for the migration, $N = 12$ for the local overwintering at the nymphal stage, $N = 32$ for the local overwintering at the adult stage.

In addition, *E. balteatus* were caught in an agricultural region in Western France (study site Pleine-Fougères, 48°32' N, -1°33' E) throughout the spring, in order (1) to distinguish long distance immigrants from local immigrants, (2) to quantify the proportion of long distance immigrants, and (3) to identify the period of immigration in this region. The Pleine-Fougères study site is part of the “Zone Atelier Armorique” experimental site and belongs to a Long Term Ecological Research network (LTER S1001201). This site was chosen to study migration dynamics because it is located on migratory routes of many insect species (Chapman et al. 2012, Stefanescu et al. 2013), and because it is far enough away from southern Europe that variations in the δD ratio between local individuals and immigrants from the south can be detected.

The individuals were captured using Malaise traps which were set up on May 11 and collected every week until the July 13. (Table 2 lists the sizes of the samples and the sampling periods.)

All the samples were manually sorted and *E. balteatus* specimens were individually stored at 4°C in Eppendorf tubes filled with 90° ethanol prior to morphometric and isotopic analyses.

Morphometric analyses

As there was a significant sexual dimorphism in wing morphology in *E. balteatus*, we analyzed wing shape separately in males and females. For studying the influence of the overwintering strategy on wing morphology, we used only females since the local overwintering strategy at adult stage only concerns females (Lyon 1967).

Photography and digitalization of landmarks.—The right wing of each specimen was cut at the base and slide-mounted in alcohol. The images of the individuals originating from the seven European sampling sites and using the three overwintering strategies were taken at the MNHN morphometric platform (Paris, France) using a Leica Z6 binocular with a 1× lens and 1.25× magnification, and a Leica DFC420 video camera. The images of the individuals collected in Pleine-Fougères during the spring 2011 were taken at the ISEM morphometric platform (Montpellier, France) using a Wild M3Z binocular with a 0.3× lens and 6.5× magnification, and an Oscar F810 video camera. For each specimen, the coordinates of 18 landmarks were recorded on the digitized wing pictures (Fig. 1) using tpsDig 2.16 software.

Variation in wing morphology among sampling sites and among overwintering strategies.—First, we compared overall wing size in each sex among

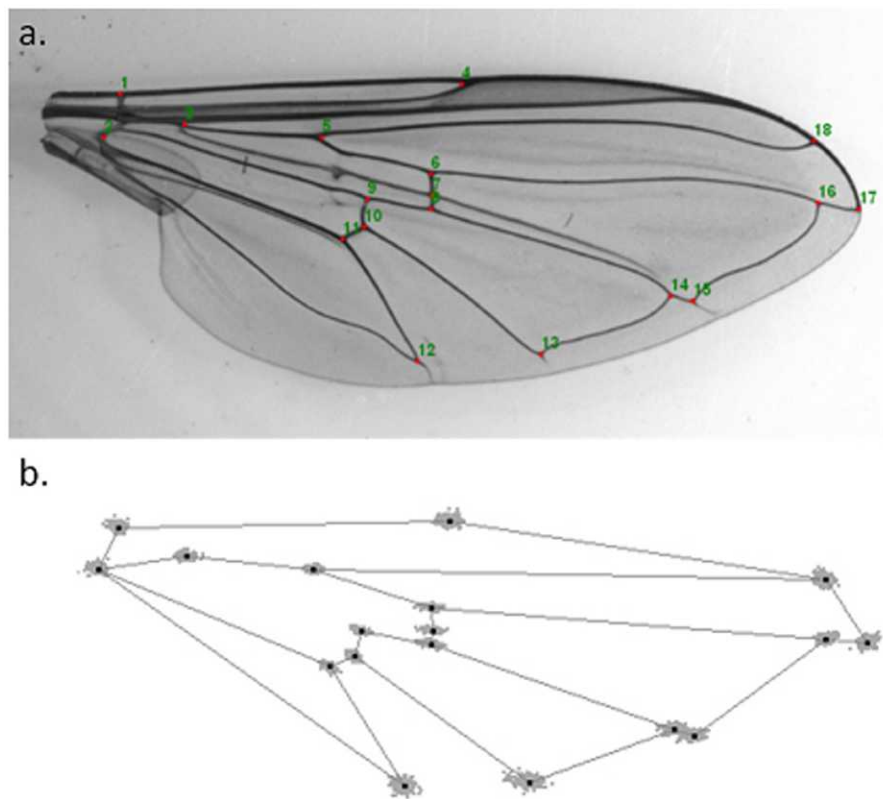


Fig. 1. The 18 homologous landmarks recorded on the *Episyrphus balteatus* right wing (a), the result of the procrustes superimposition in females (b). In (b), gray dots show the individual positions of the landmarks and the black dots correspond to the consensus positions of the landmarks in the whole sample.

