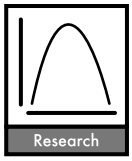

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Placing butterflies on the map – testing regional geographical resolution of three stable isotopes in Sweden using the monophagus peacock *Inachis io*

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Stable isotope analyses of tissues have been used to help delineate natal regions and routes of migratory animals. The foundations of such studies are isotopic gradients or differences representing geographic regions and habitat used by the organism that are retained in selected tissues. We sampled peacock butterflies *Inachis io* on a regional level in southern Sweden to study natural variation and the resolving power of the stable isotope method to delineate individuals from known areas on a smaller scale than has typically been used in previous studies. Hydrogen (δD), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were obtained from butterflies at seven different locations in an area of 250 × 250 km over three years (2002–2004). We found sufficient isotopic differences on this regional scale to delineate approximate origins. Of the three isotopes, deuterium showed good discrimination between sites, carbon isotopes showed weaker differentiation, whereas nitrogen isotopes proved unsuitable for small scale studies in this region due to high and unpredictable variation. We found there was enough variation in δD between years to prevent a general application of the technique to resolve sub-regional variation. Substantial part of this variation was probably caused by seasonal changes in δD of precipitation. These differences produce significant variation in δD between years in animals having short and variable tissue 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 the natural environmental variation in tissue δD cannot be controlled for. In the case of migratory species, this information is difficult to obtain, making the confidence intervals for prediction of natal areas fairly wide and probably only suitable for longer distance migration.

In recent years, stable-isotope biogeochemistry has undergone 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 isotope measurements have been used to track changes in ecosystems and climate (Flanagan and Ehleringer 1998, Kohn 1999, Staddon 2004), and to resolve questions related to habitat preferences, foraging, food-web and migration ecology in animals (Ehleringer et al. 1986, Kelly 2000, Eggers and Hefin Jones 2000, Post 2002, Webster et al. 2002, Hobson 2003, Rubenstein and Hobson 2004). In particular, the spatial tracking of individuals during migration has been especially problematic due to the inherent limitation of physical mark and recapture methods (for review e.g. Webster et al. 2002, Nathan et al. 2003). Stable isotope measurements provide an intrinsic marker of spatial origin and may resolve many open questions about butterfly and insect migration. For example, Hobson et al. (1999) showed that the stable isotopes of hydrogen ($^2H/^1H$, δD) and carbon ($^{13}C/^{12}C$, $\delta^{13}C$) in butterfly wings could be used to infer the geographical origin of adult monarch butterflies *Danaus plexippus* in North America. This was possible since butterfly wings are composed mainly of proteins and chitin, a tissue that is metabolically inert following formation (Rubenstein and Hobson 2004), and therefore reflect the isotope composition of diet and hydrological patterns at the natal area of the individual (Wassenaar and Hobson 1998). Isotope ratios found in animals are not equivalent to natural gradients in soil, air and water since metabolic and abiotic processes normally modify the isotope ratios for each step in the food chain (Kelly 2000). Fortunately, such isotopic discrimination processes are often systematic, so that the relative difference in the tissues of the studied animals' reflects the same differences found in their food sources (Hobson et al. 1999, Post 2002). Most studies of stable isotopes in terrestrial migrating animals have been made in North America (Wassenaar and Hobson 1998, Hobson et al. 2003) where both δD and $\delta^{13}C$ values differ across large geographical gradients, making it possible to determine the origin of individual animals with reasonable accuracy (Hobson 2003).

The patterns of stable isotope gradients within Europe appear to be either more complicated (e.g. δD), or less well known (e.g. $\delta^{13}C$), and the number of studies on migrating animals originating from Europe remain few (for reviews, Hobson 2003, Rubenstein and Hobson 2004, Hobson et al. 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 montane regions have large impact on δD values (Hobson et al. 2003). In Europe there are clear gradients in precipitation δD, showing decreasing values towards the northeast (Bowen et al. 2005). We are unaware of any study of stable isotopes from insects of known origin in Europe. Our objectives were to assess the regional scale at which stable isotopes (^{2}H, ^{13}C, ^{15}N) can be used together to delineate origins of insect populations in Scandinavia within the broader European context. Since no background data were available, we 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 δD, $\delta^{13}C$, and $\delta^{15}N$ measurements of 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 compositions, but also to investigate interannual variability.

