Isotope evidence for latitudinal migrations of the
dragonfly *Sympetrum fonscolombii* (Odonata:
*Libellulidae*) in Middle Asia

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Abstract. 1. *Sympetrum fonscolombii* dragonflies are believed to migrate seasonally. 
In the spring and early summer, the already-mature dragonflies arrive in Middle Asia for 
reproduction. In the late summer and autumn, summer-generation dragonflies migrate 
to the south. Their wintering places remain unknown.

2. Stable hydrogen (δ2H) and oxygen (δ18O) isotope analyses were conducted to 
confirm the migration of *S. fonscolombii* and determine the wintering area. Stable isotope 
composition of carbon (δ13C) and nitrogen (δ15N) in wings and legs was used to clarify 
the habitats in which dragonfly development took place.

3. Three cohorts of dragonflies collected in different regions of Middle Asia were used 
for analysis: (i) immigrants that arrived in the spring, (ii) residents that developed in 
Middle Asia, and (iii) transit dragonflies migrating to the south during autumn.

4. The average δ2H values in the wings were significantly higher in immigrants 
(−96‰) than in residents (−134‰) and transit individuals (−124‰). High δ18O and 
δ15N values in the tissue of immigrants confirmed their southerly origin.

5. Based on the species range and the global distribution of annual averages of δ2H and 
δ18O values in precipitation, the latitudinal migrations of *S. fonscolombii* were inferred 
to cover the area from the proposed natal regions of immigrants in South-West Asia 
(below ~36°N) to Southern Ural and the south of Western Siberia in the north (54–55°N) 
with a maximum migration distance of more than 4000 km.

Key words. Carbon-13, deuterium, isoscape, migration circuits, nitrogen-15, oxygen-18, seasonal migration.

Introduction

Despite extensive research on dragonfly migration (Corbet, 1999; Holland *et al.*, 2006; May, 2013), many puzzling aspects and questions about this phenomenon remain. One of the main problems is how to determine the region of origin of migratory dragonflies. Compared to birds and mammals, insects are small in size, which limits the use of exogenous markers – different identification marks attached to animals.

Additional limitations to exogenous markers are associated with tracking over either short duration or short distances and a low probability of re-capture (Wikelski *et al.*, 2006). Therefore, there is an obvious advantage of using naturally occurring endogenous markers, such as isotopic composition (Hobson, 2019), which makes preliminary labelling of insects no longer necessary to infer migratory movements. The isotopic composition (or isotopic signatures) of the habitats where an organism developed is reflected in metabolically inert tissue (for example dragonfly wing chitin) after its formation. The demonstration that hydrogen (δ2H) and oxygen (δ18O) isotopic composition in animal tissue is connected to δ2H and δ18O values of precipitation (δ2H_p and δ18O_p) in the regions where the development of the animals took place has been a major
breakthrough. This makes it possible to use the global isotope maps of precipitation (isoscapes) to determine the geographical origin of migrants (Brattström et al., 2010; Chabot et al., 2012).

The method of stable isotope analysis is currently widely used in the study of animal migration, including the migration of insects (van Hardenbroek et al., 2012; Hobson et al., 2018). It has also been used in the study of dragonfly migration. Hydrogen is the only element that has been used in these analyses (Hobson et al., 2012a,b; Cao et al., 2018; Hallworth et al., 2018). In this study, we used stable isotope composition of hydrogen, oxygen, nitrogen, and carbon to clarify seasonal latitudinal migrations of *Sympetrum fonscolombii* (Selys, 1840) dragonfly in the Middle Asia.

*S. fonscolombii* is common in Middle Asia and has a wide geographical range. The latter covers three continents, Africa, Europe, and Asia, but is not evenly populated (Fig. 1). In the African continent, the species is known only from arid and subarid regions in the south, east, north, and northwest, and it does not inhabit the vast region of Central and West Africa (Dumont, 1988; Clausnitzer et al., 2012). In Europe, the species is distributed northward to Scotland, Sweden, and Finland (Kalkman & Bogdanovic, 2015). In Asia, the continuous range of *S. fonscolombii* covers South-West and Middle Asia eastward to China and India. In the rest of the range up to the eastern continental margins, the species is known locally from single finds. A wide distribution of this species in the Indian subcontinent (Kalkman & Bogdanovic, 2015) is doubtful. It is known that in India this species is common only in the three northern states and the extreme south (Fraser, 1936; Babu & Nandy, 2010); it is known only from single finds in the west (Kulkarni & Subramanian, 2013). The northern limit of its distribution in Asia is the Southern Ural and the south of Western Siberia, approximately 54°–55°N (Fig. 1; Borisov, 2011; Popova & Eremina, 2016).