sampling sites and among overwintering strategies using centroid size. Centroid size is the square root of the summed squared distances of each landmark to the centroid (i.e., the point whose coordinates are the average of the x and y coordinates of the landmarks of an individual specimen). Variations in size linked to the sampling site and to the overwintering strategy were explored using ANOVA, and pair-wise comparisons between populations were made using Tukey's honestly significant difference tests for the unequal sample size. Normality and dispersion of the residuals were checked. The raw landmark coordinates of all specimens within each sex were superimposed by generalized procrustes analysis (GPA) (Rohlf and Slice 1990, Rohlf 1999) to extract information on wing shape from landmark data. The procrustes superimposition procedure removes variations that are not related to shape (variations in

landmark configurations due to scaling, position, and orientation), and the variation remaining in the procrustes coordinates contains complete information about shape variation (Dryden and Mardia 1998). Variations in wing shape between populations from different sampling sites or using different overwintering strategies were explored by performing MANOVAs on the procrustes residuals. In order to separate individuals originating from different sampling sites or using different overwintering strategies, we performed linear discriminant analyses on the procrustes residuals, in each sex. We then made classifications on the results of linear discriminant analyses using cross-validation procedures to evaluate the power of the classifications (i.e., the rate of correct classification of an individual with respect to a sampling site or to an overwintering strategy).

Variation in wing morphology during the course of

spring in western France.—In the specimens caught in Pleine-Fougères during the course of spring in 2011, we compared centroid size and wing conformation between sampling dates. In each sex, variations in size between sampling dates were explored using ANOVA, and pairwise comparisons between samples were made using Tukey's honestly significant difference tests. Normality and dispersion of the residuals were checked. After superimpositions by GPA in each sex, variations in wing shape between sampling dates were explored by performing MANOVAs on the procrustes residuals. We then projected morphometric data concerning the females collected in Pleine-Fougères from May 20, 2011 to July 13, 2011 on the plot of the first two principal components of the linear discriminant analysis performed on the females using the three alternative overwintering strategies.

All calculations were performed using R software (R Development Core Team 2005), version 2.14, and in particular Rmorph, a specialized library for geometric morphometrics developed by Michel Baylac (2010).

Isotopic analyses

Isotopic analyses in hoverfly tissues.—Wings and legs of *E. balteatus* were stored in Eppendorf tubes filled with 96% ethanol. Prior to isotopic analyses, the wings were dried in a stove at 50°C for 24 h to eliminate any ethanol and hydrogen contained therein. Hydrogen isotope analyses of insect tissues were performed by Iso-Analytical (Crewe, UK). After drying, 0.5–1 mg sample material were weighed in silver capsules (5 × 8 mm). The filled capsules were left open for a period of not less than 4 days to allow the exchangeable hydrogen in the sample chitin to fully equilibrate with the moisture in the laboratory air. The capsules containing insect tissues were sealed just prior to analysis by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS). The reference material used for δD analysis was IA-R002 (mineral oil, $\delta D_{VSMOW} = -111.2\text{‰}$). IA-R002 is traceable to NBS-22 (mineral oil, $\delta D_{VSMOW} = 118.5\text{‰}$) distributed as an inter-laboratory comparison standard by the International Atomic Energy Agency (IAEA). Samples of IA-R002, IAEA-CH-7 (polyethylene foil, $\delta D_{VSMOW} = -100.3\text{‰}$) and IA-R062 (olive oil, $\delta D_{VSMOW} = -137.06\text{‰}$) were measured along

with hoverfly samples for quality control of the samples. IAEA-CH-7 is an interlaboratory comparison standard distributed by the IAEA. The results are expressed in δ units relative to NBS-22 (mineral oil) [$\delta D_H = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$], where R is the ratio of D/H atomic.

Hydrogen isotopic ratios in precipitation.—The general trend for the variation pattern of δD values in precipitation (δD_W) in Europe is that δD_W become more negative along a northern gradient. The δD_W values also vary over the year, and this seasonal variation has to be taken into account when inferring the geographical origin of migratory organisms (Brattström et al. 2010). We used the Online Isotope in Precipitation Calculator (OIPC) (Bowen 2013) to acquire interpolated amount-weighted monthly δD_W values for the rainwater at our sampling sites (Bowen et al. 2005). We calculated δD_W values on pairs of months by averaging monthly δD_W values weighted by the quantity of precipitation that fell each month. A two-month period was chosen to cover the developmental time of *E. balteatus* and of its prey. We evaluated variations in pair-month δD_W values over the year and compared this temporal variation with the geographical variation in δD_W values. Then, for each of the seven European sampling sites, we compared the δD_W values in the precipitation for the pair of months preceding capture of the hoverflies, with the δD values measured in the hoverfly tissues (δD_H) in order to estimate the relationship between them.

