An insect isoscape of UK and Ireland

Jason Newton

SUERC, Rankine Avenue, East Kilbride, G75 0QF, UK

Correspondence
J. Newton, SUERC, Rankine Avenue, East Kilbride G75 0QF, UK.
Email: jason.newton@glasgow.ac.uk

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Rationale: The study of insect migration is problematic due to the small size of insects. Stable isotope analysis can be used to elucidate movement, either by geographic assignment of location of a species, or by simply distinguishing migrant from resident populations. There are few isoscapes of any kind in the UK/Ireland available for interrogation. Thus, I have measured stable isotope ratios (of H, C, N and S) of 299 individuals of the non-migratory Brimstone moth (Opisthograptis luteolata) collected from 93 locations around the UK and Ireland by citizen scientists.

Methods: After removing lipids, stable isotope ratios were measured by continuous flow isotope ratio mass spectrometry, using either a conventional elemental analyser (C, N and S) or a high-temperature, thermal conversion elemental analyser in reductive mode.

Results: Maps (isoscapes) were constructed that illustrate the stable isotope spatial distribution of this insect. These are the first isoscapes of H, C, N and S of biological samples covering both UK and Ireland.

Conclusions: The insect isoscape patterns can be explained from what we know of moth diet, climate and geology. Sulfur isotopes may be of particular use for distinguishing individuals from areas of unique geology. Isoscape patterns may (with care) predict isotope compositions of other, herbivorous, non-aquatic, chitinous taxa. Such isoscapes, when extended beyond the UK and Ireland, would provide a useful tool to elucidate insect migration.

1 | INTRODUCTION

Migration in insects is widespread and in some species exceeds well-known mammalian and avian migrations in terms of biomass. It is crucial to link locations used by individuals to understand their ecology, to conserve threatened species and to understand how climate change may change their distribution and numbers. Insect migration also has important implications for humans – some of these are negative, since some migratory insects are crop pests or act as disease vectors; conversely, other migrants are important pollinators and therefore provide positive impacts. Despite this, our understanding of insect migration is surpassed by that of vertebrates simply because of the small size of insects. Size is the main barrier to tracking individual insects over long distances; this is unfortunate, since many insect migrations may be multi-generational where each individual participates in only a section of the migratory circuit.

Tracking migration has involved many innovations, resulting in a multitude of techniques appropriate to the taxon in question. Extrinsic markers (e.g. tags) require both capture and recapture but this has been popular for insects given the inherent challenges of their small size. For example, the monarch butterfly migration of North America has been extensively studied using numbered adhesive tags. However, this does require considerable effort since the recovery rate of tagged individuals in such a large population is quite low, 0.046 to 1.27%. Recent technological
advances have allowed the attachment of miniaturised radio-frequency transmitters to taxa as small as 0.2 g.6,7 Such innovations have expanded our ability to track the migration of small-sized taxa; however the cost and possibility of adverse effects on flight behaviour remain problematic.

Intrinsic markers such as stable isotope analysis require only one capture to reveal potential information about origin and are therefore less costly. Despite their typically lower resolution than that of extrinsic methods, stable isotopes comprise a convenient tool with which to investigate migration.7,8 In addition, since there is no attachment of bulky equipment the technique is ideal for small taxa. Furthermore, stable isotopes can distinguish migrants from residents within the same species; important since many insect species are partial migrants. Isotopic markers from the environment are assimilated by animals and fixed into biological tissues, and thus stable isotopes in an animal's tissue record both what it has been eating and where it has been. The main requirements for successfully using isotopes for geolocation are that (i) the isotope ratios of the elements being measured have sufficient discrimination within the geographic area of interest and (ii) the analysed tissues faithfully record isotopic information.

Whilst many stable isotopes have been used to track long-distance migration of birds, examples of insects being tracked with stable isotopes are fewer. Examples are: Monarch butterflies (Danaus plexippus, δ2H, δ18O and δ13C values),9,12 Painted Lady butterflies (Vanessa cardui, δ2H values),13,14 Common Green Darner dragonflies (Anax junius, δ2H values)15 and Globe Skimmer dragonflies (Pantala flavescens, δ2H values).16,17 In addition, emergent Red Admiral butterflies (Vanessa atalanta) and Silver Y moths (Autographa gamma) from several locations in western/southern Europe have been discriminated using δ2H values,18 and six species of North American butterfly were also provenanced using δ2H values.19

To assign location based on stable isotope ratios, one requires a spatial landscape of environmental isotopic variation or “isoscape”.20 There are essentially two approaches to this: (i) for hydrogen and oxygen isotopes, using precipitation isoscapes derived from the Global Network of Isotopes in Precipitation (GNIP)21,22 and a linear transfer function which relates the stable isotope ratio of tissues synthesised at known locations to that of precipitation;2 and (ii) a direct approach using the isotope ratio of a non-migratory analogue. For insect migration, moths are ideal candidates, since moth trapping is a popular pastime and a well-chosen resident species can provide many geographically disparate points from which to interpolate an isoscape.