A characteristic feature of *S. fonscolombii* is the appearance of mature dragonflies in spring for Middle Asia. However, their origin remains unknown. The inflow of immigrants apparently continues from early April (it can be earlier during warmer years) until the appearance of dragonflies of the summer generation in June–July. In Tajikistan (Parkhara village, 37°31′N, 70°26′E, 870 m a.s.l.) on October 12. In Middle Asia, preimaginal phases of *S. fonscolombii* develop rapidly, approximately within 2 months (Borisov, 2011). In Europe, the duration of the development of summer generations is about the same – from 54 days (Hunger & Schiel, 1999) to 75 days (Lemptert, 1997).

In Middle Asia, the wintering of *S. fonscolombii* larvae is apparently not possible due to temperature conditions. Currently, there are no data on the wintering of this species (Borisov, 2011). In Europe, the northernmost points of the area where larvae of *S. fonscolombii* can survive the winter lie on the Iberian Peninsula (up to 39°36′N) (Weihrauch & Weihrauch, 2003). In the Asian part of the range, the northernmost point, where development during winter is noted, is in southern Turkey (36°17′N; Dijkstra & Kalkman, 2001). Presumably, individuals of the summer generation migrate south in the autumn from Middle Asia. Annual autumn migrations have been observed at the Chokpak Pass in the Western Tien-Shan (southern Kazakhstan, 42°N 70°E). Here, *S. fonscolombii* in transit, as well as other migratory species, fell in large numbers (maximum 3000 specimens a day) into traps set up for catching and ringing migratory birds (Borisov, 2009, 2011). The fate of migratory individuals beyond this point remains unknown. The place of origin of dragonflies that arrive to Middle Asia in spring is also unknown.

We used stable isotope analysis of hydrogen and oxygen (D/H or δ2H; 18O/16O or δ18O) to study the seasonal latitudinal migrations of *S. fonscolombii* in Middle Asia. The main aim was to compare isotopic signatures of different cohorts of dragonflies: (i) migrating to Middle Asia in the spring (immigrants); (ii) developing in the study area in the summer (residents); and (iii) migrating south from the northern parts of the range in the autumn (transit individuals). Based on the well-known patterns of the latitudinal trends in δ2H and δ18O values in precipitation (Terzer et al., 2013), we assumed immigrants would be enriched in 2H and 18O compared to residents and transit individuals, confirming its southern origin. In addition, a likelihood-based assignment approach and a comparison of the obtained data with isoscapes should indicate the probable area of origin of migrants (Bowen, 2010). Stable carbon (δ13C) and nitrogen (δ15N) isotope analyses were made for the tissue with different level metabolic activity (wings and legs) to clarify the geographical distribution of *S. fonscolombii*. 

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Table 1. Sites, dates of collection, and the \( \delta^2\text{H} \) values for *Sympetrum fonscolombii* (sample code: K, Kazakhstan; T, Tajikistan; U, Uzbekistan). Collection sites are shown on the map (Fig. 1).