Variation in the δD values in hoverfly tissues during the course of spring

We used ANOVA and post-hoc Tukey's tests to compare the δD_H values measured in hoverflies captured at Pleine-Fougères on eight different dates between May 11 and July 13, 2011. We then calculated the variation in the variance of δD_H values for the eight sampling dates. We used the linear equation linking δD_H values and δD_W values, and the calculated δD_W values in precipitation at Pleine-Fougères for captures from May to July, to infer δD_H values for individuals that developed in Pleine-Fougères during this period. We compared these inferred values with δD_H values measured in the tissues of hoverflies collected at Pleine-Fougères from May 11 to July 13, 2011.

RESULTS

Wing morphometrics

Variation in wing morphology across Europe.—In both sexes, the ANOVA on the centroid size of the wings revealed highly significant differences among sampling sites (males, ANOVA: $F = 11.28$, $P < 0.001$; females, ANOVA: $F = 7.21$, $P < 0.001$; Appendix). In males, Tukey's post-hoc pair wise comparisons revealed significantly smaller wings in specimens trapped in Toulouse (France) than in specimens from Hanover (Germany), Chize (France), Pleine-Fougères (France) and Gembloux (Belgium) ($P < 0.05$; Tukey's HSD test). In females, specimens from Toulouse also had smaller wings than specimens from Hanover, Chizé and Pleine-Fougères, and there was a significant difference in size between specimens from Chizé and Gembloux ($P < 0.05$; Tukey's HSD test). In males, the MANOVA on the procrustes residuals revealed a significant difference in wing shape among sampling sites ($P = 0.018$). However, the plot of first two principal components of the linear discriminant analysis showed that the variation was not sufficient to unambiguously assign all individuals to their populations of origin (Appendix: Fig. A2a). The cross-validated percentage of correct classification was 37.5%, with more than half the specimens being wrongly classified. In females, wing shapes also differed significantly among sampling sites (MANOVA: $P = 0.002$). However, like in males, the variation among sampling sites was not sufficient to distinguish them on the first two principal components of the linear discriminant analysis (Appendix: Fig. A2b). The cross-validated percentage of correct classification was 35.2%.

Variation in wing morphology in relation with overwintering strategy.—The ANOVA on the centroid size of the wings revealed significant differences in wing size among overwintering strategies (ANOVA: $F = 3.8$; $P = 0.03$). Tukey's post hoc pairwise comparisons showed significant smaller wings in females that overwintered locally at the nymphal stage than in females that overwintered locally at the adult stage or migrated ($P < 0.05$; Tukey's HSD test). The MANOVA on the procrustes residuals showed a slight but non-significant difference in wing shape among strategies ($P = 0.08$). The plot of

the first two principal components of the linear discriminant analysis showed that the variation was not sufficient to perfectly distinguish individuals using different overwintering strategies (Fig. 2). The cross-validated percentage of correct classification was 45.5%, with more than half the specimens being wrongly classified.

Variations in wing morphology during the course of spring in western France.—In both sexes, the ANOVA on the centroid size of the wings revealed significant differences among sampling periods (males, ANOVA: $F = 2.91$; $P = 0.01$; females, ANOVA: $F = 3.35$; $P = 0.003$). In males, Tukey's post-hoc pairwise comparisons showed significant differences in size between samples collected on July 1 and samples collected on May 25, with specimens collected on July 1 having smaller wings ($P < 0.05$; Tukey's HSD test). In females, specimens collected on July 13 had smaller wings than specimens collected on May 25 and June 3 ($P < 0.05$; Tukey's HSD test). In males, the MANOVA on the procrustes residuals revealed a significant difference in wing shape among sampling dates (males, MANOVA: $P = 0.02$). In females, we observed a slight but non-significant difference (females, MANOVA: $P = 0.06$). Projection of the data concerning females collected in Pleine-Fougères during spring on the plot of the first two principal components of the linear discriminant analysis performed with the three overwintering strategies showed that females collected from May 20 to June 10 were closer to individuals that overwintered locally at the nymphal stage whereas females collected from June 17 to July 13 were closer to migratory individuals (Fig. 3).

Hydrogen isotopic ratio analyses

Temporal variation in δD in precipitation.—The two-month δD values in precipitation (δD_W) displayed high temporal variation over the year. The δD_W values calculated for the two-month periods March–April (MA) and June–July (JJ) at the Pleine-Fougères site differed by 16‰ ($\delta D_{WMA} = -51$ ‰; $\delta D_{WJJ} = -37$ ‰). This difference was similar to the difference in δD_W values observed for a same couple of months between Pleine-Fougères and the north of Germany or between Pleine-Fougères and the center of Spain, confirming the need to take temporal variations in δD_W into account in the analyses.

