Understanding the migration ecology of European red admirals *Vanessa atalanta* using stable hydrogen isotopes

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Tracking migratory movement of small animals with variable migration patterns is difficult with standard mark–recapture methods or genetic analysis. We used stable hydrogen isotope (δD) measurements of wings from European red admirals *Vanessa atalanta* to study several aspects of this species' migration. In the central part of southern Europe we found large differences in δD values between red admirals sampled in autumn and spring supporting the hypothesis that reproduction takes place in the Mediterranean region during winter. There was also an apparent influx to southern Europe in the spring of individuals with a more southerly origin, since many samples had higher δD values and similar to those expected from coastal areas of North Africa. We found a clear seasonal difference in the δD values of red admirals sampled in northern Europe. Spring migrants arriving in northern Europe generally had high δD values that indicated a southerly origin. In autumn, δD values suggested that red admirals were mostly from regions close to the sampling sites, but throughout the sampling period there were always individuals with δD values suggesting non-local origins. The migration pattern of this species is supposedly highly variable and plastic. δD differences between individuals in the western part of Europe were generally small making migratory patterns difficult to interpret. However, butterflies from western Europe were apparently isolated from those from north-eastern Europe, since δD values in the western region rarely corresponded to those of autumn migrants from the north-east. Use of δD data for inferring butterfly migration in Europe is complex, but our study showed that this technique can be used to help uncover previously unknown aspects of red admiral migration.

Understanding the ecology of migrating animals poses a special challenge to ecologists, due to the long distances travelled, unknown routes of migration or stopover locations. To better understand the ecology of such animals we need to follow them throughout their annual cycles in the different habitats they frequent. It is also important to understand how different parts of their life histories are connected by their migratory journeys (Alerstam et al. 2003). Some of the most straightforward approaches to detect migration routes are to follow animals directly using exogenous markers such as radio or satellite transmitters and metal or colour tags (Webster et al. 2002). Endogenous markers such as morphometric, molecular and biogeochemical markers have also been used especially for animals too small for exogenous markers or when re-encounter rates are very low (Hobson 1999, Webster et al. 2002, Rubenstein and Hobson 2004, Bowen et al. 2005, Hobson and Norris 2008).

For insects, genetic studies can be difficult to use for identifying migratory patterns since interbreeding often keeps populations in different geographical regions from developing discernable genetic differences (Endler 1973). Stable isotopes of hydrogen, carbon, and nitrogen are now widely used to help delineate the origins of migrating animals (reviewed by Hobson and Wassenaar 2008). Hobson et al. (1999) used geographically predictable patterns of stable-hydrogen isotopes (δD) in precipitation to infer the origin of migratory monarch butterflies *Danaus plexippus* in North America. Further, Dockx et al. (2002) demonstrated that a previously unknown portion of the eastern US and Canadian populations of monarchs wintered in Cuba.

Ratios of naturally occurring stable isotopes often vary in systematic ways across large geographical areas (Hobson 2003, Bowen et al. 2005). There is almost always some sort of isotopic discrimination between the food and the consumer, but this is generally systematic and if the discrimination function is known, it is possible to estimate the values for the location where these isotopes were incorporated (Hobson et al. 1999; but see Wunder 2009). If metabolically inert tissues are used in isotope analyses, stable isotopes should reflect the provenance where they were incorporated into the tissues of the consumer. If the timing and turn-over rates for a tissue are known, stable isotopes can be used to infer natal areas or wintering regions for migrating animals (Hobson 1999, 2003, Hobson et al.

2004). Since chitinous wings of butterflies appears to not be part of the active metabolism after eclosion of the adults (Hobson et al. 1999), we decided to sample the wings of red admirals (and some local reference species) over parts of their European distribution to try to determine the natal area of separate individuals. Red admirals are among the best known European insect migrants with a fairly regular annual pattern of occurrence (Mikkola 2003b) and should therefore be a good candidate species for such a study. We decided to use δD as the geographic distribution of this isotope compared to other possible candidates is reasonably well known due to its tight relationship with well-established spatial precipitation patterns (Bowen et al. 2005).

The red admiral *Vanessa atalanta* is a widespread migratory butterfly that can be found in most areas of North America, Europe, western Asia and North Africa (Tolman 1997). The species' annual migration in Europe has been described based on flight directions taken at several locations (Williams 1951, Imby 1993, Benvenuti et al. 1996, Steiniger and Eitschberger 1996, Hansen 2001). Studies of winter ecology have been performed in Spain (Stefanescu 2001) and Italy (Brattström 2006), while observations of migration in relation to winds have been studied in northern Europe (Mikkola 2003a, Brattström et al. 2008a). Our knowledge about migration routes and phenology in this species, however, is still very limited.

Most of the European red admirals appear to spend winter around the Mediterranean Sea (Stefanescu 2001, Brattström 2006). Hibernation during winter does occur in the northern parts of Europe, but Pollard and Greatorex-Davies (1998) suggested it a less important strategy compared to a southward migration. We know from field observations of that migration not only occurs in a dominant direction, i.e. towards the north in spring and the south in autumn, but also that at the same time small numbers of individuals are reported flying opposite to the dominant direction (Williams 1951, Benvenuti et al. 1996, Stefanescu 2001). It has also been observed that the peak dates of the annual migration are variable (Hansen 2001, Brattström et al. 2008a). Much of this variation is likely caused by differences in environmental conditions experienced during the larval and pupal period. The developmental environment of butterflies has been shown to affect flight directions of adults in large whites *Pieris brassicae* (Spieth et al. 1998). Spieth et al. (1998) also noted that adult butterflies kept their flight direction during the whole experimental period (14 d), regardless of the environment they experienced after hatching. This suggests that the flight direction of the adult butterfly is determined before eclosion. It is therefore reasonable to suspect that the migration phenology of red admirals is highly variable and affected by variation in local environmental conditions.

In this study we were primarily interested in studying four questions about the red admiral's migration ecology that were well suited to investigation using hydrogen isotope methods: 1) are the red admirals found in southern Europe during autumn hatched in that region where they are captured, or are they immigrants from northern areas? 2) Are the red admirals that fly north from the Mediterranean region in spring hibernated individuals or the offspring of autumn migrants? 3) Does the population that breeds in northern Europe mainly consist of migrants from the south, or do large numbers of individuals successfully hibernate at northern latitudes? 4) Are there any distinct sub-groups of red admirals that utilize different migratory pathways in Europe?