Materials and methods

Study species

We rationalized our choice of the peacock butterfly *Inachis io* by the following criteria: 1) the peacock is a common butterfly in Europe and it can usually be found and sampled throughout the study region in southern Sweden. 2) The larvae are monophagus, feeding only on nettles *Urtica dioca*, and thus we would expect differences in isotopic ratios to be affected 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) Even though nettles are perennial, they have an annual re-growth of steam and leaf matter so that parts of the plant consumed by the larvae during one season are produced from nutrients taken up during the same year, thereby allowing us to examine interannual differences in stable isotope composition of both food and consumer over a limited time period. 4) We were interested in the possibilities of employing stable isotope measurements in future studies to track the movements of migrating red admiral butterflies *Vanessa atalanta* in Europe. The peacock butterfly is closely related to the red admiral (Wahlberg et al. 2005), and both species use nettles as host plants. Thus, we expected that data and insights gained from the less mobile peacock butterflies could later be used comparatively in studies aimed at delineating the natal areas of migratory red admirals, as both species have similar breeding biology and food preferences.

Sampling

Peacock butterflies were collected by hand netting at seven 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, individuals were euthanized using ethyl acetate. The wings were removed from the body and stored under dry conditions in individual envelopes.

Stable isotope analysis

All wing membrane samples were rinsed in a 2:1 chloroform-methanol solution 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 located in the Ecology Dept at Lund Univ. For these analyses ca 1 cm^2 of wing membrane (ca 0.5 mg) was cut from the distal part of the forewing and packed in tin capsules (Elements Microanalysis). Capsules were combusted in an ANCA-GSL elemental analyser (PDZ Europe) attached to a continuous flow isotope-ratio mass spectrometer (20–20 PDZ Europe). To correct for analytical error and drift during different runs, three different lab standards of known isotope composition were

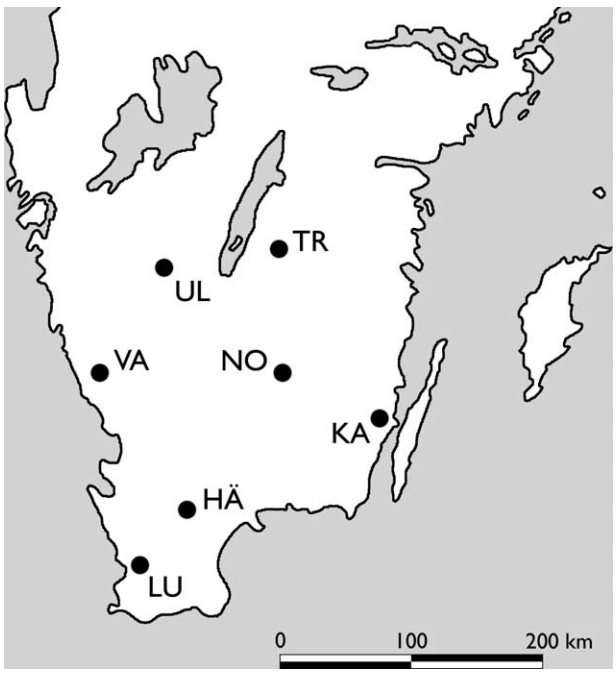

Figure 1. Position of the seven sample locations in southern Sweden. LU = Lund, HÄ = Hässleholm, KA = Kalmar, VA = Varberg, NO = Nottebäck, UL = Ulricehamn and TR = Tranås.