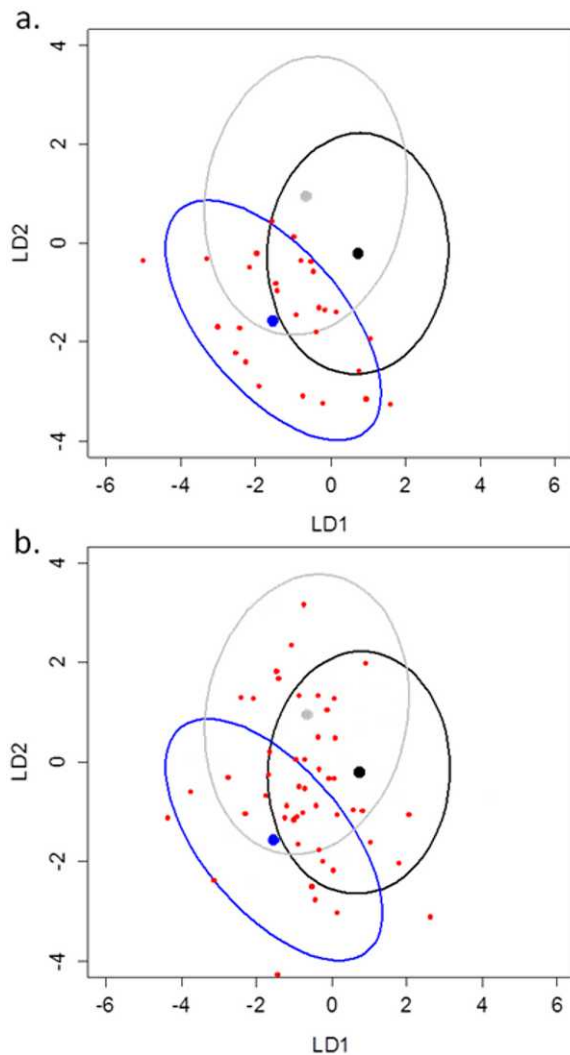


Fig. 2. Plot of the first plane of the linear discriminant analysis performed on the wing morphometric data of females using the three overwintering strategies, and projection of the wing morphometric data of the females collected in Pleine-Fougères in the beginning of the spring (a) and in the end of the spring (b). The black, blue and grey points are the centroid of 95% confidence ellipses derived from the coordinates of individuals within each overwintering strategy, each color represent a particular overwintering strategy: migration (black, $N = 55$), local overwintering at nymphal stage (blue, $N = 12$), local overwintering at adult stage (grey, $N = 32$); projection of each individual within each overwintering strategy is not represented. Red points are the projections of the females collected in Pleine-Fougères from May 20 to June 10, 2011 (a) and from June 17 to July 13, 2011 (b).

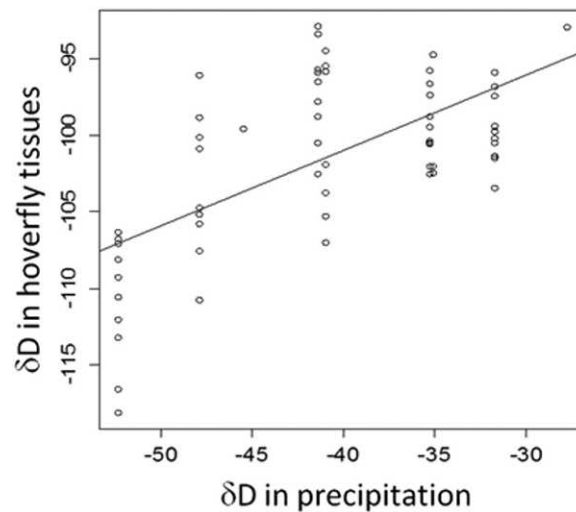


Fig. 3. Relationship between δD values measured in hoverfly tissues and δD values in precipitation averaged over the two months preceding capture from the OIPC monthly values, for the seven sampling sites in Europe. Equation of the regression line is $\delta D_H = 0.49 \times \delta D_W - 81.3$; $r^2 = 0.39$; $P < 0.001$.

Hydrogen isotopic ratio in hoverfly tissues across Europe

As expected, δD values in hoverfly tissues (δD_H) showed the same pattern of variation as the δD values in precipitation (δD_W). δD_H values were higher in the south than in the north. The maximum δD_H value (-92.9) was measured in a specimen from Toulouse (France; $43^\circ 16' N$, $0^\circ 51' E$), and the minimum (-118.1) in a specimen from Hanover (Germany; $52^\circ 22' N$, $9^\circ 42' E$). We observed a strong correlation between δD_H values and δD_W values in the two months preceding captures at each sampling site ($r^2 = 0.39$; $P < 0.001$; Fig. 3). The equation of the regression line was ($\delta D_H = 0.49 \times \delta D_W - 81.3$). This equation was used to assess the expected δD_H values for adult hoverflies caught from May to July at the Pleine-Fougères site under the assumption that they had developed locally during the two preceding months (Fig. 4).

