ECOLOGY OF RED ADMIRAL MIGRATION

Ecology of red admiral migration

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Dissertation

Lund 2007

Before I sink,
into the big sleep,
I want to hear,
I want to hear,
the scream of the butterfly

“When the Music’s Over” – Jim Morrison 1967

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Ecology of red admiral migration

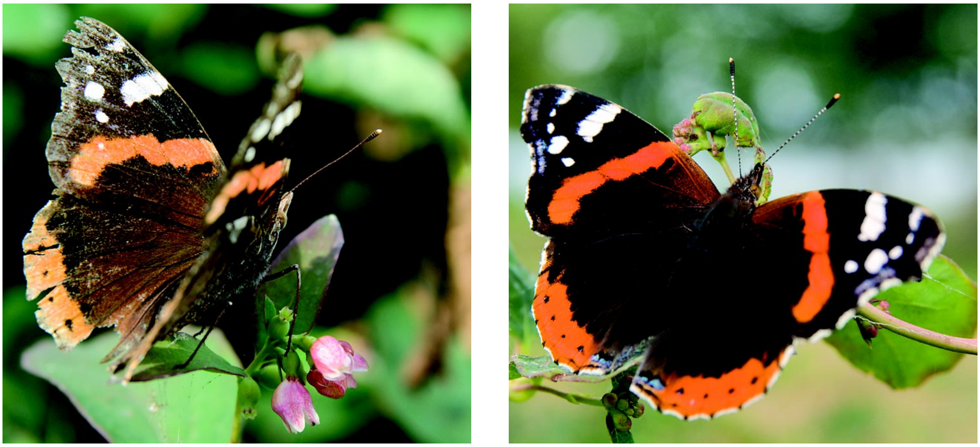
When discussing the topic of insect migration people will often mention the same behaviour in birds, since most people are unaware that many insect species migrate across continents each year. As pointed out by Williams in his classic book about insect migration (1958), these phenomena were reported in literature, and even discussed in relation to prevailing winds as early as 3000 years ago. Book of Exodus, Chapter 10, Verses 13 to 15: "and the Lord sent an east wind that blew across Egypt the rest of the day and all that night. By morning, locusts were swarming everywhere (…) The ground was black with locusts, and they ate everything left on the trees and in the fields. Nothing green remained in Egypt—not a tree or a plant." and then in verse 19: "the Lord sent a strong west wind that swept the locusts into the Red Sea". Even though the source of both locusts and wind is a matter open to debate, there is no doubt that this kind of insect migration could have substantial impact on human lives in our early history. Since butterflies do not have this great an impact on human society, we will have to move ahead more than two thousand years before we have sources mentioning their migration. And when we finally have such a reference, this is because the butterflies in question happened to interrupt something that has always been very important in our history.

From the twelfth century, we know of a report from a battlefield mentioning not just armies of men but also a huge army of butterflies, probably large whites (*Pieris brassicae*). According to this report, mentioned by Williams (1958), the butterflies were so many that they hid the light of the sun. We also find that these butterflies moved from Saxony towards Bavaria (in modern Germany); a migration route that is still today used by the same species each autumn (e.g. Spieth & Kaschuba-Holtgrave 1996, Spieth *et al.* 1998). Modern studies of butterfly migration have mainly focused on the monarchs (*Danaus plexippus*) in North America. Studies of this species' remarkable migration have been going on since 1857, and an excellent review of all monarch studies from that point up until the mid 1990s has been compiled by Brower (1995). The monarch is an exception from most other migratory butterflies as its migration is rather well known to the general public in the area where it can be observed.

From Europe, there are numerous published observations of the migration habits of both butterflies and moths, but only a few experimental (e.g. Spieth & Kaschuba-Holtgrave 1996, Spieth *et al.* 1998) or long-term studies (e.g. Stefanescu 1997, 2001). Our knowledge about butterfly migration is mostly reconstructed from snap-shots along presumed migratory routes. This has been compiled by adding together different observations of large scale migratory events, or by comparing first and last reported sightings of different species across their range throughout different years.

The study species

Among the butterflies that can be found in Europe, the red admiral (*Vanessa atalanta*) stands



No pain, no gain! The red admiral to the left looks like it has been through a lot of troubles getting there, while the one to the right looks in pristine condition. They were both photographed on 11 July 2006 at Ottenby, Öland. Since red admirals found in this area can come from various natal regions at this time of year (Paper IV) it is possible that the one to the left has flown all the way from the Mediterranean Sea while the other one might come from an area much closer to Öland. Photo: Oskar Brattström.

out among all long distance migrants showing the perhaps most regular pattern of migration. In the British Butterfly Monitoring Scheme, the average yearly change in number of red admirals seen is two-fold, while the closely related painted lady (*Vanessa cardui*), being a more irregular migrant, varies as much as six-fold on average (Pollard & Yates 1993). There are of course larger fluctuations in numbers than for most resident species but red admirals can be observed in good numbers every summer in most parts of northern Europe while many other migrants can be completely missing some years. The occurrence in the most northern parts of the distribution range is much more variable than further towards the south (Eliasson *et al.* 2005), but large numbers of red admirals can reach as far as northern Norway some years (Strann *et al.* 1995).

Because of the many reported sightings of large migrations of red admirals and a few organised studies focussing on this species in particular, we have a reasonably good knowledge about the yearly pattern of migration. But as we shall see there are still gaps in our present knowledge. The aim of this thesis is to close some of these gaps by experimental studies aimed at solving several separate open questions about the migration ecology of red admirals. Before presenting the work we have done, I will briefly present what was previously know about the red admirals' migration ecology followed by a presentation of the studies we have performed, including more detailed comparisons to previous studies focusing on the same type of questions.

The red admiral is a Nymphalid butterfly that can be found in North America, North Africa, most parts of Europe, and in the westernmost parts of Asia (Eliasson *et al.* 2005). Each spring, the European red admirals initiate large scale migrations towards the north. There are observations of these northward migratory flights from Italy (Benvenuti *et al.* 1994, 1996), Spain (Stefanescu 2001), Britain (Williams 1951) and Scandinavia (Hansen 2001) in March through June, but some studies have questioned whether the individuals leaving the Mediterranean area will reach northern Europe to any considerable extent (Roer 1961, Baker 1972). As it is known that red admirals can also hibernate, even though the significance of that behaviour is questionable, the previously mentioned studies have suggested that the species only migrate short distances as if the whole population made a short movement towards the north each spring (Fig. 1a). Lately, the common view (e.g. Mikkola 2003b, Eliasson *et al.* 2005) suggests that the migration is rather extensive and covers long distances (Fig. 1b–c), but none of the published studies have managed to prove that the Mediterranean spring migrants really reach as far north as, for example, Scandinavia each spring. Studies from Lebanon (Larsen 1976) and Spain (Stefanescu 2001) indicates that not all red admirals migrate towards the north in spring; instead some individuals fly shorter distances to reach nearby locations at high altitude. This altitudinal migration is, however, not studied in detail at other regions.

Regardless of the origin of, or the distance flown by the spring immigrants, they all appear to mate shortly after the migration flight ends and the females then lay their eggs on nettles (*Urtica dioica*). Later in the summer a new generation hatches and additional reproduction events might even occur. Red admirals are regularly observed as late as October in northern Europe (Henriksen & Kreutzer 1982, Eliasson *et al.* 2005). Each year migration in large numbers towards the south is reported in autumn throughout Europe (Willimas 1951, Roer 1991, Imby 1993, Benvenuti *et al.* 1994, 1996, Hansen 2001, Stefanescu 2001, Mikkola 2003a) but there are also reports of large scale migrations towards the north at this time of year (Rudebeck 1951, Radford 1975), suggesting a variable migratory pattern in autumn. In addition to all these reports on migration, there are also reports of red admirals in the middle of winter in the northern region (e.g. Steiniger & Eitschberger 1996) as well as on hibernated specimens in early spring before any immigrants have had time to reach these areas (e.g. Pollard & Greatorex-Davis 1998). Previously,

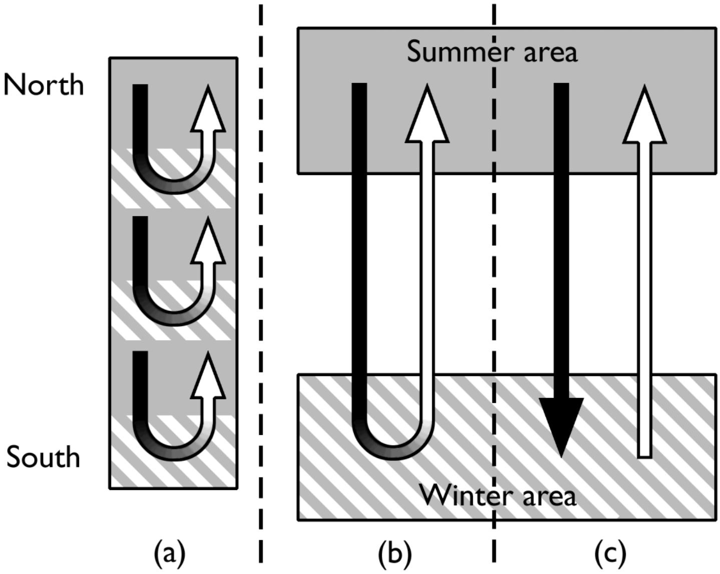


Figure 1. Schematic representations of three possible migration systems suggested for red admirals. Black arrows represent autumn migration and white arrows spring migration. Reproduction takes place at the areas indicated by the arrow points. In the first system (a) there are several different populations in a north – south axis that move a small distance towards the south each autumn to avoid the harshest of winter conditions. They then hibernate during winter and return north for reproduction during summer. The next system (b) is similar but the distance of the migration flight spans the whole distribution area. In the final system (c) the winter area is not used for hibernation, but reproduction takes place both here and in the summer area. Since insects in general are plastic migrants the number of generations produced each year can vary, but the main difference remains that the first system has many discrete groups of short distance migrants and that the last system has no hibernation at all, and year-round reproduction.

most literature considered the red admiral to be a true hibernator (e.g. Higgins & Hargreaves 1983) or at least that it remained in reproductive diapause (references in Lempke 1971) during winter in all parts of its distribution (Fig. 1b). However, observations of breeding during winter in Lebanon (Larsen 1976) and recently from Spain (Stefanescu 2001) suggest that hibernation is not a common strategy (Mikkola 2003b), at least not in the areas around the Mediterranean Sea (Fig. 1c).

When studying the relationship between the number of observed hibernated specimens in spring and the number of individuals sighted during autumn in Britain, no correlation was found suggesting that hibernation is not an important strategy, at least not in the north-western part of Europe (Pollard & Greatorex-Davis 1998). Experimental studies trying to induce hibernation in captured red admirals in Germany failed to keep any specimens alive over the winter (Roer 1961, references in Lempke 1971). The general trend has been to upgrade the red admiral to a migrant species over time. In the early 1900s it was considered to be a resident species and then a short distance migrant (Fig. 1b) fifty years later (Williams 1958, Roer 1961). The present literature (e.g. Mikkola 2003b, Eliasson *et al.* 2005) suggests, as previously mentioned, that the red admiral is a long distance migrant that does not regularly hibernate (Fig. 1c). As shall be seen, the work presented in this thesis mainly supports these most recent ideas.

Research objectives

The main objective in this thesis has been to study selected parts of the red admiral migration biology in more detail to try to link together all knowledge generated in the previously mentioned field observations. Our work has mainly been focused on five questions using different study methods. We investigated:

1) If there was any genetic structure among the red admirals in Europe that could shed light on its migratory patterns (Paper I).
2) The winter ecology of the species in the Mediterranean region to find solid evidence for either reproduction or hibernation (Paper II).
3) If it is possible to infer the natal region of red admirals sampled during migration, and if so use this knowledge to investigate the annual phenology of the species (Paper III, IV and V).
4) The flight direction and orientation mechanism used during autumn migration (Paper VI).
5) The red admiral migration intensity in autumn at a coastal site in Southern Sweden in relation to different weather parameters (Paper VII).

Methods

Estimates of genetic differentiation

When comparing samples from different locations with regard to genetic difference, originating from the geographical isolation of sub-populations of migrating animals, there are several different techniques that can be employed (Webster *et al.* 2002). We decided to use AFLP (Amplified Fragment Length Polymorphism; Vos *et al.* 1995, recent review, see Bensch & Åkesson 2005) because it is a cost efficient method that will quickly find differences, mostly within neutral parts of the DNA, and it can be used without any previous knowledge of the genome of the study species. To describe the process briefly, AFLP uses restriction enzymes to produce a large number of fragments with 'sticky ends' and specific adaptors are then ligated to these. The adaptors will only adhere to fragments with 'sticky ends' that match a known sequence of the adaptor. After that, a selective amplification of the fragments that have adhered to the adaptors is performed. To lower the number of amplified fragments, primers that will amplify only those fragments that include a known sequence are arbitrarily selected. The amplified fragments are then separated by size on a polyacrylamide gel and visualized by a labelled primer. The primers in the selective amplification are optimized to generate a number of fragments that are variable enough to find differences between individuals, but also low enough to make the gels easily analysed.

We used the AFLP protocol described in Vos *et al.* (1995), modified according to Bensch *et al.* (2002) and using a combination of three different primers (Paper I). This gave us a total of 169 polymorphic sites in the red admiral genome that could be unambiguously scored. To analyse the AFLP data, we calculated pair-wise F_{ST}-values for all our sampled groups and analysed if there was any correlation between these values and different measurements of geographic distance between the sample sites. We also analysed the genetic information from our red admirals using the software STRUCTURE 2.1 (Pritchard & Wen 2003), looking for possible grouping within our data that could not be detected when arranging the data according to the sample locations. STRUCTURE 2.1 estimates the most likely number of populations that would produce the given data set and disregards information on collection sites. It also assigns each sample a probability for belonging to either of the suggested populations.

Wing length measurements, sex determination and estimates of lipid content

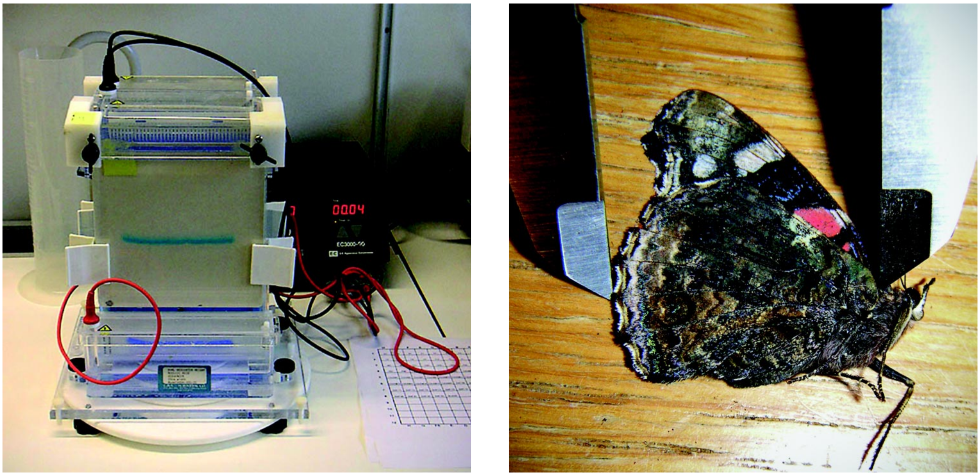
In the study of seasonal differences in the winter region (Paper II), we needed an easily acquired measurement of size of individual butterflies. The length of the hindwing was chosen for this, and was recorded (maximal distance from the base to the outer margin) to the nearest 0.5 mm with a digital calliper. Only individuals with unabraded hindwings were included in analysis using size measurement.

Lipid content was estimated using two different methods. In the study of winter ecology (Paper II), the abdomen of the collected butterflies were placed in plastic test tubes with rubber sealed caps and put in a freezer. All of the collected butterflies had their abdomen dissected to determine sex by inspection of their genitalia. After dissection, the butterflies were dried in 70°C for 24 hours and the dry mass of the abdomen was measured separately on a balance. Since butterflies feed on liquid food which contain high amounts of water, their mass can increase immediately after feeding (Christer Wiklund & Fredrik Stjernholm, personal communication), and therefore dry mass is a more reliable estimate of lipid content than wet mass. In the long term study of migration at a coastal site (Paper V), we could not keep the collected abdomens frozen and therefore we preserved the samples in 99.9% ethanol. Sex was determined as previously described, but lipid content was inspected visually using a scale consisting of six different classes.

Analysis of stable isotope ratios

Ratios of naturally occurring stable isotopes often vary in systematic ways across large geographical areas (e.g. Hobson 2003, Bowen *et al.* 2005). Since these isotopes only show a little difference in their function in biological matter they are taken up into plants and animals in a ratio reflecting the local region (e.g. Kelly 2000). There is almost always some sort of discrimination in the uptake process, but this is generally systematic and if the discrimination function is known, it is possible to estimate the natural value of the location from where the incorporated elements originate (Hobson *et al.* 1999, Paper III & IV). If we use metabolically inert tissue in our analysis, the ratios of stable isotopes should reflect the ratios of the area where the nutrients used for producing the actual tissue were acquired; a pattern that can be used to infer natal areas or wintering regions for migrating animals (Hobson 1999 & 2003, Hobson *et al.* 2004). Knowing that wings of butterflies are not part of their metabolism after eclosion of the adults, we sampled 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.

All wing keratin samples were rinsed in a chloroform-methanol solution (2:1) to remove surface oils that could affect the isotope assays, and air dried overnight. Stable nitrogen and carbon isotope analysis was performed at the Stable Isotope Laboratory in the Ecology Department at Lund University and stable hydrogen isotope analysis was conducted at Environment Canada in Saskatoon, Canada. The full details of the method is described in the papers using stable isotopes analysis (Paper III, IV & V), but in short the method works as follows: The sam-



"Behind the scenes" in the lab: To the left is the setup for size separation of genetic samples on a polyacrylamide gel that was used in the AFLP analysis. To the right red admiral "E14" has its hindwing measured after a flight session in an orientation cage. Photo: Mikael Åkesson (left), Oskar Brattström (right).

ples are pyrolysed, and the resulting gases from the combustion are carried by a stream of inert carrier gas towards a set of detectors. The detectors utilize the difference in mass between two isotopes to determine the ratio between them. Results are usually reported as a deviation from standard references (δ-values), and we follow that system. As internal references to correct for differences between runs in the carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) analysis (Paper III), we used previously calibrated glycin, homogenised great reed warbler feather keratin (*Acrocephalus arundinaceus*) and powdered bowhead whale baleen (*Balaena mysticetus*) samples. The use of previously known standards is even more important in analysis of hydrogen isotopes ($^{2}H/^{1}H$), since there is uncontrolled isotopic exchange between samples and ambient water vapour (Wassenaar & Hobson 2000). We used the comparative equilibration technique so that the values reported here (Paper III, IV & V) are equivalent to nonexchangeable hydrogen (Wassenaar & Hobson 2003).

In the study investigating the resolution of stable isotope methods (Paper III), we needed to use an alternative study species: the peacock butterfly (*Inachis io*). This was necessary since we wanted to study the values of different isotope ratios that one can expect to find in butterflies hatched at known locations. Knowledge about natural gradients and annual fluctuations in isotope ratios are necessary to be able to infer the natal region of migrating animals. The peacock is closely related to the red admiral, and uses the same host plant but in contrast to the red admiral it appears to be relatively stationary in the time period following eclosion of the adults. Since peacocks often move considerable distances in autumn to find suitable habitats for hibernation, which is the sole winter strategy of this species, we always sampled the butterflies used in this study (Paper III) as soon as possible after hatching.

Orientation experiments

To study the flight direction of red admirals during autumn migration (Paper VI), we used circular flight cages in which the movements of the butterflies could be recorded using video imaging, a type of setup previously used to study flight direction in large whites (*Pieris brassicae*) (Spieth & Kaschuba-Holtgrave 1996, Spieth *et al.* 1998). Since previous studies (Perez *et al.* 1997, Oliveria *et al.* 1998, Mouritsen & Frost 2002) have suggested that migrating butterflies mainly rely on a time compensated sun compass for orientation, we studied the flight

In the early autumn morning at the island of Öland, Sweden, the cages used in the orientation experiments are casting their shadow over the wind protection set up around the test area. At this location we studied the orientation of red admirals on autumn migration over three years (Paper VI). Photo: Oskar Brattström.

direction of a clock-shifted group in relation to a control group. All orientation experiments were conducted in late summer at Ottenby Bird Observatory, situated at the southern point of the island of Öland in southeastern Sweden (56°12´N, 16°24´E). We collected red admirals in the observatory garden and kept them indoors in cages for a minimum of three days to allow their internal clock to adapt to the new light/dark regime. In our study, the control group experienced a light/dark cycle similar to the local natural conditions while the clock-shifted group had their cycle delayed by four hours. We performed orientation experiments in the autumn of 1999, 2002 and 2005. In the last two years, we collected the tested butterflies after they had been filmed in the cages, to be able to analyse the genetic structure and isotope signature of these individuals at a later date for use in other studies (Paper I & IV).

In 1999, we did not keep the tested butterflies for later studies, instead we released them immediately after the cage experiments. This enabled us to compare these two methods of estimating flight directions; by using the same individuals in both parts of the study. We released the butterflies on a large open grass meadow close to the site where we performed the cage experiments, and after release we followed them visually using a pair of binoculars (10×40) until they disappeared out of sight. Vanishing bearing of each individual was estimated using a handheld compass, and wind direction as well as wind speed was measured using a handheld anemometer. Only individuals that flew a minimum of 50 meters before settling, or that continued flying until they disappeared out of sight were included in the analysis.

Migration counts at a coastal site

At the Falsterbo peninsula in south-eastern Sweden (55°23´N, 12°49´E), organized counts of migratory birds have been performed for more than forty years (Kjellén 2006 and earlier references there in). The people counting the passing birds have previously noted that not only birds migrate at the Falsterbo peninsula, but most autumns a large number of butterflies can also be seen on migration flights across the sea towards the Danish coast. Since we are not aware of any long term study in Europe where organised counts of butterfly migration in a standardized way has been combined with data of local weather, we decided to count the visible migration of red admirals at the same location and compare migration intensity with weather and wind parameters (Paper VII).

The observation site is situated at the eastern coastline of the point of the Falsterbo peninsula, approximately 100 meters east of the western coastline, running in a north-south direction.

In connection with the normal counts of migrating birds, the observers also recorded butterflies that were seen migrating out over the water between 1 August and 25 October during the autumns of 2004 – 2006. Individuals venturing out over the sea were followed roughly 50 meters and if they continued in the same direction they were regarded as migrants and an approximate migratory direction was then estimated using the vanishing bearings of the observed butterflies.

Data for weather variables were obtained from the Swedish Meteorological and Hydrological Institute (SMHI) recorded by a weather station located at Falsterbo. Among the variables recorded at the station we used wind direction and speed, air pressure, temperature, precipitation and cloud cover in the analysis.

Results

Genetic structure of European red admirals

If a migratory species is spread out over a large area and different parts of the population use separate migration routes, it is likely that genetic differences will be found between these subpopulations (Webster *et al.* 2002). Since the available reports on the flight direction used by red admirals in Europe suggest that the majority of the migrants follow a north-south axis, we expected that differences would be larger with increasing longitudinal (east-west axis) distance between sample sites. We used samples of red admirals from 2004 and 2005 colleted throughout Europe, and analysed the genetic structure of individuals using AFLP. Differences between sample sites were first investigated using isolation-by-distance methods, but there were no significant patterns, regardless of the kind of geographical distance used. When instead we looked at the result from the STRUCTURE 2.1 run, we found an intriguing pattern with two populations as the most likely number to produce a data set of the kind we had. When looking at the individual probabilities for belonging to either of these two population (Fig. 2), we found a clear bimodal distribution; each individual was assigned with a high probability to either of the two populations and there were strikingly few intermediate genotypes (13%). Combining this information with the geographical origin of the samples, we suggest that there are two sub-populations of European red admirals that use different wintering areas. One of these spends winter around the Mediterranean area while the other probably can be found further to the east at this time of year. In the northern part of the distribution range individuals from both of these groups can be found but there are large variations between years regarding which group is the most common, even at the same site. Interbreeding is most likely hindered by temporal isolation, but since intermediate genotypes can sometimes be found in substantial frequencies at single locations, other mechanisms of selection must also be in operation to maintain the distinctiveness of the identified clusters.

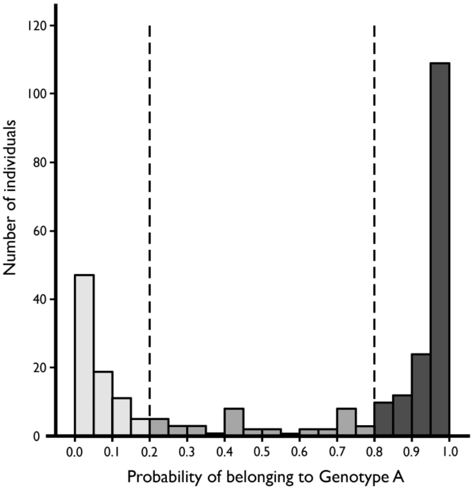


Figure 2. Histogram showing the frequency of individuals of red admirals assigned to two suggested genotype clusters that is found in Europe. The arbitrary limits (Paper I) set by us, for being assigned to each group are marked with dashed lines.

Studies of red admiral winter ecology

In the beginning of the last century, the common view on the winter habits of red admirals held that it was a resident species (reviewed in Williams 1958), and just like other resident

Nymphalides it was thought to be a hibernator. Closely related species, for example the peacock and the small tortoiseshell (*Aglais urticae*), spend winter in hibernation and then reproduce in spring (Eliasson *et al.* 2005) before the later hatching of other butterfly species that have spent winter as eggs, larvae or pupas. While the view shifted towards the red admiral not being a resident, the view on winter hibernation remained, but one can question the validity of this view. Why would red admirals migrate – a behaviour likely to be dangerous – if they do this just to reach an area were they hibernate before returning the next spring (Fig. 1b)? If closely related species can manage the winter climate in the north, and in doing so do not have to risk the rigours of migration, they would probably have an advantage over the red admirals. However, if red admirals can breed during winter (Fig. 1c) they should be able to counterbalance the cost of migration (Walker 1980). As parasites, among other factors, could be important for the evolution of animal migration systems (Alerstam *et al.* 2003), and are also a major cause of death in butterflies, it is possible that migration costs can also be balanced by a reduction in parasite prevalence. Larsen (1993) reported that resident butterfly species often have infection rates as high as 80%, while nomadic butterfly species can be almost completely free of parasites. The prevalence of a protozoan parasite (*Ophryocystis elektroscirrha*) was investigated in different sub-populations of the North American monarch and it was shown that monarchs that moved the longest distance during migration had the lowest number of parasites (Altizier *et al.* 2000).

Evidence against hibernation in the Mediterranean region has been around for a long time, Lempke (1971) mentions some old reports (1897–1953) on observations of actively flying red admirals in the region during winter, but the idea that reproduction is part of the winter ecology is rather new. The perhaps earliest report on breeding during winter came from Lebanon (Larsen 1976) and Stefanescu (2001) recently reported what was probably the first solid series of observations supporting reproduction in winter as the normal pattern in the Mediterranean area. He found that the amount of wing-wear was steadily increasing in red admirals observed in late summer up until winter, but then in spring the butterflies appeared once again to have fresh wings. Since the wings of butterflies are not replaced or maintained after hatching, this is strong support for the appearance of a new generation which, over the time spent in the winter areas, replaces the old generation that arrived to the area the previous autumn.

To gather further data on winter ecology of red admirals in the Mediterranean area, we studied differences in wing length and abdominal dry mass (i.e. lipid content) in samples colleted both in autumn and spring at Capri, Italy (Paper II). We found no difference in abdominal dry mass compared to wing length between autumn and spring. This suggests that relative lipid content did not differ between the two seasons (Fig. 3). However, the red admirals had longer wings in autumn compared to spring and this was not just due to a loss of the largest individuals from the population, but to the appearance of a whole new range of smaller individuals. These results are in line with Larsens' (1976) and Stefanescus' (2001) findings, and further support the theory that red admirals around the Mediterranean Sea do not spend winter in hibernation. The only well documented long distance butterfly migrant that is a hibernator; the monarch, has been shown to put on large amounts of lipids prior to hiberna-

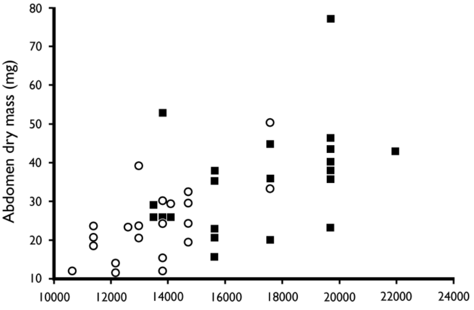
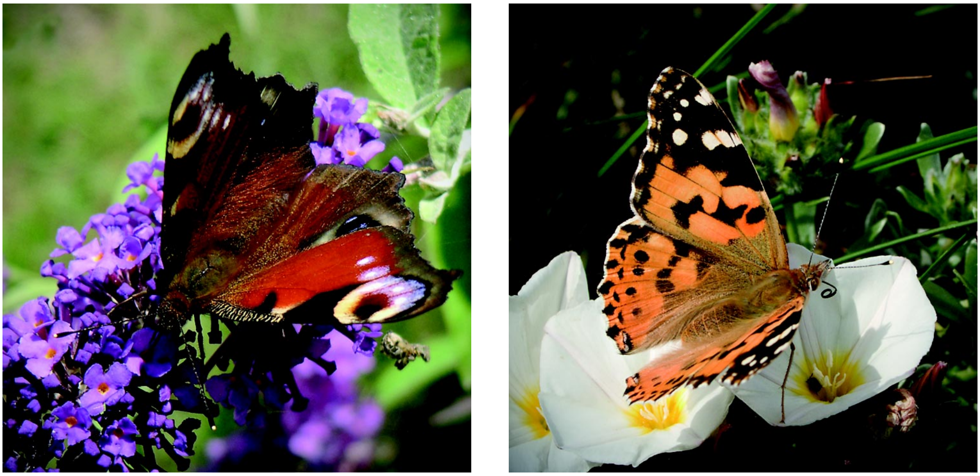


Figure 3. Relationship between hindwing length and dry mass of abdomen (an estimate of lipid content) from red admirals caught during spring (○) and autumn (■) on Capri, Italy. There is no seasonal difference in this relationship but the absolute size of the captured individuals differs between seasons.



Two other species appearing in this thesis: To the left is a peacock, a species that was used when evaluating the isotope methods (Paper III) and to the right is a painted lady, a migrant that is closely related to the red admiral but has a much more irregular pattern of appearance. The painted lady is the most cosmopolitan of all known butterflies; it can be found all over the world except in the neotropical and polar regions. Photo: Oskar Brattström.

tion (Beall 1948, Alonso-Meija *et al.* 1997), something we did not observe in our study. It would probably be maladaptive for a species that is active throughout winter to put on large amount of lipids since predators would then be able to catch them much easier (Dudley *et al.* 2002). The difference in size we observed between seasons at Capri, with smaller individuals in spring, lends further support to the idea of winter reproduction as opposed to hibernation since the mortality of hibernating monarchs has been found to be greater in the smaller size classes (Alonso-Meija *et al.* 1997). In our studies of migratory patterns in the European red admirals (Paper IV), we find further evidence supporting that reproduction takes place during winter (see below).

Annual patterns in the timing and origin of the migrants

Since we can expect high mortality during migration in butterflies, mark-recapture methods are unsuitable for finding migration routes and such methods are therefore unlikely to be of any use in studies of this species (e.g. Webster *et al.* 2002). Our study on the genetic structures of the European population found substantial differences between a presumed western and eastern group (Paper I), but could not identify distinct sub-populations that use the same migratory corridors on a regional scale. Such patterns can however be hard to identify with genetic methods if interbreeding keeps sub-populations in different geographical regions from developing discernable genetic differences (Endler 1973). In the case of red admirals, we needed to try a different approach for finding patterns and routes in the annual migration.

A study of monarch butterflies has shown that stable isotopes can be used to infer the origin of migratory butterflies (Hobson *et al.* 1999) and we therefore wanted to investigate if this method could be used for studies of migration in European butterflies. To evaluate the stable isotope technique, we sampled peacock butterflies at seven different locations in an area of 250×250 kilometers in southern Sweden during three years (2002–2004) to study the natural variation and the resolution power of three different stable isotopes (Paper III). Ratios of hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes were obtained from our sampled butterflies and the results showed that ratio of hydrogen isotopes (δD) was the most useful indicator of natal origin, as has been shown for migratory birds in Europe (Hobson *et al.* 2004). It should be noted however, that because of differences in δD values in local rain water (Fig. 4) the expected value in butterflies originating from the same location will vary over the season (Hobson 2003, Paper III). Based on our findings using peacocks, we decided to focus our following studies of stable isotopes on hydrogen ratios (^{2}H/^{1}H or δD) as found in the wings of red admirals captured during different seasons throughout Europe (Paper IV & V). Since the δD values directly reflect the geographic origin of single individuals, the technique can help identify migratory patterns across Europe even in species with large annual variation and fairly regular intermixing between sub-populations.

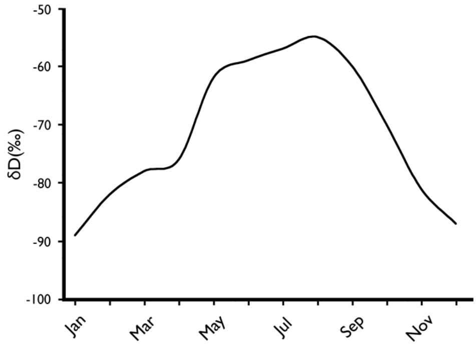


Figure 4. Monthly δD values estimated for local rain water at a sample sites in southern Sweden (Lund). This general pattern of annual variation is present all over Europe, but the difference between winter and summer is more pronounced in northern locations.