Materials and methods

Collection of red admiral samples

The red admirals used in this study were collected by the lead author and a number of voluntary collectors. The butterflies were euthanized using ethyl acetate and the wings were removed from the body and stored in glassine envelopes in a dry, dark place up until the analyses were conducted. We sampled 259 red admirals from three main regions of Europe: northern Europe (172 ind.) (southern Sweden, Denmark, Estonia and Kaliningrad in Russia), central Mediterranean (58 ind.) (Capri in Italy) and western Europe (29 ind.) (northern Spain and northern France). The sample sizes per location and collection period are given in Table 1. All sample locations are indicated in Fig. 1. Most samples were from the northern and southern regions. In order to study differences between generations, all samples from each region were divided into two groups depending on date of capture. For the data from northern Europe we used samples from 1 May until 15 July when studying spring migration and from the autumn generation we used samples from 25 August to 16 September. We used these periods not only because we had a good number of red admirals from these dates, but also because peak dates for return migration towards the south reported from Denmark were within this interval (Hansen 2001). This makes it reasonable to assume that the majority of the sampled individuals were captured while migrating.

At Capri, red admirals from the spring generation were sampled in the second week of May, while individuals from the autumn generation were sampled in the first week of October. We also collected a small set of resident species from Capri in order to evaluate the δD values found in wings of butterflies from this location. In 2004, we captured ten wall browns *Lasiommata megera* and ten southern small whites *Pieris mannii* in the second week of September and the following spring we captured an additional ten wall browns from the same period as the spring samples from red admirals. Wall browns hibernate during winter as small immature larvae (Tolman 1997), and therefore almost all the plant material contributing to the measured δD values in the wings of the adult butterflies are consumed in early spring. The samples from western Europe were of limited numbers and from different time periods, ranging from 16 April in the spring to 7 November in the autumn. We used these samples primarily to estimate the range of δD values encountered in red admirals captured in western Europe since they were too few to study the regional phenology in more detail. To compare the seasonal differences in δD values of butterflies in Spain we classified all samples up until 15 July each year as individuals emerged during spring (spring) and those captured after that date as individuals emerged during late summer and autumn (autumn). From

Table 1. Geographic position of sample locations, time period of sampling, number of butterflies sampled and mean δD values (with standard error and min/max values) measured in wings of red admirals *Vanessa atalanta*, wall browns *Lasiommata megera* and southern small whites *Pieris mannii* throughout Europe in 2003–2006.

| Species	Region	Location	Lat	Long	Year	Season	Date	N	δD ± SE (‰)	Min	Max
Vanessa atalanta	North	Ølsted, DEN	55.9°N	12.1°E	2004	Autumn	7–16 Sep	10	−106.2 ± 2.2	−119.7	−95.2
		Öland, SWE	56.2°N	16.4°E	2003	Spring	9 June	10	−87.1 ± 2.4	−104.3	−73.8
					2005	Spring	13 July	15	−100.5 ± 4.8	−145.9	−73.9
					2005	Autumn	31 Aug–6 Sep	15	−126.0 ± 2.1	−148.4	−117.5
		Lund, SWE	55.7°N	13.3°E	2003	Autumn	5–7 Sep	15	−117.5 ± 3.8	−143.6	−88.1
		Kullaberg, SWE	56.3°N	12.5°E	2004	Autumn	5 Sep	13	−118.2 ± 5.0	−116.3	−96.5
		Sandhammaren, SWE	55.4°N	14.2°E	2004	Autumn	9 Sep	15	−121.5 ± 2.6	−139.3	−105.1
		Rybachy, RUS	55.2°N	20.8°E	2004	Spring	24 June–3 July	15	−99.4 ± 2.9	−119.0	−80.0
					2004	Autumn	4–15 Sep	24	−127.5 ± 2.6	−158.6	−105.8
					2005	Spring	16 June–9 July	11	−95.9 ± 4.2	−119.9	−77.9
					2005	Autumn	31 Aug–14 Sep	17	−126.4 ± 3.1	−151.4	−107.3
		Karilatsi, EST	58.1°N	26.5°E	2004	Autumn	28 Aug	12	−128.3 ± 3.4	−140.8	−99.4
	West	Vilagarcía, SPA	42.6°N	8.8°W	2004–2006	Spring	16 Apr–22 May	7	−75.3 ± 3.8	−86.9	−56.2
					2004 and 2006	Autumn	17 July–7 Nov	11	−69.6 ± 4.6	−107.0	−54.1
		Lassy, FRA	48.1°N	1.9°W	2003–2005	Autumn	27 July–26 Sep	11	−72.6 ± 4.5	−96.8	−54.9
	South	Capri, ITA	40.6°N	14.2°E	2004	Autumn	1–6 Oct	10	−77.1 ± 5.6	−104.4	−53.9
					2005	Spring	9–12 May	10	−66.2 ± 4.5	−91.6	−44.8
					2005	Autumn	2–5 Oct	10	−99.9 ± 2.9	−116.2	−71.8
					2006	Spring	8–15 May	14	−72.0 ± 3.1	−91.3	−44.3
					2006	Autumn	2–7 Oct	14	−94.1 ± 3.6	−114.3	−71.8
Lasiommata megera	South	Capri, ITA	40.6°N	14.2°E	2004	Autumn	9–10 Sep	10	−51.3 ± 2.8	−64.5	−39.3
					2005	Spring	11–13 May	10	−82.2 ± 2.6	−95.2	−70.9
Pieris mannii	South	Capri, ITA	40.6°N	14.2°E	2004	Autumn	10–13 Sep	10	−56.3 ± 3.8	−75.7	−33.8
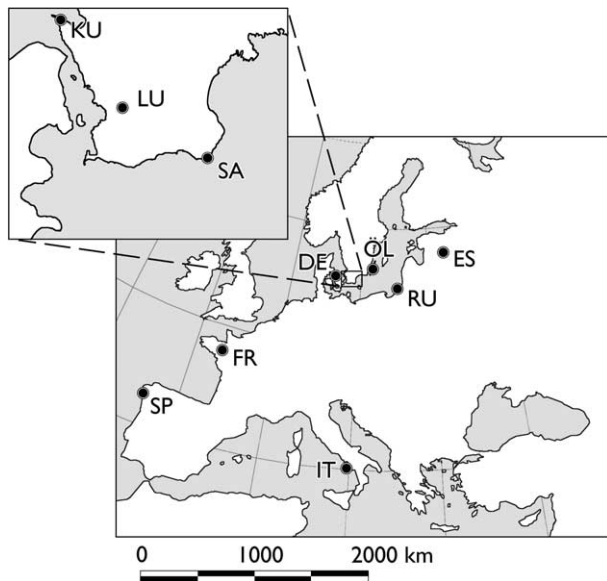
Figure 1. Position of sample locations where red admirals *Vanessa atalanta* were captured during spring and autumn 2003–2006. Locations are: Vilagarcía – Spain (SP), Lassy – France (FR), Capri – Italy (IT), Ølsted – Denmark (DE), Kullaberg – Sweden (KU), Lund – Sweden (LU), Sandhammaren – Sweden (SA), Öland – Sweden (ÖL), Rybachy – Russia (RU) and Karilatsi – Estonia (ES).