| Sample | Cohort | Locality    | Country | Altitude, m.a.s.l. | Latitude, N | Longitude, E | Date collected | Mean \( \delta^2\text{H} \) wing, ‰ | \( \delta^2\text{H} \) wing 95% CI, ‰ | Mean \( \delta^2\text{H} \) body, ‰ | \( \delta^2\text{H} \) body 95% CI, ‰ |
|--------|--------|-------------|---------|--------------------|-------------|--------------|---------------|----------------|-------------------------------|----------------|------------------------------|-------------------------------|
| K1     | Immigrant | Bayanaul  | Kazakhstan | 464 | 50°49′ | 75°45′ | 30.VI.2013 | -90.6 | -108.2; -73.0 | -52.8; -72.2 | -33.5 |
| K2     | Immigrant | Kerbulak  | Kazakhstan | 430 | 44°11′ | 76°55′ | 24.VI.2006 | -105.6 | -111.1; -100.0 | -69.3; -75.3 | -63.1 |
| K2     | Immigrant | Kurty     | Kazakhstan | 520 | 43°54′ | 76°20′ | 19.VI.2006 | -113.9 | -121.9; -112.0 | -81.6; -77.3 | -80.4 |
| K3     | Immigrant | Almaty    | Kazakhstan | 670 | 43°20′ | 77°00′ | 21.VI.2005 | -114.9 | -121.9; -112.0 | -81.6; -77.3 | -80.4 |
| K3     | Immigrant | Kyzylorda | Kazakhstan | 170 | 45°50′ | 64°55′ | 29.V.1976 | -85.7 | -121.4; -50.1 | -47.4; -86.7 | -8.3 |
| K3     | Resident  | Kyzylorda | Kazakhstan | 170 | 45°50′ | 64°55′ | 18.VIII.1976 | -116.8 | -120.6; -112.9 | -86.5; -75.8 | -77.3 |
| K4     | Transit   | Chokpak   | Kazakhstan | 1183 | 42°31′ | 70°36′ | 26.IX.2008 | -116.9 | -121.9; -112.0 | -81.7; -78.2 | -76.3 |
| K4     | Transit   | Chokpak   | Kazakhstan | 1183 | 42°31′ | 70°36′ | 15.II.2009 | -131.5 | -139.7; -123.3 | -97.8; -106.7 | -88.8 |
| T1     | Immigrant | Dushanbe  | Tajikistan  | 440 | 37°54′ | 68°51′ | 26.IV.1987 | -93.7 | -129.5; -57.9 | -56.2; -95.5 | -16.9 |
| T2     | Immigrant | Krylov    | Tajikistan  | 440 | 37°54′ | 68°51′ | 1.V.2014 | -92.1 | -129.5; -57.9 | -56.2; -95.5 | -16.9 |
| T3     | Resident  | Kayrakkum | Tajikistan  | 330 | 40°16′ | 69°47′ | 30.VII.1987 | -129.1 | -140.0; -118.1 | -95.1; -107.1 | -83.1 |
| T4     | Resident  | Parkhar   | Tajikistan  | 470 | 37°30′ | 69°23′ | 10.VII.1984 | -147.7 | -150.5; -144.9 | -115.6; -112.4 | -112.4 |
| U1     | Resident  | Shollik   | Uzbekistan  | 270 | 40°29′ | 69°07′ | 04.VI.1980 | -120.0 | -143.3; -96.5 | -85.1; -110.7 | -59.3 |
| U2     | Immigrant | Bekabad   | Uzbekistan  | 290 | 40°16′ | 69°15′ | 24.VI.1980 | -102.5 | -111.5; -93.4 | -65.9; -75.7 | -55.9 |

area of development and nutritional habits of migratory dragonflies. We expected that isotopic signatures of wings (metabolically inert tissue, therefore, a marker for ponds within which larvae developed) and legs (metabolically active tissues) would differ considerably in immigrants and transit individuals, whose preimaginal development and imaginal life have taken place in different parts of the range, whereas in residents the difference between wings and legs should be small.

Materials and methods

Sample collection

Tissues of 98 individuals of *S. fonscolombii* collected in different regions of Middle Asia were used for isotope analysis. The sampled dragonflies came from an area extending 13°19′ (more than 1500 km) in latitude. We used material collected over many years (1976–2014) because it is difficult to collect enough migratory dragonflies in different seasons [spring immigration, summer (aboriginal individuals), and autumn migration] during one or two field seasons. In accordance with other authors (Hobson et al., 2012b), we assumed that dry museum specimens stored for a long time, are suitable for isotope analysis. After capture, dragonflies were killed by ethyl acetate, dried, and stored on cotton wadding. It is known that acetone does not affect \( \delta^2\text{H} \) values in dragonfly wings (Hobson et al., 2012b). Although there is no study addressing the effect of ethyl acetate on \( \delta^2\text{H} \) values in wings, it can be argued that the chemical effects of both volatile solvents are similar. Data on location and dates of collection of dragonflies are given in Table 1 and on the map (Fig. 1). The dragonfly specimens used in this study are stored in the collection of the Institute of Systematics and Ecology of Animals of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk).