Only two GNIP stations exist in UK/Ireland – in Wallingford, England, and Valencia, Ireland, which limits hydrogen isoscape modelling. No other country-wide isoscapes exist, although there are regional published isoscapes for different matrices, e.g. a Scotland-wide freshwater lake hydrogen/oxygen isoscape,23 and a soil sulfur isoscape in Northern Ireland.24 Thus, this study represents the first large-scale isoscapes of biological samples for the four elements H, N, O and S.

2 | METHODS

The first question when considering which part of the insect to analyse, is whether the tissue is metabolically active. When adult butterflies emerge from pupae, their wings are considered inert,10 with no further tissue turnover, and so an individual's wings retain the isotopic signature of their larval origins. However, since the wings are composed primarily of the polysaccharide chitin ([(C6H11O5N)₉]), and therefore essentially free of S (bar small amounts of protein), the wings are unsuitable for δ34S analysis. Since the aim was also to investigate the sulfur isoscape of UK/Ireland, combined head/thorax were analysed for S (and N and C) isotope ratios. Although these tissues are at least partially metabolically active, the Brimstone moth is a resident, so it was assumed that the S, N and C compositions of the moth body would represent an accurate assessment of their environment.

2.1 | Sample collection

Samples were collected from around the UK and Ireland by canvassing county recorders from the National Moth Recording Scheme, and then from volunteers contacted directly to fill in the “gaps” where possible. Instructions were to collect three Brimstone moths on disparate occasions between May 1st and August 31st, although in a small number of cases samples were collected outwith those dates. The number of samples, three, collected was based on an attempt to maximise spatial coverage, explore uncertainty within a location, and minimise analysis costs. In some cases more samples were collected than requested, and in other cases, notably towards the edges of Brimstone moth distribution, only one or two samples were collected. In one location a deliberate attempt was made to collect multiple samples throughout the entire flight period, to investigate any potential seasonal isotopic change.

Moths were all collected nocturnally by light traps of variable construction. Each volunteer was asked to store each moth in a polystyrene petri dish held within a sealed polystyrene sample bag which snugly held the petri dishes, in a domestic freezer. Once the required number of samples had been collected, these were mailed to the laboratory, accompanied by silica gel sachets to reduce humidity. On arrival at the laboratory, the samples were logged in a database and freeze-dried, before storage in labelled 20-mL glass jars which were placed in an electric desiccator. When all samples had been returned, each sample was twice cleaned in 10 mL of 2:1 chloroform/methanol for 1 h, the solvent decanted, and the sample then air-dried in a fume hood. This has the effect of removing lipids which can change the %H exchangeability, the non-exchangeable δ2H value and the δ13C value of the samples. Furthermore, this may remove pigments which can interfere with the geographic δ2H signal25 and possibly the other isotopic signals,26 although in the case of the uniformly yellow Brimstone moths this is less likely to be a major concern.
### 2.2 Stable isotope measurement

For δ\(^2\)H analysis, a core of wing material was extracted from the rear wing of each individual, with care being taken not to include any of the dark-pigmented wing. This was weighed (0.101 ± 0.029 mg) into a silver capsule (5.35 mm) and loaded into a UniPrep autosampler (Eurovector, Milan, Italy)\(^{27}\) and pumped at 60°C for two 1-h periods separated by a 10-min break in a helium atmosphere. This ensures the removal of residual adsorbed moisture.\(^{27,28}\) Hydrogen in the samples was converted into H\(_2\) gas in the reactor of a high-temperature thermal conversion elemental analyser (HTC-EA). The reactor was filled with chromium powder and glassy carbon and kept at 1425°C, which prevents the formation of other hydrogenous gases such as HCN.\(^{29}\) A likely product of the thermal decomposition of keratin. The autosampler was kept at 60°C throughout the experiments.

δ\(^2\)H measurement was carried out using a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). Corrections for drift and linearity were made using 0.08 mL and 0.04–0.19 mL, respectively, aliquots of IA-R002 mineral oil (Iso-Analytical Ltd, Crewe, UK). IA-R002 has a reported δ\(^2\)H value of −111.2 ± 1.4‰ and, as a hydrocarbon, it has no exchangeable hydrogen.