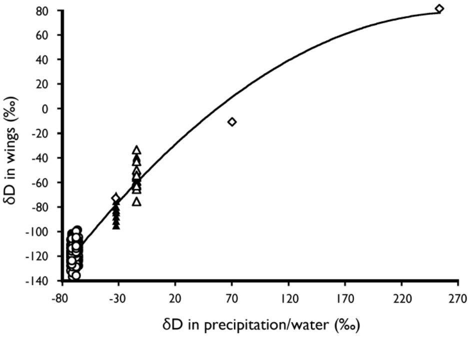


Figure 5. Relationship between measured δD values from butterfly wings and δD values in water or water of known deuterium content. Data is combined from studies of peacocks (○) in Sweden (Paper III), resident butterflies (Δ, ▲)from Italy (Paper IV), and Monarchs (◊) raised in a laboratory study (Hobson *et al.* 1999).

Using the ratio of deuterium values, we could show that δD values in locally hatched butterflies corresponded with the values in rain water (Fig. 5). Using this knowledge on isotope fractionation, we found that the red admirals appearing in spring in northern Europe most likely originate from areas much further south, and that some of them must have flown all the way from the Mediterranean area (Paper IV & V). In some red admirals captured in Italy during spring, we found δD values indicating that they originated from natal areas in North Africa (Paper IV), suggesting that the species regularly cross the Mediterranean Sea. In autumn the majority of the sampled butterflies in northern Europe are hatched locally or even further to the north (Paper IV & V), but there are always a few individuals with isotope ratios indicating a much more southern origin (Paper IV). We also found very few overlaps in δD values between the northern and western parts of Europe suggesting that migration in the western part of Europe is separated from the northern and eastern parts (Paper IV). This is in line with our findings of a predominantly east – west difference in the genetic structure of the red admirals in Europe (Paper I).

When looking in detail at the annual migration at a coastal site (Paper V), we found that the variation in origin of the captured individuals was greater than at inland sites in the same part of Europe (Fig. 6). This is probably because red admirals avoid crossing water and instead they follow the coastline as long as it does not lead them too far off their preferred migratory direction. Coastal areas will therefore be visited by individuals from many different locations that concentrate as a result of local topography (Reichholf 1978, Benvenuti *et al.* 1994, Stefanescu 2001, Paper VII). We also found a difference in δD values of samples from Capri in southern Italy between autumn and spring (Pa-

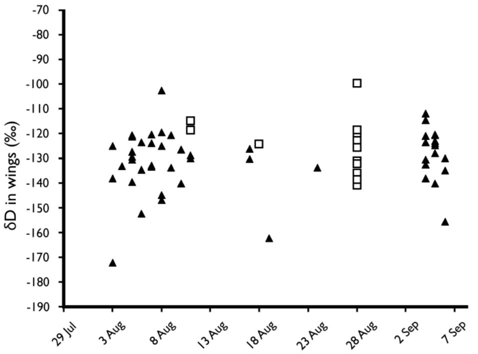


Figure 6. Measured δD values in the wings of red admirals sampled at an inland location in Estonia (☐) and samples from a coastal site in Kaliningrad, Rybachy (▲). The variation in the measurements is higher from the coastal location indicating a more diverse origin of the sampled butterflies.

per IV) once again supporting the findings that red admirals reproduce during winter in the Mediterranean region (Stefanescu 2001, Paper II).

Flight directions and orientation mechanisms

The red admirals tested in our circular cages (Paper VI) during autumn migration showed very persistent flight directions, both on an individual and group level for all three study years. The mean orientation of the control butterflies in 1999 was directed to the north while we found a completely reversed orientation; towards the south to south-west in both 2002 and 2005 (Fig. 7). In 1999, we found a clear effect of the clock-shift treatment, but in the other two years, there was no significant difference in orientation between clock-shifted and control butterflies. Thus, the red admirals in 1999 seemed to rely on a time-compensated sun compass for orientation as has been showed for other migrating butterflies (Perez *et al.* 1997, Oliveria *et al.* 1998 & Mouritsen & Frost 2002). The results of the release experiment in 1999 showed that directions recorded in cages and from the observations of the butterflies when released, corresponded rather well with each other.

The difference in control direction between the three studied autumn migrations shows once more that the migration pattern of red admirals is highly variable; differences in origin of the sampled butterflies (and presumably also annual weather differences) seem to have a sizeable impact on the flight direction. The lack of effect from clock-shifting in the other years can be caused by similar differences, it might be that the group of butterflies we tested in these years were not in a migratory state and perhaps they were fuelling up for a coming flight towards the south. Even though we found a mean flight direction towards the south, perhaps they might still not have been actively migrating, or at least not doing so under time pressure, and therefore not using their sun compass system to full extent. It is clear that to really understand the patterns in this species migration, several different methods need to be used for each studied individual. In order to fully understand the results from orientation experiments in red admirals we need to know which sub-population they belong to and from what region they originate.

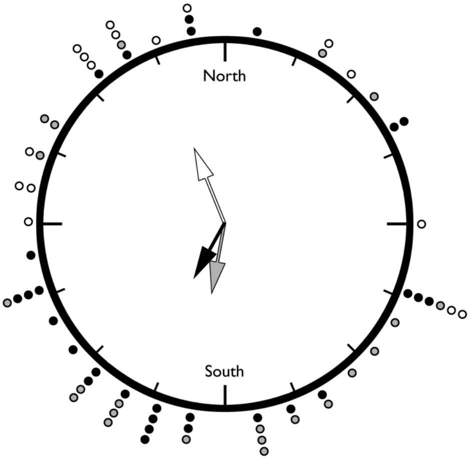


Figure 7. The mean orientation, as recorded in circular cages, for control groups of red admirals in autumn 1999 (white), 2002 (gray) and 2005 (black). Dots represent the mean direction of single individuals while the vector shows the angle of mean orientation for each of the years. Individual directions are plotted to the nearest 10° and 0° is set as geographical north.

Coastal migration in relation to local weather

There have been many studies of insect migration in relation to weather (e.g. Drake & Farrow 1988, Russell *et al.* 1998, Moskowitz *et al.* 2001), but most of them have been concerned with other insects than butterflies. Most insects migrating by flight are passively transported by the wind (e.g. Solbreck 1985), but some level of control can be gained by actively choosing to migrate when winds are favourable (e.g. Åkesson & Hedenström 2007). However, day-active butterflies and moths seem to be less affected by winds as they are often flying close to the ground in the boundary layer or on the lee-side of topographic structures, where wind speeds are lower (Taylor 1974, Walker 1985, Srygley & Oliveira 2001). Monarchs are an exception in regard to other butterflies as they are often found at (Gibo 1981) high altitude using soaring flight when wind conditions are favourable (Gibo & Pallett 1979). Red admirals have been reported to follow the general pattern for butterflies with flights close to the ground, and also to actively avoid flying out over open water. One exception to this pattern comes from a study in southern Finland, where red admirals appeared to actively gain height and to fly in suitable tail-winds as high up as 1000 meters (Mikkola 2003a). This was however at a location where they were about to cross open seas for more than 60 kilometers to reach land again in Estonia on their flight towards Continental Europe. We studied the migration patterns and the effects of weather over three autumn periods. Using a standardized count of red admirals departing the Falsterbo peninsula combined with local weather parameters (Paper VII), we were able to answer several questions on what weather conditions they chose for the flight across the sea and how they migrated relative to local topography. The results showed a large variation in the intensity of migration, not only between years but also between single days over the autumn season. We also found that just a few weather variables determined if a day was considered suitable for migration across the sea, but the intensity of migration was not dependent on the same factors. There appears to be a weather-based threshold for initiating migration, but once it is reached the intensity was not dependent on any of the weather variables we had available. This suggests that once the threshold is met, the density is more dependent on the number of red admirals that are already in the area waiting for suitable conditions for the crossing than on variations in the weather. The most important cue for initiating migration is wind direction (Fig. 8), followed by the amount of cloud cover and wind speed. A typical day with migration of red admirals at Falsterbo is characterized by winds of low speeds from the east under a clear sky. The substantial effect of wind; something that is not usually encountered when studying migrating butterflies, is probably an effect of the coastal location of the study site. The only other study of red admiral migration where winds seem to play an important role in shaping the migration was also performed at a location where the butterflies were about to cross open water (Mikkola 2003a).

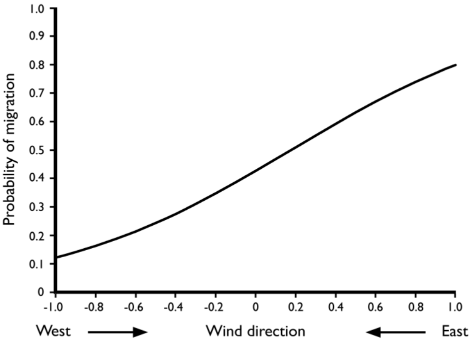


Figure 8. Relationship between predicted probability of migration at Falsterbo and the wind component in a western/eastern direction. This is the most important cue for initiation of migration. The other variables that have a significant effect for determining if a day is suitable for migration (in falling order) are cloud cover, wind speed and the northern/southern component of the wind direction.

Conclusions and directions for future studies

In this thesis I have tried to link together the many assumptions regarding red admiral mi-

Castello Barbarossa is a remarkable field station at the island of Capri, Italy. The fortification is over a thousand years old and located on a cliff towering more than 400 meters above the nearby sea. On this location we have studied the winter ecology of red admirals (II). Photo: Oskar Brattström.

gration in Europe that have been inferred from previous field observations. By using methods not previously employed in the study of this species, and in some cases not used at all on migrating butterflies in Europe, we have shown that the red admiral is a true long distance migrant and that they can clearly reproduce year round. Hibernation is probably not an important strategy for the species in the western and central parts of Europe, but some of our results indicate that differences in this pattern might well be present in the eastern part of the European red admirals' range.

When comparing our results with those from other animal migrants, for example birds, it is clear that the red admiral is much more variable in its annual pattern of migration. For example, we often find migration in two opposite directions at the same time of the year as a result of an influx of migrants from different regions to the study location. One of the reasons why we can expect differences in migratory behaviour between insects and many other animals is the lack of social learning of migratory behaviours and routes. Many animals optimize their migration by observing and learning the routes used by more experienced individuals, but for insects – being both short-lived and in the case of red admirals not social – we must assume that all the magnificent migratory movements we study are based on strictly inherited behaviours. This calls for more studies of which cues determine the migratory direction of individual butterflies, and whether that direction can change over the course of the adult life of single individuals.

It is also clear from the results that a combination of different methods, in many cases are necessary, to assist us in interpreting our results. For example, in the orientation studies there was substantial scatter in the observed directions as well as presence of individuals showing orientations opposite to the majority of the other butterflies, recorded both within and between years. Since we now know how variable our sampled groups are in regard to both population and natal origin, as shown by genetic studies and analysis of stable isotopes, it is necessary to have as detailed background information about all tested individuals as possible to be able to evaluate the results and thus to compare natural groups within a given data set.

As we have shown, the red admiral show a rather flexible and interesting migration pattern and has great potential as a representative study species for relatively regular insect migrants in Europe. However, in future studies it is important to include locations further towards the east, as many of our results indicate a difference

The author of this thesis looking for red admirals and painted ladies on the slopes of Monte Capello, Capri. Red admirals on Capri in general seem much more territorial and cautious than in the northern parts of Europe during autumn, hence the long handle of the net. Photo: Susanne Åkesson.

in migration behaviour in this part of the population compared to what we have found in central European red admirals. Moreover, it would be invaluable to have observational data over the whole migration period from several more sites in the distribution range. So far, there are only annual or at least seasonal series of reasonably standardized observations available from Spain (Stefanescu 2001), Sweden (Paper VII), Russia – Kaliningrad (Paper V) and to some extent Denmark (Hansen 2001). If possible, systematic collection of individuals from the same sites could really help to improve our knowledge about the migration system of European red admirals. It is however not easy to locate sites were one can gather reasonably sized samples of red admirals. Permanently manned biological research stations, for example bird observatories, have proved extremely useful in our studies, and a network for observations and collection of samples could probably be extended throughout Europe. Furthermore, studies of the migration of North American red admirals would be valuable for comparison to the European population. The monarch has, for understandable reasons, long been the favourite model species for butterfly studies in North America, but maybe it is time to start a more extensive comparison of its migration with other local species? The monarch, with its remarkable winter ecology is indeed a fascinating example of a migratory butterfly, but it is clearly an extreme case in a spectrum of different migratory systems. Considering the recent evidence of the ongoing global warming, studies of migrating animals and their response to climate change is potentially very important. Perhaps migrants having fixed goals for their migration, for example monarchs and many bird species, will face tougher challenges in the future compared to other migrants like red admirals showing a more variable migration pattern, which could potentially adapt much faster and easier to changes in their environment?

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Amiralens flyttningsekologi

Varje år ger sig miljontals dagfjärilar över hela jorden ut på långa flyttresor som ibland kan föra dem tusentals kilometer från deras kläckningsplatser. Ofta återvänder deras avkomma, ibland flera generationer senare, till ungefär samma platser i ett årligt återkommande mönster. Eftersom fjärilar är förhållandevis små och inte lika välkända som andra flyttande djur, t.ex. fåglar, är de inte lika välstuderade. Det mesta vi idag vet om fjärilsflyttning kommer från rapporter av amatörentomologer om fynd och observationer av olika arter, men allt mer organiserad forskning bedrivs idag inom området. Speciellt den nordamerikanska monarken (*Danaus plexippus*) är välstuderad och har varit basen för de flesta flyttningsstudier av dagfjärilar men bland de Europeiska fjärilarna finns det i dagsläget inte lika mycket forskning gjord.

I min avhandling har jag studerat amiraler (*Vanessa atalanta*), en dagfjäril som finns i hela Europa och Nordamerika samt även i delar av Nordafrika och de allra västligaste delarna av Asien. Precis som många andra europeiska fjärilar flyttar amiralen varje år norrut under våren från sina vinterkvarter i södra Europa. I Sverige brukar de första av vårens amiraler dyka upp omkring maj och juni för att lägga ägg på nässlor. Larverna växer sen till sig ganska snabbt och pupporna kläcks omkring juli–augusti. Vissa år kan amiralen vara så framgångsrik att de tillhör de vanligaste fjärilarna i Sydsverige under sensommaren. Framåt hösten drar sig sen de flesta amiralerna tillbaka söderut för att hitta områden som är milda nog för att överleva vintern. Många andra närbesläktade fjärilsarter överlever vintern genom att gå i dvala i naturliga gömslen och inte sällan inomhus på husvindar, jordkällare och garage. Tidigare trodde man att amiralen också gick i dvala över vintern fast nere i sydligare områden, något som senare tids studier börjat ifrågasätta.

Eftersom amiralen är en av de absolut vanligaste och mest regelbundna flyttfjärilarna i Europa finns det en mängd fältstudier där artens beteende har rapporterats. Vår tidigare kunskap om deras flyttmönster var en syntes av alla dessa fältobservationer. Genom att kombinera mönstret med första och sista dag som man regelbundet såg amiraler i olika länder och med hjälp av observerade flyttriktningar fastslog man hur arten flyttade men bilden av arten har förändrats genom åren. Det var först i mitten av 1900 talet som amiralen började anses som en art som årligen flyttade längre sträckor och det är endast nyligen som man börjat misstänka att de inte alls går i dvala över vintern. Mitt mål med den här avhandlingen var att försöka besvara eller bekräfta flera olika frågor om amiralens flyttningsbeteende genom att använda experimentella metoder på ett sätt som inte gjorts tidigare i studier av just amiraler. I vissa fall har metoderna inte använts överhuvudtaget i studier av flyttande fjärilar i Europa.

Det första vi gjorde var en översiktlig kartläggning av den genetiska strukturen hos amiraler över stora delar av Europa. Om det finns olika grupper inom en flyttande art som använder separata flyttvägar och förökningsområden kommer de successivt anpassa sig till sina speciella miljöer och sällan föröka sig mellan grupperna. Detta kommer med tiden att leda till att små men tydliga skillnader i deras arvsmassa (DNA) skapas. Vi fann att det i Europa finns två huvudtyper av amiraler: I Medelhavsområdet finns endast en huvudtyp i stor mängd och den blandas med en annan troligen mer östlig grupp när vi flyttar oss längre norrut. Vi har inte kunnat lokalisera huvudområdet för den östliga gruppen men det är troligen en bieffekt av hur våra insamlingslokaler varit placerade. Det som var mest slående var hur få mellanformer vi hittade, något som tyder på att starka mekanismer håller de två typerna isolerade från varandra Med tiden kan det komma att visa sig att detta är, eller håller på att bli två arter.

Genom att studera skillnaden mellan amiraler fångade på hösten och våren i Italien kunde vi visa att övervintring inte är den normala lösningen för att överleva vintern. De amiraler som fångades på våren var av en helt annan storlekskategori än de som fanns där på hösten. De hade däremot inte olika mängd av lagrat fett och eftersom andras studier gjorda med fjärilar som spenderar vintern i dvala visar att de lägger på sig stora fettlager för att överleva denna tid utan att behöva leta upp föda, tyder detta återigen på att amiraler inte övervintrar i dvala.

För att avgöra varifrån enskilda fjärilar har kommit ifrån innan vi samlat in dem på våra insamlingslokaler har vi använt oss av analyser av stabila isotoper. I berggrunden och i regnvatten finns det ofta regelbundna och storskaliga geografiska mönster för hur olika varianter (isotoper) av olika grundämnen (t.ex. kol, kväve och väte) förekommer i förhållande till varandra. Även om dessa varianter av olika ämnen på kemisk nivå är lite olika så fungerar de relativt likformigt i biologiska processer. Om växter tar upp ämnen från marken och sedan blir uppätna av ett djur så kommer förhållandet (kvoten) mellan dessa isotoper vara likadant i djuret som det var i marken och vattnet i området där växten växte. Eftersom fjärilarnas vingar inte förändras alls efter kläckningen (de hänger mest fast som ett torrt bihang på den vuxna fjärilen) så kommer förhållandet mellan olika isotoper i vingarna vara samma som området där fjärilen en gång kläcktes. Genom att mäta upp förhållandet mellan två typer av t.ex. kol i insamlade vingprover hos flyttfjärilar och sedan jämföra dem med förekomsten i hela utbredningsområdet kan man avgöra var fjärilarna växt upp. Med hjälp av denna metod har vi kunnat visa att amiralerna som fångas in i Syditalien på våren antingen är kläckta i närområdet eller i vissa fall ännu längre söder ut, ända ifrån Nordafrika. Att amiralen troligen flyttar över Medelhavet var en ny och oväntad upptäckt för oss. Vi har dessutom kunnat se att de fjärilar som kommer upp till Nordeuropa på våren antingen kommer från sydöst eller från centrala mellan Europa. Det verkar som om amiralerna som finns i sydvästra delen av Europa är relativt isolerad från dem som finns i den mer östliga delen. Med denna metod kunde vi även få ytterligare bevis för att amiraler inte spenderar vintern i dvala utan förökar sig i Medelhavsområdet under denna tid.

Djur som flyger långa sträckor har stor nytta av pålitliga navigeringssystem. Om de kan flyga närmaste sträckan mellan två punkter kommer de spara både tid och energi, värdefulla resurser för att framgångsrikt föröka sig. De flesta studierna av fjärilar visar att de verkar använda en så kallad solkompass, de använder solens riktning i förhållande till sin egen flygriktning som riktningsgivare. Eftersom solen flyttar sig under dagen kommer fjärilens kurs också ändras om de inte tar hänsyn till dessa solrörelser. Vi testade hur amiraler navigerar genom att filma deras riktningspreferenser i cirkulära burar. Genom att hålla en grupp i normal dygnsrytm (kontrollgupp) och en annan i en försenad dygnsrytm som ställde om deras ”interna klocka” (klock-skiftad grupp) i förhållande till den naturliga kunde vi studera om de tar hänsyn till solens position under dygnet. När man testar en sådan skiftad grupp parallellt med en kontrollgrupp kommer den skiftade gruppen avvika i sin flygriktning då de tror att solen står i ett annat läge än den egentligen gör eftersom deras klocka blivit omställd. Vi utförde orienteringsförsök vid Ottenby på Ölands södra udde under tre höstar. Resultaten var varierande, 1999 flög vår kontrollgrupp rakt norrut men 2002 och 2005 flög de istället söderut. Under det första året fick vi en tydlig effekt av klock-skifts behandlingen och det verkade som om amiralerna kunde kompensera för de dagliga solrörelserna. De två andra åren fick vi däremot ingen effekt alls av den behandlingen, fjärilarna flög precis likadant i båda grupperna, kanske för att de inte var lika flyttningsbenägna som första året och därför inte navigerade lika noggrant.

Det är möjligt att de fortfarande var i en fas då de lagrade upp energi för flytten och därför inte hade så bråttom och behövde använda hela sitt kompass-system fullt ut.

Det sista försöket vi gjorde var en långtidsstudie av amiraler vid Falsterbo. Flyttfåglar har länge studerats på denna plats och varje år räknas alla förbiflygande fåglar för att få kunskap om när på hösten de flyttar och ungefär hur många som finns i landet. Samtidigt som denna räkning pågick räknade observatörerna även förbiflygande amiraler under tre år (2004–2006). Amiralerna lämnar här landet och tar kurs mot närmaste land på andra sidan Öresund. Med hjälp av data från en av SMHIs väderstationer som finns vid Falsterbo kunde vi undersöka vilka väderförhållande amiralerna tar hänsyn till när de avgör om en dag är lämplig för att flyga över havet eller ej. Vi fann att lugna ostvindar kombinerat med molnfri himmel utmärkte dagar med flyttande fjärilar från dem utan, men vi kunde däremot inte finna någon skillnad bland de dagar då det fanns flyttande fjärilar. Under de dagar vi hade flyttning fanns det inga samband mellan det antal som observerades och rådande väder. Det verkar som om amiraler samlas upp på Falsterbonäset i väntan på rätt väder och när en tillräckligt bra dag dyker upp flyger alla de som finns i området över till Danmark. Precis som i de flesta andra studier av fjärilsflyttning var det ganska stora skillnader mellan åren under vilken tid på hösten flyttningen kulminerade. Då fjärilar precis som de flesta växelvarma djur är väldigt styrda av temperaturvariationer kan det ofta variera så mycket som en hel månad. Ett varmt år stannar de helt enkelt kvar längre i Sverige än vanligt men riktigt kalla år kan de nästan utebli helt.

Som helhet visar jag i den här avhandlingen att amiraler går utmärkt att använda som studieart vid undersökningar av flyttande fjärilar och att experimentella metoder kan hjälpa till att länka samman de många fältobservationer som redan finns. Vi hittade även nya samband som nu kan studeras vidare. Det som kanske närmast ligger på tur för mer studier är amiralens vinterbeteende i östra Europa samt noggrannare studier av hur deras flygriktningar avgörs. Bestäms det redan vid kläckningen i vilken riktning den vuxna fjärilen ska flytta under sitt liv eller kan de vända om miljön förändras under flyttningsperioden? Jag hoppas att den här forskningen kommer att inspirera till fler studier av flyttande insekter i Europa.

Tack!

Jag vill tacka alla de 1195 amiraler som inte kom undan håvarna…

Förutom dem är det givetvis några till som förtjänar min djupa tacksamhet

Susanne, tack för att du för nio år sen inte hindrade mig från att ge mig på att analysera orienteringsförsök med hjälp av video. Hade jag vetat hur mycket tid jag skulle lägga framför svartvita stumfilmer med suddiga fjärilar hade jag nog tvekat och då hade den här avhandlingen kanske aldrig sett dagens ljus. Din entusiasm för och kunskap om alla typer av flyttande djur är inspirerande och som handledare har du alltid stöttat mina tvära kast mellan olika metoder, resor och idéer i jakten på amiralernas hemligheter. Jag minns att jag i början av doktorerandet var rädd för din enorma kapacitet att fylla mina första trevande försök till manus med rödpenna men nu när jag vant mig ska du veta hur enormt mycket jag uppskattar all den energi du lägger ner på att gå igenom de texter vi tillsammans producerat. Utan ditt maratonengagemang i slutdelen av skrivandet hade jag aldrig blivit klar.

Thomas, du har alltid visat ett stort intresse för mina projekt och alltid tagit dig tid att diskutera det som pågår för tillfället detta trots att vår ofta planerade båttur på Vombsjön aldrig blev av. Hade det inte varit för din hjälp i tolkningen av Falsterbodatat hade jag nog aldrig vågat mig på att använda cosinus igen efter gymnasiematten. Din kunskap om äldre litteratur är oslagbar, när pappersbaserade källor kan vara svåra att få tag i är det skönt med ett levande bibliotek.

Staffan, du anslöt dig inte till handledarskaran förrän först mot slutet men du har funnits med i bakgrunden långt innan dess. Du gjorde en beundransvärd insats som fjärilsfångare (till och med framgångsrikt trots trasig håv) på Capri och när vi väl gjorde lite mer seriösa AFLP studier på amiralerna lossnade projektet rejält. Vi får inte heller glömma att jag utan din kunskap om brännmaneter nog aldrig insett tequilans fulla potential.

Åke, till Annas stora förtret har jag ropat tvärs över korridoren om jag fastnat med något eller mest för att jag skickat en fräck låt över gemtemp. Din entusiasm och engagemang är smittande och du har nästan känts som en extra handledare.

Christian, tänk att ett föredrag på Höörs naturskyddsförening skulle utmynna i sex resor till Capri! Du är en ständig källa till fantastiska historier och fick mig att förstå att man kan börja varje heldag på Barbarossasäsongen med att hänga in alpseglare på Monte Solaro, jag har i alla fall inte tröttnat än. Pedroni och Amaretto med is på Gran Caffè är en annan vana som jag utan tvekan valt att anamma.

Ulf, undrar om jag någonsin satt min fot i Nigeria utan dig? Det började med en grymt kul doktorandresa och nu börjar man känna sig som veckopendlare till Amurum. Med lite tur ska vi nog hitta en *ottossoni* till slut…

Anna, alla borde ha en rumskompis som förlåter en när man råkar ”ge bort” favoritsaxen till okända studenter, ständigt lägger vantarna på Zuperhäftaren och aldrig håller tyst. Utan din statistiska uppfriskning inför slutdelen hade jag nog försökt fula mig undan med t-test i hela avhandlingen.

Hela gänget på Ottenby, ni har gjort månadslånga fältarbeten till något man ser tillbaka på med saknad och utan er hjälp hade jag nog inte hängt in den eftertraktade ängspipen än på många år.

Sara och Michi, om man kan stå ut med varandra fem veckor i djungeln (till och med när man är osams redan innan...) måste det vara något särskilt. Tillsammans med den bitterljuva Lariamen såg vi till att gadda ihop oss mot världen, hoppa över all onödig sömn, tappa vikt motsvarande ungefär 103256,7 amiraler, undersöka hela tropiksjukdomsfloran i praktiken och ha kul under tiden. När åker vi nästa gång?

Micke, alltid lika skönt laid-back men skärpt som få. Du var en ovärderlig hjälp vid analyserna av AFLP materialet och dessutom behöver vi alla någon att hata eller hur? Nästa gång Reverend är i stan ska jag se till att köpa en t-shirt jag med.

Johan, det är skönt med någon som förstår och driver på i kampen mot fler millimeter. Ibland behövs det någon som verkligen tar sig tid att lyssna när man bara vill prata bländare, avtonade gråfilter, ultraljudsmotorer och spot-mätning. Kampen mot CanoNikon och för flest EX fortsätter…

Douglas, utan din hjälp i labbet hade AFLP jobbet inte flutit lika smidigt som det gjorde. Man behöver aldrig oroa sig för att man glömt bort protokollet för analyserna när du finns i labbet.

Gunilla L, du har sett till att det administrativa aldrig känts betungande, du är en klippa för sådana som mig, som aldrig kommer fatta det där med periodiseringar och avskrivningar.

Gunilla A, tack för att du fått de enformiga manuskripten att se ut som övertygande artiklar.

Hela personalen på biologibiblioteket, ni är alltid lika trevliga och tillmötesgående. I de flesta fall har jag kunnat lösa mina referensbehov genom den välskötta hemsidan men när hederliga papperstidskrifter behövts har ni letat fram de mest obskyra titlar med lätthet.

Thank you, all of the voluntary collectors who helped me gather samples of red admirals all over Europe. Without your help much of this work would have been impossible.

Tack Ernst, bättre fältassistent kan man inte ha. Trots att du dricker för mycket, är högljudd som få och inte allt för sällan bryter ihop totalt långt från hemorten har vi alltid lyckats reparera problemen och ta oss hem tillsammans igen. Du kommer alltid vara min favoritbil!

Mor och Far, ni har ständigt stöttat mig i vägen mot Ekologihuset. Om ni vetat att en blivande biolog måste testa alla närområdets djurarter i fångenskap där hemma minst tre gånger och att ni skulle bli väckta av hungriga kråkor flera somrar på rad hade ni kanske tvekat, men nej, det tror jag faktiskt inte. Tack för allt ert stöd, och tack för att er kampanj mot anläggandet av ”dammen” misslyckades totalt…

Linda, tack för att du finns! Du har varit den som fått mig att orka igenom allt skrivande, hos dig och Lova kan man lätt koppla bort alla jobbtankar och bara trivas. Den här avhandlingen tillägnar jag er.

Slutligen vill jag tacka Ludvig, min korrekturläsande vän. Förutom att du läser och kommenterar ett manus på en dryg timme har du även lärt mig älska semikolon; glädjen man känner när man hittat den ultimata språkliga konstruktionen (läs ursäkten för infogande av tidigare nämnt tecken) fick mig att se på grammatik med nya ögon!

1

AFLP reveals cryptic population structure in migratory European red admirals

AFLP reveals cryptic population structure in migratory European red admirals

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The genetic differentiation in a migratory butterfly, the red admiral (*Vanessa atalanta*), was investigated with the purpose of possibly establishing the patterns of migratory routes used across Europe. The AFLP profiles were significantly different between almost all sampled locations, but there was no clear pattern of isolation-by-distance. Using the software STRUCTURE 2.1, a program that identifies groups of individuals without prior knowledge of the samples' origin, we found two distinct genotype clusters present in different frequencies at all of our sites. The frequencies of these genotypic clusters also varied significantly between years within the same site. Remarkably few individuals were of mixed ancestry, indicating that some isolating mechanisms are present. We suggest that the European red admirals belong to two distinct groups that use different wintering areas. Interbreeding is most likely hindered by temporal isolation, keeping these two groups isolated. Hybrids are, however, found in substantial frequencies at some sites, suggesting that other mechanisms of selection must be operating to maintain the distinctiveness of the identified clusters. Even though most field observations of red admirals suggest a directed north-south migration, our data indicates a more variable pattern since the composition of the two genotypic clusters shows dramatic variation between sites and years in the northern part of the distribution range.

Introduction

Many animals migrate between areas of importance for survival and reproduction and their movements are influenced by their natural history, locomotion and the accessibility of different habitats (e.g. Alerstam *et al.* 2003, Åkesson & Hedenström 2007, Roff & Fairbairn 2007). To understand the biology of these animals, selection pressures and mortality have to be estimated in the different geographical areas frequented by them, including those passed during migration (Sillett *et al.* 2000). Since it has rarely been possible to study the same individuals at more than one location, migrating animals have posed a long-lasting challenge to ecologists. Linking together winter and summer ecology requires knowledge of migration routes and population structure (Webster *et al.* 2002); mark-recapture studies can be used to acquire this knowledge for most relatively large and long-lived animals. Ringing of migratory birds is the best example of this, but we still lack complete information regarding population structure and migration routes for many species of birds, as well as most other animals. Advanced telemetry methods enable tracking of sufficiently large animal migrants (e.g. Åkesson 2002, Godley *et al.* 2003, Cochran *et al.* 2004, Alerstam *et al.* 2006), but has only recently been applied in studies of smaller sized animals (Holland *et al.* 2006).

Insects make up a substantial part of animals that migrate (Williams 1958, Dingle 1996, Holland *et al.* 2006), and compared to birds they are small, fragile and short lived, making tagging projects much more difficult. To date, large-scale mark- recapture studies have been successful only in one migratory insect, the monarch butterfly (*Danaus plexippus*) (Urquhart & Urquhart 1978, 1979). Compared to other migratory insects, monarchs differ in at least two ways. First, their migration is well known to the general public, making large-scale marking possible with the help of voluntary workers (http://www.monarchwatch.org/tagmig/index.htm). Second, most of the monarchs spend winter hibernating in large clusters (Oberhauser & Solensky 2004) in very limited areas, making recaptures more likely. For most other migrating insects, other methods are needed to gather information regarding migration routes and population connectivity (Dingle 1996).