Temporal variation of δD in hoverfly tissues during the course of spring.—The average δD_H values measured in specimens collected in Pleine-Fougères at eight different dates from May to July 2011 differed significantly between sampling dates (ANOVA: $P < 0.001$). Tukey's post hoc comparison identified two subsets for the entire

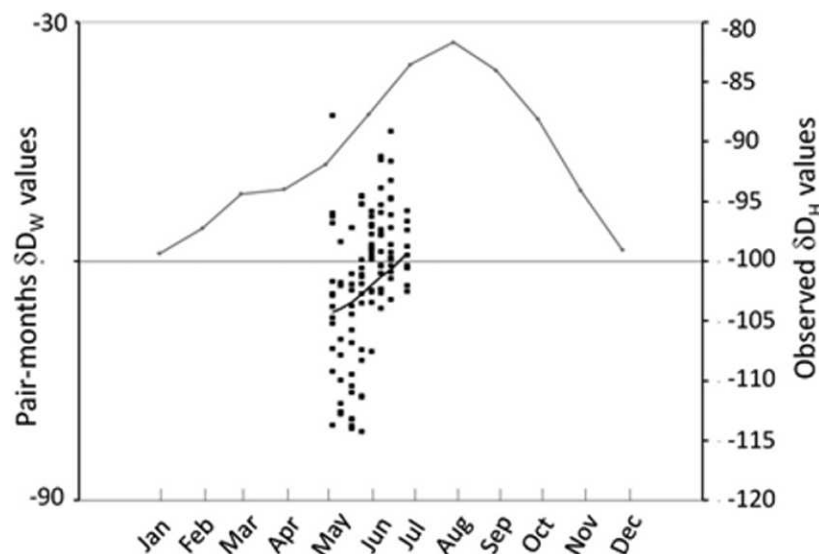


Fig. 4. Variation in δD values in precipitation (δD_W) for pairs of months and δD values in hoverfly tissues (δD_H) measured in Pleine-Fougères (France, 48°32' N, -1°33' E) over time. Dark gray line: δD_W values for pairs of months calculated from the OIPC monthly values. Black squares: δD_H values measured in *Episyrrhus balteatus* specimens collected from May 20 to July 13, 2011. Black line: δD_H values inferred from δD_W values calculated for pairs of months and from the relationship between δD_H and δD_W values ($\delta D_H = 0.49 \times \delta D_W - 81.3$).

sampling period that differed from one another. The first set contained samples collected from May 25 to June 3 and the second set contained samples collected from June 17 to July 17. The δD_H values of samples collected on May 20 and June 10 did not significantly differ from the others.

The mean δD_H values observed in specimens collected in Pleine-Fougères from May to July were close to the inferred δD_H values for hoverflies originating from Pleine-Fougères in this period but the specimens collected from May 20 to June 10 had lower δD_H values than expected, while specimens collected from June 17 to July 13 had higher δD_H values than expected (Figs. 4, 5a). There was a decrease in the variance of the δD_H values during the sampling period (Fig. 5b), and one δD_H value that was much higher than expected (-87.80) in a specimen caught during the first sampling session (May 20) (Fig. 4).

DISCUSSION

Wing morphology as a tool for the characterization of migration dynamics

Morphometric analyses of wing venation

revealed a significant variation in wing traits across Europe. Such geographical variability in size and/or shape has already been observed in *E. balteatus* (Sullivan and Sutherland 1999) but also in the migratory hoverfly species *Eristalis tenax* (L.) (Francuski et al. 2013). These morphological differences may be signatures of different environmental pressures, such as climatic conditions or trophic resources availability, experienced during the development of the larvae or even during the ontogenesis. However, compared to local inter-individual variation, morphological differentiation between sampling sites was not sufficient to accurately distinguish the different origins of the individuals. The error rates observed in cross validation tests were more than 60% in both males and females.

Our results also showed morphological variation in wing size and wing shape related to overwintering strategies. In *E. balteatus*, various overwintering strategies co-exist in Western Europe and result in different environmental pressures, such as climatic conditions or incidence of natural enemies, on the individuals that use one of these strategies. These different environmental pressures which are not associat-