France we only received data from individuals captured during the autumn period.

Stable isotope analysis

We rinsed all wing samples in a chloroform-methanol solution (2:1) to remove oils that could affect the isotope assays, and air dried the samples overnight. Samples were weighed to 0.36 ± 0.01 mg in silver capsules. Because stable-hydrogen isotope analyses of chitinous and keratinous materials are complicated by isotopic exchange between samples and ambient water vapour (Wassenaar and Hobson 2000), we used the comparative equilibration technique so that the values reported here are equivalent to non-exchangeable hydrogen (Wassenaar and Hobson 2003, Kelly et al. 2009). We assumed that properties of hydrogen exchange in chitinous materials were similar to those in keratinous samples. In short, the process involved simultaneous measurement of wing samples with replicates of three different keratin standards whose non-exchangeable δD values were known. Stable-hydrogen isotope measurements of wings and the keratin standards were performed on H_2 derived from high-temperature ($1300°C$) flash pyrolysis of wings and continuous-flow isotope-ratio mass spectrometry. We used a Eurovector 3000 (Milan, Italy) high temperature elemental analyzer (EA) to automatically pyrolyse samples to a single pulse of H_2 gas. The resolved H_2 pulse was introduced to the isotope ratio mass spectrometer (Micromass Isoprime with electrostatic analyzer) via an open-split capillary. All deuterium results are expressed in the typical delta (δD) notation, in units of per mil (‰), and normalised on the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation

(VSMOW-SLAP) standard scale. The laboratory error was estimated to be ± 3.1‰ based on a 6-month running average of within-run keratin reference materials (Wassenaar and Hobson 2006).

Deuterium ratios in precipitation

The δD values found in butterfly wings is ultimately controlled by the δD value of local precipitation (Hobson et al. 1999, Brattström et al. 2008b), but isotope discrimination processes deplete this value when going from precipitation to the larval food plants. We used the Online Isotopes in Precipitation Calculator (OIPC) (<http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html>) to acquire interpolated amount-weighted annual and monthly δD values for the rainwater at our sampling locations (Fig. 2a). The annual variation of δD values in precipitation across Europe reveals a fairly complicated picture because of local topography (e.g. the
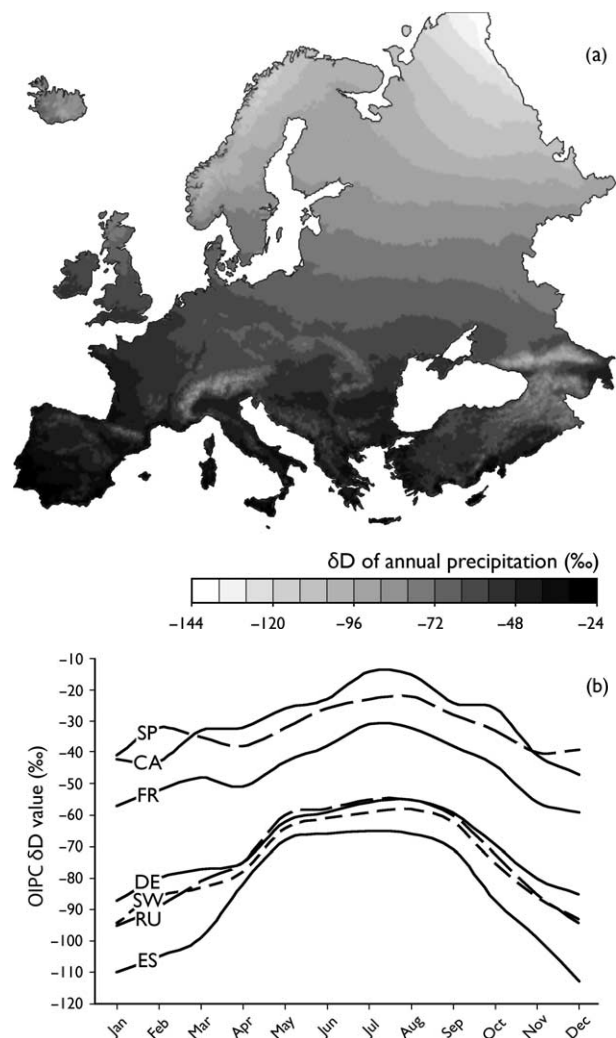

Figure 2. Interpolated δD values from OIPC (the Online Isotopes in Precipitation Calculator). (a) Mean annual values in Europe (map based on file downloaded from OIPC), and (b) monthly average values in precipitation from the different locations used in this study. The Swedish sample sites are represented by a single mean value (SW).