![Fig 2. Specimens of *Sympetrum fonscolombii* of different cohorts. Immigrants are at the top, and residents are at the bottom (males are on the left, and females are on the right). [Colour figure can be viewed at wileyonlinelibrary.com].](image-url)

Three different cohorts of dragonflies were used for the analysis: immigrants, residents, and transit dragonflies. In Middle Asia, the arrival dates of immigrants (April–June) and the emergence dates of their descendants (June–October) overlap, therefore, in June both immigrant and resident individuals are found at the same time. They were differentiated by body colouration, degree of sclerotisation of the chitinous exoskeleton, and worn-out wings (Fig. 2).

**Immigrants.** Dragonflies (n = 29), caught from April 26 to June 30, which are thought to arrive in Middle Asia from the
more southern parts of the range. These were ‘old’ individuals, often with conspicuously worn-out wings. They were different in colour from dragonflies that recently acquired wings. All specimens were characterised by a deep-red in males and brown or dark olive body colouration in females (Fig. 2). The latitudinal range of the dragonfly specimens in this group was from 37°54′N in Tajikistan to 50°49′ in Kazakhstan.

Residents. Dragonflies (n = 36) caught from June 4 to August 18. Their body colour was light-yellow in both sexes, and often with a chitinous exoskeleton that was not yet fully strong. Dry specimens had a wrinkled chitinous cover due to storage (Fig. 2). These are aboriginal individuals, developed in the locations where they were caught. The latitudinal range of these dragonfly specimens was from 37°30′N in Tajikistan to 44°50′N in Kazakhstan.

Transit specimens. Dragonflies (n = 33), caught in ornithological traps in the Chokpak Pass in southern Kazakhstan (42°31′N) from September 26 to October 11 during autumn migrations from the north to the south. In September, light yellow individuals with recently acquired wings were captured in the traps. In October, these were more ‘mature’ individuals, with a reddish body in males and olive colouration in females (Borisov, 2011).

The isotopic composition of H and O of dragonfly wings was determined. For isotope C and N analysis, wings and legs of every individual were used to identify possible cumulative differences in nutrition/habitat at the larval and imaginal phases of the dragonflies, which migrate over long distances. The C and N isotopic composition of muscle tissue reflects changes in insect nutrition and is similar to that of the legs (Tsurikov et al., 2015). Therefore, we considered the legs as a marker of the current nutrition of a mature insect. In contrast, wings are a chitinous tissue formed during larval nutrition with the heavy isotope to light isotope molar ratio. Reference material EMA-P2 (Elemental Microanalysis Catalogue No. B2205) for H and O, and USGS 40, USGS 41 (glutamic acid), and IAEA-CH-3 (cellulose) for C and N. Stable hydrogen isotope analysis was conducted using the comparative equilibrium method (Wassenaar & Hobson, 2003; Hobson et al., 2012b). In addition to USGS reference materials KHS (kudu horn, non-exchangeable $\delta^1^3^C$ = −35.3 ± 1.1‰, $\delta^1^8^O$ = 20.3 ± 0.3‰) and CBS (caribou hoof, non-exchangeable $\delta^2^H$ = −157.0 ± 0.9‰, $\delta^1^8^O$ = 3.8 ± 0.3‰), specially prepared laboratory standards were used: homogenised dragonfly wings of Crocothemis erythraea (Brullé, 1832) from Tajikistan (DS1, $\delta^2^H$ and $\delta^1^8^O$: −111.6‰ and 15.2‰, respectively), S. sanguineum (Müller, 1764) from Kazakhstan (DS2, $\delta^2^H$ and $\delta^1^8^O$: −110.1‰ and 16.2‰, respectively), S. flavoletum (Linnaeus, 1758) from DS3, $\delta^2^H$ and $\delta^1^8^O$: −110.2‰ and 15.6‰, respectively), mountain hare fur (Lepus timidus Linnaeus, 1758) from the Arctic Circle (Chukotka) (DS4, $\delta^2^H$ and $\delta^1^8^O$: −138.2‰ and 5.9‰, respectively), and human hair (DS5, $\delta^2^H$ and $\delta^1^8^O$: −70.8‰ and 13.0‰, respectively). The $\delta^2^H$ values and non-exchangeable $\delta^2^H$ values for five laboratory standards were obtained from IsoAnalytical Ltd (Crewe, Great Britain). Non-exchangeable $\delta^2^H$ values were obtained using a three-point linear calibration after equilibration with USGS42 (human hair, non-exchangeable $\delta^2^H$: −44.4 ± 2.0‰), USGS43 (human hair, non-exchangeable $\delta^2^H$: −72.9 ± 2.2‰), and Eurofins 11/2/C (casein, non-exchangeable $\delta^2^H$: −113.37 ± 3.8‰) reference materials. For the $\delta^1^3^C$/$\delta^1^5^N$ and $\delta^1^5^N$:/$\delta^1^4^N$ ratio analysis, casein was used as the laboratory standard (Elemental Microanalysis Ltd, Okehampton, U.K.). Analytical error (SD) of the determination of isotopic composition in standard materials did not exceed 2‰ for $\delta^2^H$, 1‰ for $\delta^1^8^O$, and 0.2‰ for $\delta^1^5^N$ and $\delta^1^3^C$.