The red admiral (*Vanessa atalanta*) is a migratory butterfly distributed in North America, Europe and the western part of Asia (Tolman 1997). The general pattern of the species' annual migration in Europe is fairly well documented. Each spring the red admirals spread out northwards, leaving the southern areas were they have spent the winter season, and produce at least one new generation in the summer areas. The new generation returns south during autumn and was previously thought to spend winter in hibernation. Recent studies, using estimates of wing wear (Stefanescu 2001) and wing length measurements (Brattström 2006) suggest that the red admirals found in the wintering areas, just before northward spring migration, are from a newly hatched generation produced during winter. Even though red admirals are regarded as migratory, since they are not found in the northernmost part of their distribution during winter, we still know very little about the actual migration routes. Our present knowledge is based on annual changes in occurrence and scattered observations of migratory movements. Field studies report northward flights during spring in Italy (Benvenuti *et al.* 1996), Great Britain (Williams 1951) and Denmark (Hansen 2001). A study made in Spain, reported no clearly identifiable northward flights, but areas with numerous records during early spring were almost depleted of red admirals during summer (Stefanescu 2001). Return flights towards the south in autumn are reported from Finland (Mikkola 2003), Sweden (Imby 1993), Denmark (Hansen 2001), Great Britain (Williams 1951), Germany (Roer 1991, Steiniger & Eitschberger 1996), The Netherlands (in Lempke 1971), Italy (Benvenuti *et al.* 1996), Croatia (Reichholf 1978) and Spain (Stefanescu 2001). In late autumn, flight directions recorded in Italy are scattered, without a well defined migratory direction (Benvenuti *et al.* 1996), suggesting that red admirals do not migrate during the winter season in this region. Stefanescu (2001) reported that breeding is observed in Spain during winter, again suggesting that red admirals do not migrate during this time of the year. It should also be noted that some studies suggest that red admirals only migrate short distances, and that hibernation, even in the northern part of the distribution is a common strategy (Roer 1961, Baker 1972, Pollard & Yates 1993). So far, no study has been able to clearly demonstrate that red admirals regularly migrate long distances all over Europe each year, even though this is the view favoured by most studies.

Webster *et al.* (2002) reviewed several possible methods for studying migratory connectivity. If a migratory species is found over a large area, and different subpopulations use separate migration routes, it is likely that genetic differences will be found between these groups. The red admiral, thought to be the most regular long distance migratory butterfly in Europe with a large continuous distribution, is a good candidate species for studying migration routes using genetic analyses. Based on present knowledge about this species' annual migration, we set up three hypotheses. First, if red admirals are long distance migrants, but lack any general migration pattern, we expect no large genetic differences between sites in Europe since the constant mixing of individuals from different areas will keep the gene flow large enough to prevent local differentiation. Second, if red admirals are only migrating short distances, without mixing over large areas, we expect to see an increase in genetic difference relative to distance from the sample location regardless of compass direction. Third, if red admirals do migrate long distances, and the migration is performed mainly along a north-south axis with only short distance movements during winter when flight directions are reported to be scattered, we expect to find genetic differences relative to distance along an east-west axis but no, or at least smaller differences along a north-south axis. We used genotype data (AFLP) from nine locations in order to distinguish between these hypotheses.

Materials and Methods

Collection of samples

Adult red admirals were collected in the field during 2004 and 2005. Sampling was carried out by the main author (OB) and by volunteers; in total we collected 277 individuals from nine different locations in Europe (Table 1). After capture the butterflies were killed with ethyl acetate and the heads were stored in 99.9% ethanol until extraction of DNA was performed, using a standard phenol chloroform protocol (Sambrook *et al.* 1989).

Generating AFLPs

We used amplified fragment length polymorphism (AFLP) to generate a large number of polymorphic markers randomly distributed throughout the genome. This method is useful for studies of population differences and requires no previous knowledge of the genome of the study species. We used the AFLP protocol described in Vos *et al.* (1995), and modified according to Bensch *et al.* (2002). Fragments were separated in 6% polyacrylamide gels and scanned with a Typhoon 9200 (Amersham Biosciences) after 70 minutes, and then again after an additional 60 minutes. We used three combinations of selective primers: A ($E_{TGA} \times M_{CAG}$), B ($E_{TGA} \times M_{CGA}$), and C ($E_{TCG} \times M_{CAA}$). These primer combinations amplified a total of 199 bands that could be unambiguously scored.

Data analysis

Gels were analysed manually, and only clearly visible bands that could be scored easily for presence/absence in all gels were included in the analysis. Arlequin 3.1 (Excoffier *et al.* 2005) was

Table 1. Red admiral sampling locations and number of individuals analysed during 2004–2005. All individuals were collected in late summer, except for the Italian sample from 2005 which was divided in two groups since we sampled both in early spring and late summer from two clearly different generations

	Coordinates		Number of individuals	
Sampling location	Latitude	Longitude	2004	2005
Denmark, Ølsted	55.92° N	12.07° E	10	9
Estonia, Karilatsi	58.07° N	26.55° E	15	–
Italy, Capri	40.55° N	14.23° E	15_{autumn}	$15_{\text{spring}}/15_{\text{autumn}}$
Russia (Kaliningrad), Rybachy	55.16° N	20.84° E	42	42
Poland, Czestochowa	50.81° N	19.12° E	–	29
Sweden, Kullaberg	56.30° N	12.47° E	15	–
Sweden, Ottenby	56.20° N	16.40° E	–	45
Sweden, Sandhammaren	55.38° N	14.20° E	15	–
Spain, Vilagarcía	42.57° N	8.76° W	10	–
Total			122	155

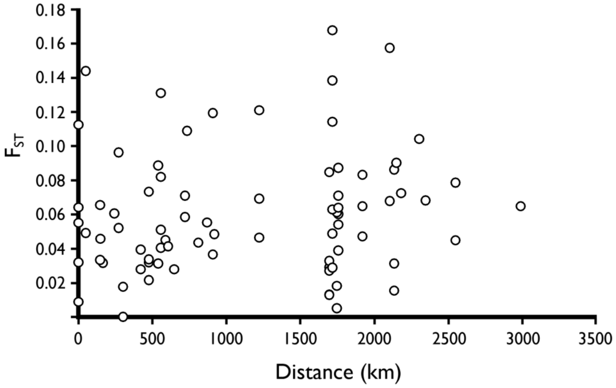

Figure 1. F_{ST}-values from pair-wise comparison between red admirals sampled at nine different locations in Europe during 2004–2005. Genetic population divergence shows no significant evidence of isolation-by-distance (Mantel-test; $r = 0.138$, $p > 0.05$).

used to calculate pair-wise F_{ST}-values as well as performing an AMOVA to calculate a global F_{ST}-value for differences between all sampled groups. We used a Mantel test to estimate p-values for correlations in the isolation-by-distance analyses for both geographical distance between sites and relative to the sites' east-west and north-south distances. We used STRUCTURE 2.1 (Pritchard & Wen 2003) in order to estimate the most likely number of genetic populations, disregarding information on collection site. For individuals having the presence allele, the dominant nature of the AFLP method makes it difficult to separate between heterozygotes and homozygotes (Bensch & Åkesson 2005). We therefore arranged the data file as for diploid organisms with missing values at the second position for each marker. Each run had a burn-in of 10,000 iterations, followed by 10,000 iterations of data collection. We ran the analysis for up to ten possible populations (K = 1 – 10) with five different runs at each level. We used an admixture model, providing no prior information about the origin of our samples. To determine the most likely number of populations, we analysed the STRUCTURE 2.1 output using the methods described in Evanno *et al.* (2005).

Sex determination

To make sure that sexual differences would not bias our results, a total of 84 individuals were dissected to determine sex. This enabled us to exclude genetic markers that are exclusively present in only one sex making local differences in sex-ratios appear as population differences. This is important, since the sex-ratios in a sample from a single location can be extremely skewed (Brattström 2006). Sexual differences in behaviour, for example hill-topping (Brown & Alcock 1990), can make catching one sex rather than the other more likely, depending on sampling technique.

Results

In total we identified and scored 199 AFLP loci, of which 169 were polymorphic (30 for primer combination A, 69 for primer combination B and 70 for primer combination C).

The AMOVA revealed a significant genetic difference between the sampled groups ($F_{ST} = 0.048$, $p<0.001$). The pair-wise F_{ST}-values ranged from 0.00024 to 0.17, and most of these (73 of 78) were significant. From the three sites where we had samples from both years (two samples each for Denmark and Russia, and three different samples from Italy) separate pair-wise F_{ST}-values were calculated using only samples from one site at a time in each comparison. Four of the five comparisons were significant with F_{ST}-values ranging from 0.03 to 0.10. The sampled European red admirals are clearly not part of the same large panmictic population. The correlation between pair-wise F_{ST}-values and geographic distance was non significant (Mantel test; $r = 0.138$, $p > 0.05$, Fig. 1), showing no isolation-by-distance effect. The same test was performed, checking for differences in an east-west (Mantel test; r = 0.155, $p > 0.05$), and then north-south (Mantel test; r = 0.072, $p > 0.05$) axis. These were also not significant.

Using STRUCTURE 2.1 and following the method for calculating ΔK described in Evanno *et al.* (2005), two populations were most likely to have produced the given dataset. The mean likelihood values (Ln P(D)) were actually higher for both three or four populations (Fig. 2a) to produce our data, but the variation between runs was larger than for two populations so the resulting ΔK value showed a marked peak at K = 2 (Fig. 2b) supporting two populations as the most likely situation to produce a data set like the one we got from the AFLPs. The geographical subgroups from the original dataset were divided into two new subsets based on the suggested population for the majority of the individuals within each subgroup. These two new datasets were run separately, since strong genetic differentiation at a high level can mask the same at lower levels in STRUCTURE 2.1 (Evanno *et al.* 2005). No further informative sub-structuring was found within these two groups.

When examining the output from STRUCTURE 2.1, it was clear that most individuals (86%) could be assigned with high probability (>0.8) to either of the two suggested genotype clusters (Fig. 3). Among the dissected specimens no single marker was present exclusively in one sex, making it clear that the suggested clustering was not a sexual difference. To further test that sex was not a factor behind grouping of the data, we performed a χ^2-test comparing sex with the most likely genotype for the individuals that were dissected. There was no significant relationship between these two factors ($\chi^2_{1,84} = 1.31$, $p = 0.25$).

The geographic distribution of the individuals from the two different clusters, and those of

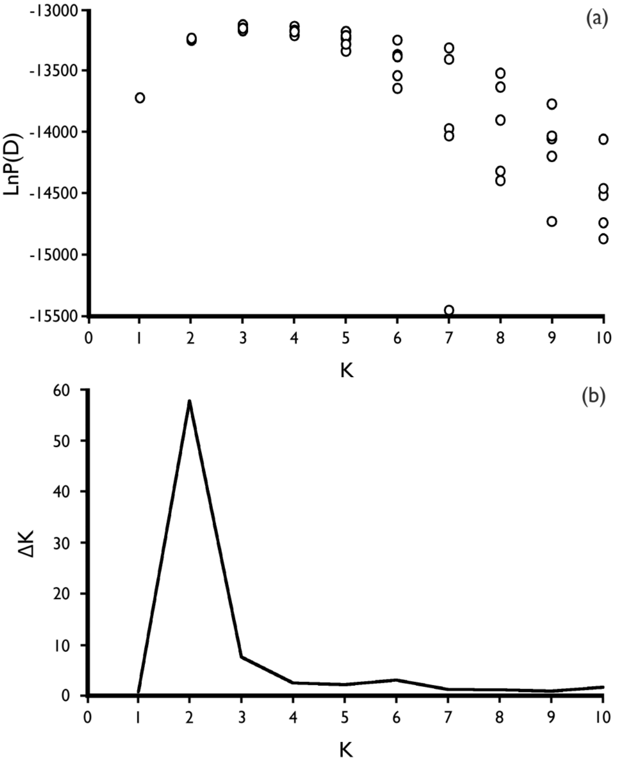

Figure 2. Outputs from testing of red admiral data using STRUCTURE 2.1 software.
a) Probability LnP(D) of producing the given red admiral data set from different number of populations (K) over 5 runs for each K value. Highest probability is reached for 3 populations. b) Taking variation of data into account, the calculated level of ΔK for each level of K, result in two populations being the most likely to produce our genetic data.

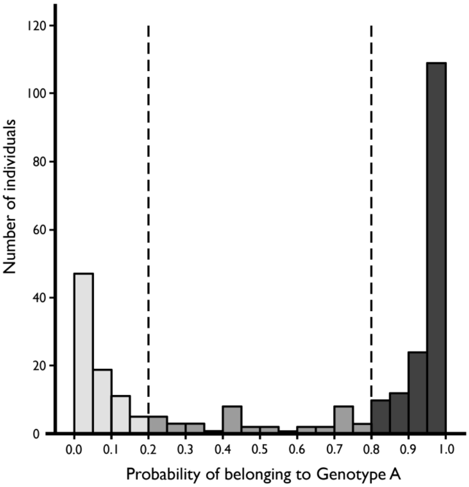

Figure 3. Histogram showing the frequency of individuals of red admirals belonging to two suggested genotype clusters. Probability of belonging to genotype A was estimated by STRUCTURE 2.1 software using an admixture model and two suggested populations. The three groups (A, mixed ancestry and B) used in figure 4 are marked with the same shade of grey as used in figure 4. The arbitrary limits set by us, for being assigned to each group are marked with dashed lines.

mixed ancestry can be seen in Figure 4. We found no clear difference in distribution of the two genotypes at our sample sites, but variation was greatest in northern Europe, even between the two years at the same site. During further discussion we will refer to the different clusters (Figs. 3 & 4) as A (Dark grey), B (Light gray) and A/B (Medium gray). The selected limit of minimum probability for being assigned to either cluster A or B (0.8) is purely arbitrary, but it is clear from Figure 3 that altering the cut-points will not change the proportion between the groups to any large extent.

Discussion

Our results show substantial genetic differentiation between red admiral populations within Europe, and there is also significant variation at the same sites between two consecutive years. Based on reported observations of flight directions following mainly a north-south direction (for references see above), we set up three hypotheses to test if genetic variation showed any correlation with distance between our sampling sites. None of these three hypotheses, however, were supported by our data. If the first one had been true, that there is a constant mix of individuals between sites in a random fashion, we would not see any significant differences between sites regardless of distance. In contrast, almost all of the pair-wise F_{ST}-values were significant, showing substantial genetic differences between the sampling sites.

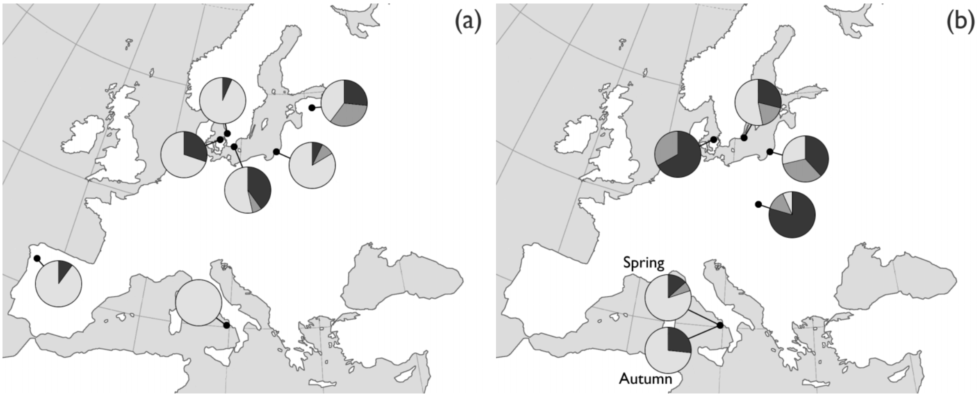

Figure 4. Circular graphs showing the relative proportion of sampled red admirals belonging to two different genotypes (Dark (A) and light (B) grey) or being of mixed ancestry (medium grey, less than 0.8 probability of belonging to either of the two main genotypes) from 2004 (a) and 2005 (b). Both genotypes are present each year and individuals with mixed ancestry are rare.

We could also reject the second hypothesis, which stated that if migration flights only cover short distances, we would expect a clear isolation-by-distance effect. We found no evidence of such a pattern. Instead, the same sites showed significant differences (F_{ST}-values from 0.032 to 0.11) between the two study years, demonstrating a presence of temporal instability. This indicates that individuals captured at the same site, but in different years, have at least partly different origin. A similar pattern of large and unstructured genetic differences between populations has been found in, for example, studies of fishes, Turbot (*Psetta maxima*) in the Baltic Sea (Florin & Höglund 2007).

Our third hypothesis, that the migration mainly follows a north-south axis each year, would result in a pattern where genetic difference increase with distance only in an east-west axis. Again, we found no evidence for such an isolation-by-distance effect.

We found no correlations between genetic and geographic distances in our study. Despite this, most pair-wise comparisons between sampling sites show significant F_{ST}-values within the range from 0.013 to 0.17. The reason for these patterns appears to be the occurrence of two different clusters of genotypes, whose frequencies varies significantly between sites and years (Fig. 3 & 4). The patterns of genetic variation among individuals captured at our sample locations show, that the migration of European red admirals is not as regular as previous field observations suggest (e.g. Williams 1951, Benvenuti *et al.* 1996, Hansen 2001, Mikkola 2003). Looking at the distribution of the two genotypes (Fig. 4) we can see that genotype A is always uncommon in the samples from Spain and Italy. In northern Europe both of the two genotypes are found but there is a large variation among the different sites. It might be that we have two different populations, one western population (B) that mainly spends winter in the central and western Mediterranean areas, and a second population (A) wintering in a non-sampled area, presumably further towards the east.

Despite the co-occurrence of these genotypes, we find remarkably few (13%, calculated as mean proportion among sites) individuals of mixed ancestry. Such a low proportion of individuals with intermediate genotypes suggests that there are mechanisms at work that hinder interbreeding between the two main populations. If the two genotypes reflect adaptations for different wintering areas, individuals with intermediate genotypes might have lower winter survival rates, since weather conditions during winter in the western Mediterranean (possible winter location for Genotype B) are probably different from those in regions further towards the east (possible winter location for Genotype A). If hybrids do worse during winter, it is reasonable to expect that selection has favoured assortative mating with a preference for mating only with individuals originating from the same population, possibly mediated by pheromones or visual cues. Speaking against this is the fact that we sometimes see a large proportion of intermediates (e.g. Estonia in 2004 and Russia in 2005); this would not be the case if active avoidance was a strong isolating mechanism. Another explanation of this pattern could be indirect temporal isolation during breeding. Assuming that the individuals of the two genotypes use different wintering areas, individuals from each of these groups are more likely to arrive in the northern breeding areas together since they most likely follow similar routes during northward migration. Most migrating insects seem to reproduce soon after a migratory flight (Dingle 1972), so even though other individuals end up in the same area in summer, they will not likely interbreed since the earlier arriving individuals have already reproduced when the late ones arrive. Interbreeding will still occur if individuals from both winter areas arrive simultaneously at the same breeding location. Even though the resulting hybrids produced at these occasions may have a lower fitness (see above), direct selection for assortative mating can not be strong if indirect factors are already at play, keeping the number of hybrids low.

We see a large variation in observed frequencies of the two genotypes between years at our northern sites, suggesting that the origin of the majority of red admirals breeding there varies between years. Many field observations (references above) suggest a relatively constant migratory direction following a north-south axis, but our data suggests that the migratory track is not the same each year. Which of the genotypes become more successful in the northern parts of Europe in a given year could possibly depend on the prevailing wind directions during spring migration. Being a fairly small animal, wind directions during migration supposedly have a strong effect on both flight direction and distance (Alerstam 1990, Dingle 1996). Also, differences in the reproductive success of both populations during the previous winter will be important.

Isolation between populations is a strong factor that can lead to formation of new species. The European red admirals have never been subject to splitting into subspecies or species, and only minimal individual variation of morphology is reported in literature (e.g. Henriksen & Kreutzer 1982, Eliasson *et al.* 2005). Our results suggest that the red admirals in Europe have features that resemble the situation in the bath white complex, *Pontia daplidice/edusa*. These two species of butterflies are virtually impossible to separate without molecular analysis and they co-occur in some parts of their distribution range in Europe (Porter *et al.* 1997). Just like the red admiral, both species of bath white are highly migratory, but they are much more unpredictable in their occurrences, occasionally forming permanent populations further north than they are normally found (Eliasson *et al.* 2005). Unlike red admirals, the bath white complex has narrow hybrid zones at most places of co-occurrence (Porter *et al.* 1997), and hybrids are reported as being both unviable (Geiger *et al.* in Porter *et al.* 1997) and viable (Büchi 1996 in Porter *et al.* 1997). In a first step to understand the patterns we have seen in the present study, samples are needed from the potential wintering area of the A genotype cluster. We expect future studies, combining biometric analyses and with molecular data will be most rewarding when disentangling the enigmatic migration behaviour of the red admiral.

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11

Is there seasonal variation in size and mass of Red Admirals *Vanessa atlanta* on Capri, Italy?

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Is there seasonal variation in size and mass of Red Admirals Vanessa atalanta on Capri, Italy?

Finns det någon säsongsberoende variation i storlek och vikt hos amiraler Vanessa atalanta på Capri, Italien?

OSKAR BRATTSTRÖM

Abstract

I present data on seasonal variation in wing length and mass of Red Admirals Vanessa atalanta captured on Capri, Italy, during spring and autumn. The Red Admiral is a migratory butterfly that migrates north throughout Europe each year and then heads back south in the autumn. During the winter they are mostly found in the northern Mediterranean area where they previously were thought to hibernate, but recent data suggest that a new generation is produced before spring migration. The Red Admirals captured on Capri showed no difference in dry mass between the two seasons, when taking size in account, but had significantly longer wings in autumn. This suggests that a new generation is produced during winter.

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Introduction

The Red Admiral *Vanessa atalanta* is one of the most regular long distance migrants among the European butterflies (Pollard & Yates 1993). It shows a clear bi-directional migration, heading north from the Mediterranean area in spring (Benvenuti et al. 1996) before reproducing (Henriksen & Kreutzer 1982). In late summer it returns south, as reported from field studies made at different locations in northern Europe (Williams 1951, Hansen 2001, Mikkola 2003). The numbers found in the northern parts of Europe are fluctuating, with smaller populations in cold summers, but the Red Admiral is much more regular there than most other migrating butterflies, for example the closely related Painted Lady *Vanessa cardui*. In England, where small numbers of Red Admirals regularly hibernate, the numbers found in early spring before the first true migrants arrive are not correlated with numbers found in the following autumn, suggesting that the population is completely dependent on immigration in the northern parts of Europe (Pollard & Greatorex-Davies 1998). It was initially believed that European Red Admirals migrated short distances and hibernated during winter, and that the same individuals returned north again in spring to produce the next generation (Roer 1961). This was later questioned, as Red Admirals were found to be poorly adapted to hibernation (Lempke 1971). Recently, Stefanescu (2001) found that in north-eastern Spain, reproduction occurred during the winter, and he suggested that the Mediteranean area as a whole is a breeding area rather than a wintering area for the adults of migrant populations of the Red Admiral.

Most studies on butterfly migration have been made on American Monarchs *Danaus plexippus*, that migrate across North America each year (Brower 1996). The majority of the monarch population spends the winter in Mexico, hibernating without additional feeding. To survive the winter, they need to build up large lipid stores before they reach the hibernation areas and the difference in lipid content can therefore vary dramatically during the year. Some individuals have lipid stores as large as 134% of their lean mass, compared to newly hatched individuals which have a lipid store of about 30%, which still is more than butterflies in general (Beall 1948). Not much is known about lipid accumulation in other species of migrating butterflies. The monarch studies show that large variations are likely to occur based on the current need of the butterfly. From these studies we also know that mass is often closely correlated with lipid content if size is controlled for (Brown

& Chippendale 1974). Also in studies where size was not controlled for, lean mass was fairly constant, compared to lipid content which showed much larger differences (Beall 1948). Therefore, mass looks promising as a quick and easy way for measurements of lipid stores in butterflies.

In this paper, I will present some data on size and dry mass differences in Red Admirals caught during spring and autumn on Capri, Italy. Since Capri is located well inside the winter range of the species, seasonal differences between individuals found here might tell us more about the winter ecology in this species.

Materials and methods

Red Admirals were captured on Capri 1–10 October 2004 and 9–20 May 2005. The butterflies were primarily caught to be used in orientation experiments; therefore measurements of hindwing length and dry mass were taken after these experiments, to avoid adverse effects on the orientation results from the handling. Prior to the orientation experiments, usually three days, all the butterflies had constant access to fructose solution which they fed on during the time in captivity. Immediately after the experiments the butterflies were killed with ethyl acetate. The length of the hindwing was recorded to the nearest 0.5mm with a digital calliper (DCA-150, Velleman components) and the head was removed for use in DNA studies. Only individuals with unabraded hindwings were included in the analysis. The thorax and abdomen were placed in plastic test tubes with rubber sealed caps and put in a freezer. The abdomen of all the collected butterflies was dissected to determine sex by inspection of their genitalia. After dissection the butterflies were dried in 70°C for 24 hours and the dry mass of thorax and abdomen was measured separately on a balance (Mettler Toledo AG 245). Since butterflies feed on liquid food which contain high amounts of water their mass can increase much after feeding (Christer Wiklund & Fredrik Stjernholm, personal communication), therefore dry mass is a more reliable estimate of lipid content than wet mass.

Statistics

To get a linear fit between the length of the hindwing and dry mass the wing measurements were raised to the power of three. To analyse differences in dry masses of thorax and abdomen between seasons, Analysis of Covariance (ANCOVA) with length of hindwing3 as covariate was used. To investigate differences in the Red Admirals' length of hindwing between the two seasons I used Mann-Whitney U-Test, since the residuals were not normally distributed. All statistical calculations were performed with Statistical Program for Social Sciences (SPSS) 11.0 for Windows.

Results

A total of 42 Red Admirals were captured, 21 in spring and 21 in autumn. All of the individuals were males. There was a significant correlation between the cube of hindwing length and dry mass of abdomen, and no significant effect of season (Table 1a). The same relationship was found for thorax dry mass (Table 1b) but the variation (Fig 1b) was smaller than for abdomen dry mass (Fig 1a). From the plotted data (Fig 1a & b) it was clear that the autumn individuals have longer hindwings. This difference in mean hindwing length was highly significant (Mann-Whitney U test, U = 65.5, $N_1 = 21$, $N_2 = 21$, $p < 0.0001$). The mean hindwing length and standard deviation was 23.79 ± 1.04 mm for the spring individuals compared to 25.71 ± 1.31 mm for the autumn individuals.

Table 1. Results of the ANCOVA test on dry mass of abdomen and thorax from male Red Admirals caught during spring and autumn on Capri, Italy. The interaction between hindwing length and season was nonsignificant (p>0.05) in both cases and thus removed from the final calculations.

Resultat av ANCOVA-test avseende torrvikt för abdomen och thorax hos hanar av amiraler fångade under vår och höst på Capri, Italien. Interaktionen mellan bakvingens längd och säsongen var inte signifikant (p>0,05) i något av fallen och togs därför bort från den slutliga beräkningen.

Variable	S.S.	df	F	P
Abdomen				
Hindwing length^3	1393.3	1	12.61	0.001
Season	15.9	1	0.14	0.707
Error	4307.8	39		
Total	44327.1	41		
Thorax				
Hindwing length^3	2615.2	1	49.48	<0.001
Season	0.2	1	0.04	0.951
Error	2061.4	39		
Total	100509.2	41		

Discussion

There are at least three possible explanations to the difference in hindwing length found in spring and autumn: abrasion, differential mortality or the appearance of a different generation. First, only individuals with undamaged wings were included in the analysis, and therefore abrasion is a highly unlikely cause of the seasonal difference in hindwing length. Second, the difference in hindwing length could be caused by a higher mortality rate during winter for larger individuals. If this is the case the mean value of hindwing length would decrease but the range of lengths found would look similar between the two seasons. The hindwing length of many of the spring individuals was well below the smallest individuals found in autumn (Figure 1). Further, a study of monarchs showed that smaller individuals died in larger proportions during winter (AlonsoMejia et al. 1997). Third and last, the autumn migrants might produce a new generation during winter in areas near Capri, which is the most likely explanation for the difference found in hindwing length between the seasons. This is also supported by the fact that there was no difference in dry mass when size was taken in account. Studies on monarchs that hibernate without extra energy intake during the winter period show that they build up large lipid reserves before hibernation (Beall 1948, AlonsoMejia et al. 1997. To build up large lipid reserves would be maladaptive for a butterfly that is active during winter, since bird predators would catch them easier (Dudley et al. 2002).

Stefanescu (2001) found that Red Admirals start to lay eggs as soon as they arrive in north-eastern Spain in late autumn. The larvae develop throughout the winter, and a new generation of adults appears in early spring. Stefanescu noted that the Red Admirals had very worn wings in autumn, but in spring the wings of the observed individuals were fresh, also suggesting that a new generation had been produced. The result from my study on Capri, adding a new site with a spring generation, supports the suggestion by Stefanescu (2001) that migrant populations of the Red Admiral all over the Mediterranean area are not hibernating but instead breeding during the winter.

One large difference with my study compared to other studies of lipid accumulation is that the butterflies have been allowed to feed in captivity before their mass was recorded. The data might therefore not show natural variations of lipid content. But on the other hand if the butterflies have an optimum level of lipid reserves, results might have looked different using freshly caught individuals since they might not have gained the lipid reserves they were trying to accumulate.

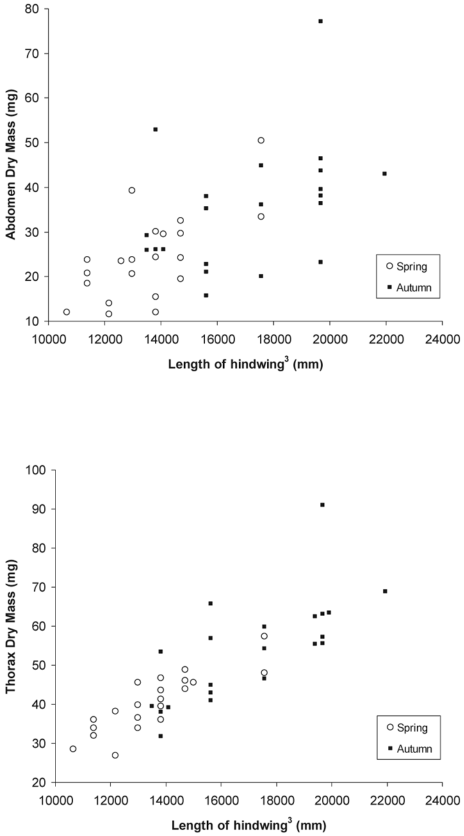

Figure 1. Relationship between hindwing length and dry mass of abdomen (a) and thorax (b) from male Red Admirals Vanessa atalanta caught during spring and autumn on Capri. To make all data points visible, a few of them have been slightly adjusted.
Förhållandet mellan bakvingens längd och torrvikten av abdomen (a) och thorax (b) hos hanar av amiral Vanessa atalanta fångade under vår och höst på Carpri. För att göra alla punkter synliga har ett fåtal flyttats något.

All butterflies in my study were males. The explanation is probably that male Red Admirals are territorial and perform hilltopping behaviours (Brown & Alcock 1990). They often perch on rocks, making them easy to spot in the field. In the future it would be most interesting to include females and analyse the relationship between dry mass and lipid content in detail.

Acknowledgements

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Sammanfattning

Många dagfjärilar flyttar i likhet med fåglar långa sträckor varje år. Hur fåglar lagrar upp fett inför flyttningen är välstuderat men hos fjärilar är det endast den amerikanska monarken *Danaus plexippus* som är relativt välstuderad. Monarker övervintrar i Mexico och flyttar norrut på våren genom större delen av Nordamerika för att senare återvända på hösten. Under den årliga flyttcykeln varierar mängden fett, precis som hos fåglar, kraftigt beroende på behovet av lagrad energi. I Europa flyttar flera fjärilar långa sträckor men det finns knappast några studier av årliga variationer i fettupplagring. Amiralen *Vanessa atalanta* klarar inte av vinterklimatet i norra Europa utan spenderar huvudsakligen vintern i norra Medelhavsområdet. Under våren sprider de sig norrut genom hela Europa och återvänder sedan söderut på hösten och de individer som observeras på Capri övervintrar troligen i närområdet. I samband med en orienteringsstudie av Amiraler togs vingmått på individer från både höst (1–10 oktober 2004) och vår (9–20 maj 2005), mellankroppen och bakkroppen sparades för kontroll av kön och torrvikt. Endast individer med oskadade vingar togs med i analysen. Studier gjorda på Monarker har visat att större delen av variation i vikt hos individer av samma storlek beror på skillnad i lagrat fett varför vikt kan fungera som ett fettmått. Fjärilarna i den här studien hade haft fri tillgång till fruktoslösning under tiden i fångenskap (runt 3 dagar) så vikten representerar inte exakt vad en ren fältstudie hade producerat utan snarare den mäng fett som fjärilarna strävar efter att uppnå. Totalt samlades 42 amiraler in, 21 på våren och 21 på hösten och alla visade sig vara hanar. Resultaten visade att amiralerna hade längre bakvingar på hösten men det fanns inte någon skillnad i torr vikt i förhållande till storlek (Figur 1). Störst variation fanns hos

bakkroppsvikten (Figur 1 b). Det finns tre möjliga förklaringar till skillnaden i vinglängd: slitage, högre vinterdödlighet för stora individer eller att individerna som fångas på våren tillhör en ny generation. Slitage är inte någon trolig förklaring eftersom endast individer med oskadade bakvingar är medtagna i analysen. Att större individer skulle ha högre dödlighet är inte heller troligt eftersom en stor del av de individer som fångats på våren är mindre än de minsta höstindividerna. Den troligaste förklaringen är att amiralerna förökar sig under vintern och att vårindividerna kommer från en helt ny generation. Detta stöds även av att det inte fanns några säsongsberoende skillnader i vikt. Om amiralerna är aktiva under vintersäsongen behöver de inte lagra upp fett, en stor fettdepå skulle göra dem långsammare och därmed ett lättare byte för predatorer. Slutsatsen är alltså att amiralerna i områdena runt Capri är aktiva och förökar sig under vintern. Individer från den nya generationen som flyttar norrut nästa vår är mindre än de som anlände norrifrån på hösten.