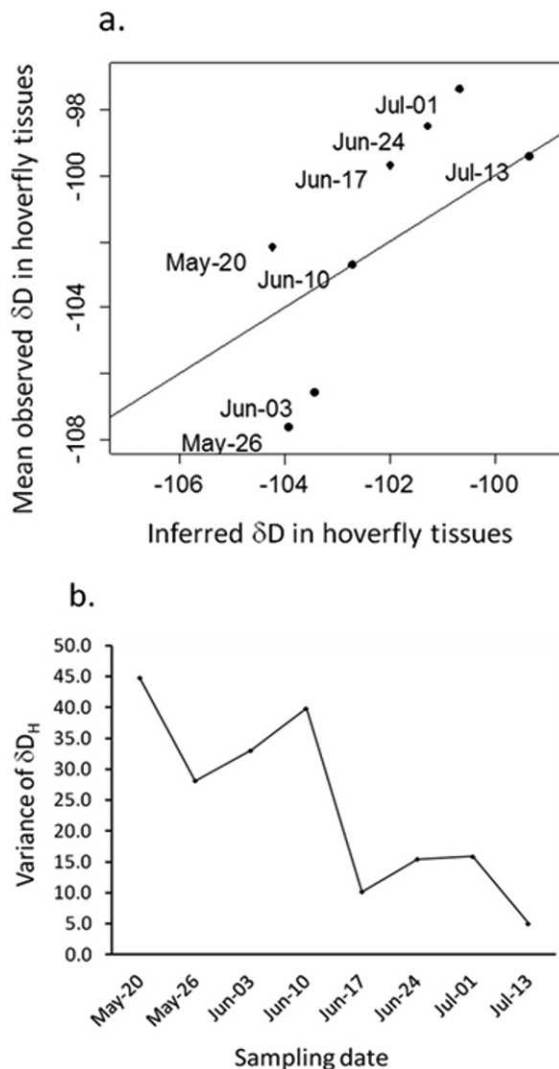


Fig. 5. Variation in δD values in hoverfly tissues (δD_H) between sampling dates for *Episyrphus balteatus* specimens caught in Pleine-Fougères (France, 48°32' N, -1°33' E) between May and July 2011. Comparison between inferred and observed mean δD_H values (a). Changes in the variance of the δD_H values at each sampling date during the sampling period (b).

ed with genetic differentiation (Raymond et al. 2013a) may explain the morphological differences observed in wing venation in relation with overwintering strategy. In other taxa, morphological variations have already been reported in relation with some life history traits, such as migration, that do not necessarily match geographical origin (Dockx 2007, Johansson et al.

2009). Our findings showed that overwintering at the nymphal stage had a stronger signature than migration on wing morphology (size and shape), as there was no significant difference either in wing size or in wing conformation between individuals that migrated and individuals that overwintered locally as adults. In any case, as for the geographical origin, morphological differentiation between overwintering strategies was not sufficient to accurately distinguish the individuals using the different strategies.

Several factors could explain the absence of complete separation between our samples. The environmental conditions at the different sampling sites may not be sufficiently contrasted. Indeed, for the geographical origin, we studied individuals from summer generations, and climatic conditions during the summer months in Europe are relatively homogeneous. Moreover, the double influence on wing morphology of life history traits and of the conditions at the location where the individual developed creates confounding effects and reduces the power of wing morphology to determine the geographical origin. Finally, morphological differences are also often associated with genetic structure (Villemant et al. 2007, Francoy et al. 2011, Vicente et al. 2011, Neto et al. 2013). However, previous studies found no evidence for genetic structure either in relation with geographical origin (Hondelmann et al. 2005, Raymond et al. 2013b), or with overwintering strategy (Raymond et al. 2013a) in *E. balteatus*. This lack of genetic structure may reduce the degree of morphological variation between individuals originating from different regions or using different overwintering strategies.

Pattern of variation in the hydrogen isotopic ratio in hoverfly tissues in Europe

Our results confirm that in *E. balteatus* the δD in the tissues is correlated with the δD in precipitation in the area in which the individual developed (Ouin et al. 2011). This relationship has previously been shown in many bird species in Europe (Hobson et al. 2009, Marquiss et al. 2012) and in some insect species (Brattström et al. 2010). The work of Ouin et al. (2011) was the first one to establish this relationship in a third trophic level consumer. The water-tissue relationship we found in our samples $\delta D_H = 0.49 \times$

$\delta D_w - 81.3$) is close to that found by Hobson et al. (1999) in the monarch butterfly (*Danaus plexippus*) in North America ($\delta D_{\text{butterflies}} = 0.62 \times \delta D_{\text{water}} - 79$). The greater fractionation rate between water and hoverfly tissues (0.49) than between water and butterfly tissues (0.62) may be due to the difference in their trophic levels. Conversely, the coefficients we found were very different from that found by Ouin et al. (2011) in *E. balteatus* ($\delta D_{\text{hoverflies}} = 1.04 \times \delta D_{\text{water}} - 25.2$). This difference could be due to the use of different values of δD_w in the two studies. Indeed, we used mean pluri-annual interpolated δD_w values whereas Ouin et al. measured the δD_w value directly in the water for three sampling groups from different origins. The use of different tissues (i.e., wings plus legs or wings plus chitin fragments) might also influence the results of the two studies.

Since *E. balteatus* is a migratory hoverfly, the water-tissue relationship we found is uncertain because it is very difficult to be sure where the sampled individuals developed. In our European wide sample, we limited the risk of catching immigrants by collecting specimens out of the probable migration periods. The variability in the δD_H values among individuals within sampling sites was similar to the inter-individual variability observed in other studies in sampling groups whose geographical origins were known. In our study, the standard deviation associated with δD_H values at a particular sampling site for a given month ranged from 2.25 to 5.18, similar to the standard deviations observed in other studies on insect species (Hobson et al. 1999, Brattström et al. 2010). This suggests that the individuals collected at each sampling point in our study mostly originated from a location that was not far away, and included no—or a very low proportion of—long-range immigrants.