Likelihood-based assignment of geographic origins

We estimated the geographic origin of the dragonflies using IsoMAP, a framework that allows to model, predict, and analyse stable hydrogen isoscapes (Bowen et al., 2014; https://isomap.r.cac.purdue.edu/isomap/). We created a geostatistical model of $\delta^2^H$ values in precipitation ($\delta^2^H_p$) as a function of latitude, and squared latitude over the years 1960–2010 (IsoMAP job key: 77950). We included precipitation isotopic data collected from January to December as S. fonscolombii develops in different parts of the range throughout the year. This model was used to estimate the spatial distribution of $\delta^2^H_p$ values across the S. fonscolombii range within a longitudinal range of 19.5°W to 93.3°E and a latitudinal range of 36.6°S to 59.7°N (IsoMAP job key: 77953). Following Bowen et al. (2014), the $\delta^2^H$ values for wings were converted into the precipitation hydrogen equivalents by applying the calibration equation ($\delta^2^H_{wing} = 0.91*\delta^2^H_p − 42.54$‰, Hobson et al., 2012b). We calculated the precipitation hydrogen equivalent in each of the wing samples. In this preliminary analysis, the efficiency of the likelihood-based assignment method was checked by estimating the probable origin of the resident dragonflies of known origin. For this, we generated a geographic probability assignment for the mean $\delta^2^H$ value for each collection site (12 sites in total; Table 1) by including it and a standard deviation of 14.7‰ estimated from the residuals of the regression linking $\delta^2^H_{wing}$ and $\delta^2^H_{precip}$.
MigrationsofdragonfliesinMiddleAsia

Fig 3. The δ²H values for wings of immigrants, residents, and transit specimens of *Sympetrum fonscolombii* from different locations, and the average values for cohorts (within the cohort, the locations are aligned in order of increasing latitude). Significant differences between the three cohorts are indicated by different upper case letters, and significant differences within the cohort are indicated by different lower case letters (unequal n Tukey HSD, *P* < 0.05). Means and 95% confidence intervals are shown.

Data analysis

Data met assumptions for parametric analysis and were, therefore, analysed using one-way ANOVA and Tukey HSD test for unequal sample sizes. Relationships between variables were determined using Pearson correlation. The difference Δδ = δₑₑₑ − δₑₑₑ was calculated for each individual to compare isotopic signatures of carbon and nitrogen in different tissue. Statistical analysis was conducted using SPSS 16.0. The average value and 95% CI are given as the central trend and variation.

Results

Isotopic composition of hydrogen

The three cohorts of dragonflies differed significantly in the isotopic composition of non-exchangeable hydrogen in wings (ANOVA, *F*₂,₉₅ = 60.1, *P* < 0.0001): δ²H value for wings was the highest for immigrants [−96.1‰ (−100.7‰; −91.5‰), range from −123.1 to −72.6‰] and significantly lower among residents [−134.3‰ (−139.6‰; −129.0‰), range from −156.2 to −100.9‰] and transit individuals [−123.6‰ (−128.6‰; −118.5‰), range from −169.4 to −99.8‰; Fig. 3].

The δ²H values for wings of immigrants collected in different locations were similar and did not depend on the date and geographical latitude of the collection (Table 1; Fig. 3).

Transit dragonflies that were caught at the Chokpak Pass during autumn migrations in September 2008 had higher δ²H values for wings than those that were caught in October 2009 [−116.9‰ (−121.9‰; −112.0‰) and −131.5‰ (−139.7‰; −123.3‰), respectively; Fig. 3].
Fig 4. Dependence of the $\delta^2$H values for wings of Sympetrum fonscolombii residents on the latitude of sites of capture in Middle Asia. The designations of localities are as in Table 1.