Alps) but the general trend is that δD in precipitation becomes more negative along a north eastern gradient (Fig. 2a, see also Hobson 2003). The deuterium values in precipitation also changes depending on the season (Fig. 2b). Since red admirals are migratory butterflies, we can rarely be sure of the hatching location of sampled individuals. To determine the expected local level of δD in butterfly wings, we therefore used data from resident butterfly species collected at Capri in Italy during spring and autumn as well as peacock butterflies *Inachis io* developed in Sweden (Brattström et al. 2008b). For the samples from Capri we used the OIPC values for the two months preceding the capture date to cover the time when the butterflies developed. For the peacocks we used the interval April–June, since this was a period corresponding to the season when peacock larvae develop in Sweden (Brattström et al. 2008b). We used a narrower time period for the Capri material since butterflies in general develop faster at warmer, more southern locations compared to the north. We compared measured δD values from wings of butterflies with known origin against the OIPC data to determine if we could find a relationship similar to the one reported for North American monarchs (Hobson et al. 1999).

Statistical analysis

All samples from the same location and season were pooled into one group regardless of year as we had no year for which we had data from all sampling sites. For each location with several years sampled, we found similar δD values between years (except autumn 2004 in Italy, see Discussion). We used a two-way ANOVA to study effects of season and sample location on the data from northern Europe, after first having tested that year had no significant result. This was done using a general mixed model including year as a random factor. For the samples from Capri, France and Spain we used a t-test to determine if spring and autumn individuals had different δD values. We also used a t-test to analyse if autumn individuals from the two local butterfly species at Capri captured during the autumn season differed in mean δD values. We used linear regression to analyse if there was a relationship between measured δD in the butterflies' wings and the OIPC values for the months prior to the hatching of the butterflies. In all comparisons, when considering mean values we present them as population mean ± standard error. SPSS 15.01 was used for all statistical calculations except the general mixed model (PROC MIXED) which was calculated using SAS 9.2.

Results

Results from the analysis of δD are presented as mean, standard error and range of δD values for all of the sampled locations and time periods in Table 1.

There was a strong linear relationship ($r^2 = 0.87$; $p < 0.001$; Fig. 3) between measured δD values in the wings of local butterflies from Capri and peacock butterflies from southern Sweden in relation to the OIPC values for
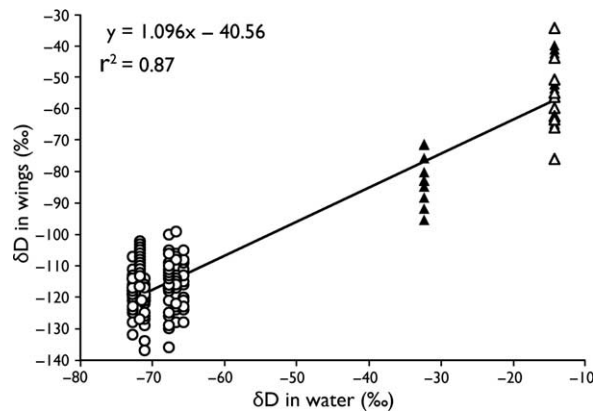

Figure 3. Relationship between measured δD values for local butterflies captured at Capri, Italy and in Sweden (for details see Brattström et al. 2008b) in relationship to local δD values in precipitation (derived from OIPC data) during the developmental time period of the larvae.

their region and season. The equation of the regression line was ($δD_{WING} = 1.1 \times δD_{PRECIPITATION} - 41‰$).

The mean δD value in wings of red admirals from Capri was higher in spring ($-69.6 \pm 2.6‰$) compared to in autumn ($-90.8 \pm 2.8‰$) (t-test, $t = 5.31$, $N_{Spring} = 24$, $N_{Autumn} = 34$, $p < 0.001$). The local butterfly species collected at Capri instead showed lower δD values in spring ($-82.2 \pm 2.6‰$) than in autumn ($-53.8 \pm 2.4‰$) (t-test, $t = -7.45$, $N_{Spring} = 10$, $N_{Autumn} = 20$, $p < 0.001$). There was no difference between the two species of local butterflies included in the autumn group (t-test, $t = 1.07$, $N_{L. megera} = 10$, $N_{P. mannii} = 10$, $p = 0.299$). The δD values of individual butterflies captured at Capri and the OIPC curve for mean monthly δD in precipitation at this location are presented in Fig. 4.
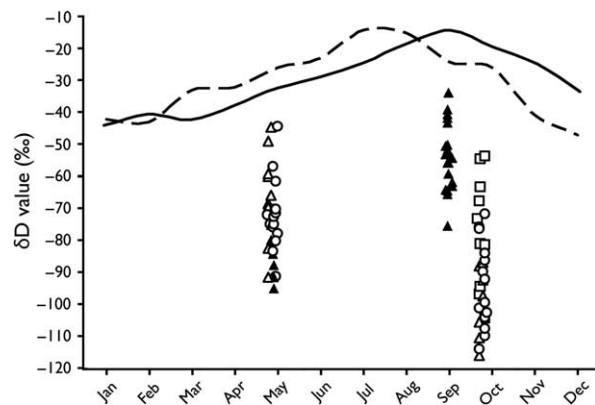

Figure 4. Mean δD values measured in wings of butterflies captured during spring and autumn at Capri, Italy. The included species are: two species of local butterflies (▲), wall brown *Lasiommata megera* and southern small white *Pieris mannii* and the migratory red admiral *Vanessa atalanta*. Red admirals are presented separately for each year: 2004 (□), 2005 (△) and 2006 (○). The broken line is the mean monthly δD values in the local precipitation as calculated by using the OIPC and the solid line is the mean values of the previous two months δD values (time period estimated to match the development period of the sampled butterflies). See text for further explanations.

The two-way ANOVA showed that both location and season had a significant effect on the mean δD values measured in red admiral wings in northern Europe (Table 2). There was no interaction between these two covariates. Samples from the spring season showed higher δD values (-96.6 ± 2.0‰; pooled mean across all locations) than those from the late summer generation (-122.5 ± 1.2‰; pooled mean across all locations) (Fig. 5). A Tukey post-hoc comparison identified two subsets among the sample locations in late-summer season that were different from each other ($p < 0.05$; Fig. 5). Denmark stands out among the locations with the highest δD values in autumn. Furthermore, we found a general decrease in δD values along an eastern gradient.