III

Placing butterflies on the map – testing the regional geographical resolution of three stable isotopes in Sweden using a monofagus Nymphalid, the peacock (*Inachis io*)

Placing butterflies on the map – testing the regional geographical resolution of three stable isotopes in Sweden using a monofagus Nymphalid, the peacock (*Inachis io*)

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Stable isotope studies have been used to find natal regions and migratory routes for animals in many taxa. These studies are usually based on samples from distant regions, making the expected differences in observed isotope ratios relatively large. We have sampled peacock butterflies (*Inachis io*) on a regional level (Southern Sweden), to study the natural variation and the resolution power of the stable isotope method to separate natural samples from known areas on a much smaller scale than what is commonly used. Ratios of hydrogen ($^{2}H/^{1}H$), carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes were obtained from butterflies at seven different locations in an area of 250×250 km during three years (2002–2004). We show that there is variation enough even on this regional scale to find significant relationships between isotope ratios and latitude/longitude. Of the three isotopes we analysed, deuterium ratios are the most useful for discriminating between our sites. Carbon isotope ratios show similar but weaker relationships, while nitrogen isotopes are unsuitable for small scale studies in this region because of high and unpredictable local variation. Even though we find differences on a smaller scale there is still substantial variation between years, sometimes making our sites indistinguishable from each other. Much of this variation is probably caused by the seasonal changes in deuterium ratios of rainwater. These differences produce large natural variation between years in animals with short and variable development times, and are difficult to estimate in natural situations. We conclude that stable isotopes are potentially powerful predictors for studies of migratory butterflies in Europe. However, without good knowledge about the sampled individuals' previous life-history, a lot of natural environmental variation cannot be controlled for. In the case of migratory species, this information is very hard to obtain, making the confidence intervals for prediction of natal areas fairly wide.

Introduction

In recent years, stable-isotope biogeochemistry has enjoyed a dramatic expansion as a result of successful analytical advancement enabling reconstruction of ecological events on different time scales (West *et al.* 2006). For instance, stable isotopes have been used for tracking effects of ecosystems and climate change (e.g. Flanagan & Ehleringer 1998, Kohn 1999, Staddon 2004), and to resolve questions related to habitat preferences, foraging, food-web and migration ecology in animals (e.g. Ehleringer *et al.* 1986, Eggers & Hefin Jones 2000, Kelly 2000, Post 2002, Webster *et al.* 2002, Hobson 2003, Rubenstein & Hobson 2004).

In particular, the spatial tracking of individuals during migratory movement has been particularly problematic due to the inherent limitation of physical mark and recapture methods (for review e.g. Webster *et al.* 2002, Nathan *et al.* 2003). The use of stable isotopes provides an intrinsic marker of spatial origin and promise to resolve many open questions about butterfly and insect migration. For example, Hobson *et al.* (1999) showed that the stable isotopes of hydrogen ($^2H/^1H$) and carbon ($^{13}C/^{12}C$) in butterfly wings could be used to infer the geographical origin of adult monarch butterflies (*Danaus plexippus*) in North America. This is possible since butterfly wings are composed mainly of keratin, a tissue that is metabolically inert (Rubenstein & Hobson 2004), and therefore reflect the isotope composition of diet and hydrological patterns at the natal origin of the individual. The isotope ratios of these tissues are preserved even in individuals that are captured far away during or after migration (Wassenaar & Hobson 1998). Most studies of stable isotopes in terrestrial migrating animals have been made in North and Central America (Wassenaar & Hobson 1998; Hobson *et al.* 2003) where both hydrogen and carbon in large regions form transverse gradients, making it possible to place the origin of individual animals on the map with a reasonable accuracy (e.g. Hobson 2003).

The pattern of isotope gradients within Europe appears to be either more complicated (e.g. deuterium), or less well known (e.g. carbon) and the number of studies on migrating animals originating from Europe remain few (for reviews, see Hobson 2003, Hobson *et al.* 2004; Rubenstein & Hobson 2004, Newton *et al.* 2006). Spatial gradients in deuterium are tightly linked to patterns of precipitation, temperature and elevation (Hobson 2003, Bowen *et al.* 2005) and therefore mountain regions have large impact on hydrogen isotope values (Hobson *et al.* 2003). In Europe there are clear gradients in deuterium showing decreasing values towards northeast (Bowen *et al.* 2005). We are unaware of any study of stable isotopes from insects of known origin in Europe. Our objective here is to assess the regional scale at which stable isotopes of hydrogen, carbon and nitrogen can be used together to delineate insect populations in Scandinavia within the broader European context.

We were interested in employing stable isotopes to track the movements of migrating red admiral butterflies (*Vanessa atalanta*) in Europe. Since no background data were available, we first conducted an evaluation of the resolution of the isotope method by studying the natural and spatial variation of stable isotopes in a sedentary species, the peacock butterfly (*Inachis io*) in Sweden. For this analysis we worked on a regional scale in southern Sweden using the relative composition of hydrogen ($^2H/^1H$), carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes analysed from wing samples collected from local populations of the peacock butterfly. We collected samples over a three-year period to compare regional and spatial differences in isotope composition, but also to investigate interannual variability.

Materials and Methods

Study species

We rationalized our choice of the peacock butterfly (*Inachis io*) using the following criteria:

1) The peacock is a common butterfly that can usually be found and sampled throughout the study region in Southern Sweden and in Europe.
2) The larvae are monofagus, feeding only on nettles (*Urtica dioica*), and thus we would expect differences in isotopic ratios to be af-

fected mainly by geographic location and the local environmental growth conditions of the plants and therefore unaffected by uncontrolled differences in food choice of the butterflies.

3) Nettles are annuals, thereby allowing us to examine interannual differences in stable isotope composition of the food and the consumer over a limited time period.
4) The peacock butterfly is closely related to the migratory red admiral (*Vanessa atalanta*) (Wahlberg *et al.* 2005), a species that also use nettles as host plants. Thus, we expected that data and insights gained from the less mobile peacock butterflies could be used comparatively to study the natal areas of migratory red admirals that have similar breeding biology and food preferences.

Sampling

Peacock butterflies were collected by hand netting at seven different locations in Southern Sweden (Fig. 1) during the summers of 2002–2004. Peacocks show some seasonal migratory tendencies, but move only within a very restricted geographical range (Eliasson *et al.* 2005). They fly to habitats suitable for wintering late in summer and return to suitable breeding locations early in spring (Eliasson *et al.* 2005). Each year collections were made on a single day per location immediately following eclosion of the adult butterflies. We selected this time for sampling to ensure we captured individuals in close proximity to the natal site. Observations supporting local origin of adults were that larvae of peacocks were often observed at the same locations. After capture, the wings of the butterflies were removed from the body and stored dry in individual envelopes.

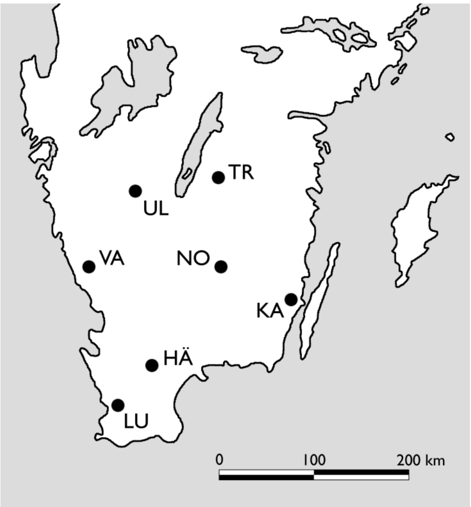

Figure 1. Position of the seven sample locations in southern Sweden. LU = Lund, HÄ = Hässleholm, KA = Kalmar, VA = Varberg, NO = Nottebäck, UL = Ulricehamn and TR = Tranås.

Stable isotope analysis

All wing keratin samples were rinsed in a chloroform-methanol solution (2:1) to remove surface oils that could affect the isotope assays, and air dried overnight. Stable nitrogen and carbon isotope analysis were performed at the Stable Isotope Laboratory in the Ecology Department at Lund University. For these analyses approximately 1 cm^2 of wing keratin (~ 0.5 mg) was cut from the distal part of the forewing and packed in tin capsules (Elements Microanalysis Limited). Capsules were combusted in an ANCA-GSL elemental analyser (PDZ Europe Limited) attached to a continuous flow isotope ratio mass spectrometer (20-20 PDZ Europe Limited). To correct for analytical error and drift during different runs, three different lab standards of known isotope composition were incorporated in each batch of peacock samples. We used previously calibrated glycin, homogenised great reed warbler feather keratin (*Acrocephalus arundinaceus*) and powdered bowhead whale baleen (*Balaena mysticetus*) as local references included in each run. Stable isotope ratios are reported as δ-values showing deviation from standard references, atmospheric air for nitrogen isotopes and Pee Dee belemnite (PDB) for Carbon isotopes. Our laboratory error for these analyses by repeated measurements was <±0.2 ‰ for nitrogen and <±0.1 ‰ for carbon. Stable hydrogen isotope analyses were conducted at Environment Canada in Saskatoon, Canada. Because stable-hydrogen isotope analyses of keratinous materials are complicated by uncontrolled isotopic exchange between samples and ambient water vapour (Wassenaar & Hobson 2000), we used the comparative equilibration technique so that the values reported here are equivalent to nonexchangeable hydrogen (Wassenaar & Hobson 2003). Briefly, the process involves the simultaneous measurement of wings with replicates of three different keratin standards whose nonexchangeable δD values are known and which span the range of expected values. Stable-hydrogen isotope measurements of wings and the keratin standards were performed on H_2 derived from high-temperature flash pyrolysis of wings and continuous-flow isotope-ratio mass spectrometry. A Eurovector 3000 (Milan, Italy) high temperature elemental analyzer (EA) with autosampler was used to automatically pyrolyse wing 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. Based on within-run measurements of intercomparison material and consideration of within feather variance (Wassenaar & Hobson 2006), we estimate our laboratory error to be ±2 ‰.

Precipitation data

The δD values in rainwater are influenced not only by location and altitude, but also by season. As precipitation is the main factor shaping known geospatial differences of deuterium ratios in biological studies, we needed to obtain estimates of expected δD values in precipitation from our study region. Using the Online Isotopes in Precipitation Calculator (OIPC) (http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html) we acquired interpolated annual and monthly δD values for the rainwater at our sampling locations. The deuterium content of the precipitation varies over the season with increasing δD values as the spring progresses into summer (Fig. 2). Since nettles are annual plants with rapid growth, and knowing that peacock larvae spend about one month feeding before pupation (Eliasson *et al.* 2005), we calculated a set of local δD values for the time period 1 April to 30 June. The OIPC generates one mean δD value per month which we assigned to the middle third of each month and then interpolated the δD values for the first and third part of each month. We then used precipitation data obtained from nearby weather stations operated by the Swedish Meteorological and Hydrological Institute (SMHI) to calculate the proportion of total rain water that fell during each of these time periods at all of our locations. Combining these data we calculated a new set of yearly local "precipitation weighted" δD values for the Peacock Larvae Growth Season (PLGS) letting the different parts of the month contribute to the PLGS deuterium values in relation to the proportion of water during each time period during April – June.

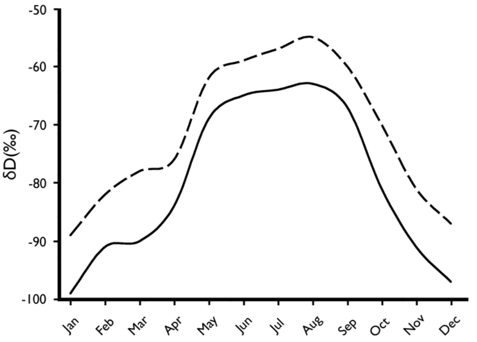

Figure 2. Monthly δD values (obtained from OIPC) for the two most distant sample sites in South Sweden (Lund (LU), broken line and Tranås (TR), solid line). All sites follow the same trend and are located between the two most extreme sites included in the graph.

Data analysis

We separated our analysis of variation in the three isotopes between location and year by three different ANCOVAs with measured isotope ratios as dependent variable and year as a fixed factor. Latitude and longitude of the sample locations were used as covariates. We included all possible two-way interactions and removed non-significant variables and interactions in a backward elimination procedure. To analyse the effect on δD values from altitude of the sample locations we performed linear regression using the residuals for δD values (from the final ANCOVA model) as dependent variable and altitude of the sampling locations as predictor. To analyse if the annual OIPC values and our calculated PLGS values were related to the measured δD values from the collected peacock wing samples we used linear regression with measured δD values as dependent variable and our calculated values as predictors. We tested each year and predictor variable separately.

Results

Sampling

We tried to obtain at least 10 individuals per location each year, but this was not always possible despite repeated visits if the first visit was unsuccessful. In total, we collected 145 peacocks over three years. The location of the sample sites, date of capture, and numbers of peacocks caught each year are given in Table 1. The date of capture varied somewhat between years since hatching date is dependent on weather conditions and we selected to collect butterflies close to eclosion. Peacocks are heat loving butterflies and our locations were close to their northern limit (Bryant *et al.* 1997), which possibly also affected the variation in numbers of individuals found each year.

Distribution of isotope values

All 145 samples were analysed for δD, but due to sample limitations 4 of the 145 butterflies were not measured for δ^{15}N and δ^{13}C. When we examined the data for the three different isotopes, it was clear that several isotope values could be considered as extreme outliers. While it was not clear if this was solely due to analytical error, we excluded two δ^{13}C, four δ^{15}N and two δD values. All these outliers belonged to different individuals that otherwise showed normal values for the two other isotope ratios.

Table 1. Sampling locations of Swedish peacock butterflies. Number of individuals caught and date of capture are given separately for each location and year used in the analysis.

Location				2002		2003		2004	
Name	Altitude	Latitude	Longitude	Date	N	Date	N	Date	N
Lund	66 m	55.72° N	13.27° E	7 Aug	7	30 July	10	–	–
Hässleholm	52 m	56.14° N	13.76° E	5 Aug	10	7 Aug	10	–	–
Varberg	34 m	57.05° N	12.45° E	–	–	6 Aug	10	23 Aug	10
Nottebäck	222 m	57.10° N	15.18° E	13 Aug	8	7 Aug	8	22 Aug	5
Kalmar	7 m	56.78° N	16.43° E	–	–	31 July	8	17 Aug	4
Ulricehamn	170 m	57.80° N	13.40° E	4 Aug	10	6 Aug	10	21 Aug	10
Tranås	220 m	58.01° N	15.00° E	15 Aug	5	5 Aug	10	20 Aug	10
Total					40		66		39

Plots of the isotope ratios from all groups and years are presented in Figure 3. The $\delta^{15}N$ values varied substantially with a higher coefficient of variation (mean bias-corrected CV from all sites and years) (CV_N = 27.5%) compared to $\delta^{13}C$ (CV_C = 2.5%) and δD (CV_D = 4.5%).

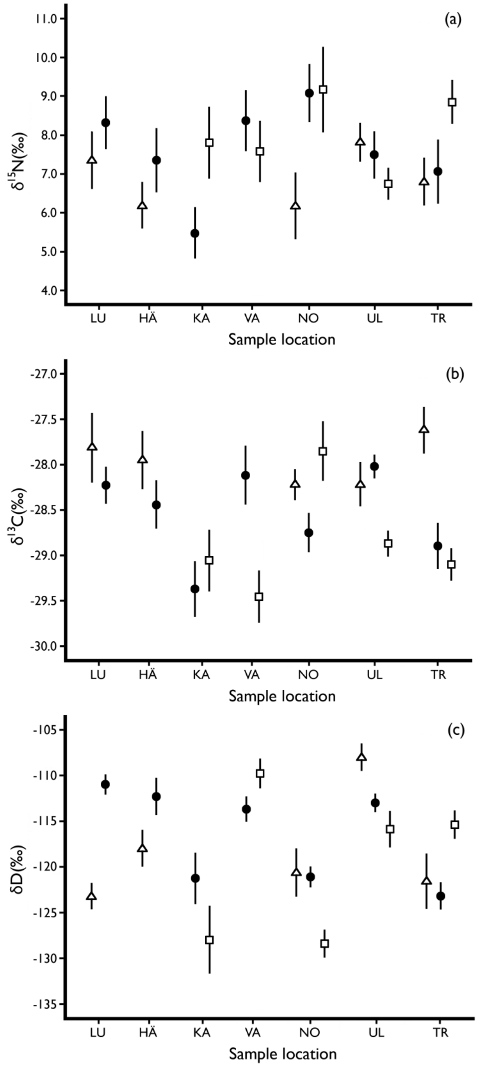

Figure 3. Mean values of (a) $\delta^{15}N$, (b) $\delta^{13}C$ and (c) δD in wings of peacock butterflies sampled at seven different locations in southern Sweden (names and position of the locations are given in Fig. 1 and Table 1). Measurements are from 2002 (Δ), 2003 (●) and 2004 (□). Bars indicate ±1 standard error of the mean value.

Analysis of individual isotopes' geographical pattern

For $\delta^{15}N$ there was a significant effect of year and a significant interaction between year and longitude (Table 2a). A separate analysis of the three years showed that a longitudinal effect was only apparent in 2003 (Fig. 4a), showing decreasing $\delta^{15}N$ values with increasing longitude. The correlation was very weak (r^2 = 0.064) and that is most certainly because we found large variation in $\delta^{15}N$ values between individuals at each sample site. The result of the final model is presented in Table 2a.

For $\delta^{13}C$ we found similar results as we found for $\delta^{15}N$ (Table 2b) with a longitudinal correlation present only in 2003 showing decreasing values with increasing longitude (Fig. 4b). However, the relationship for $\delta^{13}C$ was stronger (r^2 = 0.268) than for $\delta^{15}N$.

For δD, on the other hand, we found a stronger longitudinal correlation and it was present in all three years (Table 2c). However, the pattern was still relatively weak in 2002 (r^2 = 0.137) compared to in 2003 (r^2 = 0.352) and 2004 (r^2 = 0.422; Fig. 4c–e). There was also a significant effect of latitude in 2002 and 2003 (Fig. 4f, g), but the direction of the relationship was different among the years. In 2002 increasing latitudes resulted in increasing δD values (r^2 = 0.138), but in 2003 the relationship (r^2 = 0.165) was reversed (Fig. 4f, g).

We found no significant correlation between the δD residuals and altitude of the sampling locations.

OIPC/PLGS data

The long-term annual δD values calculated by the Online Isotopes in Precipitation Calculator (OIPC) and the recalculated Peacock Larvae Growth Season (PLGS) δD values showed a significant relationship with measured δD values in the peacocks, but only in 2003 (r^2_{Annual} = 0.245, r^2_{PLGS} = 0.333). The significant relationships of 2003 were stronger when using PLGS values (Fig. 5a) than annual OIPC values (Fig. 5b). Results from all the correlations are given in Table 3.

Table 2. Results of the ANCOVA test on measured δ-values for $^{15}N/^{14}N$ (a), $^{13}C/^{12}C$ (b) and $^{2}H/^{1}H$ (c) from peacock butterflies collected during three years 2002–2004 at different locations throughout southern Sweden. The presented results are the final model that remains after non significant main effects and interactions have been removed in a backward fashion.

a) Dependent variable: $\delta^{15}N$

Variable	S.S.	df	F	p
Study Year	31.3	2	3.65	0.029
Longitude	5.4	1	1.25	0.265
Study Year * Longitude	34.0	2	3.96	0.021
Error	562.7	131		
Total	44327.1	137		

b) Dependent variable: $\delta^{13}C$

Variable	S.S.	df	F	p
Study Year	10.8	2	9.89	<0.001
Longitude	0.5	1	0.89	0.35
Study Year * Longitude	9.9	2	9.07	<0.001
Error	72.6	133		
Total	112961.6	139		

c) Dependent variable: δD

Variable	S.S.	df	F	p
Study Year	877.2	2	13.11	<0.001
Longitude	2420.8	1	72.35	<0.001
Latitude	246.2	1	7.36	0.008
Study Year * Latitude	872.2	2	13.03	<0.001
Error	4550.5	136		
Total	1954713.0	143		

Discussion

In our study, ratios of deuterium show the most consistency both spatially and interanually of the three isotopes analysed. We found a clear and significant longitudinal correlation for all years for deuterium. Although a somewhat weaker effect was present in 2002, this could be explained by a lower number of sample sites that year. In 2002 we did not obtain samples from our eastern and westernmost localities, so the range of longitudes available for the analysis was smaller than in 2003 and 2004. For deuterium, we also observed effect of latitude in both 2002 and 2003 but the direction of this relationship was different between these years. Our data shows that even in a reasonably controlled situation natural, variation of deuterium ratios in one location (ex. Lund 2002–2003) can be as large as 10‰, which is consistent with populations of local songbirds (Langin *et al.* 2007). From the interpolated means of deuterium ratios in rain water in Sweden (Bowen *et al.* 2005), this translates to an expected mean difference between two locations about 400 km apart. In Scandinavia a pattern of decreasing values for deuterium ratios is predicted towards northeast caused by the variation in tempera-

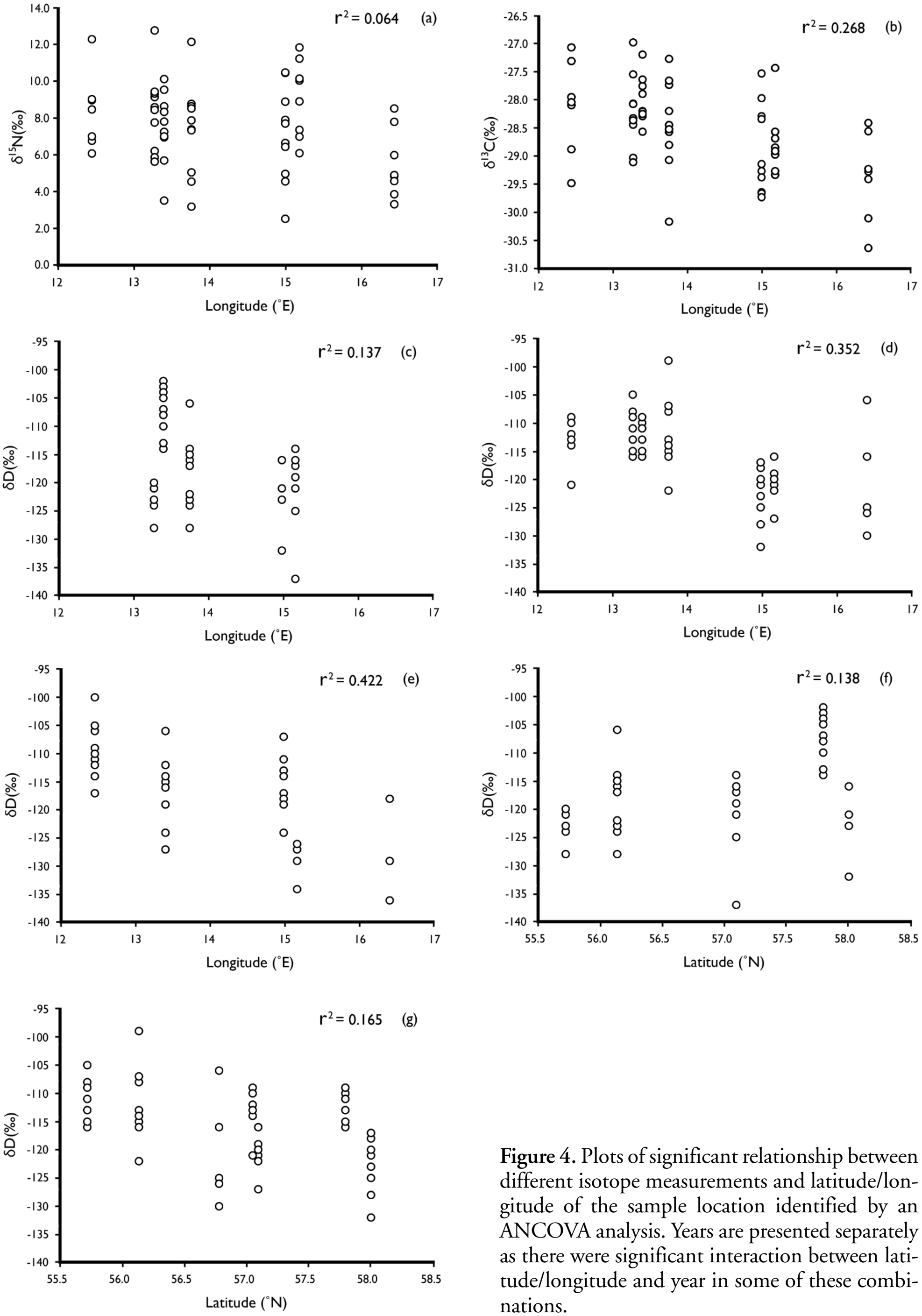

Figure 4. Plots of significant relationship between different isotope measurements and latitude/longitude of the sample location identified by an ANCOVA analysis. Years are presented separately as there were significant interaction between latitude/longitude and year in some of these combinations.

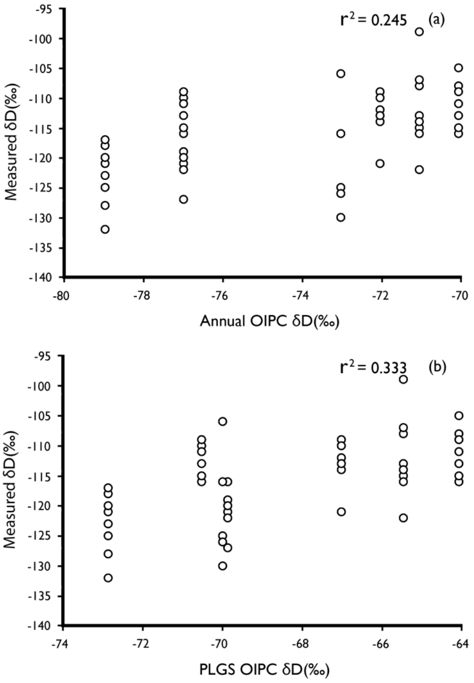

Figure 5. Relationship between measured δD values and (a) annual mean δD values in local rainwater obtained from OIPC and (b) mean δD values for the time period April–June weighted in relation to the amount of rain during different parts of that time period (see text for explanation). Significant relationships were only found in 2003.

ture and precipitation (Bowen *et al.* 2005), and thus both a longitudinal and latitudinal effect could be expected; as was observed in our study. A similar trend of deuterium ratios decreasing towards northeast have also been reported for stationary birds in Europe (Hobson *et al.* 2004). However, Hobson *et al.* (2004) did not have any samples from Sweden in their study, so it is not directly comparable to ours. We found no significant effect from the difference in altitude of our sampling locations but considering the relatively small differences (7–222 m above sea level) between our sites this is not surprising. The OIPC calculated δD values change with altitude at about –1.5 ‰ / 100 m in the study region.

It is clear that 2003 stands out as a comparatively stable year over the two other years with respect to the relationship between spatial location and isotope values. In 2003 all of the measured isotope ratios (δN, δC and δD) showed predictable patterns in relation to their geographical location, but in 2002 and 2004 there were predictable patterns for deuterium only. The deuterium relationship was much weaker for 2002 and 2004 compared to in 2003. There are two important reasons why 2003 might have produced the clearest geospatial patterns in distribution of isotope ratios. First, 2003 was the only year that we were able to sample from all of our locations giving us a broader range of both latitude and longitudes in our models. Second it was a year with the most typical climate situation. Mean temperatures from all of our sample locations show a gradual decline in temperature with increasing latitude during spring in 2003 (Fig. 6) (except for Varberg (VA) which was always relatively warm, probably because of relatively high solar radiation). This

Table 3. Correlations between measured δD values in wings from peacock butterflies and annual mean δD values of local rainwater (OIPC) and mean δD values of rainwater during the peacock larval growth season (PLGS). Data are from samples collected during three years 2002–2004 at different locations throughout southern Sweden.

Year		N	OIPC	PLGS
2002	Pearson Corr.	38	–0.221	–0.113
	Sig. (2-tailed)		0.183	0.498
2003	Pearson Corr.	66	0.495	0.577
	Sig. (2-tailed)		< 0.001	< 0.001
2004	Pearson Corr.	39	0.173	0.112
	Sig. (2-tailed)		0.294	0.498

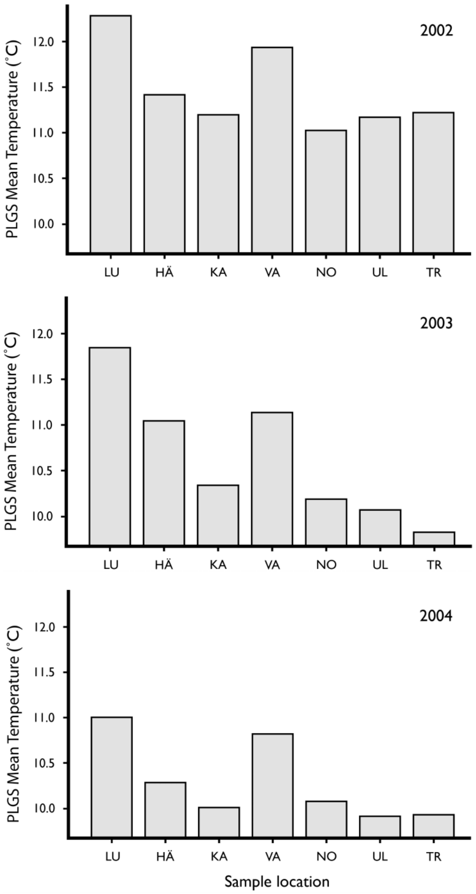

Figure 6. Yearly mean temperature for April – June at each of the sample locations. 2003 shows the typical pattern for the region with a generally decreasing temperature with increasing latitude. In 2002 and 2004 we have more atypical temperature patterns for the region.

probably resulted in a more predictable period of development for the peacock larvae. In 2002 and 2004 the local temperatures showed a far more unpredictable pattern between regions which might have affected the local development periods in different directions. Since the yearly pattern of the deuterium ratios in precipitation changes so much during the time of the year when the peacocks are developing on their host plants (Fig. 2), differences in development periods of a few weeks likely have a substantial effect on the deuterium ratios we later measure in the wings. Since the development time of butterflies is highly variable and depends to a large extent on variations in temperature (Eliasson *et al.* 2005) this is an important factor that can produce substantial local variation.

Carbon isotopes in animals reflect the natural variation of isotopes in the diet used to produce the tissue that is analysed (Hobson 1999, Kelly 2000, Staddon 2004). Carbon isotopes often show more depleted values in cooler and moister climates, caused by differences between plants carbon isotope discrimination among C_3, C_4 and CAM plants (Oleary 1988, Rubenstein & Hobson 2004, Staddon 2004). The different ratios of these plants in relation to climate affect the carbon isotope values in tissues from animals higher up in the food chain, but when studying a species feeding on a single food plant we would not expect any large differences. Plant populations adapted to drier and warmer habitats show higher discrimination against heavy isotopes (Lajtha & Marshall 1994) related to difference in water use efficiency, leading to differences among plant population of the same species even in common garden experiments (Lauteri *et al.* 1997). This could explain why we find differences in 2003 with depletion in carbon values with increasing longitude. The climate is drier and warmer towards the east in the whole southern part of Sweden because of rain-off effects and most weather systems containing large amounts of rain originate from the west or the southwest. Noting that this effect was only found during one year, it is probably caused by plasticity of the plants (Lauteri *et al.* 2004) than by genetically differentiated plant populations.

How useful is the method for studying natal origin in monofagous and migrating insects?

Since the introduction of stable isotope methods in ecological studies, large efforts have been focused on long distance animal movement (for reviews, see e.g. Hobson 1999, 2003, Rubenstein & Hobson 2004). Animals from different geographical origins have been studied to find migration routes, winter areas and natal regions. Most of these studies to date have been focused on individuals with the sampled tissues originating from far apart, making the isotopic differences sizeable (e.g. Hobson *et al.* 2004). Instead, our study focused on a smaller regional scale to test the limits of the methods' resolution power using natural samples. Even though we worked on a smaller scale where isotopic differences were anticipated to be less pronounced, we did observe geographical isotopic differences between our sample sites (mostly longitudinal) but only during certain years. While it is not possible to delineate all of the locations, the geospatial extremes were sufficiently different to be distinguished even though our sample sizes were not very high. Most important were the large isotopic differences that were sometimes present between two different years at the same location. The interannual difference at a single site could be larger between two consecutive years than over the whole study area during one single year. Large inter-individual variation in δD has been reported from known populations of migratory songbirds in North America, further suggesting that caution is needed when interpreting the results for migrating animals (Langin *et al.* 2007).

Even though we observed a large variation in isotope values, samples from the same region and year (excluding nitrogen) were often close to each other. This reveals that sampling errors are not a likely explanation for the large variation of our carbon and deuterium values. Since $\delta^{15}N$ is measured simultaneously with $\delta^{13}C$ it is likely that the larger variation in nitrogen is caused by a high natural variation and does not represent an artefact of our analytical method. The larval food plants (i.e. nettles) are nitrogen loving plants often found in the vicinity of farms, where artificial fertilizers further complicate the nutrient picture (e.g. Robinson 2001, Hobson 2005). The only relationship we found when looking at nitrogen isotopes was a weak longitudinal relationship (in 2003) and it cannot be said to be a very useful indicator for studies in areas were fertilizers and other non-natural substances are used.

Among the three isotopes used in our study deuterium was clearly the most useful isotope for spatially delineating the samples from our Swedish locations (maximum distance in north-south or east-west direction was about 250km). Carbon isotope ratio was potentially a good predictor as it shows the same but slightly weaker pattern as deuterium. Carbon might be used in combination with deuterium or over ranges showing larger natural variations in carbon values than we find in our study area (e.g. Wassenaar & Hobson 1998). The predictive power of carbon is likely to increase even in our study area if a species with a more varied diet was studied than our study species, the monofagus peacock butterfly.