Likelihood-based assignment of geographic origins

To validate the geographic assignment method based on the $\delta^2$H isoscape (Fig. 5), we estimated the natal regions of S. fonscolombii residents, which are known to develop in situ in Middle Asia. The probability surfaces of origin for residents did not coincide with their collection sites (Figure S1a–d). In the Middle Asian region, the predicted areas of origin of residents are shifted along the plains to the north and towards the nearest mountains (Figure S1a–d). This fact significantly complicates the use of the likelihood-based assignment method for assessing the geographical origin of immigrants (Figure S1e–j) and transit individuals (Figure S1k, l).

Isotopic composition of oxygen

The correlation between $\delta^2$H and $\delta^{18}$O values for wings was positive for immigrants ($r^2 = 0.769$, $P < 0.0001$) and residents ($r^2 = 0.901$, $P < 0.0001$); however, such a correlation was absent for transit individuals ($r^2 = 0.05$, $P > 0.05$; Fig. 6). Significant differences in $\delta^{18}$O values for wings were found between dragonfly cohorts ($F_{2,95} = 77.10$, $P < 0.0001$). The $\delta^{18}$O values were high, with relatively low variability, in immigrants [18.8‰ (18.0‰; 19.5‰)] and transit dragonflies [17.8‰ (17.3‰; 18.3‰)], but the values were low in residents [11.1‰ (9.7‰; 12.4‰)], with high variability (Figs 7 and 8a).

Isotopic composition of carbon

The $\delta^{13}$C values for wings of immigrants and transit dragonflies [–23.2‰ (–24.7‰; –21.7‰) and –23.8‰ (–24.7‰; –22.9‰), respectively] were significantly lower than the $\delta^{13}$C values for wings of residents [–20.6‰ (–21.5‰; –19.7‰); $F_{2,96} = 11.35$, $P < 0.0001$; Fig. 8a].

Isotopic composition of nitrogen

Imigrants had wing $\delta^{15}$N values with a range nearly twice as wide (2.9–14.2‰) as the range for residents (5.0–11.3‰) and transit dragonflies (4.1–10.3‰). The average $\delta^{15}$N value for wings of immigrants [9.4‰ (8.3‰; 10.5‰)] was higher than those of residents and transit dragonflies [6.9‰ (6.3‰; 7.6‰) and 7.3‰ (6.8‰; 7.8‰), respectively; $F_{2,94} = 12.71$, $P < 0.001$; Fig. 8b].

A comparison of $\delta^{13}$C and $\delta^{15}$N values for the wing and leg tissue

The isotopic signatures of wings and legs of dragonflies were highly correlated ($\delta^{13}$C: $r^2 = 0.929$, $P < 0.0001$; $\delta^{15}$N: $r^2 = 0.925$, $P < 0.0001$). The difference in $\delta^{13}$C values between wings and legs of immigrants was on average small but varied significantly [mean $\Delta\delta^{13}$C: 0.1‰ (–0.4‰; 0.6‰); the range of $\Delta\delta^{13}$C values was 6.3‰, which was 1.5 times higher than that for residents and transit dragonflies (4.0‰). Residents differed from transit dragonflies by a lower average $\Delta\delta^{13}$C value [–0.2‰ (–0.4‰; 0.0‰) and 0.4‰ (0.1‰; 0.7‰), respectively; $F_{2,92} = 3.19$, $P < 0.05$; Fig. 9]. According to the range and average values for the difference in $\delta^{13}$N between wings and legs, the three dragonfly cohorts were similar [$\Delta\delta^{15}$N: –0.1‰ (–0.3‰; 0.2‰) for immigrants, –0.3‰ (–0.5‰; –0.1‰) for residents, and 0.0‰ (–0.5‰; 0.3‰) for transit dragonflies; $F_{2,92} = 2.44$, $P = 0.09$; Fig. 9].
Fig 6. The relationship between $\delta^{2}$H and $\delta^{18}$O values for dragonfly wings of different cohorts of *Sympetrum fonscolombii*.