A separate ANOVA analysing samples from the island of Öland in the Baltic Sea only from three distinctly different time periods), showed that δD values of red admirals from 9 June (-87.1 ± 2.4‰), 13 July (-100.5 ± 4.8‰) and 31 Aug–6 Sep (-126.0 ± 2.1‰) were significantly different depending on sampling period (Table 3). A Tukey post-hoc comparison showed that all three time periods were significantly different from each other. Figure 6 shows the distribution of deuterium values from this subset of individuals.

There was no significant difference in mean δD values from red admirals captured in northern Spain during the first (-75.3 ± 3.8‰) and second (-69.6 ± 4.6‰) (t-test, $t = -0.88$, $N_{Spring} = 7$, $N_{Autumn} = 11$, $p = 0.392$) part of the year. Nor were there any significant differences in mean δD values between northern Spain (-71.8 ± 3.1‰; spring and autumn combined) and France (-72.6 ± 4.5‰) (t-test, $t = -0.16$, $N_{Spain} = 18$, $N_{France} = 11$, $p = 0.876$). The mean δD values for the western part of Europe are presented in Fig. 7.

Discussion

Movement patterns and timing of migration of red admirals across Europe is currently based on inferences from observational data and hence still relatively unsubstantiated. We found significant differences in δD in wings of red admirals from different sites in Europe. Even though the considerable natural variation in δD values can make predictions about sample origin difficult (Pain et al. 2004, Langin et al. 2007, Brattström et al. 2008b), especially when studying migratory animals with multiple generations, our data is the first evidence that can be used
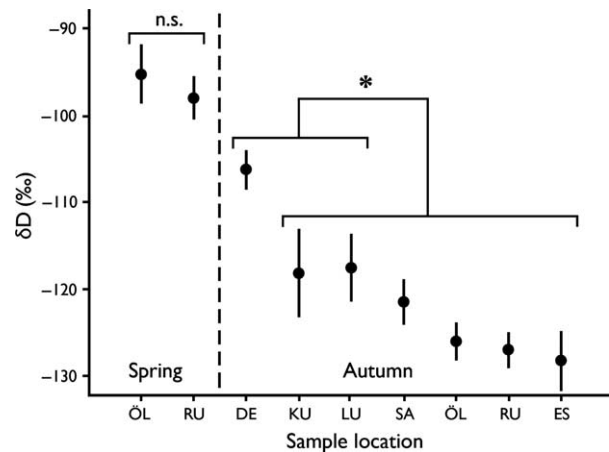

Figure 5. Mean δD values measured in wings of red admirals captured at seven locations in northern Europe during spring and autumn migration. Locations are presented in order of their geographical position along a longitudinal gradient. The values from spring were significantly different from autumn samples. There was no significant difference between the mean values with data from the spring season. Results from a Tukey post-hoc test show that the significant effect of sample location during autumn is composed of two subsets.

to answer some fundamental questions about the patterns and timing of migration for red admirals in Europe.

Relationship between deuterium content of precipitation and butterfly wings

We found that the δD of butterfly wings from Swedish peacock butterflies and non migratory butterflies from Capri were depleted by ca -40‰ (presumably due to isotopic discrimination) compared to the estimated local precipitation during the larval development period, similar to that of many birds (Hobson and Wassenaar 2008). Whereas the water-tissue relationship we found for our samples do not match the results presented by Hobson et al. (1999) from a study of monarchs raised in a controlled environment, it should be noted that their study used water with a deuterium content that occurs outside the range of δD values naturally occurring in our study region. Another potentially important factor that has to be taken into account when interpreting δD values in migratory organisms is the possible impact of seasonal variation of δD values in precipitation. Studies of moth larvae developing on leaves of different age classes showed that the fitness of the adults were highest when the larvae fed on buds and young leaves compared to older leaves (Virtanen and Neuvonen 1999). Studies of peacock butterflies in Sweden

Table 2. Results of the ANOVA test on measured δD values for red admirals *Vanessa atalanta* collected at different locations in northern Europe during spring (1 May–15 July) and late summer (25 August–16 September) season. The presented result is the final model that remains after a non-significant interaction terms have been removed.
Dependent variable: δD

Variable	S.S.	DF	F	p
Sample location	4797.6	6	4.70	<0.001
Season	22379.8	1	131.51	<0.001
Error	27907.9	164		
Total	2323809.4	172		

Table 3. Results of the ANOVA test on measured δD values for red admirals *Vanessa atalanta* collected at three different time periods at Öland, southern Sweden.
Dependent variable: δD

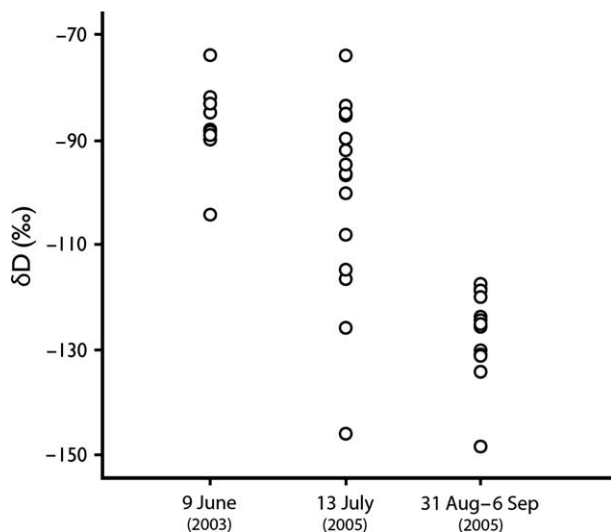| Variable	S.S.	DF	F	p
Time period	10008.2	2	28.89	<0.001
Error	6408.9	37		
Total	472115.7	39		

Figure 6. Measured δD values in wings from red admirals *Vanessa atalanta* captured on the island of Öland (southern Sweden) at three different time periods during summer. The early summer individuals have isotopic signature matching a southerly origin while the last group have values matching what would be expected for hatching close to Öland, or even further north. The mid group (July) individuals show a very wide range of values, suggesting that at this time of the year there is a mix of local individuals hatched and migrants from southerly areas.

found that δD values in wings of adults corresponded better with the δD values in local precipitation for the months prior to hatching of the pupae than the annual mean values (Brattström et al. 2008b). Red admirals are closely related to peacock butterflies and utilise the same host plants and are therefore expected to show a similar pattern. If we only looked at the annual amount-weighted δD values in precipitation (Fig. 2a) without considering development time, host plants and their water use when trying to determine origins of individual butterflies, we would estimate the origin of our sampled butterflies incorrectly. Butterflies from a spring sample that have developed during a period with lower-than-average local precipitation δD values will be assigned to a region further towards the northeast. For autumn samples the error will be reversed, i.e. they will be misplaced further towards the southwest.