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IV

Understanding migration patterns of European red admirals (*Vanessa atalanta*) using stable hydrogen isotopes

Understanding migration patterns 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 the ratios of stable hydrogen isotopes ($^2H/^1H$ or δD) measured in wings from red admirals (*Vanessa atalanta*) to study patterns in the species' migration. A clear seasonal difference in the isotopic signatures of red admirals in northern Europe was found with an inferred southern origin of the spring migrants. In autumn, δD measurements suggest that red admirals are mostly from regions close to the sampling sites, but throughout the sampling period there were always individuals with δD values indicating non-local origins. The migration pattern of this species is supposedly highly variable and plastic. Isotopic differences between individuals in the western part of Europe are generally small making migratory patterns difficult to interpret. However, butterflies from the western parts of Europe are apparently isolated from those from the north-eastern part, since δD values found in the western region only rarely correspond to those of autumn migrants from the north-east. In the central part of southern Europe we found large differences in δD values between spring and autumn samples supporting presence of a local reproducing population during winter. There is also a presumed influx in the spring of individuals with an even more southern origin since we find δD values matching coastal areas of North Africa. Use of stable hydrogen isotope data for studying butterfly migration in Europe is clearly complex, but our study shows that this technique can be used to uncover previously unknown aspects of red admiral migration and be a powerful tool in combination with other available methods to unravel patterns of butterfly migration.

Introduction

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' yearly migration in Europe has been described with focus on flight directions taken at several locations (e.g. Williams 1951, Imby 1993, Benvenuti *et al.* 1996, Steiniger & 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 North Europe (Mikkola 2003, Brattström *et al.* submitted a). In addition to these, a study revealing the presence to two largely genetically separated but often co-occurring populations of red admirals in Europe have given new interesting insights to red admiral migration in Europe (Brattström *et al.* in prep b). Our knowledge about migration routes and phenology in this species however, are still very limited.

Most of the European red admirals seem to spend winter around the Mediterranean Sea (Stefanescu 2001, Brattström 2006), and possibly also further east (Brattström *et al.* in prep b). Hibernation during winter is not uncommon in the northern parts of Europe, but it is probably not an important strategy compared to migration towards the south to avoid harsh winter conditions (Pollard & Greatorex-Davies 1998). A genetic study of European red admirals indicate that there are two distinct groups with primarily western and eastern distributions, but with some mixing present, at least in some years in the northern part of Europe (Brattström *et al.* in prep b). In this study, we did not find any support for different isolated migration routes, indicating that there are no simple north-south routes used year after year by different sub populations of red admirals in Europe. Such patterns can be hard to identify with genetic methods since interbreeding, even though it does not occur every year, keeps populations in different geographical regions from developing discernable genetic differences (Endler 1973). We know from observations of flight direction in the field that migration often occurs simultaneously in both a dominant direction, i.e. towards the north in spring and the south in autumn, and at the same time; small numbers of individuals are reported flying opposite the dominant direction (Williams 1951, Benvenuti *et al.* 1996, Stefanescu 2001, Brattström & Åkesson in prep a). We also know that the yearly peak dates of migration are variable (Hansen 2001, Brattström *et al.* submitted a). 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 days), regardless of the environment they experienced after hatching. This indicates that the flight direction of the adult butterfly is already 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 small animals, where we can suspect high mortality during migration, mark-recapture methods are unsuitable for finding migration routes and such methods are therefore unlikely to be of any use in studies of red admirals (e.g. Webster *et al.* 2002). A study of monarch butterflies (*Danaus plexippus*) in North America has shown that stable isotopes can be used to infer the origin of migratory butterflies (Hobson *et al.* 1999). We therefore decided to study stable hydrogen isotope ratios ($^2H/^1H$ or δD) found in the wings of red admirals captured during different seasons throughout Europe. Since δD values directly reflect the geographic origin of single individuals the technique can help identify patterns across Europe even in species with large annual variations and fairly regular intermixing between sub populations.

Materials and Methods

Collection of red admiral samples

The red admirals used in this study were collected by Oskar Brattström and voluntary collectors. The butterflies were killed with ethyl acetate and the wings were then removed from the body and stored in glassine envelopes in a dry, dark place up until the analyses were conducted. The samples were from three main regions of Europe: North (Southern Sweden, Denmark, Estonia and Kaliningrad in Russia), South (Capri in Italy) and West (Northern Spain and Northern France). All sample locations are given in Figure 1. The main part of the sample is 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, Italy, 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 more resident species from Capri to be able to estimate the natural level of δD values found in the wings of butterflies from this location. In 2004, we captured ten wall browns (*Lasiommata megera*) and

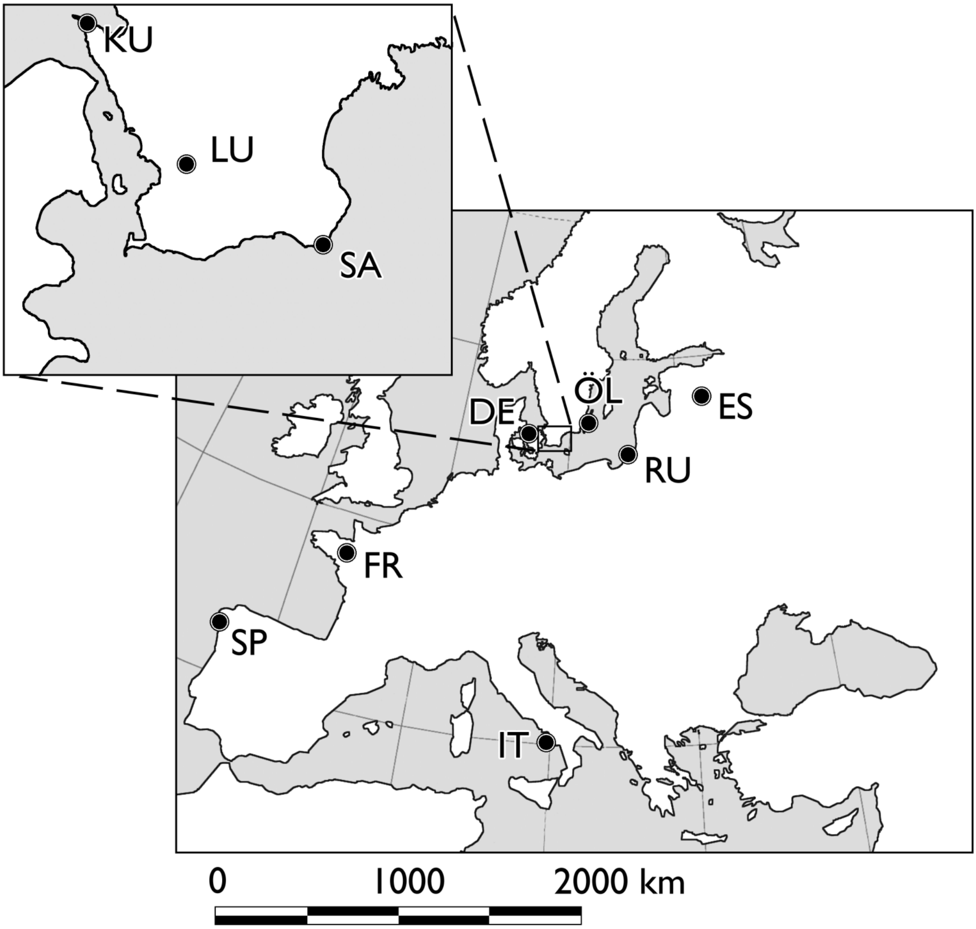

Figure 1. Position of sample locations where red admirals were captured during spring and autumn 2003–2006. Locations are: Vilagarcía – Spain (SP), Lassy – France (FR), Capri – Italy (IT), Ølsted – Denmark (DE), Kullaberg – Sweden (KU), Lund – Sweden (LU), Sandhammaren – Sweden (SA), Öland – Sweden (ÖL), Rybachy – Russia (RU) and Karilatsi – Estonia (ES).

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 second week of May. The samples from western Europe were of limited numbers and from different time periods, ranging from 16 April in spring to 7 November in 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 closer detail. To compare the seasonal differences in δD values we denoted all samples up until 15 July each year as individuals emerged during spring (spring) and those captured after that date we considered as individuals emerged during late summer and autumn (autumn).

Stable isotope analysis

We rinsed all wing keratin samples in a chloroform-methanol solution (2:1) to remove surface oils that could affect the isotope assays, and air dried the samples overnight. Samples were weighed to 0.36 ± 0.01 mg, packed in silver capsules at the Department of Animal Ecology, Lund University, and shipped to Canada for analysis. Stable hydrogen isotope analysis was conducted at Environment Canada in Saskatoon, Canada. Because stable-hydrogen isotope analyses of keratinous materials are complicated by uncontrolled isotopic exchange between samples and ambient water vapour (Wassenaar & Hobson 2000), we used the comparative equilibration technique so that the values reported here are equivalent to nonexchangeable hydrogen (Wassenaar & Hobson 2003). In short, the process involves the simultaneous measurement of wing samples with replicates of three different keratin standards, whose nonexchangeable δD values are known and which span the range of expected values. Stable-hydrogen isotope measurements of wings and the keratin standards were performed on H_2 derived from high-temperature flash pyrolysis of wings and continuous-flow isotope-ratio mass spectrometry. We used a Eurovector 3000 (Milan, Italy) high temperature elemental analyzer (EA) with autosampler to automatically pyrolyse wing 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. We have estimated our laboratory error to be ±2 ‰ based on within-run measurements of intercomparison material and consideration of within sample variance (Wassenaar & Hobson 2006).

Deuterium ratios in precipitation

The δD values found in butterfly wings is dependent on the δD value of local precipitation (Hobson *et al.* 1999; Brattström *et al.* submitted b), but fractionation processes deplete this value when going from precipitation to the larval foodplants. We used the Online Isotopes in Precipitation Calculator (OIPC) (http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html) to acquire interpolated annual and monthly δD values for the rainwater at our sampling locations (Fig. 2a). The variation of δD in rainwater across Europe reveals a fairly complicated picture because of local topography (e.g. the Alps) but the general trend is that δD in precipitation becomes more depleted along a north eastern gradient (Fig 2b, see also Hobson 2003). 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 more 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.* submitted b). 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 is a time period corresponding to the season when peacock larvae develop in Sweden (Brattström *et al.* submitted b). We used a narrower time period for the

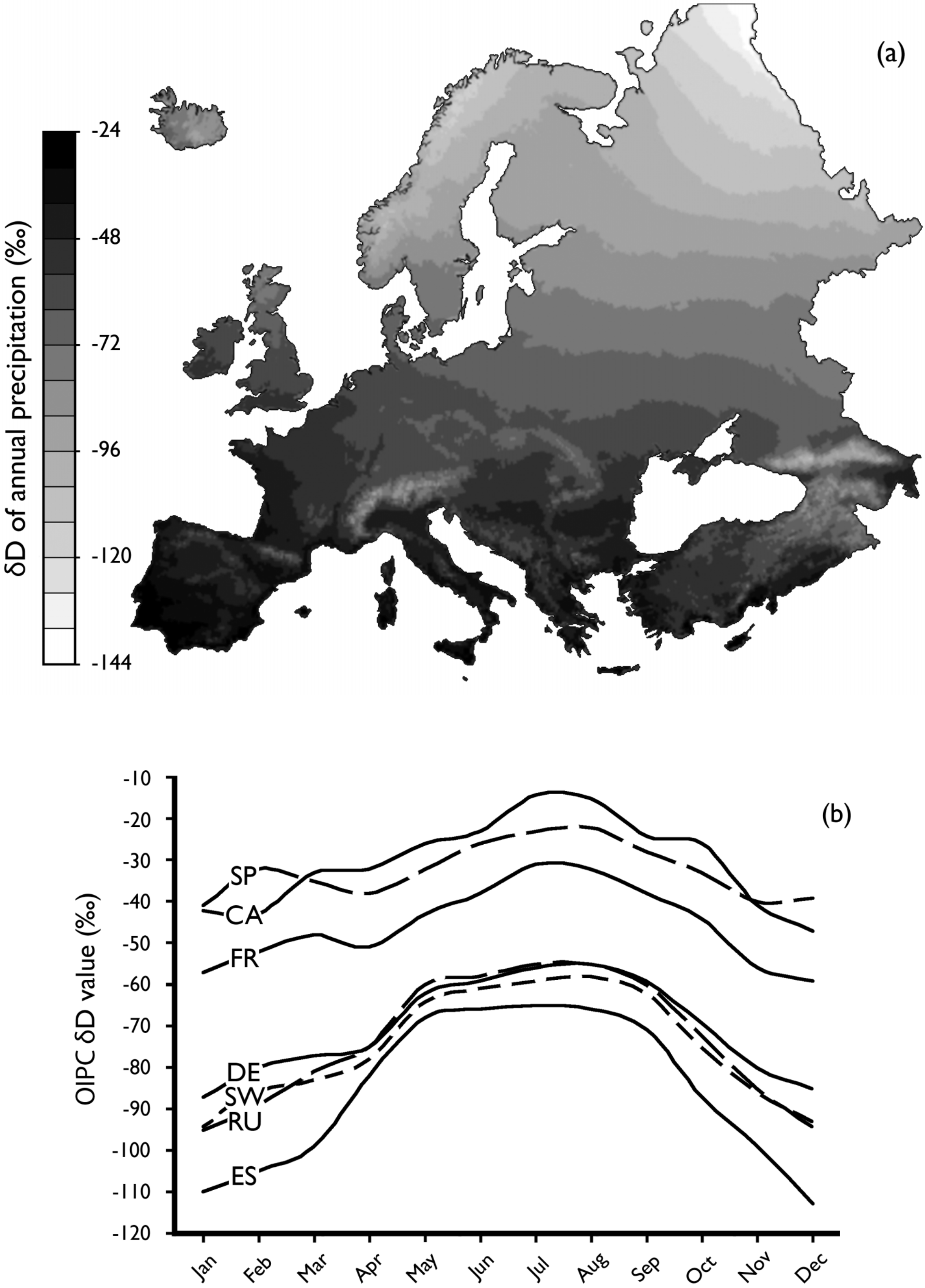

Figure 2. Interpolated δD values from OIPC (the Online Isotopes in Precipitation Calculator). a) Mean annual values in Europe (map based on file downloaded from OIPC), and b) monthly average values in precipitation from the different locations used in this study. The Swedish sample sites are represented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

Capri material since butterflies in general develop faster at warmer, more southern locations compared to the north. We compared the 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 since we had no year with data from all sites. For each location with several years sampled, we found very similar δD values between years (expect autumn 2004 in Italy, see discussion). We used an ANOVA to study effects of season and sample location on the data from northern Europe. For the samples from Capri, France and Spain we used a t-test to study if spring and autumn individuals had different deuterium ratios in both of the sample sites. 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 ratios. 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.

Results

Numbers of sampled butterflies, collection period, mean, standard error and range of δD values of the sampled individuals are presented for each site and time period in Table 1. The 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). 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. 3). A Tukey post-hoc comparison identified two subsets among the sample locations in late-summer season that were significantly different from each other ($p<0.05$; Fig. 3). Denmark stands out among the locations with the highest δD values in autumn. Furthermore, we found a general decrease in δD values with increasing longitude.

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

There was no significant difference in mean δD values from red admirals captured in north-

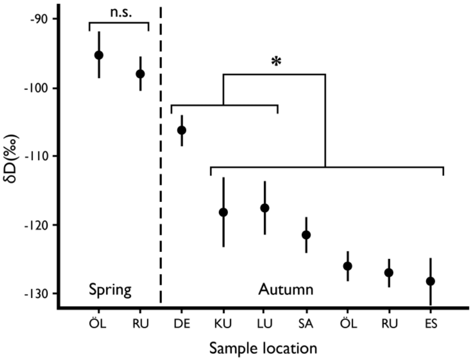

Figure 3. Mean δD values measured in wings of red admirals captured at seven locations in northern Europe during spring and autumn migration. Locations are presented in order of their geographical position along a longitudinal gradient. The values from spring were significantly different from autumn 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.

Table 1. Geographic position of sample locations, time period of sampling, number of butterflies sampled and mean δD values (with ± 1 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	2000, 2005	Spring	16 Apr – 22 May	7	–75.3 ± 3.8	–86.9	–56.2
					2003 – 2005	Autumn	17 July – 7 Nov	11	–69.6 ± 4.6	–107.0	–54.1
		Lassy, FRA	48.1°N	1.9°W	2004 – 2006	Spring	26 May – 18 June	5	–64.1 ± 4.8	–81.7	–54.9
					2004, 2006	Autumn	27 July – 18 Sep	7	–80.0 ± 5.1	–96.8	–55.8
	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

Table 2. Results of the ANOVA test on measured δD values for red admirals 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		

ern 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. In Northern France there was an almost significant difference in mean δD between the first (–64.1 ± 4.8 ‰) and second (–80.0 ± 5.1 ‰) (t-test, $t = 2.18$, $N_{Spring} = 5$, $N_{Autumn} = 7$, $p = 0.055$) part of the year. The mean δD values for the western region in spring and autumn are presented in Figure 5.

There was a significant linear relationship ($r^2 = 0.87$; $p < 0.001$; Fig. 6a) 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 their region and season. The equation of the regression line ($\delta D_{WING} = 1.1 * \delta D_{PRECIPITATION} - 41$) is different from the one presented by Hobson *et al.* (1999) ($\delta D_{WING} = 0.5 * \delta D_{WATER} - 53$). When combining the data from the previous study by Hobson *et al.* (1999) and δD values for butterflies of known origin and OIPC values from our study we found a good fit for a quadratic linear relationship between wing δD and precipitation or water δD ($\delta D_{WING} = -0.0017 * \delta D_{PRECIPITATION/WATER}^2 + 0.91 * \delta D_{PRECIPITATION/WATER} - 44.85$; $r^2 = 0.91$; $p < 0.001$; Fig. 6b).

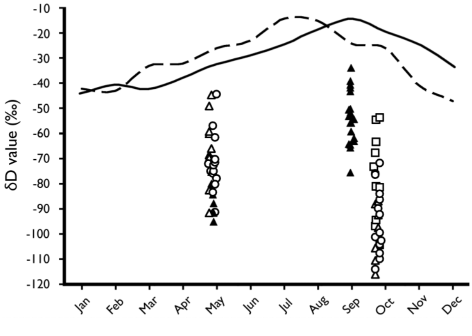

Figure 4. Mean δD values measured in wings of butterflies captured during spring and autumn at Capri, Italy. The included species are: Two species of local butterflies (▲), wall brown and southern small white and the migratory red admiral. Red admirals are presented separately for each year: 2004 (□), 2005 (Δ) 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.

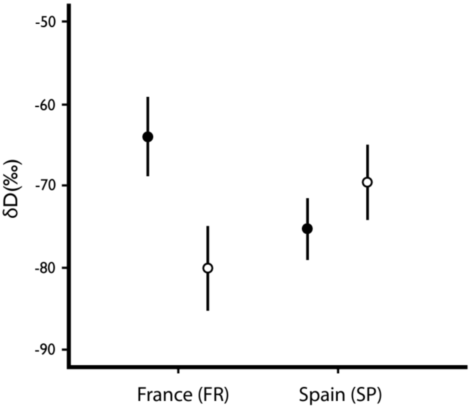

Figure 5. Mean δD values measured in wings of butterflies sampled in northern Spain and northern France from 2003 to 2006. The migration periods are divided in spring (before 15 July) and autumn seasons (after 15 July).

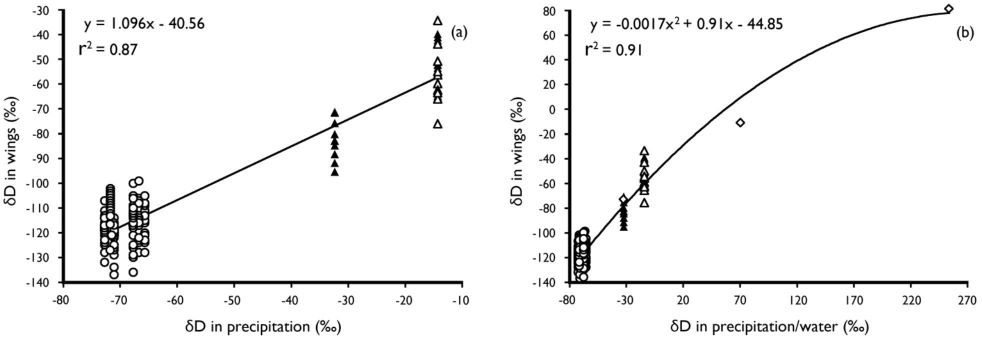

Figure 6. Relationship between measured δD values from butterfly wings and OIPC derived δD values in water or water of known deuterium content from laboratory experiments. a) δD values for local butterflies captured at Capri, Italy and in Sweden (for details see Brattström *et al.* submitted b) in relationship to local δD values in precipitation during the developmental time period of the larvae. The relationship seems to be linear in the first order. In b) samples from a laboratory study of monarchs (Hobson *et al.* 1999) using water of known deuterium content which also reported a similar linear relationship but with a much shallower slope are included with the samples of butterflies of know geographic origin from our study. A combination of the data sets result in a quadratic relationship.

Discussion

The movement patterns and timing of red admirals migrating across Europe is still relatively unknown and based on inferences from observational data. Earlier studies suggest that stable isotopes, and particularly deuterium, could be used to define area of origin for migrating animals (e.g. Webster *et al.* 2002, Hobson 2003, Rubenstein & Hobson 2004). We found sizeable differences in δD values measured in wings of red admirals from different sites in Europe. Even though the natural variation in these values can make good predictions about sample origin cumbersome (Langin *et al.* 2007; Brattström *et al.* submitted b), our data can be used to answer general questions about the timing and migration patterns, i.e. between which regions red admirals migrate in Europe.

Red admirals captured during spring migration

Southern region

Our data shows that the local butterfly species collected at Capri must have been hatched during the same spring season in which they were collected. By assuming that the larval foodplants of these species contain similar δD values to those being the food sources for the locally hatched red admirals, we can use this 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 captured during spring at Capri show similar δD values as the local butterfly species, but a fraction of the individuals show slightly higher values indicating a more southern origin. The minimum value is almost identical (Table 1) and thus it seems like the Capri spring population of red admirals is made up of both locally hatched individuals and those originating from further south in Italy, and possibly even from the north coast of Africa. If the fractionation of δD values from rainwater into wing material in the European region is around –40‰, as our data indicates (Fig. 6a), the individuals with the highest δD values corresponds to values found in Libyan coastal regions, suggesting that many of the red admirals collected in spring at Capri came from further south.

Northern region

The samples from the spring migrants found in the northern part of Europe show values around –90‰ which is lower than the mean of the

Capri spring samples. The highest values observed in northern Europe overlap with those observed at Capri in spring showing that it is possible that at least some of the red admirals that arrive in northern Europe originate from Italy or nearby areas. However, the majority of the butterflies arriving in spring to northern Europe must originate from areas much further north and north east. Some of them even have values close to the expected value for locally hatched butterflies, which means that they are either the offspring of individuals that have spent winter in the area or that they originate from areas further east of the study region. We must also consider the possibility that there can be individuals from an intermediate spring generation produced in the central parts of Europe on their way north. Support for this can be gained from the fact that individuals from the earliest spring sample from Öland in Sweden (2003) show the highest δD values (and thus possibly originate from the most southern locations). Migration systems where different parts of the migration route are covered by different generations are well known for North American Monarchs (e.g. Brower 1995), and suggest that this type of migration could also have evolved in other insects species.

Red admirals captured during autumn migration

Northern region

The samples of red admirals in autumn from northern Europe, as well as those from Capri, are clearly different from those found in spring. The samples from northern Europe show similar mean δD values to the ones found in peacock butterflies from Sweden (Brattström *et al.* submitted b). However, the range of values from each location (except Öland 2005) contains at least some individuals with δD values corresponding to those expected in individuals from more southern areas. Even though the general migration in the northern parts of Europe is reported towards the south during autumn (e.g. Imby 1993, Hansen 2001, Mikkola 2003) there are butterflies in our sample with an isotope signature that suggests a more southern origin. These individuals might be on a northward migration despite the late season, and because of this we find a mixture of individuals with different migratory origin and directions at the same location at this time of year. Still, the majority of the migrants seem to be from the local region or further north, probably on their way towards the south. We know from orientation studies performed with red admirals during autumn migration in Southern Sweden that they generally have a southern flight direction during late summer (Brattström & Åkesson in prep a), but individuals flying towards the north are always encountered during the same time period. Other studies of free-flying migratory red admirals in southern Europe (Benvenuti *et al.* 1996, Stefanescu 2001) and Britain (Williams 1951) also report a few individuals flying in an opposite direction compared to the mean migratory direction.

We found a general trend towards lower mean 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). This difference in our samples is larger than expected from the OIPC data for the locations, so there are probably individuals from areas further north and north-east encountered in Sweden, Kaliningrad and Estonia. Since we deal 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 compared to the OIPC data (Brattström *et al.* submitted b).

One potentially important factor that has to be taken into account when interpreting δD values is the annual variation of these values in precipitation, which is especially pronounced in northern locations (Fig. 2b). Since the red admirals captured during autumn have developed during a time of the year when the isotope ratio in the precipitation is at its highest peak, the wing δD values may not be directly comparable to individuals from spring. If we only look at the annual mean gradients of δD values in precipitation (Fig. 2a) when trying to determine origins of individual butterflies, we will estimate the origin of our sampled butterflies incorrectly. The spring samples that have developed during a period with lower than average local precipitation δD values (Fig. 2b), will be assigned to a region further towards the north-east than where they actually come from. In autumn, when the sampled individuals have developed during summer with relatively high local precipitation δD values (Fig. 2b), we will instead tend to place them further towards the southwest. In the present study this has not been a serious problem, since we have focused on comparing samples from different regions to each other rather than trying to pin-point their natal areas. With larger and more complete data sets, preferably with many samples from local and resident butterflies, it should be possible to broadly identify natal areas. Because of the variation within each single year (Fig. 2b), and variation between different years at the same location, however (Brattström *et al.* submitted b), detailed predictions will be difficult to obtain even with extensive data.

Southern region

Red admirals encountered at Capri in autumn show different δD values from the ones found during spring. The δD values were lower, suggesting that these samples originated from further north. The mean δD values were lower than those found by us in northern Europe during the autumn migration, so the majority of red admirals spending winter in the Italian region must be from areas not too far to the north, or else from further to the west. It is possible that the Alps act as a migration divider (e.g. Alerstam 2001, Alerstam *et al.* 2003, Åkesson & Hedenström 2007), so that red admirals reaching the western coast of Italy originate mainly from summer regions west of the Alps. It is likely that red admirals are affected by local topography 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 (e.g. Liechti & Bruderer 1986, Bruderer & Jenni 1988, 1990; see also Brattström *et al.* submitted a). The main migratory direction of birds has been estimated toward southwest in Central Italy (Fig. 1 in Bruderer 1980) as a result of predominating winds and local topography. However, since the δD values we found corresponded to several areas in different geographic directions north of Capri, we cannot be completely sure of the origin of the red admirals that arrive there in autumn. Visual observations at a coastal location north of Capri suggest that red admirals follow the coast towards south east in autumn (Benvenuti *et al.* 1996), but this can at least in part be a migration direction affected by local topography (see above). The δD values we found at Capri in autumn do not correspond to the δD values we found at the northernmost locations. Therefore, we can at least be sure that the majority of the autumn migrants arriving at Capri come from other regions than our northern sample locations. Furthermore, in 2004 the δD values found at Capri in autumn were higher than the other two years, suggesting that there are yearly variations in recruitment areas, and possibly also in δD values within the same region (Brattström *et al.* submitted b).

Red admiral migration in the western part of Europe

We found an almost significant difference in δD values for red admirals between spring and autumn in France, but not in Spain. Since the numbers of individuals sampled were few and span a longer time period, it is not possible to draw any solid conclusions from these differences. One additional result, making isotope studies in that region hard to interpret, is the small natural variation in hydrogen isotope ratios (Fig. 2) because of the large effect on the precipitation pattern in the region due to the vicinity of the Atlantic Ocean (e.g. Hobson 2003, Rubenstein & Hobson 2004). Despite these limitations of the data, we can still gain one very important insight into red admiral migration from these samples. The lowest deuterium value found in this region was –107‰, a value much higher than what would be expected in red admirals hatched during summer and autumn in the northern region (mean: –122.5 ± 1.2‰), especially in the north eastern part (Kaliningrad) where we regularly find values lower than –140‰. The samples from the western region were collected over a long period of time, and if migration movements of red admirals between the northern and western region of Europe were common, we would expect to find several individuals in autumn in the western region with low (< –110 ‰) δD values. In our study there are no such samples, suggesting that red admirals originating from North Europe do not reach France and Spain in autumn, at least not in high numbers. A study of genetic differences in Europe has suggested that there are two distinct types of red admirals in Europe with a primarily western and eastern distribution with some mixing in the northern region (Brattström *et al.* in prep b).

One additional factor making studies based on δD data troublesome is the altitudinal migration of red admirals that have been observed in Spain (Stefanescu 2001) and possibly also occurs in other regions around the Mediterranean Sea. Each spring when the number of individuals observed in the lowland areas where red admirals are known to breed during winter decrease, the numbers instead increase at nearby high altitude locations (Stefanescu 2001). This suggests that red admirals in their efforts to track suitable larval resources not only engage in annual north-south migration to reach suitable regions for summer reproduction, but that part of the population performs altitudinal migration for the same purpose. Since these populations most likely utilize the same winter areas, autumn samples from the southern region can include individuals from both of these groups. As mountain regions in general have more depleted δD values (e.g. Hobson *et al.* 2003, Rubenstein & Hobson 2004), individuals that have hatched there will have δD values that can be incorrectly interpreted as coming from more northern regions. More studies of the altitudinal migration are needed before we can determine how much this affects our results. If the two behavioural groups do not interbreed during winter, however, it should be possible to separate them using genetic methods when isotope differences can not be used.

Conclusions

Despite the extensive natural variations in butterfly δD values caused by variation in local topography and precipitation δD values in Europe, it is possible to find some general patterns among the red admiral samples in our study. First, there is no doubt that a migration with a strong north-south component is present in northern Europe and we have indications that the spring migrants come from not only the Mediterranean area, but also form areas further to the north. We also found that individuals from southern areas can still be encountered at our collection sites in northern Europe in autumn. Most of the individuals captured at Capri, both in spring and autumn seem to belong to another migratory population than those captured in northern Europe. Only the most extreme values from each season overlap between northern Europe and Italy. Our samples from the western region suggest that this area is isolated form the northern parts of Europe. Stable hydrogen isotopes are powerful tools in studies of migratory butterflies in Europe, especially when combined with field observations and genetic analysis.

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V

Annual patterns in migration of red admirals (*Vanessa atalanta*) at Rybachy, a coastal site in northern Europe (Kaliningrad)

Annual patterns in migration of red admirals (*Vanessa atalanta*) at Rybachy, a coastal site in northern Europe (Kaliningrad)

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We present flight season data from a two-year time series of migrating red admirals (*Vanessa atalanta*) captured in Rybachy, Kaliningrad, in the northern part of Europe. Red admirals that passed our study site were found during two distinct time periods, with a period of low activity in mid summer. Analysis of stable hydrogen isotopes (δD) showed that the spring samples were of a southern origin, while those caught in August or later in the autumn originated from the sample region or areas further to the north. All females caught during spring had developing eggs in their abdomen, but no eggs were found in females caught in late summer/autumn. Findings of a male-biased sex ratio that occurred during autumn, and a difference in lipid content between years were unexpected. When comparing Rybachy samples with data from inland locations in the same part of Europe, it was clear that the range of δD in red admirals was greater in Rybachy than the reference sites. This is likely due to the coastal location of Rybachy, since migrating red admirals follow local topography and individuals from different natal regions are funnelled to a higher extent into Rybachy than to inland locations.

Introduction

The red admiral (*Vanessa atalanta*) is perhaps the species showing the most regular migration pattern of all the European butterflies that are considered as long-distance migrants (e.g. Pollard & Yates 1993). Migration is variable in intensity, timing and origin, but red admirals almost always reach northern Europe in good numbers each spring. These immigrants reproduce and give rise to a new generation that allegedly migrate south later in the season to reach areas suitable for reproduction during winter, although parts of the population apparently spend winter hibernating. Most reports of red admiral hibernation are from the northern region, but the behaviour seems to be of little importance for the new generation that hatches the following summer (Pollard & Greatorex-Davis 1998). It is however possible that the individuals from more eastern parts of the population are specialised hibernators (Brattström *et* *al.* in prep a). Since most insect migrants are far more variable in their migration than for example birds (e.g. Dingle 1996, Alerstam *et al.* 2003) we lack long-term studies of insect migrants from the same location. There are few locations where one can be sure to observe large numbers of red admirals during most years, which is a requirement in order to obtain sufficient data for analysis of differences in migration intensity both within and between years. Coastal locations are better than inland areas for observing red admirals (e.g. Reichholf 1978, Benvenuti *et al.* 1994, Hansen 2001, Brattström *et al.* submitted a), because higher numbers are seen as a consequence of the funnelling effects of topography and this species' tendency to avoid crossing of large water bodies.

The objective of this study has been to obtain a continuous series of red admiral samples from one location in order to obtain detailed insights into the yearly migration patterns. Most other studies of migrating red admirals represent snap-shots in time (e.g. Imby 1993) or have been reported from different locations (e.g. Hansen 2001), and even though they have given us knowledge about the variable migration of the species, no study has followed yearly changes at a single site in a systematic way. We sampled red admirals as extensively as possible at one fixed coastal location, Rybachy, where large numbers of this species can usually be seen. Sampled butterflies were then analysed with respect to stable hydrogen isotopes (δD) (in order to determine area of natal origin), sex ratios, breeding status, and lipid content.