Fig 7. The $\delta^{18}$O values for wings of three cohorts of *Sympetrum fonscolombii* dragonflies from different locations and the average $\delta^{18}$O values for the cohorts (within the cohort, the locations are aligned in order of increasing latitude). Significant differences between the cohorts are indicated by different lead letters, significant differences within the cohort are indicated by different block letters (unequal n Tukey HSD, $P < 0.05$). Means and 95% confidence intervals are shown.

**Discussion**

The use of isotopic signatures as natural markers of the region of origin is the most relevant method for studying insect migration. The small size of migrants and the negligible chance that they would be caught repeatedly limit the use of any extrinsic methods of marking, especially for insects, which can migrate large distances of thousands of kilometres. For instance, *Vanessa cardui* butterflies migrate from Europe to Africa in the autumn, crossing the Sahara (Stefanescu et al., 2016), and *Pantala flavescens* dragonflies migrate from the north of India to East Africa, crossing the Arabian Sea (Hobson et al., 2012a). Previously, mainly hydrogen has been used for studying insect migration by isotope analysis. In our study of dragonfly migration, we simultaneously used the content of stable isotopes of four biogenic elements (H, O, C, N) in different tissue.

Determination of natal origins of migratory dragonflies in Asia using data on the distribution of $\delta^{2}$H in precipitation ($\delta^{2}$H$_{p}$) causes difficulties associated with the proximity of mountains and deserts (Bridge et al., 2014) and the lack of Global Network of Isotopes in Precipitation (GNIP) stations (Pekarsky et al., 2015). In particular, in Middle Asia, it is difficult to use $^{2}$H and $^{18}$O content as markers of the sites of development of amphibiont insects due to the overlap in latitudinal and altitudinal patterns of distribution of $\delta^{2}$H$_{p}$ and $\delta^{18}$O$_{p}$ values.

Here, high mountain systems are co-located with vast plains. According to the altitudinal pattern, values for $\delta^{2}H$ and $\delta^{18}O$ are low in the mountains, and therefore, vastly different from those in the neighbouring lowlands (Bowen, 2010; Terzer et al., 2013). In fact, the lowest values for $\delta^{2}H$ outline the configuration of the Tien-Shan and the Pamir-Alay mountain systems in Middle Asia, as well as the Hindu Kush and the Himalayas. The $\delta^{2}H$ and $\delta^{18}O$ values in the mountains of Middle Asia are virtually equal to those in the lowlands of Asia, which are located far to the north (50–60°N; Terzer et al., 2013). In the arid climate of Middle Asia with low rainfall in the summer, in the lowlands, amphibiont insects develop in water bodies, which feed on different sources, including mountain rivers. Additional variation in $\delta^{2}H$ and $\delta^{18}O$ values associated with local variability in the size of water bodies, evapotranspiration, hydrology, and water source is difficult to consider. The influence of these factors on the predicting of natal origins of aquatic insects according to their $\delta^{2}H$ and $\delta^{18}O$ values requires future examination.

Isotopic composition of $H$ and $\delta^{2}H$-based assignment of geographic origins

In the case of residents, the trend in the increase of the $\delta^{2}H$ value for wings in a northward direction (Figs 3 and 4) deserves special attention. This is contrary to the well-known regularity of latitudinal changes of $\delta^{2}H$ of meteoric waters in the lowlands;
the northern areas usually have lower δ²Hₚ values (Bowen et al., 2005; Terzer et al., 2013). We associate this trend with the fact that the closer the visitors are to the sites of dragonfly capture, the lower are the δ²H values for dragonflies wings (Figs 1 and 4). In our case, the southernmost site of residents (T4, 37°N; the average δ²H value for wings was −148‰) is in close proximity to the Western Pamir and the Hindu Kush mountain systems, and the northernmost site (K3, 44°N; the average δ²H value for wings was −117‰) is in the Turan Lowland at some distance from the mountains. It should be emphasised that in this case the leading role is played by the proximity of the high-mountain river basins, but not the absolute altitude of the residents’ locations.

Low summer precipitation and mountain sources feeding temporary ponds of dragonfly development in Middle Asia may explain the discrepancy between the probability surfaces of origin for residents and the collection sites, especially for residents from T3 and U1 locations (Figure S1b, d). To a lesser extent, the mountain influence explains the northward deviation of the assigned origins for residents from the remote location K3 (Figure S1a) and T4 location, where according to isoscape (Fig. 5) places with such low δ²Hₚ values are rare (Figure S1c).