incorporated in each batch of peacock samples. We used previously calibrated glycine, homogenised feather keratin (*Acrocephalus arundinaceus*) and powdered bowhead whale baleen (*Balaena mysticetus*) as laboratory 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 $< \pm 0.2‰$ for nitrogen and $< \pm 0.1‰$ for carbon. Stable hydrogen isotope analysis was conducted at Environment Canada in Saskatoon, Canada. Because stable-hydrogen isotope analyses of complex organic materials are complicated by uncontrolled isotopic exchange between samples and ambient water vapour (Wassenaar and Hobson 2000), we used the comparative equilibration technique so that the values reported here are equivalent to nonexchangeable hydrogen (Wassenaar and Hobson 2003). Briefly, the process involves the simultaneous measurement of wing membrane with replicates of three different keratin standards whose non-exchangeable δD values are known, and which span the range of expected values. Stable-hydrogen isotope measurements of wing membranes and the keratin standards were performed on H_2 derived from high-temperature (1250°C) 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 and Hobson 2006), we estimate our laboratory error to be $\pm 2‰$.

Precipitation data

The δD values in rainwater are influenced not only by temperature, 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. By 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 rainwater at our sampling locations. The deuterium content of 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–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 Inst. (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.

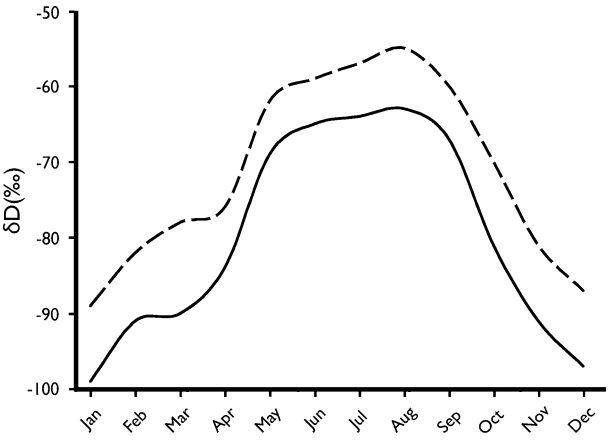

Figure 2. Monthly δD values (obtained from OIPC) for the two most distant sample sites in south Sweden (Lund, broken line and Tranås, solid line). All sites follow the same trend and are located between the two most extreme sites included in the graph.

Data analysis

We separated our analysis of variation among 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. Location of the sample sites, date of capture, and numbers of peacocks caught each year are given in Table 1. Date of capture varied somewhat between years since hatching date is dependent on weather conditions and we chose 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 small sample size limitations, 4 of the 145 butterflies were not measured for $\delta^{15}N$ and $\delta^{13}C$. When we examined the data for the three different isotopes, it was clear that several isotope results could be considered as extreme outliers. While it was unclear whether this was solely due to analytical error, we excluded two $\delta^{13}C$, four $\delta^{15}N$ and two δD values. All these outliers belonged to different individuals that otherwise showed normal values for the two other isotope ratios. Plots of the isotope ratios from all groups and years are presented in Fig. 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 ($CV_D = 4.5\%$).

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 this 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). The pattern was however, still relatively weak in 2002 ($r^2 = 0.137$) compared to 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 and 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 and 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.

Discussion

Values of δD showed 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 can be explained by a lower number of sample sites that year. In 2002 we obtained no samples from the 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 an 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 natural situation, variation of δD values 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 ca 400 km apart. In Scandinavia a pattern of decreasing values for deuterium ratios is predicted towards northeast (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

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

Location name	Altitude	Latitude	Longitude	2002		2003		2004	
				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