Materials and Methods

Study location and collection of red admirals

Rybachy (55°2′N, 12°8′E) is situated on the Curonian spit, a long, thin and curved peninsula at the eastern shore of the Baltic Sea (Fig. 1). Red admirals were collected in a large permanent trap of the "Rybachy-type"; a trap built out of net and shaped like a large funnel with an opening in the direction from which the migrants arrive, passively catching migrating birds, but also insects. In the far end of the trap is a collecting box. The dimensions of the trap are 70*35*15 meters (Lengh*Width*Height) and it is operated daily between 1 April to 1 November every year. A sample of the red admirals that were caught in the trap were euthanized using ethyl acetate and the wings were then removed for δD analyses. The wings were stored in glassine envelopes and stored in a dark and dry place up until analysis. The head and the body were stored in 99.9% ethanol for later analysis of sex, breeding status and lipid content. We captured red admirals over two complete spring and autumn seasons in 2004 and 2005. On days with large numbers of red admirals we sampled fewer individuals (5–10). We recorded exact numbers captured each day to be able to estimate yearly differences in intensity and median date of migration.

We analysed hydrogen isotope ratios in red admirals captured at coastal and inland sites by comparing our samples collected at Rybachy with two nearby sites in Northeast Europe. These samples were represented by 15 individuals captured in Estonia (Karilatsi) between 11 and 28 August in 2004, and 20 individuals collected in 2005 between 14 September and 2 October at two locations in southern Poland (Czestochowa and Ktomnice) (Fig. 1).

Analysis of hydrogen isotopes

All red admiral wing samples were rinsed in a chloroform-methanol solution (2:1) to remove surface oils that could affect the isotope assays, and air dried overnight. Stable hydrogen isotope analyses were conducted at Environment Canada in Saskatoon, using the comparative equilibration technique so that the values reported here are equivalent to nonexchangeable hydrogen (Wassenaar & Hobson 2003). Stable-hydrogen isotope measurements of wings and the calibrated keratin standards were done on H_2 derived from high-temperature (1300 °C) flash pyrolysis of wings and continuous-flow isotope-ratio mass spectrometry. 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.

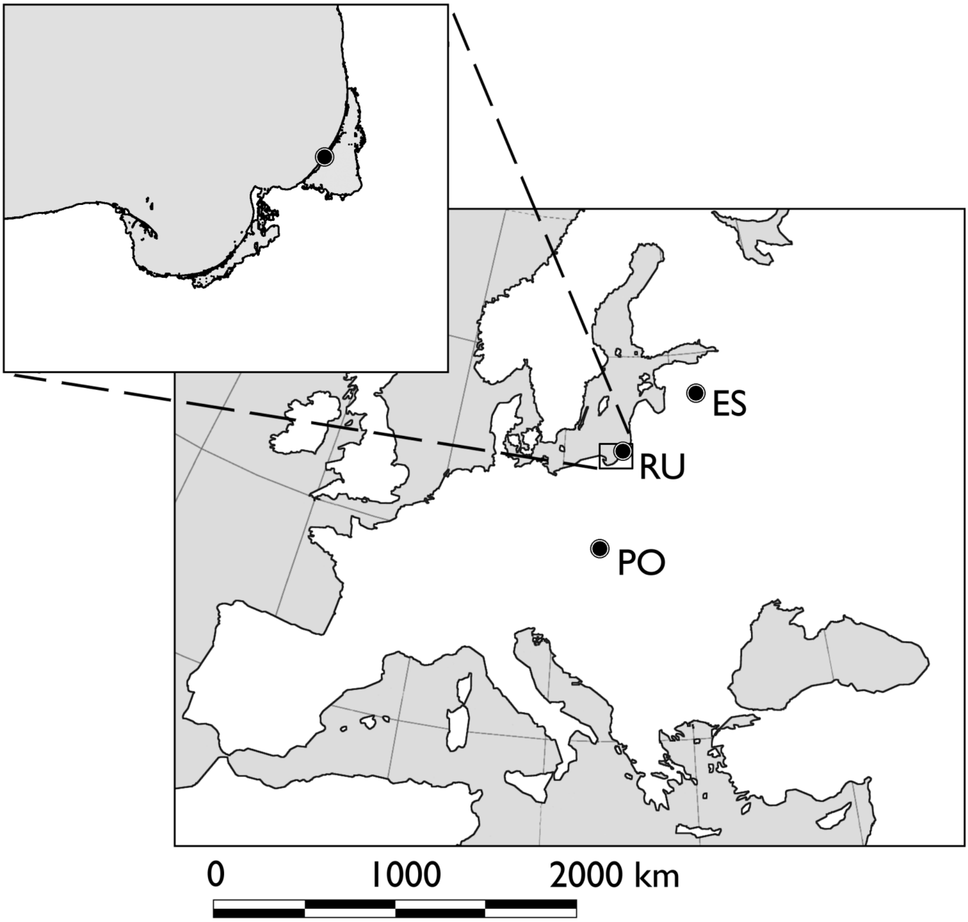

Figure 1. The map shows the location of the sites were red admirals were captured in 2004 and 2005. Most red admirals in this study came from Rybacy in Kaliningrad – Russia (RU), located at the eastern shore of the Baltic Sea. Some additional material was also collected in Karilatsi – Estonia (ES) and in Czestochowa and Ktomnice – Poland (PO). The capture sites in Estonia and Poland were inland locations, and were compared with the coastal site at Rybachy.

Based on within-run measurements of intercomparison material and consideration of within sample variance (Wassenaar & Hobson 2006), we estimate our laboratory error to be ±2 ‰.

Precipitation data

We acquired interpolated monthly δD values for the rainwater at Rybachy by using the Online Isotopes in Precipitation Calculator (OIPC) (http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/oipc.html). Previous studies have shown that the δD values encountered in butterfly wings are related to the δD values in precipitation at their natal site (for details see, Brattström *et al.* in prep b). We used the OIPC data on precipitation δD values to estimate the expected δD values for butterflies of local origin in the Rybachy region over the whole sample period. This approach allowed us to determine whether captured red admirals were of local origin or if they had migrated to the Rybachy area from either north or south.

Sex, breeding status and lipid class determination

To be able to determine the sex of individual butterflies as well as to evaluate the amount of lipid reserves, we dissected the abdomen of the red admirals captured at Rybachy. Sex was determined by visually inspecting the genitalia under a microscope. To estimate lipid content we cut the wall of the abdomen open along one side using a fine pair of scissors and visually scored the amount of lipids in the abdominal cavity. When the red admirals use up their lipid resources a clear cavity began to form in the stored abdominal fat. We estimated lipid reserves using a scale with six different classes from 0 (no fat) to 5 (abdomen cavity completely filled with fat). The criterion for each class is given in Table 1. In females, we also checked for the presence or absence of developing eggs to get an indication of breeding status.

Statistical analysis

Since there was a clear drop in the number of captured red admirals around mid July we analysed early (up until 31 July, spring) and late (from 1 August, autumn) samples separately. We did not remove δD outliers in any analyses, since the large natural variation in dD makes it impossible to be sure outliers (as long as they are within a possible biological range) are not recruits from other populations. SPSS 15.01 assigned only 7 δD values as "outliers" when looking at the data divided according to study year and season. Analyses were not affected by the presence or absence of the outliers. SPSS 15.01 was used for all statistical calculations.

We used a t-test to analyse differences in δD between 2004 and 2005 in the autumn group. Differences in yearly mean date of passage during both seasons were analysed using a t-test. Differences in sex ratio between the two years as well as deviations from an equal ratio of males and females both in the whole data set and separately for spring and autumn were analysed using χ^2-tests. Differences in mean date of capture for the different sexes in both of the seasons were compared using t-tests. To analyse if there was any difference in sex ratio between days with low and high migratory intensity, we divided the autumn material into two groups (the spring data was not analysed because of the low numbers of individuals available). In 2004 we designated days with more than ten individuals captured as high intensity days and in 2005 we lowered the limit to more than five since total numbers observed was smaller that year. Differences between the two groups were analysed using a χ^2-test.

To analyse differences in lipid reserves we performed an ANOVA with lipid class as dependent variable and used sex, study year and

Table 1. Criteria for division of samples from red admirals into different classes based on the abdominal lipid content. Proportion and number of individuals assigned to each class in the two years of the study is also presented.

Lipid Class	Criteria for inclusion	% of total (N) 2004	% of total (N) 2005
0	Abdominal compartment completely emptied of lipids	9% (11)	9% (8)
1	Large cavity in the abdominal lipid reserve	32% (38)	10% (9)
2	Small cavity in the abdominal lipid reserve	24% (28)	10% (9)
3	No visible cavity in lipid reserve, abdomen of normal size	31% (37)	25% (23)
4	Abdomen larger than normal because of substantial amounts of lipid, but not completely filled	1% (1)	17% (15)
5	Abdomen much larger than usual and completely filled with lipid	3% (3)	29% (26)

sample season as fixed factors. We included all possible two-way interactions and removed them in a backward fashion. Since we had a significant interaction between season and sex we performed separate ANOVA tests for males and females.

Results

Timing and intensity of migration

Spring migration

The total number of red admirals captured during spring migration was 82 in 2004 and 15 in 2005. The median date of passage for the spring migration was 4 July in 2004 and 6 July in 2005 (Fig. 2a). Since we have very few recorded individuals in 2005 this value may be somewhat unreliable, although it was very close to the passage date noted the previous year. The median date of passage for the autumn migration was 2 October in 2004 (N = 341) (Fig. 2b) and 25 September in 2005 (N = 125) (Fig. 2c). There was no significant difference between years for the mean passage date in spring (t-test, t = 1.21, $N_{2004} = 82$, $N_{2005} = 15$, p = 0.246) passage. The mean date of passage in the autumn was however significantly different (t-test, t = –2.93, $N_{2004} = 341$, $N_{2005} = 125$, p = 0.004).

Measured and OIPC estimated δD values

Rybachy

The measured δD values in the wings of the sampled red admirals are presented in Figure 3. There were differences between the spring and autumn individuals in both years. The expected δD values for locally developed butterflies (for details see Brattström *et al.* in prep b) show that all individuals from the autumn samples are of either a local or more north-eastern natal origin. The spring individuals in 2004, especially those sampled later in that time period corresponds both with locally and more southern origins. In 2005, all spring individuals had δD values corresponding to more southerly origins. The mean δD values measured during autumn was –129.7‰ ± 1.39‰ (SE) in 2004 and –136.3‰ ± 1.82‰ (SE) in 2005 and this difference was significant (t-test, t = 2.86, $N_{2004} = 86$, $N_{2005} = 79$, p = 0.005)

Estonia and Poland

The δD values in samples from our two inland reference locations are shown in Figure 4, plotted in relation to the samples from Rybachy captured at a similar time period. The range of δD values is greater in the Rybachy samples (2004: min = –172.1‰, max = –102.4‰, range = 69.7‰, and 2005: min = –180.8‰, max = –107.3‰, range = 73.5‰) than for the inland locations (Estonia, 2004: min = –140.8‰, max = 99.4‰, range = 41.4‰ and Poland, 2005: min = –117.3‰, max = –74.4‰, range= 42.9‰.

Differences in sex ratios

There was no significant difference in sex ratio between the two years ($\chi^2_{1, 208} = 0.21$, p = 0.89) and the pooled data from both years showed a sex ratio that was significantly biased towards more males being captured in the trap ($\chi^2_{1, 208} = 4.33$, p = 0.038). When analysing the spring and autumn material separately, it was clear that this difference was only present in the autumn (spring: $\chi^2_{1, 43} = 0.23$, p = 0.88; autumn: $\chi^2_{1, 165} = 5.10$, p = 0.024). There was no difference in mean date of capture between sexes during spring (t-test, t = –0.16, $N_{Males} = 22$, $N_{Females} = 21$, p = 0.874) or autumn (t-test, t = 0.46, $N_{Males} = 97$, $N_{Females} = 68$, p = 0.645), nor was there any difference in sex ratio depending on the intensity of the migration ($\chi^2_{1, 165} = 0.78$, p = 0.38).

Breeding status of females

All of the females sampled during the spring season had developing eggs in the abdomen ($N_{2004} = 16$ (Date: 24 June – 12 July), $N_{2005} = 5$ (16 June – 8 July). During the late summer/autumn season we found no visible eggs in any of the sampled females ($N_{2004} = 35$ (Date: 3 August – 4 October), $N_{2005} = 33$ (19 August – 14 October).

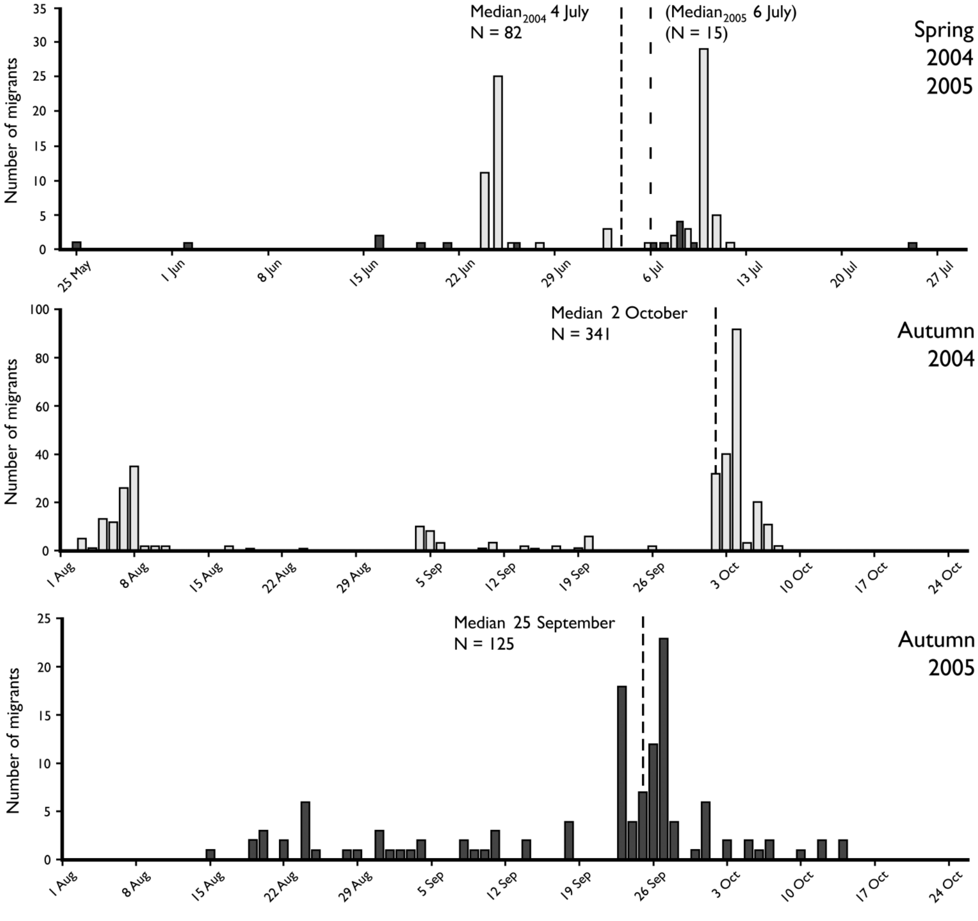

Figure 2. The number of red admirals caught each day in traps over two complete seasons (1 April to 1 November) 2004 and 2005. The recorded captures from a) both spring seasons, b) autumn 2004, and c) autumn 2005. Note the different scale on the y-axis. The highest number of red admirals was captured in autumn compared to in spring and 2005 had a much higher number caught than 2004.

Differences in lipid content

The ANOVA analysis on visually estimated lipid classes showed a significant effect of study year for males (Table 2a), and for females we had significant effects of both study year and sample season (Table 2b). Both sexes were assigned to higher lipid classes in 2005, but for females there was less effect when comparing only spring samples (Fig. 5).

Discussion

It is clear from our study that the red admirals that pass the Curonian spit on migration do so during two distinct time periods, with a clear drop in migratory activity occurring in mid summer. The median capture date was very similar over the two studied years for both spring and autumn. Even though there was a significant difference between the times of the

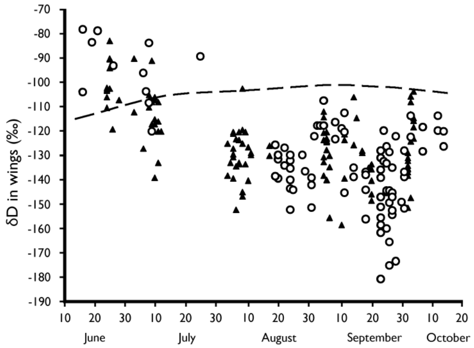

Figure 3. Measured δD values in the wings of red admirals sampled from the trap captures at Rybachy in 2004 (▲) and 2005 (○). The broken line shows the expected value for locally hatched individuals from the Rybachy region. For details on calculation of this estimate see text and Brattström *et al.* (in prep b).

autumn passage the absolute difference was not more than a week, suggesting a fairly regular migration pattern between the two years.

The ratio between stable hydrogen isotopes has been shown to correlate with geographic location, decreasing in value from south-west to north-east in Europe (Hobson 2003, Bowen *et al.* 2005, Rubenstein & Hobson 2004). This isotopic gradient can be used to interpolate natal origin over relatively limited geographical regions for a proxy species, the peacock (*Inachis io*), in southern Scandinavia (Brattström *et al.* submitted b). This indicates that δD can be used to define origin of red admirals from Europe over a larger geospatial range. We found distinct differences in admiral δD values between the spring and autumn seasons at Rybachy, and therefore presumably also their natal areas. The range of δD was also wider at Rybachy compared to Estonia and Poland, suggesting a larger variation of origin at the coastal site compared to the inland sites. In spring, every female that was captured had developing eggs in the abdomen, but during autumn not a single egg was found. We also found an unexpectedly skewed sex ratio in favour of the males, but only during the autumn migration. Difference in lipid content was also present, but this difference was more pronounced when comparing years than seasons. In 2005, the red admirals carried considerably larger lipid reserves than the preceding year.

Timing of migration

In Denmark, the median date of observed migration of red admirals over six years (1995–2000) was 26 September the earliest date with observed migration was 13 August, and the latest 30 October (Hansen 2001). Thus, the difference between earliest and latest mean date of migration during Hansen's (2001) study was more than six weeks. At Falsterbo in southern Sweden the median passage of red admirals was studied by visual counts of migrating butterflies between 2004 and 2006 (Brattström *et al.* sub-

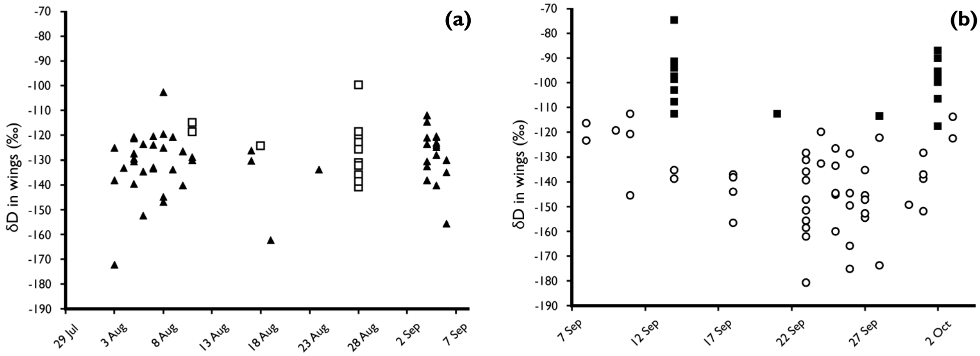

Figure 4. Measured δD values in the wings of red admirals sampled at a) Estonia (□) in 2004 and b) Poland (■) in 2005. Values are plotted together with the Rybachy values from the same time periods.

Table 2. Results of the ANOVA test of effects from study year and sample season on the assigned lipid class for males and female red admirals sampled in spring and late summer/autumn in 2004 and 2005 at Rybachy, Kaliningrad. The presented results are the final model that remains after the non significant interaction between the two factors have been removed.

a) Males

Variable	**S.S.**	**df**	**F**	**p**
Sample season	3.4	1	1.64	0.202
Study Year	38.9	1	18.76	<0.001
Error	240.6	116		
Total	956.0	119		

b) Females

Variable	**S.S.**	**df**	**F**	**p**
Sample season	12.1	1	8.41	0.005
Study Year	48.3	1	33.6	<0.001
Error	123.5	86		
Total	760.0	89		

mitted a), and showed median dates (2004: 6 September, 2005: 31 August and 2006: 24 September) somewhat earlier than in Denmark. The median dates of our captured individuals at Rybachy were similar to the other reported dates from northern Europe (see above), but since we only have data from two seasons we can not say if variation at this location is as large as have been observed in Denmark (Hansen 2001) and at a nearby site in southern Sweden (Brattström *et al.* submitted a). The difference between the two years in this study at Rybachy was significant, but only encompasses a week. It is clear from the different types of studies of red admiral migration that variation in many parameters of butterfly migration (for example timing and origin of migrants) can be great, and likely heavily dependent on yearly differences in weather, winds and reproductive success (e.g. Hansen 2001, Mikkola 2001, Brattström et al submitted a, Brattström *et al.* in prep b). Since data used to calculate these median dates were obtained using different methods; reported sightings (Hansen 2001), standardized counts (Brattström *et al.* submitted a) and passive trap captures (this study), they are probably not directly comparable to each other, but they all were within the same approximation making it safe to assume that the main migration from northern Europe occurs around September most years. Evaluations of three different methods for counting monarch butterflies at the same location stressed the importance of using

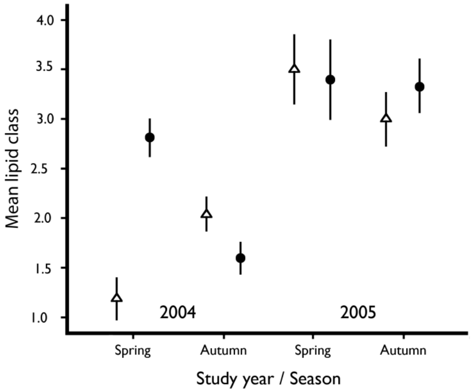

Figure 5. Mean lipid class assigned to male (Δ) and female (●) red admirals captured at Rybachy in two different seasons 2004 and 2005. The difference between the years was significant and there was also a significant effect from season in the females. The errorbars represent ± 1 Standard Error.

standardized counts to make comparative studies easier (Davis & Garland 2002).

When looking at the pattern of captured numbers over the season, it was evident that red admirals do not pass Rybachy continuously with an even intensity, or within one single migration period. Instead there can be many days without a single captured individual, followed by peaks in the capture data when half of the sampled individuals from one year are captured over the course of a single week (Fig. 2). This is especially pronounced in autumn. We found a similar temporal pattern of migration at Falsterbo in southern Sweden recorded by visual observations of migrating red admirals (Brattström *et al.* submitted a). What is the reason for these dramatic differences in migratory intensity? Studies of the effect of weather on migration of red admirals at coastal sites showed that wind direction is important to initiate large-scale migration (Mikkola 2003, Brattström *et al.* submitted a). It should be noted that these studies were performed at locations where the red admirals are about to cross open water and therefore possibly more dependant on favourable winds than usual. Such wind sensitive departures have also been shown for migrating songbirds at coastal sites (Åkesson & Hedenström 2000, see also Åkesson *et al.* 2002, Zehnder *et al.* 2001).

At Rybachy, the migrants follow a coast that does not deviate much from the preferred main migratory direction and hence they may not be as reliant on choosing optimal days for migration. On the other hand Rybachy, being a narrow peninsula with few nectar sources is not a good location for breeding or fuelling before migration flight. Thus, red admirals are not expected to be encountered here other than during active migration periods, and especially when winds from the east have concentrated large numbers at the coastline (see below). The high numbers could also be an effect of large-scale hatching events in the nearby region. When a period of bad weather prevents pupas from hatching, a few warm days may start mass hatchings of butterflies that have been forced to wait for good weather. Both of these explanations are weather dependant but in different ways. One is mostly affected by wind speed and direction during migration, while the other is more affected by temperatures at development.

Skewed sex ratios

In autumn of both years, we found more males than females in our samples from the Rybachy migration site. In many butterflies the males hatch before the females (Eliasson *et al.* 2005) but we found no difference in mean date of capture for the two sexes of red admirals during autumn migration at Rybachy. Theoretical modelling of protandry suggest that butterfly males in species that hibernate before reproduction should not hatch before females (Wiklund & Fagerström 1977) and this development pattern would also most likely be the case with migratory species. In butterflies, more males than females are often encountered in the field, but the actual sex ratio as determined by observing hatching individuals is 1:1 (Adamski 2004). A reason for this is that the males move around more than females and are therefore observed more often (Adamski 2004). Brattström (2006) found skewed sex ratios when sampling red admirals in Italy during late autumn/early spring where the red admirals are breeding. However, this skewed male sex ratio was interpreted as a sexual difference in hill-topping behaviour (Brown & Alcock 1990). When skewed sex ratios that are not just artefacts of sampling techniques (Adamski 2004, Brattström 2006) are encountered in butterflies and moths these are almost exclusively female-biased, and are caused by large scale infection of male-killing *Wolbachia* parasites (e.g. Werren *et al.* 1995, Kageyama & Traut 2004). There exists in at least one butterfly species a system were a male-biased sex ratio is the norm (Underwood & Shapiro 1999), but it seems unlikely in our study.

Differences between sexes as well as age groups in migration distance and wintering areas is common for migratory birds (e.g. Ketterson & Nolan 1976, 1983, Gauthreaux 1982, Münster 1996, Alerstam *et al.* 2003). It is however questionable if sexual difference in wintering areas is a reasonable explanation for the sex ratio we found among the red admirals at Rybachy. We found developing eggs in all females captured in spring, but not in any females during autumn. This shows that red admirals at Rybachy that were supposedly hatched in the north eastern parts of Europe will most likely not breed in this region but migrate south before reproducing. If this is true, there is no reason to expect behavioural differences between males and females at Rybachy during autumn migration. Both sexes most likely have the same goal and flight behaviour since they are still in a pre-reproductive state. Migrating insects in general show less difference between sexes during the migratory flight period than during reproduction. After the flight phase, changes in juvenile hormone levels reshape the insects from migratory to breeding states were sexual differentiation are more marked (for review, see Dingle & Winchell 1997). Since we know that red admirals that migrate in the autumn to the Mediterranean area reproduce there during winter, males and females must share wintering areas in this region. Mating before migration is unlikely, knowing that migration and subsequent breeding are two distinctly different phases in the life cycle of migratory insects (Dingle 1972). We have, however, clues that the eastern red admiral population is genetically distinct compared to the western region because of differences in wintering behaviour (Brattström *et al.* in prep a). Could it be that the sexes in a sub-population of winter-hibernating red admirals use different winter regions and that this is reflected in our Rybachy data? We can be fairly certain that males captured at Rybachy do not visit the region for hibernation, but must instead be enroute to a suitable winter habitat. Experimental studies of hibernating red admirals showed that mortality rates increased dramatically if the butterflies were kept in a moist environment (Lembke 1971). Being a coastal location, Rybachy has high humidity throughout the winter, making it a bad choice for hibernation. The skewed sex ratio found is interesting but puzzling; it deserves more attention. There is one report from Denmark reporting on sex ratios in the painted lady (*Vanessa cardui*), a species closely related to the red admiral, but in this study the sex ratio was female-biased (Münster-Swendsen 1980). The observation period for this study, however, was just two weeks. It would be interesting to know if this is a common phenomenon in migrating insects occurring only in the northern range of their distribution, or if the red admiral data from Rybachy represent something rare and exclusive.

Differences in fat content between years

Even though it has been known for a long time that lipid content can vary extensively in migrating monarch butterflies (*Danaus plexippus*) (Beall 1948), and that red admirals can store large amount of lipids (Hansen 2000), the difference in lipid content between years found in this study was surprising. We also observed a higher number of individuals in 2004, indicating that that was a year with more successful breeding in the northern part of the range. The high number can not be due to a second influx from the south later in the season, since most individuals found during autumn showed δD values of local or more northern origin. One possibility is that the large number of red admirals produced during 2004 meant that food sources were limited thereby, and individuals were therefore leaner than in 2005? Perhaps the foraging situation prior to capture at Rybachy was more favourable in 2005 compared to 2004, enabling red admirals to put on larger fuel stores. A study from Denmark suggested that red admirals in northern Europe can even die from starvation in large numbers if weather conditions during autumn limit the available food and time for feeding (Hansen 2000). The red admirals in 2004 could also have been under time pressure and forced to leave the northern regions with lower fat reserves (for discussion of time-minimized migration and departure fat loads, see Alerstam & Lindström 1990), but this remains to be shown. Red admirals captured in autumn 2005 had lower mean δD values than in 2004, indicating a more northern origin than 2004. Thus, it could be that in 2004, we primarily sampled locally hatched and not migratory individuals, but in 2005 we captured a majority of migrants with lipid stored ready for long distance flight.

If the fat stores found in the migrating red admirals is important for reproduction, we would expect them to be at their largest prior to reproduction in spring, and in our samples from 2004 we see that females, even tough they were leaner than in 2005, have larger lipid reserves than the males. A study from Italy comparing fat content between autumn, before reproduction and spring, before migration found no difference between these two groups (Brattström 2006), but that study included only males. We can not resolve this question with our available data, but the presence of differences in lipid content between years, and also to some extent between sexes, is something that has not previously been reported in European migrating insects butterflies.

Effects of coastal location

We found a wider range of δD values in the Rybachy samples than samples from the inland locations of Estonia and Poland. This qualitatively indicates that red admirals at coastal sites stem from a more diverse catchment area, while the majority of individuals sampled at inland locations are from the local surrounding areas. Hansen (2001) reported higher numbers of red admirals from coastal locations in Denmark compared to inland locations during autumn migration. Studies of flight behaviour of red admirals (Reichholf 1978, Benvenuti *et al.* 1994) as well as monarchs (Schmidt-Koening 1985) in coastal regions show that butterflies tend to follow coastlines, and avoid flying over open water if possible. This behaviour will lead to a concentration of individuals from diverse locations ending up at coastlines that roughly follow the migratory direction, just as is shown in our samples from Rybachy.

Conclusions

There are few locations in Europe where it is possible to sample migrating butterflies over complete seasons in relatively high numbers. Rybachy in Kaliningrad represents such a location, where our data clearly demonstrate that new insights on timing of migration, source areas, and fat content of migrating individuals as well as sex ratios can be gained for red admirals even during a two year study period. Unfortunately, since no similar data sets recording migrating insects systematically in Europe are available we cannot make comparisons with species collected at other locations. Coastal sites with channelling topography seem to attract migrating butterflies both during spring and autumn enabling sufficient sampling with passive traps. Hopefully this study can inspire more bird observatories over Europe to apart from birds also record the migration of insects and butterflies.

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VI

Flight direction of red admirals (*Vanessa atalanta*) during autumn migration in southern Sweden – results from orientation cages and release experiments

Flight direction of red admirals (*Vanessa atalanta*) during autumn migration in southern Sweden – results from orientation cages and release experiments

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Each year large numbers of red admirals (*Vanessa atalanta*) leave the northern parts of Europe to reach areas further south that are suitable for spending winter. Field observations from all over Europe have reported consistent flight directions towards the south during autumn. We studied flight directions in this species using large flight cages which recorded movement by video imaging. This allowed us to study the normal flight direction in a controlled situation, without the effect of wind and possibly also less influenced by escape reactions compared to release experiments. It also made it possible to manipulate the internal clock of an experimental group to study if red admirals use a time-compensated sun compass. Such an orientation mechanism has been found in several species of migrating butterflies by using clock-shift procedures and different techniques for monitoring the following flight directions. Our results from the red admirals are as variable as the species' migration system; we find evidence of migration towards the north in the autumn in the control group of one year but towards the south in the other two years. There was a significant effect of the clock-shift treatment but only in the year with migration towards the north. It is evident that, to fully interpret results in orientation experiments of red admirals, one needs access to additional information about the tested individuals' natal regions and genetic structure. The flight cage method however, seems to work well to record preferred orientation and was compared with results from a release experiment performed during one year using the same individuals that were first tested in the cages.

Introduction

Early field observations of migrating butterflies (Baker 1968a, 1968b, 1969) and recordings of orientation in flight cages (Kanz 1977) revealed that butterflies mainly rely on the sun for orientation. Later Perez *et al.* (1997) reported evidence of a time-compensation mechanism for the sun compass (e.g. Schmidt-Koening 1990) by recording vanishing bearings in release experiments of clock-shifted monarchs (*Danaus plexippus*). From further release experiments it was later shown that a sun orientation mechanism based on time-compensation was also used by two other migratory butterfly species *Aphrissa statira* and *Phoebis argante* (Oliveira *et al.* 1998). The most recent account of the use of a time-compensated sun compass in monarchs was based on experiments with butterflies tethered to a flight recorder (Mouritsen & Frost 2002, Froy *et al.* 2003), in which deviations in orientation relative to the controls was demonstrated after both advanced and delayed clock-shifts. Despite a relatively high number of studies of sun orientation none of the studies that found evidence for time-compensation were able to determine if the butterflies' sun compass is matched to the local ephemeris function or if they rely upon an averaging estimate of the solar movements (for discussion see Srygley & Oliveira 2001, see also Brattström & Åkesson 2005).

Up until today, there has been no experimental study on sun-compass orientation in any migratory day-active butterfly in Europe. In a study of large whites (*Pieris brassicae*), the flight directions recorded in cages were found to be constant throughout the day (Spieth *et al.* 1998). However, since the butterflies in these experiments were not manipulated, a time-compensation mechanism could not be excluded since other orientation mechanisms, like the geomagnetic compass (e.g. Wiltschko & Wiltschko 1995), could also have been used to keep a constant geographic course.

We decided to study the preferred migratory orientation and the sun compass orientation mechanism in the red admiral (*Vanessa atalanta*) migrating at Ottenby in southeastern Sweden in autumn. The red admiral is a common butterfly in Europe and it occurs in southern Scandinavia each year, but in somewhat variable numbers. The species shows a fairly regular annual pattern of migration throughout Europe (Henriksen & Kreutzer 1982, Higgins & Hargreaves 1983). We studied the flight directions and sun compass orientation of red admirals in southern Sweden during three autumn migration periods, and while doing so evaluated the use of circular flight cages for studies of butterfly orientation. During the first year we also compared the orientation recorded in our circular flight cages with the departure directions recorded in release experiments using the same individual butterflies. To study if red admirals use a time-compensated sun compass, we clock-shifted one group of butterflies and compared their orientation to a group of butterflies kept in the local light/dark regime (controls).