Since chitin and proteins contain many N–H and O–H bonds, a proportion of the samples will readily exchange with ambient water vapour. To counter this, I used a comparative equilibration\(^{30}\) technique using the reference materials CBS and KHS (−157.0 ± 0.9 and −35.3 ± 1.1‰, respectively)\(^{28}\) run in quadruplicate in each of nine experimental runs over 4 weeks. Since CBS and KHS are not matrix-equivalent to lepidopteran wings (although the H exchangeabilities are equivalent),\(^{28}\) I also added two powdered butterfly wing samples in each experiment. These are: YBWC, powdered Phoebus spp. raised on avocado in East Kilbride, UK, by F. Elliott, and UKRA, powdered Vanessa atalanta raised in York, UK, by C. Jewell. The δ\(^2\)H values of YBWC and UKRA over the nine experiments were −59.60 ± 2.13‰ and −80.57 ± 1.89‰, respectively; uncertainties reflect one standard deviation from all measurements (nine experiments with standards run in quadruplicate).

Wing tissue is protein-poor and hence too low in S concentration for an accurate δ\(^34\)S analysis. Thus, for same-sample consecutive measurement of δ\(^15\)N, δ\(^13\)C and δ\(^34\)S values, a combined head and thorax of each moth (2.5–5 mg) was added to a tin capsule (3 × 5 mm), combusted on a Pyrocube elemental analyser (Elementar UK Ltd, Cheadle Hulme, UK) and the separated sample gases analysed on a Vision isotope ratio mass spectrometer (Elementar UK Ltd). Analyses of laboratory reference materials methanesulphonamide/gelatine (MSAG2), methionine/alanine/glycine/ gelatine (M2) and sulfanilamide/alanine/gelatine (SAAG2) were repeated after every 10 samples and were used to correct for linearity and instrument drift over two long measurement cycles. Each of the laboratory reference measurements is checked regularly against international standards USGS40, USGS41 (for δ\(^15\)N and δ\(^13\)C values), and IAEA-S1, -S2 and -S3 (for δ\(^34\)S values). All international standards (except USGS41) are also measured as part of each analytical cycle (n = 4 per cycle). The precision, as indicated by the standard deviation of the 91 analyses of MSAG2 over the two experiments, is: δ\(^15\)N: ±0.13‰; δ\(^13\)C: ±0.10‰; δ\(^34\)S: ±0.62‰.

### 2.3 Data exploration/analysis

It is general practice to record minimum overnight temperature for moth recording (UK Garden Moth Scheme instructions); however, only two-thirds of the samples had these temperatures recorded. Instead, daytime temperature was extracted from the CEDA archive.\(^{31}\) Linear relationships were explored between daytime temperature, date of trapping, latitude and longitude, and isotope ratios (Table S1, supporting information). A relationship between the δ\(^2\)H value and date of trapping was not expected since wing δ\(^2\)H is a larval signature. No a priori predictions were made for the head/thorax signatures of the δ\(^15\)N, δ\(^13\)C and δ\(^34\)S values. A linear transfer function which exploits the relationship between the measured wing δ\(^2\)H value and the mean annual δ\(^2\)H value of precipitation (from the Online Isotopes in Precipitation Calculator\(^{21,32,33}\) was calculated. Stable isotope compositions at each location are presented in Data S1.

A generalised additive model (GAM) was used to generate isocapes using the mgcv package\(^{34}\) in R.\(^{35}\) In each case two-dimensional thin plate regression splines were fitted to the samples by location using the structure s(longitude, latitude), the method GCV, Cp, and default basis dimension k = 30. After fitting, the GAM residuals of all four isotope models showed approximately normal distributions. This allowed visual exploration and interpretation of four isocapes. Coefficient tables are provided in Table S2 (supporting information) and the rationale for choice of basis dimension and performance metrics for the GAMs in Table S3 (supporting information).