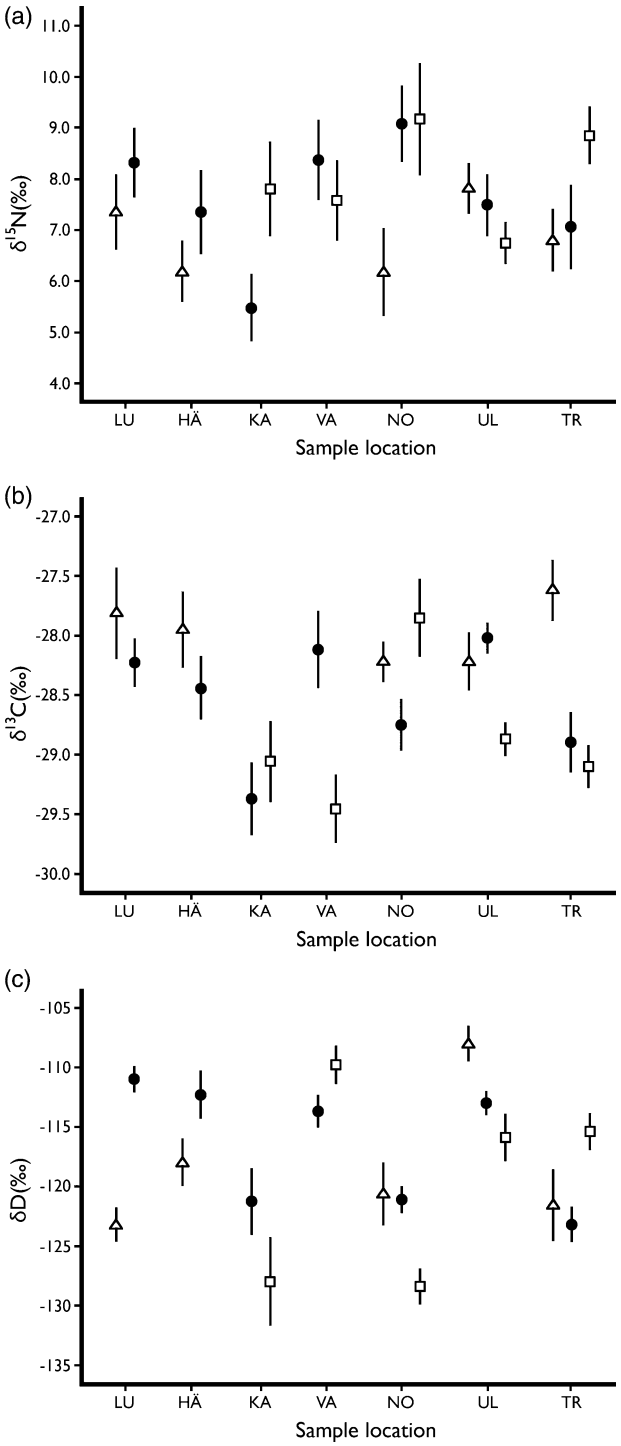

Figure 3. Mean values of (a) δN^{15}, (b) δC^{13} and (c) δD in wings of peacock butterflies *Inachis io* sampled at seven different locations in southern Sweden (names and position of the locations are given in Fig. 1 and Table 1). Measurements are from 2002 (Δ), 2003 (●) and 2004 (□). Bars indicate ±1 SE of the mean value.

δD values decreasing towards the northeast has also been reported for resident 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 altitudinal effect for our sampling locations, but considering the relatively small elevation differences between our sites (7–222 m a.s.l.), this is not surprising. The OIPC calculated δD values change with altitude at about −1.5‰/100 m in the study region.

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 *Inachis io* 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: δN^{15}.

Variable	SS	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: δC^{13}.

Variable	SS	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	SS	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		

It is clear that 2003 stands out as a comparatively stable year over the other two with respect to the relationship between spatial location and isotope values. In 2003 all of the measured isotope ratios ($\delta^{15}N$, $\delta^{13}C$ 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 weaker for 2002 and 2004 compared to 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 showed a gradual decline in temperature with increasing latitude during spring in 2003 (Fig. 6) (except for Varberg (VA) which was always relatively warm). This 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 δD values 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 δD values we later