Materials and Methods

Experimental setup and procedure

We performed orientation cage experiments with red admirals in 1999, 2002 and 2005. The experiments were conducted in late summer at Ottenby Bird Observatory, situated at the southern point of the island of Öland in south-east Sweden (56°12′N, 16°24′E). Red admirals were collected in the observatory garden and kept indoors in cages (35×35×40 cm) for a minimum of three days in separate rooms to allow their internal clock to adapt to the new light/dark regime. All butterflies that were caught during each day were put indoors under artificial light conditions well before sunset on the same day. The light was supplied with fluorescent lamps, with a spectrum similar to daylight (Philips TLD 965, CRI>90, Colour temp. 6500K). A study of the role of the circadian clock in monarch migration behaviour showed that artificial light (lacking UV) were sufficient for effective clock-shifting (Froy *et al.* 2003). In our study the control group experienced a light/dark cycle similar to the local natural conditions while the clock-shifted group had their cycle delayed by four hours.

To record the flight direction of individual red admirals we used circular flight cages. In 1999 and 2002 we used cages constructed from fine meshed plastic net, fixed by a thin ring of stainless steel and a wooden frame. The frame also supported a small video camera placed over the net roof of the cage, continuously recording the flight path of individual butterflies from above. Six identical cages were used, each with a diameter of 200 cm and a height of 135 cm. The cages were placed in an open area outside the observatory buildings. A cloth screen was arranged outside the cages to act as wind protection and also covering landmarks that would otherwise have been visible from inside the cages. We regularly measured wind speed with a handheld anemometer inside the arena to make sure the screen worked efficiently and no wind entered the cages. In 2005 we used smaller cages with a diameter of 100 cm and a height of 70 cm. Both cages and video cameras were mounted on an aluminium frame. This type of setup using cages for free-flying butterflies have been used previously to study flight directions in large whites (Spieth & Kaschuba-Holtgrave 1996, Spieth *et al.* 1998). To avoid interaction between individuals we kept only one red admiral in each cage during each filming session. Red admirals from both treatment groups were filmed continuously during one hour. In 1999 we filmed butterflies from the control group between 0830h – 0930h, 1100h – 1200h and 1130h – 1230h and clock-shifted butterflies between 1100h – 1200 and 1330h – 1430h. In 2002 butterflies from both groups were filmed between 1100h – 1200h, 1330h – 1430h and 1600h – 1700h. In 2005 butterflies from both groups were filmed between 1300h – 1400h and 1430h – 1530h. All time notations are given as Swedish normal time (Daylight saving 1 hour). Total number of filmed individuals in each time period are given in Table 1.

Table 1. Time periods, number of tested individuals, and mean directions for pooled groups of red admirals tested in flight cages during autumn migration in southern Sweden over three years. The clock-shifted group experienced a four hour delay of their internal clock. Most of the original groups from each time period had a sample size too small to produce significant results, and thus data were pooled. To take effect from the movement of the sun into account, we present directions with both geographic north (Geo) and the suns azimuthal position (Sun) set to 0°. Mean directions were calculated using the Rayleigh test (Batschelet 1981) and differences were tested with Mardia-Watson-Wheeler test (Batschelet 1981).

Year	Date	Film period	Treatment	N	α Geo/Sun	r / W Geo/Sun	P Geo/Sun
1999	25 Aug – 5 Sep	08:30–09:30	Control	9			
		11:00–12:00	Control	6			
			Clock-shift	4			
		11:30–12:30	Control	2			
		13:30–14:30	Clock-shift	11			
		Total	Control	17	339°/205°	0.43/0.45	0.039/0.032
			Clock-shift	15	71°/238°	0.68/0.77	<0.001/<0.001
			Difference		+92°/+33°	8.96/8.38	0.011/0.015
2002	5 Aug – 22 Aug	11:00–12:00	Control	5			
			Clock-shift	7			
		13:30–14:30	Control	9			
			Clock-shift	9			
		16:00–17:00	Control	8			
			Clock-shift	9			
		Total	Control	22	190°/335°	0.39/0.48	0.032/0.005
			Clock-shift	25	203°/359°	0.42/0.45	0.011/0.006
			Difference		+13°/+23°	1.01/2.28	0.60/0.32
2005	23 Aug – 9 Sep	13:00–14:00	Control	16			
			Clock-shift	10			
		14:30–15:30	Control	12			
			Clock-shift	17			
		Total	Control	28	208°/3°	0.35/0.37	0.031/0.020
			Clock-shift	27	202°/353°	0.37/0.38	0.022/0.017
			Difference		–6°/–10°	0.42/0.31	0.81/0.86

Release experiments

In 1999 we released all the butterflies that had been tested in orientation cages, soon after they had been filmed. Releases were conducted on a large open grass meadow close to the observatory garden with at least 100 meters of grazed grass area before nearest tree or other large object. Wind direction (recorded as the direction toward which the wind was blowing) as well as wind speed were measured using a handheld anemometer to the nearest 0.1 m/s and the butterflies were then released by hand and followed visually using a pair of binoculars (10×40) until they disappeared out of sight. The vanishing bearing of each individual was estimated to the nearest 10 degrees using a handheld compass. Only individuals that flew a minimum of 50 meters before settling, or that continued flying until they disappeared out of sight were included in further analyses.

Data analysis

Video sequences were analysed by dividing the cages into sectors, in which registrations were taken only when the butterfly entered the area consisting of the outer 40% of the cage radius in each sector as seen from above. Recordings were made within the outer sectors, in such a way that notes were made each time the red admirals entered a new sector. We used the total numbers of recordings in all sectors as a measurement of activity. For the groups filmed during 1999 and 2005, we used 24 sectors covering 15° each. Since those analyses were very time consuming, in 2002 we instead used 8 sectors (45° each) for 43% of the individuals (20 films). We analysed several individuals with both methods to make sure that they yielded similar mean directions before switching method, and found no difference in mean orientation for those individuals compared.

We removed individuals with very low flight activity resulting in only a few registrations and individuals with very high numbers of registrations combined with an irregular flight pattern (see below) from the flight direction analysis. Individuals that just moved a few times were not showing migratory activity, and were considered inactive. The individuals that by contrast moved very much, often showed erratic flights with small "jumps". They rarely showed prolonged flight attempts in a general direction and their flights were clearly of another type than the persistent migratory flight patterns other butterflies showed in the cages. We set the limits for inclusion in the analysis to at least 100 registrations, but no more than 500. Since we used different methods and cage sizes, we first had to normalize the N values to be able to compare them. The butterflies analysed with 8 sectors had an average of 58% less recordings so we multiplied their number of recordings with 2.4 to be able to compare activity between different cage designs. Since we used smaller cages in 2005, we divided the number of recordings by 2. We also removed individuals with very low concentration ($r<0.3$, $n=4$) of their individual mean flight direction.

Statistical analysis

The mean flight direction of each individual, as well as the mean orientation for each group of red admirals was calculated using circular statistics (Batschelet 1981). We calculated both a mean orientation (α) relative to geographic north, with zero degrees being north, and a mean orientation in relation to the position of the sun in the middle of the 60 minutes test period (set as zero degrees). The vector length (r) describes the scatter of the circular distribution (ranges between 0 and 1, the scatter being inversely related to the vector length). The Rayleigh test was used to test if a circular distribution differed significantly from random at both the individual and group level (Batschelet 1981). To compare the mean orientation between groups, we used Mardia-Watson-Wheelers test (Batschelet 1981). Since the number of individuals from each time period in most cases was too small to produce mean directions that significantly differed from random, we pooled all data for each treatment and year into one group. To analyse the relationship between directions recorded in cages and vanishing bearing during later release we used circular correlation (Batchelet 1981). The same method was also used for analysing the relationship between vanishing bearings and prevailing winds during the release experiments. Only individuals that showed a significant mean orientation in the cages (see criteria given above) and a clear vanishing bearing during the release were included in this analysis. All analysis of circular data were performed using the computer software Oriana 2.0 for Windows (Kovach 2003).

Results

Orientation in relation to geographic North

All red admirals meeting the criteria for inclusion in the analysis (normalized N = 101 – 500 and $r > 0.3$) showed significant individual mean orientations in the cages (Rayleigh test: r ranging between 0.38 and 0.99 (mean $r = 0.70$), $p < 0.001$). A summary of the results is given in Table 1.

The mean angle of orientation for the control group in 1999 was 339.3° ± 42.3° (mean ± 95% CI; N = 17; Rayleigh test: $r = 0.43$, $p = 0.039$; Fig. 1a) and for the clock-shifted group it was 70.8° ± 25.9° (mean ± 95% CI; N = 15; Rayleigh test: $r = 0.68$, $p < 0.001$, Fig. 1a). The mean angular difference between the groups was 91.5° and the mean geographical orientation of the two groups was significantly different from each other (Mardia-Watson-Wheeler test: $W = 8.96$, $N_1 = 15$, $N_2 = 17$, $p = 0.011$).

The mean angle of orientation for the control group in 2002 was 189.8° ± 41.3° (mean ± 95% CI; N = 22; Rayleigh test: $r = 0.39$, $p = 0.032$; Fig. 1c) and for the clock-shifted group it was 202.9° ± 36.1° (mean ± 95% CI; N = 25; Rayleigh test: $r = 0.42$, $p < 0.011$, Fig. 1c). The mean angular difference between the groups was 13.1° and the mean geographical orientation of the two groups was not significantly different from each other (Mardia-Watson-Wheeler test: $W = 1.01$, $N_1 = 22$, $N_2 = 25$, $p = 0.60$).

The mean angle of orientation for the control group in 2005 was 208.0° ± 41.5° (mean ± 95% CI; N = 28; Rayleigh test: $r = 0.35$, $p = 0.031$; Fig. 1e) and for the clock-shifted group it was 202.3° ± 39.6° (mean ± 95% CI; N = 27; Rayleigh test: $r = 0.37$, $p < 0.022$, Fig. 1e). The mean angular difference between the groups was 5.7° and the mean geographical orientation of the two groups was not significantly different from each other (Mardia-Watson-Wheeler test: $W = 0.42$, $N_1 = 28$, $N_2 = 27$, $p = 0.81$).

Orientation in relation to the sun

The mean angle of orientation in 1999 relative to the sun's position for the control group was 204.7° ± 40.9° (mean ± 95% CI; N = 17; Rayleigh test: $r = 0.45$, $p = 0.032$; Fig. 1b) and for the clock-shifted group it was 238.2° ± 23.1° (mean ± 95% CI; N = 15; Rayleigh test: $r = 0.77$, $p < 0.001$, Fig. 1b). The mean angular difference between the groups was 33.5° and the mean orientation in relation to the sun was significantly different between the two groups (Mardia-Watson-Wheeler test: $W = 8.38$, $N_1 = 15$, $N_2 = 17$, $p = 0.015$).

The mean angle of orientation in 2002 relative to the sun's position for the control group was 335.2° ± 33.0° (mean ± 95% CI; N = 22; Rayleigh test: $r = 0.48$, $p = 0.005$; Fig. 1d) and for the clock-shifted group it was 358.6° ± 33.7° (mean ± 95% CI; N = 25; Rayleigh test: $r = 0.45$, $p < 0.006$, Fig. 1d). We found that the mean angular difference between the groups was 23.4° and the mean orientation in relation to the sun was not significantly different between the two groups (Mardia-Watson-Wheeler test: $W = 2.28$, $N_1 = 22$, $N_2 = 25$, $p = 0.32$).

The mean angle of orientation in 2005 relative to the sun's position for the control group was 3.3° ± 38.9° (mean ± 95% CI; N = 28; Rayleigh test: $r = 0.37$, $p = 0.020$; Fig. 1f) and for the clock-shifted group it was 353.4° ± 38.2° (mean ± 95% CI; N = 27; Rayleigh test: $r = 0.38$, $p = 0.017$, Fig. 1f). The mean angular difference between the groups was 9.9° and when we compared the mean orientation in relation to the sun there was no significant difference between the two groups (Mardia-Watson-Wheeler test: $W = 0.31$, $N_1 = 28$, $N_2 = 27$, $p = 0.86$).

Release experiments

We found a clear correlation between both the recorded orientation in the flight cages and vanishing bearing upon departure ($r = 0.54$, N = 15,

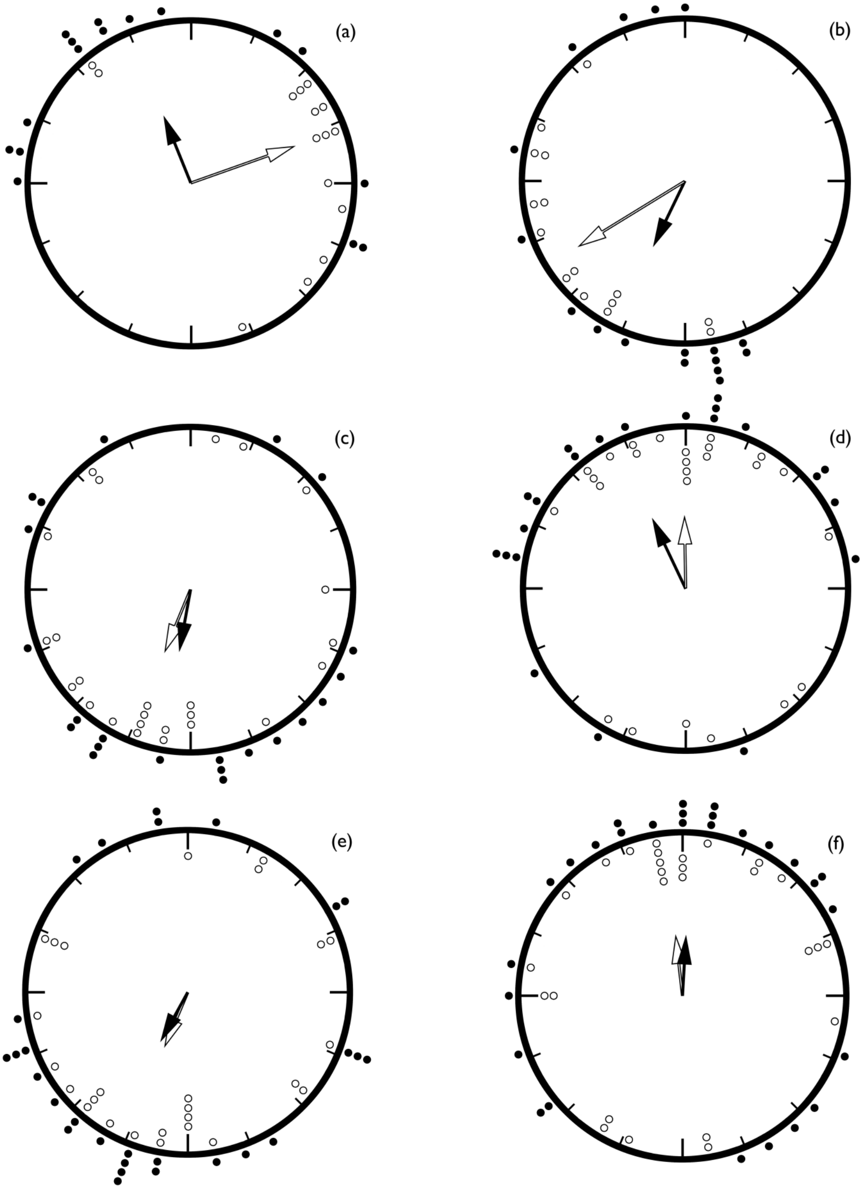

Figure 1. The mean orientation recorded in circular cages for groups of control and clock-shifted red admirals in autumn 2002 (a, b), 2002 (c, d) and 2005 (e, f). Solid (●➡) and open (○⇨) symbols represent the control and clock-shifted groups respectively. Dots represent the mean direction of single individuals while the vector shows the angle of mean orientation for each group. The length of the arrow is a measure of the scatter of the distribution ranging from 0 to 1, being inversely related to the angular scatter. Individual directions are plotted to the nearest 10°. Graphs to the left (a, c, e) are plotted with 0° set as geographical north while those to the right (b, d, f) has 0° equating the suns azimuthal position as experienced by each tested individual.

$p < 0.05$, Circular correlation, Batschelet 1981, Fig. 2a) as well as between the vanishing bearing and wind direction during release ($r = 0.72$, $N = 15$, $p < 0.05$, Circular correlation, Batschelet 1981, Fig. 2b). All releases were performed in winds of very low speed; below 2.2 m/s.

Discussion

We found that the red admirals show very persistent flight direction in our cages, both on an individual and group level. The mean orientation recorded for the control butterflies in 1999 was directed to the north and differed completely from the south to south-western orientation found in 2002 and 2005. We also found an effect of the clock-shift treatment in 1999, but not in the other two years that showed no differences in orientation between clock-shifted and control butterflies. Thus, the red admirals in 1999 seemed to rely on a time-compensated sun compass for orientation, although the compensation did not completely match the expected shift in orientation, while this could not be seen in the experiments in 2002 and 2005. Why did both the red admirals' mean orientation and the reaction to our clock-shift differ between years? We will first deal with the different preferred orientation between years.

Annual differences in migratory direction

For each of the years we found significant mean directions for the control groups, directed north in 1999 and south to southwest in 2002 and 2005. Since flight directions of red admirals recorded in the field at several locations in Europe is reported to be along a general north-south axis (e.g. Williams 1951, Benvenuti *et al.* 1996, Hansen 2001, Stefanescu 2001), our control directions seem to be a natural orientation response and not just an experimentally induced effect without biological significance. The close correspondence between the orientation recorded in cages and by release experiments in 1999, further support the interpretation that the behaviour is natural and preferred by the butterflies. Previous observations of flight direction in free-flying red admirals in northern Europe show that they mainly migrate towards the south in autumn (Imby 1993, Hansen 2001, Mikkola 2003); it is therefore surprising to find a very persistent mean orientation towards the north at Ottenby in 1999.

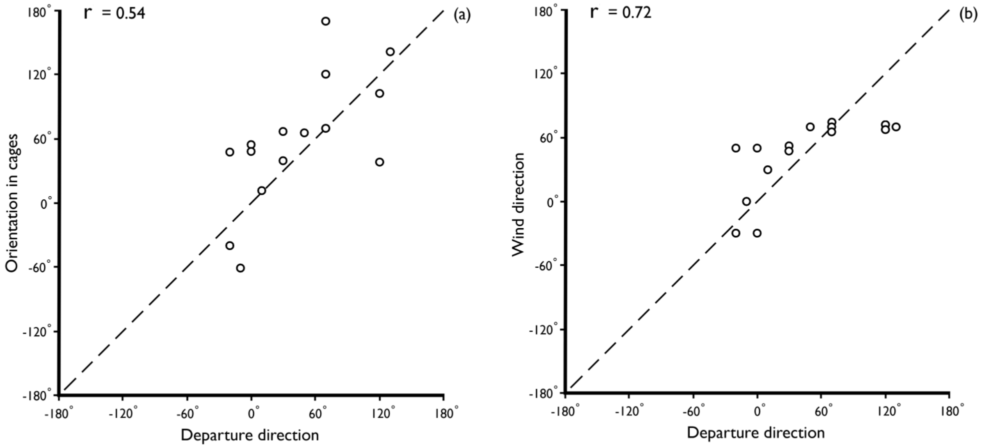

Figure 2. The relationship between a) the butterflies' direction of flight upon release and orientation recorded in the cages, and b) the direction of flight at release in relation to wind direction during release. Geographic North is equating 0° and wind directions are given as the direction the wind is blowing toward. The correlation coefficient and p-value refers to a circular correlation (Batschelet 1981).

A low proportion of reverse migrants has also been reported from observations of natural flight directions of red admirals in Britain (Williams 1951), Italy (Benvenuti *et al.* 1996) and Spain (Stefanescu 2001), which is also consistent with our data. In all our data from Öland we see a small number of individuals flying in a direction opposite to the majority of the population (Fig. 1 a, c, e).

There exists a very early report on mass migration of red admirals at Falsterbo in south-western Sweden, where the butterflies were observed migrating north in autumn (Rudebeck 1951), clearly demonstrating that the orientation in autumn can deviate from the expected. It has been suggested that these reverse flights by the red admirals at Falsterbo are a response to approaching bad weather from the south (Rudebeck 1951). Reverse migration opposite to the expected main migratory direction is well-known in migratory birds and have been observed in both spring and autumn (e.g. von Haartman 1945, von Haartman *et al.* 1946, Koskimies 1947, Evans 1968, Able 1977, Richardsson 1978, Lindström & Alerstam 1986, Åkesson *et al.* 1996). In spring it is usually performed in response to bad weather by early spring migrants, such as lapwings (*Vanellus vanellus*) and skylarks (*Alauda arvensis*), while autumn reverse migration have been reported from coastal observation sites as a response to a migration barrier (Åkesson *et al.* 1996, see also Åkesson 1999). Orientation errors and wind drift are other explanations to these deviating migration flights. At Falsterbo these temporary reverse migrations are wide-spread among a number of passerine migrants and are performed shortly after capture (Åkesson *et al.* 1996) presumably to reach better foraging areas and safer inland stop-over sites (e.g. Alerstam 1978, Lindström & Alerstam 1986). Reverse migration predominantly occur in birds with low fat reserves (Sandberg 1994, Åkesson *et al.* 1996, see also Sandberg *et al.* 1988) and are guided by the their magnetic compass (Sandberg 1994, Bäckman *et al.* 1997, Sandberg *et al.* 2002). Whether the red admirals in our study in 1999 was performing reverse migration of the same type observed in birds we cannot say for sure, since we do not have any measurements of the butterflies' fat content that year. However, we believe it would be worthwhile to further investigate the connection between fat content and orientation in butterflies, especially at coastal sites since this is a very clear response in migratory birds (e.g. Sandberg 2003) and the fat content of migrating butterflies can vary extensively (Brattström 2006, Brattström *et al.* in prep c).

Studies of stable isotopes have reported a variable pattern of migration for this species throughout its northern range, and especially related to the timing of autumn migration (Brattström *et al.* in prep. b, c). The stable isotope studies showed that red admirals captured at the same site in northern Europe in autumn can come from many different presumed natal locations (Brattström *et al.* in prep b) and this variation in individual origin is even larger at coastal sites (Brattström *et al.* in prep c). This mixed origin is likely to also be present in our tested red admirals possibly causing the variation in preferred individual migratory direction found between years. If the tested red admirals have different origin, do the time of year for our experiments support this assumption?

The median date of passage in Denmark was 23 September in 1999 (Hansen 2001), this being much later than we performed our experiments at Ottenby (Mid August to first days of September) suggesting that we potentially used butterflies from a different source area or with a different preferred orientation. In 2005, we performed orientation cage experiments at Ottenby during the same time period (Mid September) in which peak migration was observed at a nearby site at Falsterbo in south-western Sweden (Brattström *et al.* submitted). For 2002, we have no data from nearby sites on the timing of migration to compare with, but the time period is the earliest of all the three years so it was likely a year with early autumn migration from our study region since the red admirals in our study migrated towards the south already in the beginning of August.

With all this evidence of plasticity in the annual migratory pattern of European red admirals, it is not unlikely that the red admirals tested at Ottenby in 1999 were from a large influx of southern individuals on their way north. This would have been possible to test with stable isotope analysis (e.g. Hobson 2003, Rubenstein & Hobson 2004, Brattström *et al.* in prep b), but during the present year we did not sample the red admirals after the cage experiments, since they were also used in release experiments.

Orientation mechanisms

To our surprise, the results of the clock-shift experiments showed completely different responses between years. In 1999 we found a significant effect of the treatment but in 2002 and 2005 we could not find any significant effect at all. The geographical orientation of the butterflies suggests that we have a clear overcompensation as a result of the clock-shift. However, since the mean time of day for the two treatment groups were different in 1999, we have also compared the red admirals' flight direction in relation to the sun. The mean orientation of the clock-shifted red admirals indicates that they had their internal clocks delayed by 4 hours and under-compensated their orientation (i.e. showed a smaller angular difference in mean orientation relative to the controls compared to what was expected).

Smaller angular differences as a response to clock-shift, and also no response at all have been reported for migratory birds (Muheim & Åkesson 2002, and references therein). Clearly the effect of all these clock-shifts have yielded very different responses in the birds studied, such as predicted shifts, increase in scatter, shifts into unexpected direction, or larger shifts than expected (for discussion see, Muheim & Åkesson 2002). It is not clear under what conditions the birds respond with predicted shifts, but recent cue-conflict exposure experiments have indicated a close interaction between the sun and the geomagnetic compass, in which the geomagnetic compass used during flight (Cochran *et al.* 2004) is calibrated by the polarisation pattern of the sky (i.e. the sun compass) close to the horizon (Muheim *et al.* 2006, see also Able & Able 1996). We cannot exclude that a similar system of compass calibration in present also in other migrants, including butterflies.

Why do we find an effect from clock-shift in only one year and not in all? There are several possible explanations to the lack of response to the clock-shift. It could be that the red admirals tested in 1999 were all coming in from the south and actively migrating towards the north using a sun compass, while the individuals studied in 2002 and 2005 might be of a local origin and ready to start migration over the sea with a general course towards the south. It is possible that they were still fuelling up before setting off on a long migratory flight to the south, not yet ready to migrate and use their sun compass or preferred to use a geomagnetic compass. Evidence of the presence of a magnetic compass have been demonstrated for butterflies (Perez *et al.* 1999, Srygley *et al.* 2006) and moths (Baker & Mather 1982, Baker 1987), suggesting they can use such orientation cues during migration. It is also interesting to note the difference in expected (or experienced) migration across land to the north or over sea to the south that red admirals departing from Ottenby will face given the migration course selected. It might be that the red admirals reaction or absence of reaction to the clock-shift somehow was connected to their previous experience with migration over land or water, respectively, resulting in different use of compasses before capture.

Conclusions

We see clear limitations and benefits of all alternative methods to study orientation in insects, and in this study we have tried to apply methods that cause as limited a disturbance to the butterflies as possible. It is clearly possible to test the migratory direction of red admirals during autumn migration using flight cages. The variable nature of the species' migratory pattern is mirrored in the variation both between and within years in this study. There seem to be variations both in mean migratory direction as well as the orientation cues that are used during different time of the year. To fully understand the mechanisms of orientation, we need to analyse the individual butterflies with respect to genetic structure (Brattström *et al.* in prep a) and stable isotopes signature (Brattström *et al.* in prep b). Even though there seem to be many complex factors shaping the variable migration pattern in this species it is probably possible to answer most questions about the autumn migration by combing different analytical techniques.

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VII

Effects of wind and weather on red admiral (*Vanessa atalanta*) migration at a coastal site in southern Sweden

Effects of wind and weather on red admiral (*Vanessa atalanta*) migration at a coastal site in southern Sweden

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Each autumn, large numbers of red admirals migrate throughout northern Europe, flying towards south, to reach areas with conditions suitable for surviving the winter. The part of the population that breeds in Scandinavia cannot reach their wintering areas without crossing the Baltic Sea. We have observed the visible butterfly migration at Falsterbo peninsula, the south-westernmost point in Sweden, where large numbers of red admirals are seen most autumns flying towards the Danish coast on their way to more southern parts of Europe. Different weather parameters from a local weather station made it possible to analyse what cues are important for red admiral migration across the sea. Our results show that wind direction is the most important cue for the initiation of migration at this coastal location, while most other studies of butterfly migration report no large effect of wind direction. The explanation behind this difference is most likely that the butterflies in our study are about to cross open sea for more than 20 kilometres compared to most previous studies that are from inland locations where butterflies can avoid wind effects by flying close to the ground or on the lee side of topographic features. Other important cues for red admiral migration at Falsterbo are low wind speed and clear skies. The flight direction at Falsterbo is mainly towards the west, which is in contrast to the directions towards the south generally reported during autumn migration in this species. This is most likely because the red admirals follow both the local topography and the closest route to land on the other side and therefore make a deviation from the normally preferred direction to minimize the flight distance over open water.

Introduction

Insect migration is a widespread phenomenon all over the world and in different taxonomic groups (e.g. Williams 1958, Dingle 1996). Many insect species cover long distances during migration either flying actively or drifting with the wind, using flight only to stay aloft. Weather conditions, especially winds, have a large impact on their flight (e.g. Brown 1970, Mikkola 2003) as well as on the decision to migrate or not (Wikelski *et al.* 2006). Passively transported insects are completely dependent on winds and active flyers can save energy and gain speed by choosing the right day and altitude for their migration flight (Åkesson & Hedenström 2007).

Among Lepidopterans there seems to be a large difference in flight altitude between moths and butterflies (Walker 1980). Most moths seem to migrate high above the ground and they are often strongly affected by both wind direction and speed. Butterflies on the other hand are mostly found migrating in the boundary layer close to the ground (Taylor 1974, Walker 1985, Srygley & Oliveira 2001) where wind speeds are often lower than their own air speed making it possible to control their track direction relative to the ground. They can also adjust their flight path to follow leading lines that protect them from strong side winds (Nielsen 1961).

There are often concentrations of migratory land birds and insects at coasts and peninsulas pointing in the migratory direction (e.g. Alerstam 1990, Bruderer & Liechti 1998, Garland & Davis 2002). This is especially true and well studied in bird species that use thermal soaring, concentrating for instance at Gibraltar and the Bosporus. The passage of bird migration has been studied since the 1940's at Falsterbo, located in southernmost Sweden, where birds concentrate in autumn before crossing the sea towards Denmark (Fig. 1) (Rudebeck 1950, Ulfstrand *et al.* 1974). In 1973 the Swedish Environmental Protection Agency started systematic counts of the bird migration passing the Falsterbo peninsula as part of their Bird Monitoring Programme (Kjéllen 2006 and earlier references there in). During these studies it has also been observed that insects to some degree concentrate at the Falsterbo peninsula in autumn. This behaviour has been seen in butterflies, dragonflies and hymenopterans. A large migration of red admiral (*Vanessa atalanta*) was observed at this location as early as 1946 (Rudebeck 1951), but no systematic counts of any migratory insects have been performed at Falsterbo before our study. To shed some light on the migratory behaviour in relation to weather conditions we counted migrating butterflies at Falsterbo during three autumn migration periods 2004–2006.

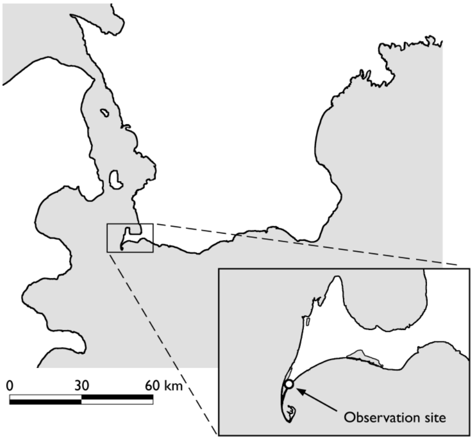

Figure 1. The observation site (marked with a circle) at Falsterbo is located at the south-western most point of Sweden. Shortest distance to Denmark from our study location is 25 km towards west-southwest.

The timing of migration and the number of individuals vary between years in butterflies. A large part of this variation is most likely caused by plasticity of most insects' life-histories as a response to differences in temperature and other weather related factors. Using the Falsterbo 2004–2006 count data for the red admiral (the most abundant migratory butterfly species) and weather data from the same site we address three different questions. First, what is the underlying factor behind the difference in migratory period between years? Second, during the migratory period, which factors make certain days suitable for migration while others are not? Third, on days with observed migration, which factors influence the numbers seen each day?

Materials and Methods

Migration counts

Red admirals were counted at the Falsterbo peninsula (55°23´N, 12°49´E) (Fig. 1) when they left Sweden flying out over the sea. At this location, organised counts of the bird migration passing the south-westernmost point (Nabben) of the peninsula have been conducted each autumn since 1973. From 2001 these counts are performed by two observers recording all migrants from dawn until 1400 hours (standard time) between 1 August and 20 November. The observation site is situated at the eastern coastline of the point of the Falsterbo peninsula, approximately 100 meters east of the western coastline, running in a north-south direction.

The environment consists of low, sparsely vegetated sand dunes and a golf course. It is not particularly rich in flowers and thus does not attract large numbers of butterflies for foraging. Typical resident butterfly species are queen of Spain fritillary (*Issoria lathonia*), rock grayling (*Hipparchia semele*) and small copper (*Lycaena phlaeas*).

In connection with the normal counts of migrating birds the observers also recorded butterflies seen migrating out over the water between 1 August and 25 October during the three autumns of 2004 – 2006. Butterflies passing the observation site were followed to determine a departure direction. If they stopped to feed or followed the coastline they were not followed further. Individuals venturing out over the sea were followed for roughly 50 meters and if they continued in a straight direction they were regarded as migrants. An approximate migratory direction was estimated based on the vanishing bearing to the nearest 45 degrees. In general most individuals held the same course throughout the whole day, and days when flight was observed in more than one sector it was always in two adjacent sectors. On such days we used the mean direction of both of these sectors.