### 3 RESULTS

There was a considerable range of stable isotope composition among moth samples (Table 1). Exploration of the relationships between date of trapping, temperature and isotope ratios yielded only two significant results (Table S1, supporting information). For δ\(^13\)C values, there was a positive relationship (P <0.01) with temperature; over the

| Variable | Mean | s.d. | Range |
|----------|------|------|-------|
| δ\(^2\)H | −87.28 | 10.09 | −124.26 to −52.66 |
| δ\(^15\)N | +6.12 | 2.98 | −1.71 to +15.27 |
| δ\(^13\)C | −29.13 | 1.48 | −32.73 to −23.00 |
| δ\(^34\)S | +4.37 | 5.16 | −18.07 to +15.10 |
16.8 °C range of daytime temperatures this equates to an increase of 1.18‰ in the δ13C value over the entire dataset. For δ15N values, there was a negative relationship (P < 0.01) with day of trapping; over the 184 nights of trapping, this equates to a drop of 2.38‰ in the δ15N value.

In terms of latitude and longitude, several significant relationships were discovered (Table S1, supporting information). The δ2H values followed a weak negative relationship (P < 0.001) with latitude; the δ15N values followed a weak positive relationship (P = 0.01) with longitude; the δ13C values followed a weak negative relationship (P = 0.003) with latitude and a weak positive relationship (P = 0.03) with longitude; and the δ34S values showed a weak positive relationship (P = 0.01) with latitude, and a weak negative relationship with longitude (P < 0.001). These relationships are unlikely to be simply linear and therefore better explored with the spatial GAM (Figures 1–4).

The linear transfer function which explores the relationship between the measured wing δ2H value and the δ2H value of precipitation is:

$$\delta^{2}H = -71 + 0.30 \delta^{2}H_p \quad (P < 0.01, r^2 = 0.02).$$

Uncertainties in all isoscapes reflect the paucity of sampling in western Ireland, East Anglia and northern Scotland (Figure 1). For δ2H values the isoscape (Figure 2) is mainly flat, with deviations toward less negative values in eastern Ireland and southern England (the latter influenced by three samples collected on Alderney), and towards more negative values in NW Scotland. The δ15N isoscape (Figure 3) tends towards lower values in north Wales and Merseyside, and including a broader area including Ireland. The highest δ15N values predicted by the isoscape are along the eastern coast of mainland Britain. In a similar (but not coincidental) pattern to the δ15N values, the δ13C isoscape (Figure 4) tends towards more negative predicted values towards the north-west. The δ34S isoscape (Figure 5) is broadly flat, with the most positive values towards the west and northern coasts of mainland Britain, and negative δ34S values predicted around east-central England.

4 | DISCUSSION

Four UK/Ireland isoscapes are presented here based on non-migratory moth wing (δ2H values) and head/thorax (δ13C, δ15N, δ34S values). The following largely attempts to explain the isoscape patterns and their uses for further work, such as the possibility of detecting migrants within the UK.

The δ2H moth isoscape (Figure 2) mirrors published precipitation (δ2Hp) isoscapes derived from GNIP data,21,22 i.e. that of generally decreasing δ2Hp values towards the north; however, the locations of moth sampling far outnumber the GNIP stations within the area of interest. This concurs with other work on non-aquatic fauna whereby the δ2H values of tissue largely reflect the δ2Hp values36 and, by extension, the δ2H value of wing tissue is largely controlled by latitude, longitude and temperature. δ18O values were not considered given the small amount of material available and that the isoscape would largely mirror that of the δ2H values. This omission was further vindicated following recent measurements on Monarch butterflies in North America with considerable variance in wing δ18O values.12 The linear transfer function is significant, comparable in slope to, but slightly lower, than those of North American Lepidoptera10,37 but with a lot of scatter. Whilst the δ2H wing isoscape follows that of δ2Hp, one source of noise in the data revolves around Brimstone moth phenology.38 In the north of the UK the larvae are found around summer/autumn and precipitation in these months would be reflected in the wing tissue δ2H values. However, in the south, caterpillars can be found in almost all months, which might cause extra “seasonal” noise in the more southern δ2H moth data. This may explain why the most obvious gradient in δ2H values is in the far NW where the caterpillar season is much shorter; δ2H patterns in the south are more likely to be masked by seasonal effects from the previous season when the larvae were feeding.