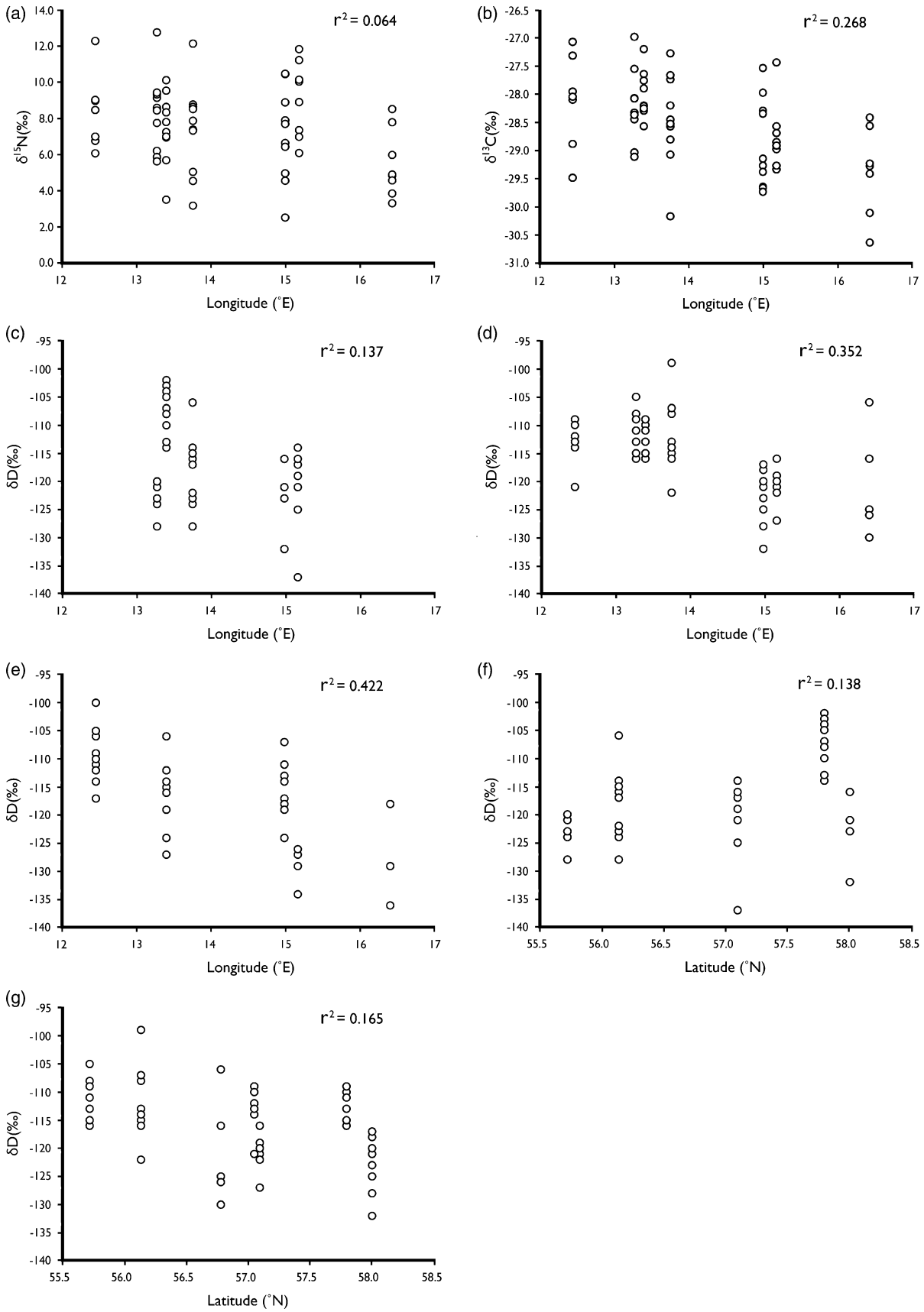

Figure 4. Plots of significant relationship between different isotope measurements and latitude/longitude of the sample location identified by an ANCOVA analysis. Years are presented separately as there were significant interaction between latitude/longitude and year in some of these combinations.