Winter ecology of red admirals in the Mediterranean region

The local butterfly species collected at Capri must have been hatched during the same season in which they were collected, and the larvae should mainly have consumed food-plant material within a time period of a few months before capture. By assuming that the larval food-plants of these species contain similar δD values to those being the food sources for possibly locally hatched red admirals; we can use these data as an indicator of the expected δD values for red admirals originating from the same area in Italy at the same time of year.

The red admirals collected at Capri in autumn showed lower δD values, different from the autumn brood of local butterflies, suggesting that the region is re-colonized each
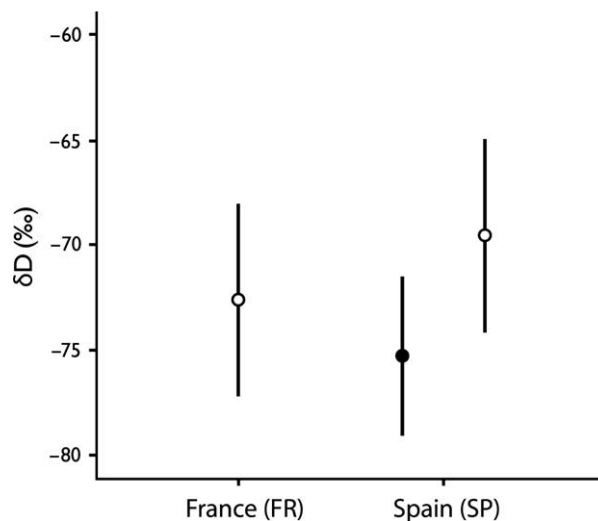

Figure 7. Mean δD values measured in wings of butterflies sampled in northern France and northern Spain from 2003 to 2006. The migration periods are divided in spring (before 15 July, filled symbol ●) and autumn seasons (after 15 July, open symbol ○).

autumn by individuals arriving from northerly regions. This is further supported by field studies showing late autumn migration in a southerly direction in Italy (Benvenuti et al. 1996) and former Yugoslavia (Reichholf 1978). In Spain, southerly movements are also observed and here it is coupled with a substantial increase in numbers of observed individuals in the regional standardized butterfly monitoring programme, further suggesting an annual recolonization in autumn (Stefanescu 2001).

Red admirals captured during spring at Capri showed similar δD values as the spring samples of local sedentary butterflies, but a fraction of the individuals showed slightly higher values suggesting a more southerly origin. The minimum δD value was almost identical between local resident butterflies and red admirals so the Capri spring populations of red admirals was probably made up of both locally hatched individuals and others originating from further south. The individuals with the highest δD values did not correspond to values that would be expected in wings from butterflies hatched anywhere in Italy, and they could therefore possibly be as far south as from the north coast of Africa, a region known to have populations of red admirals (Tolman 1997). Examining the profile of the North African coast and looking for the most southern location possible (also with a higher winter-time mean δD value in local precipitation) it is possible that some of the red admirals were hatched as far south as the coastal regions of Libya. There is no detailed data on the ecology of red admirals in the North African part of their distribution and it would be very interesting to further study their connectivity with the European population.

Our data suggest that red admirals that spend the winter in the Mediterranean region do not migrate to this southerly region just to find suitable conditions for hibernation, but instead reproduced in the area before the spring migration the following year. Wintertime reproduction has previously been suggested in Spain where wing wear index noted during monitoring suggested that a new

generation was appearing in spring (Stefanescu 2001). Also a previous study from Capri that measured the change in the range of wing lengths encountered among red admirals in spring and autumn supported the same hypothesis (Brattström 2006). The finding that some red admirals in Europe could well originate as far south as the North African coast has previously not been reported, but we know that a closely related species, the painted lady *Vanessa cardui*, crosses the Mediterranean Sea when flying from North Africa each spring on their migration towards Europe (Stefanescu et al. 2007).

Phenology of red admirals in northern Europe

The samples of red admirals collected in spring in the northern part of Europe resulted in δD values around $-90‰$ which is lower than the mean of the Capri spring samples. However, the mean δD values were still higher than expected for locally hatched individuals. The highest δD values observed in northern Europe overlapped with those observed at Capri in spring, suggesting that it was possible that some of the red admirals that arrived in northern Europe originated from the Mediterranean region. However, the majority of the butterflies in our study arriving to northern Europe in spring must have originated from areas further north or north east of Italy. Some of these red admirals showed δD values close to the expected value for locally hatched butterflies, which means that they were either the offspring of individuals that spent winter in the area or that they originated from areas well further east of the study region (Fig. 2a).

The autumn samples of red admirals from northern Europe were clearly different from those found in spring. The mean δD values were similar to those found in local peacock butterflies from Sweden (Brattström et al. 2008b). However, the range of values from most locations contained some individuals with δD values corresponding to those expected to be found in individuals from more southern areas. We found a general trend towards lower mean δD values in the sample locations towards the east. Hydrogen isotope variation in feathers of birds from different locations in Europe also shows a similar spatial pattern, with a general decrease in δD values along a southwest to northeast geographical gradient (Hobson et al. 2004, 2009, Pain et al. 2004, Newton et al. 2006). The spatial trend in our data was larger than expected from the OIPC data for the sampling locations, so there were probably individuals from areas further north and north-east encountered in Sweden, Kaliningrad and Estonia. Since we dealt with migrating as opposed to local butterflies, we have to expect larger recruitment areas for our samples and this is probably the reason for the observed mismatch between red admiral δD values compared to the expected local values from that region as inferred from the OIPC data.