Our results highlight the importance of seasonal variations in δD in precipitation that need to be taken into consideration when interpreting δD values in migratory organisms. Brattström et al. (2010) showed seasonal variation in δD values in red admiral butterflies between spring and fall. Our findings support the hypothesis of a significant variation even over a shorter period of time, i.e., only during spring, in *E. balteatus*. The rapid response of δD values in hoverfly tissues to variations in δD values in precipitation could be

due to the trophic regime of *E. balteatus* larvae. Aphidophagous larvae feed on aphids which have a short life span, and these aphids feed on plant sap, which is mostly composed of water. In contrast to butterfly larvae, which consume all the vegetative parts of their host plant and may therefore incorporate the isotopic signature of the entire period of development of the plant, *E. balteatus* larvae may incorporate the isotopic signature of a shorter period corresponding to aphid development. The temporal variation in δD in hoverfly tissues during spring is equivalent to the geographical variation between individuals from remote areas located hundreds of miles from one another. This temporal variation is a source of inter-individual variability in spring populations because δD values are likely to vary depending on the age of the individuals collected at the same place at the same date.

To establish a more robust relationship between δD values in hoverfly tissues and in precipitation, the response of δD in hoverflies to variations in δD in precipitation over time could be experimentally assessed using laboratory reared hoverflies and controlled values of δD in the water.

Spring migration dynamics of *E. balteatus*

Despite the uncertainties concerning the δD water-tissue relationship discussed above, this work provides interesting insights into the migration dynamics of *E. balteatus*. Our results clearly show an increase in δD values measured in hoverfly tissues sampled in Pleine-Fougères throughout the spring that could reflect the arrival of migrants from areas located further south. However, this variation is in agreement with the expected variation based on the assumption that the δD in hoverfly tissues depends on the δD in precipitation averaged over the two month period preceding capture. As we did not observe increased variance of δD values in hoverfly tissues, this supports the hypothesis that the increase in δD in hoverfly tissues is mainly due to the increase in δD in precipitation over the course of spring (Bowen et al. 2005, Bowen 2013).

However, we observed δD values in hoverflies lower than expected at the beginning of the sampling period (from May 20 to June 10) and δD values higher than expected in the following

sampling period (from June 17 to July 13). The lower values could be explained by the emergence of overwintering individuals that developed in fall, and thus incorporated an isotopic signature in the fall months which corresponds to lower δD values in precipitation than the signature of the spring months. The higher values in June and July may be due to the arrival of migratory individuals from regions located further south but nevertheless not far from the study site. These hypotheses are supported by the results of our analyses on wing morphology. Indeed, we observed that in terms of wing morphology, individuals collected before June 17 were close to individuals that overwintered at the nymphal stage, whereas individuals collected after this date were closer to migratory individuals. Finally, the observation of a particularly high δD value in one individual caught on May 20, 2011 suggests that *E. balteatus* may arrive from remote southern areas but that the number of these continental-scale spring immigrants is insignificant with respect to the overall population.

Our results support the hypothesis that spring populations of *E. balteatus* in western France are mostly composed of local individuals at the beginning of spring with the possible arrival of immigrants from overwintering sites located in southern Europe. The size of the spring population is subsequently increased by the arrival of individuals from southern areas located a few hundred kilometers from the study site, which supports the hypothesis of a gradual return to northern Europe by migratory individuals in spring taking place over several generations of hoverflies.

Conclusion and applications

Wing morphometrics and stable isotopes revealed some differentiation between *E. balteatus* populations originating from different regions of Western Europe. These tools thus provide valuable information for studying the large scale population dynamics of this species in Europe. However, the use of these tools is complicated by uncertainties and confounding effects.

The evaluation of the δD ratio and wing morphology in *E. balteatus* individuals collected over the course of spring in an agricultural area in Western France showed that this population

was probably not the result of the massive arrival of immigrants from southern Europe. Rather, our results suggest that the spring population is mostly comprised of local individuals with the possible arrival of individuals from nearby areas located further south, and support the hypothesis of a step by step northward return of *E. balteatus* in the spring. This implies that landscape management measures aimed at optimizing the biological control of aphids should mostly focus on hoverfly populations that overwinter locally. Agricultural practices should thus aim to preserve populations that overwinter at immature stages in the fields and to enhance biological control in autumn, for example by limiting phytosanitary treatments in the fall and mechanical weed control operations in the winter (Raymond et al. 2014). In addition, management of forest edges should aim to facilitate the overwintering of adult females and egg laying in early spring (Sarhou et al. 2005; Alignier et al. 2014). Increasing the predation on young aphid colonies in the fall and in the early spring and the synchronization between the dynamics of aphids and hoverflies may enhance the efficiency of the biological control.