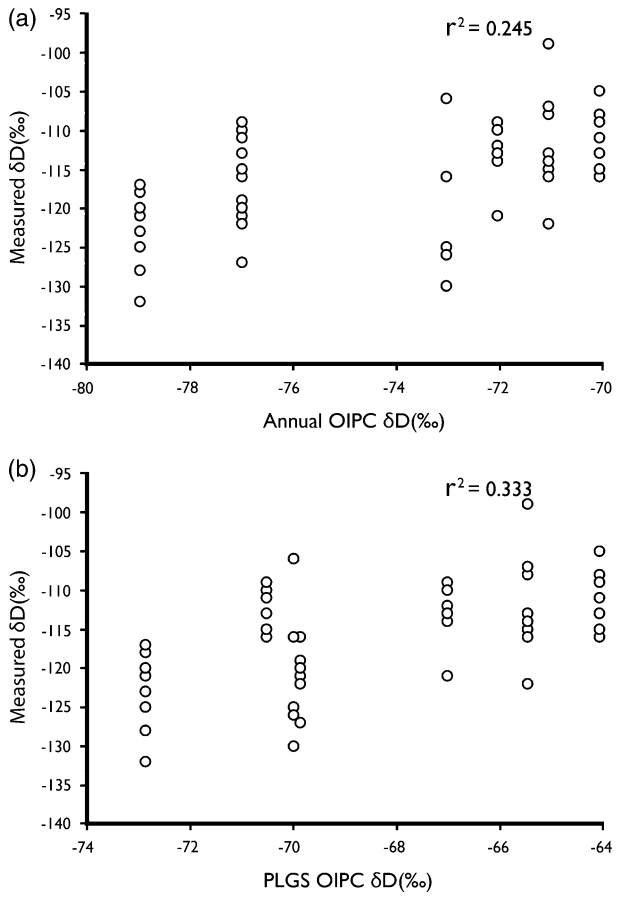

Figure 5. Relationship between measured δD values and (a) annual mean δD values in local rainwater obtained from OIPC and (b) mean δD values for the time period April–June weighted in relation to the amount of rain during different parts of that time period (see text for explanation). Significant relationships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

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.

Stable carbon isotope values in animals reflect those in their diets (Hobson 1999, Kelly 2000, Staddon 2004) and are often more depleted in cooler and moister climates, caused by differences between plants carbon isotope discrimination among C_3, C_4 and CAM plants (Oleary 1988, Staddon 2004, Rubenstein and Hobson 2004). This difference cannot explain the variation we found, since the peacocks rely exclusively on one species of host plant. However, plant populations adapted to drier and warmer habitats show less discrimination against ^{13}C (Lajtha and Marshall 1994) related to difference in water use efficiency, leading to differences among plant populations of the same species even in common garden experiments (Lauteri et al. 1997). This could explain why we found differences in 2003 with depletion in ^{13}C with increasing longitude. The climate is drier and warmer towards the east in the whole southern part of Sweden because of rain-out effects and most weather systems containing large amounts of rain originate from the west or the southwest. Noting that this

Table 3. Correlations between measured δD values in wings from peacock butterflies *Inachis io* 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
	Sign. (2-tailed)		0.183	0.498
2003	Pearson corr.	66	0.495	0.577
	Sign. (2-tailed)		<0.001	<0.001
2004	Pearson corr.	39	0.173	0.112
	Sign. (2-tailed)		0.294	0.498