Weather data

Data for weather variables were obtained from the Swedish Meteorological and Hydrological Institute (SMHI) for the weather station located at Falsterbo. Among the variables recorded at the station we used wind direction and speed, air pressure, temperature, precipitation and cloud cover. Data for all of these variables except precipitation are recorded every three hours. Precipitation is recorded in twelve hour intervals from 0600 to 1800 hours and 1800 to 0600 hours. Since counts of migrating red admirals are given as one value per single day we calculated a daily mean value for all our weather variables. For variables believed to directly influence the flight of the red admirals (wind direction and speed as well as cloud cover) we used the mean value recorded between 0600 and 1500 hours, the time of day when they are actively flying. Variables like air pressure and temperature are more likely to influence the decision to migrate, making it necessary to include data from the evening and night before the actual observation day. For air pressure measurement we used the mean from the evening before each observation day (1800 hours) up until the end of the flight period the actual day (1500 hours) and for temperature measurement we selected minimum temperature during the preceding night (1800 to 0600 hour) as well as daily mean temperature. For both air pressure and temperature we also calculated the change from the preceding day. To make the wind direction more suitable for linear analysis we calculated the sine and cosine values from the circular variable obtained from the weather station (corresponding to the East/West and North/South component, respectively, of the wind direction). All wind directions are given as the direction the wind is blowing from.

Statistical analysis

In this paper we exclude the data from 2004 since that year had very low numbers of butterflies compared to the other two years. We also focus only on red admirals since it is a common species, easy to identify in the field, and well known for its migratory behaviour. Before analysing the data with respect to factors important for migration we excluded days before and after the main migration period. It would not make sense to study factors influencing migration outside the normal migratory window. For this analysis, we therefore excluded all days up until one week before the main migration period began (when higher densities of butterflies were observed), and all days after the last observation. In 2006 we had a few outlying observations with a total of 20 red admirals observed before the start of the main migration. These individuals occurred up to one month before the main migration started and were not considered representative for the migration as a whole and therefore excluded from further analyses. The remaining days for each year were 9 August – 18 October 2005 and 5 September – 20 October 2006.

We tested if there was any significant difference in mean date of passage of red admirals between the two years by performing a Mann-Whitney U-test on the date of passage. In order to take differences in migratory intensity between days into account, each observed individual was represented by one value. To analyse if temperatures were different between the two years we used an ANCOVA, examining the relationship between daily mean temperatures recorded during the two study years.

We used the Rayleigh test (Batschelet 1981) to analyse if there was a mean population direction in observed migratory directions and the Watson-Williams F-test (Batschelet 1981) to analyse if there was any difference in orientation between the two years. To analyse if wind drift was present during days with side-winds we compared the mean orientation (also calculated using the Rayleigh test) on days with northerly or southerly winds, again using the Watson-Williams F-test.

To avoid autocorrelation between weather variables we computed a bi-variate correlation matrix between all the calculated variables. Mean air pressure and precipitation were both heavily correlated with most of the other variables and therefore removed from the analysis. Mean and minimum temperature was naturally correlated with each other so only one temperature measurement was used at the same time in each analysis.

We used a logistic regression analysis with observed migration/no migration as dependent variable. Year was included as a factor and we used wind direction (sine and cosine), wind speed, air pressure change, cloud cover and change in minimum temperature as covariates. The choice of using temperature change instead of a mean daily value was important since mean temperature naturally show a decreasing trend over the migratory period as do numbers of red admirals seen since fewer individuals are prepared to migrate. This would lead to a correlation without a biological significance. Change in minimum temperature has also been shown to be an important cue for initiating migration in dragonflies (Wikelski *et al.* 2006). The statistical significance of independent variables in the logistic regression was assessed by the change in deviance, G, which is approximated by a χ^2-distribution with one degree of freedom (Sokal & Rohlf 1995). We removed variables stepwise in a backward procedure until no variable could be removed without changing the model in a significant way ($p < 0.1$).

To analyse what factors influence the numbers of observed red admirals during days with migration we removed all days with no observation of red admirals from the dataset. Multiple regression was then performed using the logarithm of the observed admirals as dependent value. Year was included as factor and wind direction (sine and cosine), wind speed, air pressure change, cloud cover and change in minimum temperature were included as covariates.

Results

Migratory behaviour as observed during counting

Red admiral migration rarely took place under cloudy conditions or in wind speeds exceeding 5 m/s. Most red admirals were migrating below two meters above the ground. Upon reaching the waterline the majority gained height and continued at a height of 3–10 meters above the sea. On a few occasions, while scanning for birds with the binoculars, butterflies were discovered flying in a western direction at greater height (up to roughly 20 meters). Although this was rare, individuals travelling high obviously stand a much smaller chance of being spotted. Generally red admirals hesitated less than most other butterfly species when reaching the coastline. Most other butterflies only ventured out a few meters over the water, before quickly returning to the shore.

Annual migration period of red admirals

We observed 222 and 311 red admirals in 2005 and 2006 respectively, while only 35 individuals were observed in 2004. The timing of migration was different between 2005 and 2006 (Fig. 2) with a difference of 24 days in median date of passage (31 August in 2005 compared to 24 September in 2006). The difference in mean passage date of all the individual migrants were significantly different between 2005 and 2006

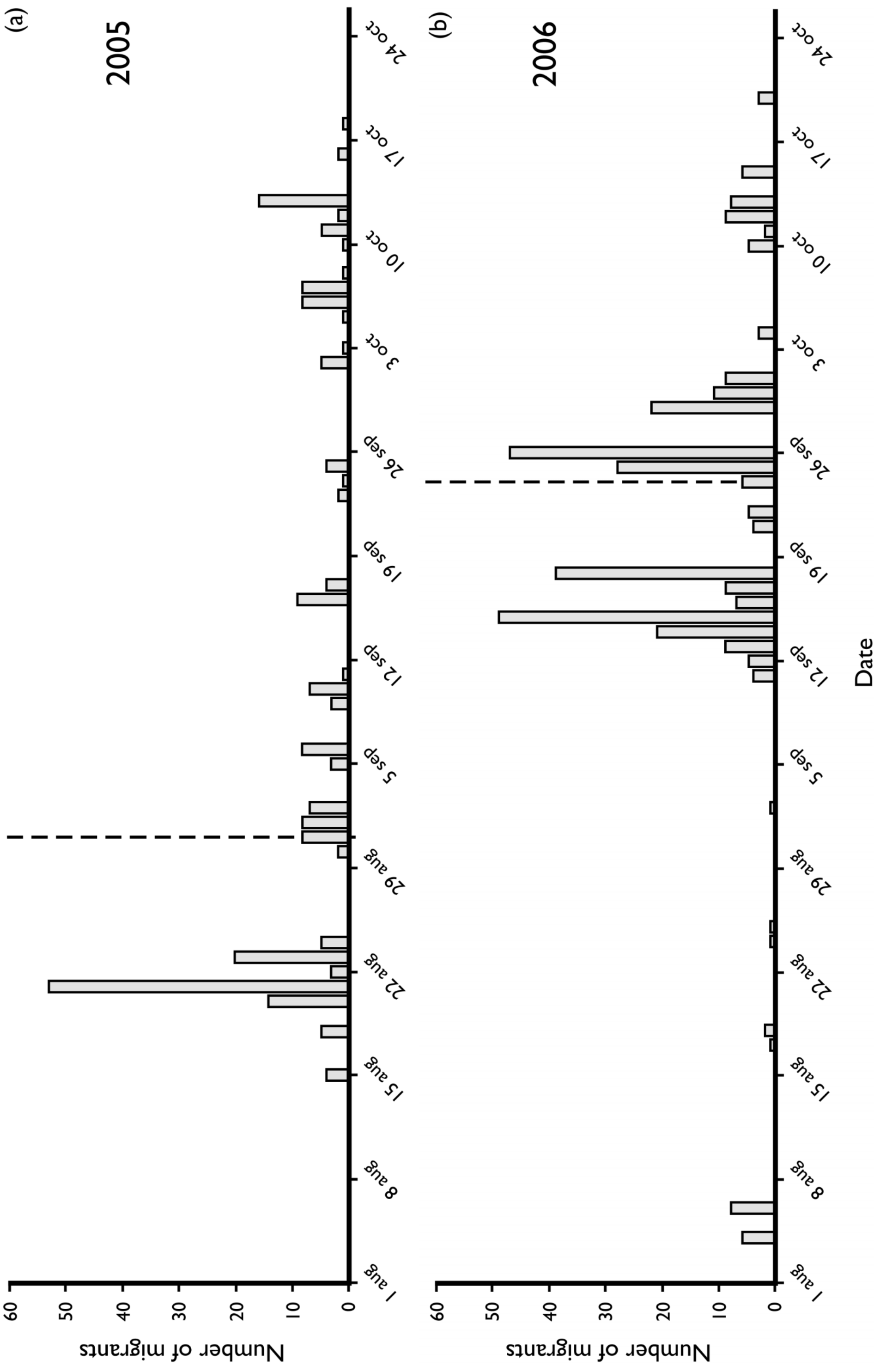

Figure 2. Numbers of red admirals migrating out over sea at Falsterbo each day during autumn 2005 and 2006. A broken line indicates median date of passage for each year and this difference is significant.

(Mann-Whitney U test, U = 16996.0, N_{2005} = 222, N_{2006} = 311, $p < 0.001$).

The ANCOVA using mean temperature as dependent variable and day from start of counting period as covariate and year as factor showed that we had a significant effect of both year and date. No interaction between date and year was found. The difference in the intercept of the regression line between the years is 1.52 °C with 2006 as the warmer year and the slope of temperature change per day is –0.074 °C (Fig. 3). This translates to the mean temperature for a day being reached about 21 days later during 2006.

Yearly flight directions and effects from side winds

The mean daily flight direction was similar and highly significant in both years with limited variation (Fig. 4a). In 2005 the mean flight direction was 265° ± 5.9° (95% CI), N = 33 (Rayleigh test: $r = 0.96$, $p < 0.001$) and in 2006 it was 255° ± 6.5° (95% CI), N = 22 (Rayleigh test: $r = 0.96$, $p < 0.001$. There was no significant difference in flight direction between the two years (Watson-Williams F-test: $F_{1,53} = 3.56$, $p = 0.065$). One day in 2006 was removed from these analyses since there was no recorded flight direction from that day.

The mean daily flight directions observed when prevailing winds had southern component was 261° ± 5.5° (95% CI), N = 42 (Rayleigh test: $r = 0.95$, $p < 0.001$) (Fig. 4b) and for a days with a northern component the direction was 258° ± 11.0° (95% CI), N = 10 (Rayleigh test: $r = 0.97$, $p < 0.001$) (Fig. 4b). These directions were not significantly different from each other (Watson-Williams F-test: $F_{1,50}$ = 0.17, $p = 0.68$) indicating that there was no wind drift. Three more days were excluded from these analyses since the wind direction was directly from the east or west.

Since all individuals from the same day in general are reported having the same estimated flight direction, these values might be somewhat unrealistic and scatter was probably more pronounced if individual directions were available, but there is still no doubt that the directions are highly similar both between days and years. Out of a total of 55 days with migration with recorded flight directions, these were reported from only one 45 degree sector in 43 (78%) of these days. During the remaining 12 days with more scattered flight direction, flight was always observed in two adjacent 45 degree sectors.

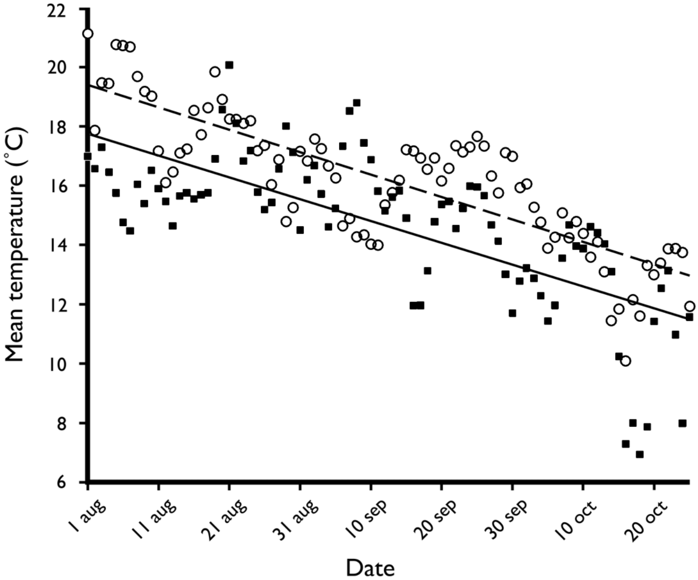

Figure 3. Daily mean temperature recorded at Falsterbo during autumn 2005 (filled boxes/solid line) and 2006 (open circles/broken line). The difference in mean temperature trend translates to 2006 being 21 days warmer than 2005.

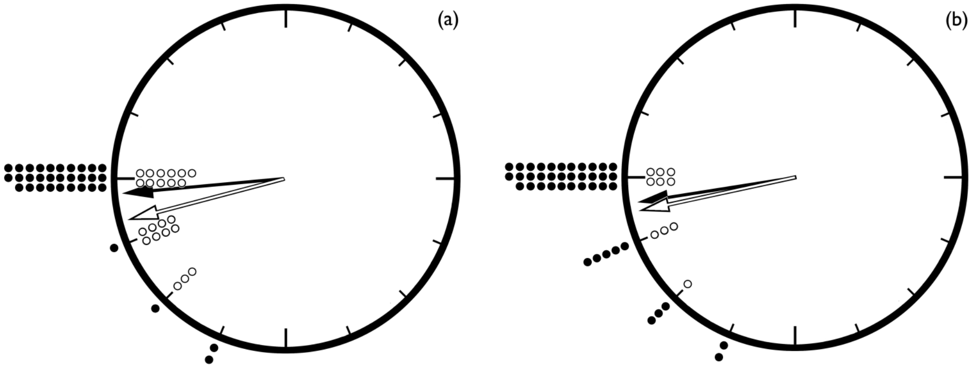

Figure 4. Circular diagrams showing the mean directions of migrating red admirals recorded at Falsterbo in autumn. a) Shows the mean orientation of the two study years (solid symbols = 2005, open symbols = 2006 and b) shows mean directions depending on direction of side winds (solid symbols = northern winds, open symbols = southern winds). Each dot represents a daily mean flight direction for all individuals observed during one observation day. The arrows show the mean direction for each group and the length of the line is related to the scatter of the distribution (r).

Weather factors affecting migration

The logistic regression found four parameters to be significant as predictors for days with or without migration. The final model predicted the correct outcome in 79.5% of the cases. The variables left in the model in order of explanatory power are: sine of wind direction (E/W component), cloud cover, wind speed and cosine of wind direction (N/S component). Probability of migration is highest when a north-eastern wind of low speed is combined with a clear sky. The results are presented in Table 1 and plots of predicted probabilities at different levels of the final model parameters are shown in Figure 5 a–d.

The multiple regression found no significant factors (except study year) explaining the difference in numbers seen migrating during days with at least one migrating individual (Table 2). The effect of study year was expected since we had a larger amount of migrating red admirals in 2006 compared to 2005. However, the daily

Table 1. Probability of migration of red admirals at Falsterbo in relation to daily local weather variables and year of study estimated by logistic regression. Non significant variables were removed step-wise in a backward procedure and reported values for the removed variables are from the last step in which they were still included in the model.

Final logistic regression model (and variables not entering the model)	Estimate	df	G	p†
Final Model		112		
Constant	4.03	1		
Sine of wind direction	1.68	1	26.44	< 0.001
Cloud cover	–0.50	1	19.50	< 0.001
Wind speed	–0.39	1	8.97	0.003
Cosine of wind direction	1.02	1	4.70	0.030
(Air pressure change)	–0.10	1	2.16	NS
(Observation Year)	–0.37	1	0.46	NS
(Difference in minimum temperature)	–0.02	1	0.01	NS

† NS indicate p > 0.1.

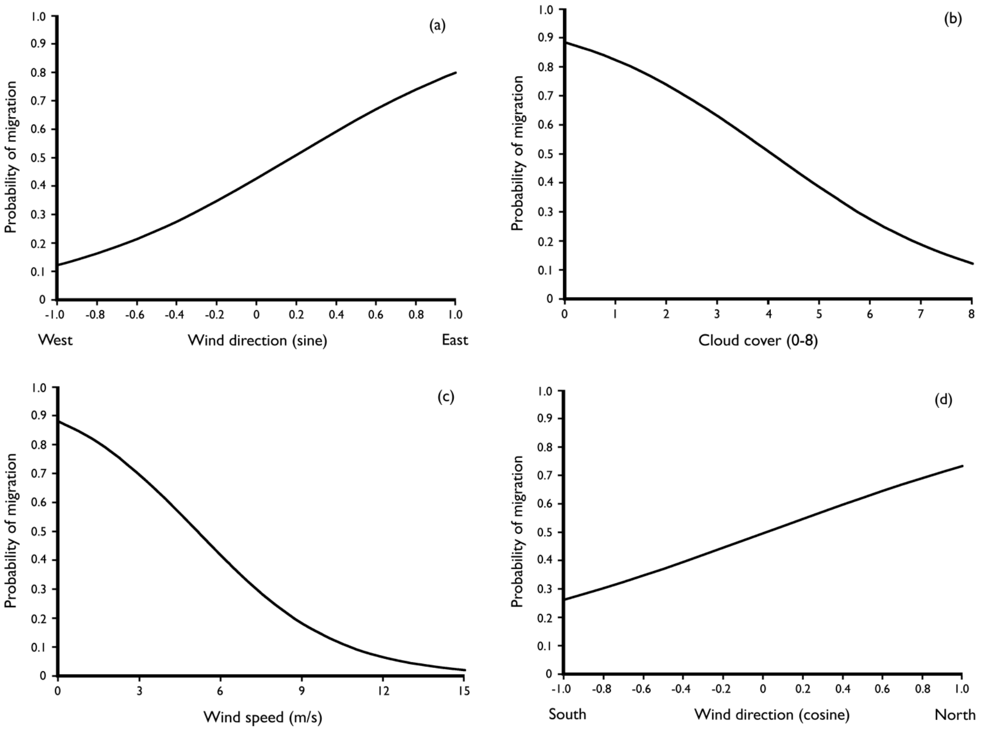

Figure 5. Relationship between predicted probability of migration and recorded weather variables. The variables that have a significant effect for determining if a day is suitable for migration are (in decreasing importance): a) Sine-component of wind direction, west (–1) –east (+1) b) Cloud cover c) Wind speed and d) Cosine-component of wind direction, south (–1) – north (+1).

variation in numbers seen on migration could not be explained by the weather variables included in our analysis.

Discussion

Timing of migration

Our results show that there is considerable plasticity in the timing of migration of red admirals at Falsterbo between years. Considering the fact that temperature is one of the few variables that can give a reasonably good indication of how far the autumn season is progressing and the fact that we found a temperature difference that matches the difference in median date of passage at Falsterbo (Fig. 3) temperature must be considered as a likely factor triggering the start of migration in red admirals. Since we have data for large red admiral migrations from only two years, it is not possible to statistically analyse this effect. A study of red admirals in Denmark also reported differences between years in peak dates of autumn migration ranging from 25 August to 8 October (1995–2000) (Hansen 2001). The same has been reported for the monarch (*Danaus plexippus*) in North America (Meitner *et al.* 2004), considered to be the most regular migrant of all butterflies. Thus, in general butterflies and other insects are more variable in their migration phenology compared to many other animals, and especially relative to birds (e.g. Edelstam 1972, Ulfstrand *et al.* 1974, Enquist & Petterson 1986). However, there are a few bird species dependent on variable food sources, like berries and pine cones, that show quite exten-

Table 2. Results of the multiple regression on migration intensity of red admirals (*Vanessa atalanta*) at Falsterbo (log-transformed values from days with observed migration only) in relation to daily local weather variables and study year. Year of study is the only variable having a significant effect on number of red admirals seen on migratory days.

Variable	S.S.	df	F	p
Year	1.19	1	9.62	<0.01
Wind speed	0.18	1	1.48	0.23
Air pressure change	0.17	1	1.36	0.25
Difference in minimum temperature	0.12	1	0.96	0.33
Cloud cover	0.07	1	0.60	0.44
Sine of wind direction	0.04	1	0.30	0.59
Cosine of wind direction	<0.01	1	0.03	0.86
Error	5.94	48		
Total	47.44	56		

sive variation in timing of autumn migration between years, like the greater spotted woodpecker (*Dendrocopus major*), fieldfare (*Turdus pilaris*) and common crossbill (*Loxia curvirostra*) (e.g. Enquist & Pettersson 1986). Many migratory birds have reasonably fixed locations of breeding and wintering while insects in general migrate until suitable areas are reached and the location of these areas change between years because of prevailing weather and climate conditions. The insects' migratory program is most likely made up of a set of simple rules with external cues initiating and terminating migration (Wikelski *et al.* 2006), so differences between years in wind and temperature can have large effects on the distribution of migratory species of insects each year. The number of red admirals seen during monitoring programs in Great Britain is fluctuating in average two-fold between years and even more irregular migrants, like the painted lady (*Vanessa cardui*) shows on average six-fold fluctuations between years (Pollard & Yates 1993). This again indicates a migration program where both direction and length of migratory flights presumably are highly variable.

Flight behaviour and effects of wind

Migratory red admirals that reach the sea at Falsterbo avoid venturing out over open water if the conditions are not favourable for flight in a primarily western direction. Most studies of butterfly migration find no large effect of wind direction since the butterflies generally stay close to the ground and fly on the lee-side of topographic features to avoid strong cross winds. At Falsterbo, days with migration are characterized by north-eastern winds of low speeds and clear skies. Since the absolute numbers seen migrating is not related to the weather situation as long as it is good enough to allow migration (Table 2) it seems like most butterflies present in the area during a suitable day for migration will cross the sea. The decreasing trend over the migratory period is probably caused by a continuous depletion of individuals that has still to leave the region. The positive correlation between migration and following winds in our study is probably caused by the fact that the red admirals are flying out over water where they cannot avoid the effect of winds and they will not be able to land if conditions turn for the worse. Most other studies (see above) have been performed at inland locations where the migration can be safely aborted at any time. One report on the other hand suggested a large migration of red admirals, traced by radar, to be observed as high up as 1000 meters or more (Mikkola 2003). Mikkola (2003) also reported sightings of red admirals gaining altitude by thermal soaring and disappearing out of view at high altitudes. This was in an area were the butterflies are about to cross an area of open water for at least 60 kilometers. However, the red admirals at Falsterbo have only 24 kilometers of flight over open water before reaching the coast of Denmark. The only migrating butterfly regularly reported to fly at high altitudes (Gibo 1981), and also use thermal soaring during migration (Gibo & Pallett 1979), is the monarch in North America.

While most observations of butterfly migrants (except monarchs) matches ours, reporting a low flight level with no sightings more than a few meters above ground (e.g. Williams 1976, Walker 1985, Benvenuti *et al.* 1994, Srygley *et al.* 1996, Srygley 2001) we cannot be sure that red admirals at Falsterbo do not migrate higher up, out of view of the observers. However, it is still likely to assume that the major migratory movements in this area are performed at lower altitudes. This is supported by the fact that the observers only rarely saw any red admirals at medium altitudes, even though they continuously scanned higher up with binoculars looking for birds. The same was reported for red admirals migrating in north-western Italy where the butterflies stayed close to the ground and, just like at Falsterbo, they often gained height if they flew out over water (Benvenuti *et al.* 1994). Presumably the butterflies make this small ascent to have a safety margin to the sea surface. Also they do not need the safety of being close to the ground for predator avoidance (Walker 1980) over the sea since they can not land anyway. Higher flight altitudes also increase the range of vision, so that the closest land could be detected more easily. If the red admirals were mainly relying on soaring flight during the sea crossing at Falsterbo we would probably see lower numbers at the observation sight during the most favourable days for thermal flight. In studies of monarchs, well known for their soaring behaviour during migration, most individuals are observed during partly overcast days. When the skies are perfectly clear the numbers drop (Meitner *et al.* 2004), presumably because they are flying well out of sight for the ground based observers. Even though our observations are only applicable for migrants flying low enough to be seen by the observers the above facts support our observations that the main migration at Falsterbo is performed at lower levels than those observed in Finland.

Flight directions and orientation

The direction of the red admiral migrants observed at several different locations in Denmark (Hansen 2001) were towards the south, so the migratory direction reported at Falsterbo might have been selected to cross the sea at the shortest distance or, alternatively, follow the local topographical features. Since red admirals have fluctuating migration patterns without locally adapted subpopulations (Brattström *et al.* in prep), it is unlikely that they have evolved a preference for a specific migratory direction connected to a geographic location of the route used to cross water efficiently. It is more likely that they can somehow detect the presence of the Danish coast and minimize the distance of the sea-crossing or react to local topography. The coast is visible from Falsterbo during reasonably clear days for a human observer, but we can not tell for sure if the red admiral butterflies have visual capacities enabling them to see the coast directly at the same distance as humans. Light reflected against water surfaces become linearly polarized (Wehner 2001, Horváth & Varjú 2003) and this can be detected over longer distances, even when the water itself is not visible above the horizon, through an optical phenomenon, caused by fog, called "water-sky" (Hegedüs *et al.* 2007). Linearly polarized light can be used by insects to detect water surfaces (Schwind 1991, Shashar *et al.* 2005), but such a behaviour has not been studied in butterflies, and we do not know if they can use this information to see how wide a water body is or rather to detect closest land. The fact that our red admirals select a more westerly migratory direction than expected coinciding with the shortest route of sea-crossing to Denmark suggests the possibility that they can detect the coastline on the other side and orientate towards it. However, this orientation also coincide with the local topography suggesting that the red admirals observed at Falsterbo react to the nearby coastline as both diurnal (Alerstam & Ulfstrand 1974) and nocturnal (Åkesson 1993) bird migrants do. Many birds seem to follow the eastern coastline of the southwesternmost part of the Falsterbo peninsula and continue their flight across open water towards west to southwest (Alerstam & Ulfstrand 1974, Åkesson 1993, Åkesson *et al.* 1996). We found no difference in flight direction during days with northern winds compared to days with southern winds suggesting that the butterflies compensate for wind drift while flying past the observation site. Other studies looking explicitly at drift compensation in single individuals of migratory butterflies, found some compensation in several species when flying over water (Srygley *et al.* 1996), even when there were no visible landmarks available to the butterflies (Srygley 2001). When butterfly migration is studied at inland locations, direct effect of wind direction seems to be of less important since the migrants fly close to the ground (e.g. Srygley & Oliveira 2001) where wind speeds are lower and they can find protection from cross-winds behind topographical features (Nielsen 1961). However, even under these conditions some wind compensation can still be present (Walker 1985). Nocturnal passerine migrants, flying at low altitudes, have been shown to follow the coastline of the Falsterbo peninsula and to completely compensate for wind drift (Site 2 in Åkesson 1993). By doing so, their orientation might deviate slightly from the mean orientation recorded for high altitude migration passage at Falsterbo (Zender *et al.* 2001), suggesting that low flying birds react to and fly along local topography to a higher extent, than the majority of the birds flying at higher altitude during migration flights. Ringing recoveries reported shortly after ringing at the Falsterbo peninsula (Falsterbo Bird Observatory) further suggest that at least for diurnal migrants, like the blue tit (*Cyanistes caeruleus*), a west to southwesten course, very similar to what we observed for our red admirals, are kept all the way across the sea to Denmark (Åkesson *et al.* 1996). It is interesting to note that blue tits also select very similar weather conditions for their migration at Falsterbo (Nilsson *et al. 2006*) as red admirals do.

Our observations were conducted on a narrow coastal peninsula and, thus, we cannot tell how representative our observations at Falsterbo are for the migration of red admirals across the whole southern part of Sweden. Migrating butterflies generally avoid crossing large bodies of water and follow the coastline as long as this do not lead them too far from their preferred migratory direction (Reichholf 1978). If this is true, the coastline of south Sweden would act as a leading line to Falsterbo (Fig. 1). However, one observation that contradicts this general idea was made at Falsterbo as early as 1946 (Rudebeck 1951), when an exceptionally large migration was observed on 25 and 28 August. On both of these days, high numbers (estimated to be more than a thousand individuals) of red admirals were observed arriving at the Falsterbo peninsula from west to southwest across the sea. The mean flight direction was towards East-Northeast, exactly the opposite from our observations and the butterflies were observed migrating in direct headwinds. This observation is exceptional since, during the regular bird surveys, there has not been any mass movement of red admirals in a northern direction for more than twenty years (Nils Kjellén, personal observations). From where did these butterfly migrants arrive? Did they arrive from the coast of Denmark or did they come from other areas? Could there be other possible explanations to these unpredicted mass movements? If these red admirals had left the coast of Denmark, assuming they have held a reasonable straight course over water, they would most probably have initiated their sea-crossing at a point were the normal behaviour of following leading lines would have led them much further north before they would cross the water to reach Sweden. This is more likely to occur almost a hundred kilometres north of Falsterbo. Can the mass migration instead be a return migration common among bird migrants meeting bad weather during migration (e.g. Evans 1968)? It is possible that these red admirals were returning to shore after they had met unfavourably weather conditions on migration. Even though the weather was favourable for migration towards Denmark during the two days when the observations were made, Rudebeck (1951) noted that cloud cover were increasing during the day until the point when he first started to observe red admirals coming in from the sea. Can the butterflies he observed indeed have left South Sweden, or even the Falsterbo area, for Denmark at higher altitudes and then decided to head back when visibility became worse because of the approaching low pressure system and increasing cloud cover? This type of return migration with outbound flight at high levels and return flights at low ones have been observed in migrating monarchs leaving the coast in a tail-wind and then aborting migration, heading back close to the sea surface to minimize effects of the head-wind (Schmidt-Koenig 1985). Even though red admiral migration has only rarely been observed at high altitudes (Mikkola 2003), it is still possible that the main migration from Southern Sweden is carried out along the entire south coast at altitudes; too high up to be observed. The numbers observed during our study are somewhat low, and it is possible that large numbers of butterflies leave Sweden each autumn from other locations. Our study, however, shows that the red admirals passing Falsterbo in autumn select to migrate on clear, sunny days with eastern winds of low speed, and that they seem to select an adaptive migratory direction, to minimize the sea crossing.

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The following is a list of Doctoral theses (Lund University, Sweden) from the Department of Animal Ecology (nos. 1–78, from no. 79 and onwards denoted by (A)) and Theoretical Ecology (T). (E) refers to Doctoral theses from the Department of Chemical Ecology/ Ecotoxicology during the years 1988–1995.

1. CHARLOTTE HOLMQVIST. Problem on marine-glacial relicts on account on the genus *Mysis*. 6 May 1959.
2. HANS KAURI. Die Rassenbildung bei europäischen *Rana*-Arten und die Gültigkeit der Klimaregeln. 9 May 1959.
3. PER DALENIUS. Studies on the Oribatei (Acari) of the Torneträsk territory in Swedish Lapland. 14 May 1963.
4. INGEMAR AHLÉN. Studies on the history of distribution, taxonomy and ecology of the Red Deer in Scandinavia. 21 May 1965.
5. STAFFAN ULFSTRAND. Bentic animal communities of river Vindelälven in Swedish Lapland. 8 May 1968.
6. SAM ERLINGE. Food habits, home range and territoriality of the otter *Lutra lutra* L. 6 May 1969.
7. GUNNAR MARKGREN. Reproduction of moose in Sweden. 17 May 1969.
8. ARNE BERGENGREN. On genetics, evolution and history of the heath-hare, a distinct population of the arctic hare, *Lepus timidus* L. 17 October 1969.
9. HÅKAN HALLANDER. Habitats and habitat selection in the wolf spiders *Pardosa chelata* (O.F. Müller) and *P. pullata* (Clerck). 20 March 1970.
10. ULF SCHELLER. The Pauropoda of Ceylon. 29 May 1970.
11. LEIF NILSSON. Non-breeding ecology of diving ducks in southernmost Sweden. 2 December 1970.
12. RUNE GERELL. Distributional history, food habits, diel behaviour, territoriality, and population fluctuations of the mink *Mustela vison* Schreber in Sweden. 30 March 1971.
13. INGRID HANSSON. Skull nematodes in mustelids. 3 June 1971.
14. STURE ABRAHAMSSON. Population ecology and relation to environmental factors of *Astacus astacus* Linné and *Pacifastacus leniusculus* Dana. 3 June 1971.
15. LENNART HANSSON. Food conditions and population dynamics of Scandinavian granivorous and herbivorous rodents. 26 November 1971.
16. SVEN-AXEL BENGTSON. Ecological segregation, reproduction and fluctuations in the size of duck populations in Iceland. 21 April 1972.
17. STEN ANDREASSON. Distribution, habitat selection, food and diel activity of Swedish freshwater sculpins (*Cottus* L.). 5 May 1972.
18. KERSTIN SVAHN. Coccidian blood parasites in Lacertids. 17 May 1972.
19. RUTGER ROSENBERG. Macrofaunal recovery in a Swedish fjord following the closure of a sulphite pulp mill. 13 April 1973.
20. SVEN ALMQVIST. Habitat selection and spatial distribution of spiders in coastal sand dunes. 25 May 1973.
21. TORSTEN MALMBERG. Population fluctuations and pesticide influence in the rook *Corvus frugilegus* L., in Scania, Sweden 1955-1970. 25 May 1973.
22. ANDERS SÖDERGREN. Transport, distribution, and degradation of organochlorine residues in limnic ecosystems (defended at the Dept of Limnology). 23 May 1973.
23. BERITH PERSSON. Effects of organochlorine residues on the whitethroat *Sylvia communis* Lath.. 7 December 1973.
24. PLUTARCO CALA. The ecology of the ide *Idus idus* (L.) in the river Kävlingeån, South Sweden. 23 May 1975.
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26. BO W SVENSSON. Population ecology of adult *Potamophylax cingulatus* (Steph.) and other Trichoptera at a South Swedish stream. 15 October 1975.
27. STEN NORDSTRÖM. Associations, activity, and growth in lumbricids in southern Sweden. 6 April 1976.
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29. CHRISTIAN OTTO. Energetics, dynamics and habitat adaptation in a larval population of *Potamophylax cingulatus* (Steph.) (Trichoptera). 9 April 1976.

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59. CHRISTER LÖFSTEDT. Sex pheromone communication in the turnip moth *Agrotis segetum*. 30 November 1984.
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