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SUPPLEMENTAL MATERIAL

APPENDIX

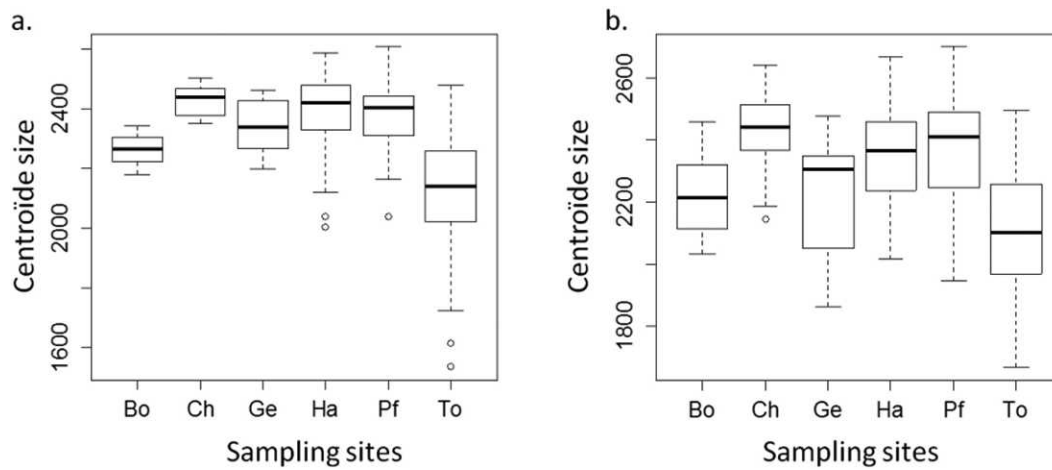


Fig. A1. Variation in the size of the right wing between sampling sites in (a) males and (b) females. Boxplot of centroid size with median, first and third quartiles.

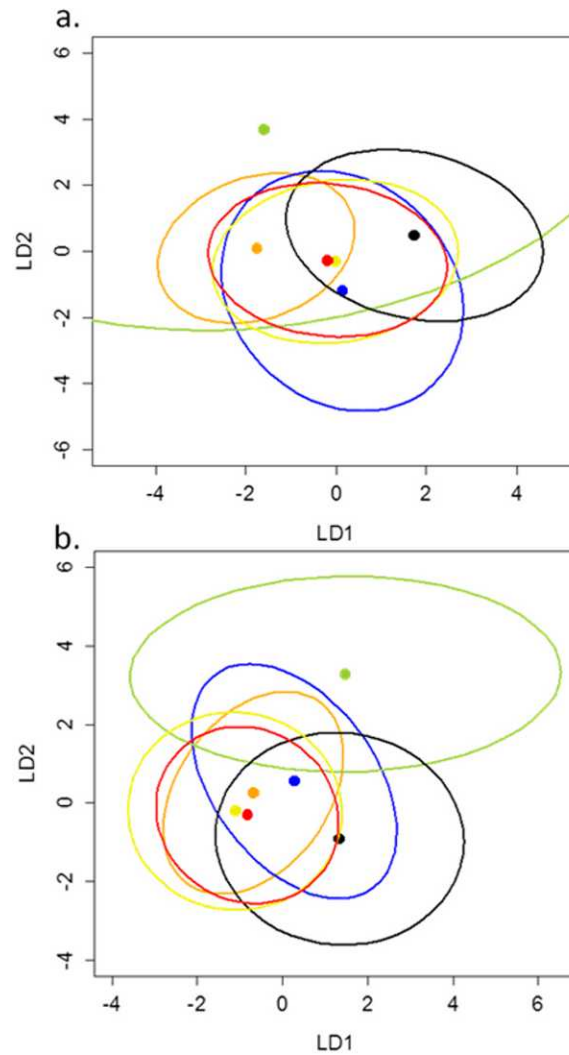


Fig. A2. Plot of the first plane of the linear discriminant analysis performed on the wing morphometric data of (a) male and (b) female individuals from the seven European sampling sites. The points are the centroid of 95% confidence ellipses derived from the coordinates of individuals within each locality, each color represent a particular locality: Hanover (orange, $N_{\text{males}} = 27$, $N_{\text{females}} = 27$), Gembloux (blue, $N_{\text{males}} = 15$, $N_{\text{females}} = 18$), Pleine-Fougères (yellow, $N_{\text{males}} = 25$, $N_{\text{females}} = 30$), Chizé (red, $N_{\text{males}} = 7$, $N_{\text{females}} = 13$), Toulouse (black, $N_{\text{males}} = 32$, $N_{\text{females}} = 32$), Bologne (green, $N_{\text{males}} = 3$, $N_{\text{females}} = 7$); projections of each individual within the localities are not represented.