Even though the general migration in the northern parts of Europe is reported towards the south during autumn (Imby 1993, Hansen 2001, Mikkola 2003a) there were butterflies in our autumn samples with δD values that suggested a more southerly origin. These individuals could be on a northward migration despite the late season, and

because of this we found a mixture of individuals with different migratory origin and directions at the same location at this time of year. The range of δD values from Öland in southern Sweden clearly demonstrated this pattern with only low values in the beginning of summer, indicating a southerly origin. Later in the season our samples from this location had a very broad range suggesting that the collected butterflies were a mix of southern immigrants and locally hatched individuals. Later in autumn we only found values corresponding to locally hatched individuals. Field observations of orientation of red admirals during migration supported our findings as they generally reported a main flight direction (north or south depending on season), but small numbers of individuals flying towards the opposite direction were always encountered during the same time period (Williams 1951, Benvenuti et al. 1996, Stefanescu 2001).

Large-scale patterns of migration in Europe inferred from stable isotope values

We found no differences in δD values for red admirals between spring and autumn in Spain or between Spain and France. Since the sample sizes were small and spanned a longer time period, it was not possible to draw any conclusions from this. However, since we know that there is large variation in δD values even within a set of samples of resident butterfly species from single locations (Brattström et al. 2008b) and only a limited natural variation in precipitation δD values in this European region due to the proximity of the Atlantic Ocean (Hobson 2003, Rubenstein and Hobson 2004), our isotope values from western Europe were harder to interpret. At the same time of the year the difference in monthly precipitation δD values at these locations differs only ca $10–20‰$, despite the large geographical distance (Fig. 2b). Despite these limitations, we gained one very important insight into red admiral migration from these samples. The lowest red admiral δD value found in this region ($-107‰$) was much higher than what would be expected in red admirals hatched during summer and autumn in the northern region, especially in the northeast (Kaliningrad) where we regularly found values lower than $-140‰$. The number of butterfly samples from the western region were small, but collected over a long period of time (four years), so if migration movements of red admirals between the northern and western region of Europe are common, we would expect to find several individuals in autumn in the western region with more negative ($< -110‰$) δD values. In our study there were no such samples, which suggested that red admirals originating from north Europe did not reach France and Spain in autumn, at least not in high numbers.

Red admirals encountered at Capri in autumn had higher δD values than those found in northern Europe during the autumn migration, so the majority of red admirals spending winter at this site likely originated from areas north of Italy in central Europe or from further to the west. This pattern could be the effect of a migratory divide (Alerstam 2001, Alerstam et al. 2003, Åkesson and Hedenström 2007) breaking up the normal migratory

direction that is reported to be along a north-south axis. It is possible that the red admirals reaching the western coast of Italy and Capri originate mainly from western European regions because of the location of the Alps in central Europe, well known to affect the migratory directions of birds (Liechti and Bruderer 1986, Bruderer and Jenni 1988, 1990, Liechti et al. 1996, Erni et al. 2005). We already know that features of local topography affects red admirals in a similar way to migratory birds, channelling their migration temporarily in directions more or less deviating from the preferred migratory direction of the season (Reicholf 1978, Benvenuti et al. 1996, Brattström et al. 2008a). However, since the red admiral δD values we found corresponded to several areas in different geographic directions somewhere north of Capri, we cannot be sure of the origin of the red admirals that arrived there in autumn. It still seems more likely that they arrive from westerly directions as visual observations at a coastal location north of Capri suggest that red admirals avoid flying over water and therefore follow the coast towards south east in autumn (Benvenuti et al. 1996). Furthermore, we know that red admirals on the eastern side of the Adriatic Sea (west of Italy) also follow the coastline (Reicholf 1978), so red admirals that pass the Alps on the eastern side during the autumn migration would be more likely to be funnelled into the Greek Peninsula rather than ending up in Italy. In 2004 the δD values found at Capri in autumn were higher than the other two years, suggesting annual variations in recruitment areas.

Conclusions

We found general patterns in butterfly δD values among the red admirals sampled in our study. The spring population in northern Europe was made up of immigrants arriving from more southern areas. There also seemed to be several different source areas for the spring immigrants, since δD values found during summer and early autumn covered a very broad range of inferred natal areas. It was also clear that red admirals, at least in the central Mediterranean region reproduced during winter and that hibernation, even if it seems to occur at low frequencies, is not a common strategy in northern Europe.

There was no doubt that north-south migration exists in Europe, as previous field observations have suggested (Benvenuti et al. 1996, Stefanescu 2001, Mikkola 2003a, b), but our data suggest that the spring migrants originated not only from the Mediterranean area, but possibly from areas further to the north or east. Most of the individuals captured in the western region and at the island of Capri in Italy, both in spring and autumn appeared to belong to another migratory population than those captured in northern Europe. Only the most extreme butterfly δD values from each season overlapped between northern Europe and the other areas suggesting that the western and central southern region of Europe were to some extent isolated form the northernmost areas. It was clear that hydrogen isotopes are powerful tools in studies of migratory butterflies and other migrating insects and animal migrants in Europe. If used in a multivariate

approach with other potentially useful isotopes (e.g. $\delta^{13}C$, $\delta^{15}N$), field observations, genetic analyses, and other available methods we may be able to get to a more detailed understanding of large scale annual movements of red admirals in Europe that to date have been impossible to study using standard monitoring techniques.

References

Åkesson, S. and Hedenström, A. 2007. How migrants get there: migratory performance and orientation. – Bioscience 57: 123–133.

Alerstam, T. 2001. Detours in bird migration. – J. Theor. Biol. 209: 319–331.

Alerstam, T. et al. 2003. Long-distance migration: evolution and determinants. – Oikos 103: 247–260.

Benvenuti, S. et al. 1996. Directional preferences in the autumn migration of the red admiral (*Vanessa atalanta*). – Ethology 102: 177–186.

Bowen, G. J. et al. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. – Oecologia 143: 337–348.

Brattström, O. 2006. Is there seasonal variation in size and mass of red admirals *Vanessa atalanta* on Capri, Italy? – Ornis Svec. 16: 69–73.

Brattström, O. et al. 2008a. Effects of wind and weather on red admiral, *Vanessa atalanta*, migration at a coastal site in southern Sweden. – Anim. Behav. 76: 335–344.

Brattström, O. et al. 2008b. Placing butterflies on the map – testing the regional geographical resolution of three stable isotopes in Sweden using a monofagus Nymphalid, the peacock *Inachis io*. – Ecography 31: 490–498.