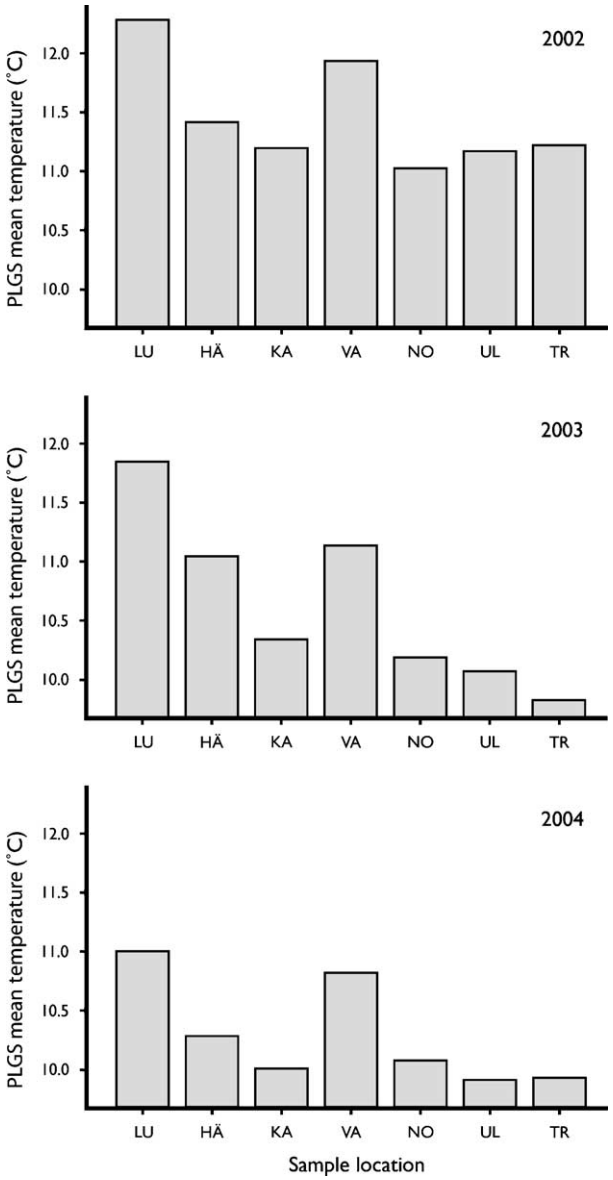

Figure 6. Yearly mean temperature for April–June at each of the sample locations. 2003 shows the typical pattern for the region with a generally decreasing temperature with increasing latitude. In 2002 and 2004 we have more atypical temperature patterns for the region. (Names and positions of the locations are given in Fig. 1 and Table 1.)

effect was only found during one year, it is probably caused by plasticity of the plants (Lauteri et al. 2004) rather than by genetically differentiated plant populations.

How useful is the stable isotope method for determining natal origin in monophagous and migrating insects?

Since the introduction of stable isotope methods in ecological studies, many efforts have been focused on long-distance animal movement (for reviews, Hobson 1999, 2003, Rubenstein and Hobson 2004). Animals from different geographical origins have previously been studied to find migration routes, winter areas and natal regions. Most of these studies to date have been based on measurements using tissue samples from individuals that have grown the sampled tissues in geographical locations that have been very far apart; this has produced substantial isotopic differences (Hobson et al. 2004). In our study on the other hand, we chose to focus on samples from a smaller region to test the limits of the resolving power of these methods. Even though we worked on a smaller scale where isotopic differences were expected to be less pronounced, we observed geographical isotopic differences among our sample sites (mostly longitudinal) but only during certain years. While it was not possible to delineate all of the locations, the geospatial extremes were sufficiently different to be distinguished even though our sample sizes were small. 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. 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 stable isotope values (excluding $\delta^{15}N$), samples from the same region and year were often close to each other. This reveals that sampling errors are not likely to explain the large variations of our $\delta^{13}C$ and δD values. Since $\delta^{15}N$ and $\delta^{13}C$ values are measured on the same sample, it is likely that the larger variation in $\delta^{15}N$ 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 (Robinson 2001, Hobson 2005). The only relationship we found when looking at $\delta^{15}N$ values 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 this 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 ca 250 km). Carbon isotopes can potentially be a good predictor as it shows a similar but slightly weaker pattern as deuterium. Carbon isotope measurements might be used in combination with deuterium or over ranges showing larger natural variations in $\delta^{13}C$ values than we found in our study area (Wassenaar and Hobson 1998). The predictive power of $\delta^{13}C$ measurements is likely to have increase in our study area if a species with a more varied diet had been used than our study species, the monophagus peacock butterfly.

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