Given the deciduous tree leaf diet of Brimstone moths, their δ15N values will reflect that of host plants, and trophic enrichment can be negated since it will be the same for all samples. Globally, leaf δ15N values largely reflect the soil δ15N values,39 but also integrate factors such as fractionation due to differing mycorrhizal associations40 and uptake of atmospheric reactive N deposition.41

From large analyses of global leaf data, the δ15N value increases with mean annual precipitation and, above ~0.5 °C at least, increases with mean annual temperature39,40; in other words, much of the δ15N variation in leaves has climatic controls. It appears
impossible to disentangle these conflicting effects on $\delta^{15}$N values in a way that would explain the moth isoscape (Figure 3). Although anthropogenic reactive nitrogen is increasing in the atmosphere,\textsuperscript{42} anomalies due to the proximity of traps to anthropogenic sources such as roads or industrial sources would be difficult to unpick from the data here.
The $\delta^{13}C$ values of moth tissue will be driven by the C3 leaf $\delta^{13}C$ values, and thus different photosynthetic pathways can be excluded as a reason for variance. That said, the predicted $\sim 2.5\%$ decrease in $\delta^{13}C$ values from the south and southeast to the northwest (Figure 4) requires explanation. This is similar to one of the early studies of the canopy effect in temperate deciduous woodlands\textsuperscript{43} requires explanation. This is similar to one of the early studies of the canopy effect in temperate deciduous woodlands\textsuperscript{43} which might suggest a geographic distributions of these species\textsuperscript{45} which might suggest a height. Furthermore, there are no obvious differences in the substrate.\textsuperscript{56} Plants may uptake S as sulfate, sulfide or as atmospheric $SO_2$.\textsuperscript{34S} values of plants generally reflect those of the S-containing substrate.\textsuperscript{55} on land plants which may have variable, but generally much lower, $\delta^{34}S$ values ($\pm 20\sim 13\%)$.\textsuperscript{52,54,56} Since there is negligible fractionation during plant uptake of S, the $\delta^{34}S$ values of plants generally reflect those of the S-containing substrate.\textsuperscript{54} Plants may uptake S as sulfate, sulfide or as atmospheric $SO_2$.\textsuperscript{56} Sulfides, although toxic to plants even in low concentrations, may be abiotic in origin or be formed from bacterial reduction of sulfate, which causes characteristically negative $\delta^{34}S$ values.\textsuperscript{57} Sulfur isotope fractionation with trophic level is small but of variable sign depending on the tissue,\textsuperscript{58} with the sole measurement for insects (Gypsy moth, Lymantria dispar) being $\pm 1.4\%$ from diet to tissue.\textsuperscript{59}

The $\delta^{34}S$ moth isoscape (Figure 5) is flat in Ireland compared with mainland UK. In the latter, there is a veneer of higher $\delta^{34}S$ values on the western (windward coast), indicative of the seaspray effect. In central England there is a pronounced area of low $\delta^{34}S$ values. Further interrogation of the individual locations suggests that there are several discrete localities in this area with negative $\delta^{34}S$ values. Significantly, all these locations (e.g. Thrapston, Northamptonshire) are on or near ironstones and mudstones, both of which have disseminated sulfide, and often evidence of bacterial sulfate reduction, which would result in negative $\delta^{34}S$ in bedrock, soils and groundwater – major sources of sulfur for both plants and herbivores.

One aspect of these isoscapes so far undiscussed is that of temporal (interannual) reproducibility. Without practically reproducing the data in subsequent years, this may be difficult to answer. The most reproducible of the isoscapes may be that of sulfur, given its tendency to simply reflect proximity to the coast and to map certain unusual lithologies. The tendency of the other stable isotope ratios to correlate with climatic parameters, such as temperature and precipitation, would imply that the absolute delta values might change; however, I suggest that the patterns and trends of these isoscapes are more robust to interannual climatic changes.

5 | CONCLUSIONS

Isoscapes of four elements are presented here following isotope analysis of a resident insect, with the aim of investigating the efficacy of spatial stable isotope patterns in elucidating migration. Patterns of stable isotope ratios are broadly as expected, although the lack of variation in some isotopes and/or areas limits utility. An insect of a different isotopic composition outwith the British or Irish range might be taken at face value as evidence that it is a migrant; however, we have no information on how the isoscapes presented here differ from those in a nearby country such as France. Despite this, one might
want to concentrate on areas in UK/Ireland where the isoscape is relatively unique, in order to ascertain whether individuals of a particular species in those areas are migrant or resident. The isoscape patterns (if not perhaps actual delta values) ought to be transferable to other chitinous taxa, with the exception of aquatic species where the δ²H value is likely to be more related to the particular water body. In addition to (non-aquatic) insects, the isoscapes also, however, offer the opportunity of investigating the migration (or otherwise) of other taxa, for instance insectivorous birds. I advocate the use of citizen scientist projects of this nature for building isoscapes, as the data would be otherwise unattainable by a single researcher.

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DATA AVAILABILITY STATEMENT
The data that supports the findings of this study are available in the supplemental material of this article.

ORCID
Jason Newton https://orcid.org/0000-0001-7594-3693

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