Bruderer, B. and Jenni, L. 1988. Strategies of bird migration in the area of the Alps. – In: Quellet, H. (ed.), Acta 19th International Ornithological Congress. Univ. of Ottawa Press, pp. 2150–2161.

Bruderer, B. and Jenni, L. 1990. Migration across the Alps. – In: Gwinner, E. (ed.), Bird migration. Springer, pp. 60–77.

Dockx, C. et al. 2002. Do North American monarch butterflies travel to Cuba? Stable isotope and chemical tracer techniques. – Ecol. Appl. 14: 1106–1114.

Endler, J. A. 1973. Gene flow and population differentiation. – Science 179: 243–250.

Erni, B. et al. 2005. The role of wind in passerine autumn migration between Europe and Africa. – Behav. Ecol. 16: 732–740.

Hansen, M. D. D. 2001. Observations on migrating red admirals (*Vanessa atalanta* L.) in Denmark 1995–2000. – Flora Fauna 107: 1–5.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. – Oecologia 120: 314–326.

Hobson, K. A. 2003. Making migratory connection with stable isotopes. – In: Berthold, P. et al. (eds), Avian migration. Springer, pp. 379–391.

Hobson, K. A. and Norris, D. R. 2008. Animal migration: a context for using new techniques and approaches. – In: Hobson, K. A. and Wassenaar, L. I. (eds), Tracking animal migration using stable isotopes. Academic Press, pp. 1–19.

Hobson, K. A. and Wassenaar, L. I. 2008. Tracking animal migration using stable isotopes. – Handbook of Terrestrial Ecology Series, Academic Press.

Hobson, K. A. et al. 1999. Stable isotopes (delta D and delta C-13) are geographic indicators of natal origins of monarch butterflies in eastern North America. – Oecologia 120: 397–404.

Hobson, K. A. et al. 2004. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. – Oecologia 141: 477–488.

Hobson, K. A. et al. 2009. Stable isotopes (δD) delineate the origins and migratory connectivity of harvested animals: the case of European woodpigeons. – J. Appl. Ecol. 46: 572–581.

Imby, L. 1993. South migrating red admirals (Vanessa atalanta L.) on southern Öland. – Entomol. Tidskr. 114: 115–116.

Kelly, J. F. et al. 2009. A test of comparative equilibration for determining non-exchangeable stable hydrogen isotope values in complex organic materials. – Rapid Commun. Mass Spectrom. 23: 2316–2320.

Langin, K. M. et al. 2007. Hydrogen isotopic variation in migratory bird tissues of known origin: implications for geographic assignment. – Oecologia 152: 449–457.

Liechti, F. and Bruderer, B. 1986. Einfluss der lokalen Topographie auf nächtlich ziehende Vögel nach Radarstudien am Alpenrand. – Ornitol. Beobachter 83: 35–66, in German with English summary.

Liechti, F. et al. 1996. The Alps, an obstacle for nocturnal broad front migration – a survey based on moon-watching. – J. Ornithol. 137: 337–356, in German with English summary.

Mikkola, K. 2003a. Red admirals Vanessa atalanta (Lepidoptera: Nymphalidae) select northern winds on southward migration. – Entomol. Fenn. 14: 15–24.

Mikkola, K. 2003b. The red admiral butterfly (Vanessa atalanta, Lepidoptera: Nymphalidae) is a true seasonal migrant: an evolutionary puzzle resolved? – Eur. J. Entomol. 100: 625–626.

Newton, I. et al. 2006. An investigation into the provenance of northern bullfinches Pyrrhula p. pyrrhula found in winter in Scotland and Denmark. – J. Avian Biol. 37: 431–435.

Pain, D. J. et al. 2004. Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler Acrocephalus paludicola. – Oecologia 138: 168–174.

Pollard, E. and Greatorex-Davies, J. N. 1998. Increased abundance of the red admiral butterfly Vanessa atalanta in Britain: the roles of immigration, overwintering and breeding within the country. – Ecol. Lett. 1: 77–81.

Reichholf, J. 1978. Migration patterns of great white (Pieris brassicae) and red admiral (Vanessa atalanta) in Adriatic. – Dtsch. Entomol. Z. 25: 191–194.

Rubenstein, D. R. and Hobson, K. A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. – Trends Ecol. Evol. 19: 256–263.

Spieth, H. R. et al. 1998. Flight directions in the migratory butterfly Pieris brassicae: results from semi-natural experiments. – Ethology 104: 339–352.

Stefanescu, C. 2001. The nature of migration in the red admiral butterfly Vanessa atalanta: evidence from the population ecology in its southern range. – Ecol. Entomol. 26: 525–536.

Stefanescu, C. et al. 2007. Migration of the painted lady butterfly, Vanessa cardui, to north-eastern Spain is aided by African wind currents. – J. Anim. Ecol. 76: 888–898.

Steiniger, H. and Eitschberger, U. 1996. Cynthia cardui and Vanessa atalanta 1994. – Atalanta 27: 29–32.

Tolman, T. 1997. Butterflies of Britain & Europe. – HarperCollins.

Virtanen, T. and Neuvonen, S. 1999. Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. – Oecologia. 120: 92–101.

Wassenaar, L. I. and Hobson, K. A. 2000. Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. – Environ. Sci. Technol. 34: 2354–2360.

Wassenaar, L. I. and Hobson, K. A. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. – Isotopes Environ. Health Stud. 39: 211–217.

Wassenaar, L. I. and Hobson, K. A. 2006. Stable hydrogen isotope heterogeneity in biological tissues: isotope-ratio mass spectrometry and migratory wildlife sampling strategies. – Rapid Commun. Mass Spectrom. 20: 2505–2510.

Webster, M. S. et al. 2002. Links between worlds: unravelling migratory connectivity. – Trends Ecol. Evol. 17: 76–83.

Williams, C. B. 1951. Seasonal changes in flight direction of migrant butterflies in the British Isles. – J. Anim. Ecol. 20: 180–190.

Wunder, M. 2009. Using isoscapes to model probability surfaces for determining geographic origins. – In: Bowen, G. J. et al. (eds), Isoscapes: isotope mapping and its applications. Springer, in press.