Additional reasons for the discrepancy in the prediction of origin for residents may relate to incomplete geostatistical model data in Asia (one GNIP station in Middle Asia, 51 stations in South-West Asia, mostly near the Mediterranean coast; Pekarsky et al., 2015) and peculiarities of seasonal variation in δ²Hₚ value (Terzer et al., 2013). Equally important for the likelihood-based assignment of geographic origins is that the calibration equation linking δ²Hₚ and δ²H values we used was developed on several species of dragonflies from North America (Hobson et al., 2012b) and was tested in the study of dragonfly migrations through Hindustan (Hobson et al., 2012a). The development of the δ²Hₚ/δ²H calibration equation for amphibious insects originating from Eurasia, including Middle and South-West Asia, will improve the accuracy of determining their likely origin in the future.

Immigrants had noticeably worn-out wings, which, as well as features of colouration, indicates an older age (Fig. 2; Borisov, 2011). The absence of differences in the δ²H values for wings of immigrants collected in different locations (Fig. 3) suggests that dragonflies, which arrive in both the southern and northern regions of the study territory (Fig. 1) in the spring and early summer, originate approximately from one region and may belong to a single ‘wave’ of immigrants. The significantly higher δ²H values in immigrants compared to those in residents show that they developed in different geographical areas. Probability surfaces of origin for immigrants covered the bulk of Europe, West and South Asia (Figure S1e–j), which is highly questionable as the wintering of S. fonscolombii larvae north of 36–39°N is apparently not possible due to temperature conditions (Dijkstra & Kalkman, 2001; Weihrauch & Weihrauch, 2003; Borisov, 2011). This limited southern part of probability surfaces of origin for immigrants includes the northern part of the Arabian Peninsula, the Levant, Iran, Afghanistan, and Pakistan (Figure S1e–j). Nevertheless, the application of the likelihood-based assignment approach to assess the origins of dragonflies in Asia is difficult and needs to be improved. It may be inferred that the proposed natal regions of S. fonscolombii immigrants lie in South-West Asia below ~36°N.

To date, there have been only four studies of dragonfly migration using isotope analysis. Two relate to migrations in North America (Hobson et al., 2012b; Hallworth et al., 2018), and the remaining two focus on migrations of P. flavescens in Asia (Hobson et al., 2012a; Cao et al., 2018). Based on the hydrogen isotopic signatures of P. flavescens, which were caught in the Maldives in November–December, it has been assumed that they developed in the northern part of the Indian subcontinent, and, probably, even further north and east (Hobson et al., 2012a). The δ²H value for the wings of these dragonflies were −117±16‰ with a high variation (n = 49, range from −151 to −83‰). The significant overlap of δ²H values for wings in P. flavescens from the Maldives and in S. fonscolombii residents from Middle Asia (range from −156 to −101‰) is noteworthy. In our view, it may indicate that P. flavescens individuals, which develop in much a wider territory than the north of the Indian subcontinent, including, probably, Middle Asia where this species is common, are centred in the Maldives during autumn migrations (Borisov, 2015).

Isotopic composition of O, C, and N

There are similarities between the patterns of the global distribution of δ²H and δ¹⁸O (Bowen, 2010). Therefore, the patterns of δ²H and δ¹⁸O values in S. fonscolombii should be similar, as has been shown for other aquatic insects (Myers et al., 2012; Hobson et al., 2012b). Indeed, a positive correlation between δ²H and δ¹⁸O in S. fonscolombii wings was found for immigrants and residents, although it was absent in transit individuals (Fig. 6). The δ¹⁸O values for the wings of immigrants were on average 8‰ higher than those of residents (Fig. 8a). Based on the global distribution of the values for δ¹⁸O with more enriched values in the Arabian Peninsula and East Africa compared to collection sites (Terzer et al., 2013), this data confirmed the southerly origin of immigrants that arrived in the spring to Middle Asia.

The differences in carbon isotopic signatures between S. fonscolombii cohorts may be associated with the ecological features of natal habitats of dragonflies rather than with their different spatial origin. Residents had higher δ¹³C values for wings than did immigrants (Fig. 8a). These differences appear not to be related to the spatial distribution of C3 and C4 plants (Still et al., 2003). It is more likely that in different regions, ponds, where S. fonscolombii larvae developed, could differ in size, lifetime, degree of eutrophication, contributions of macrophytic and detrital resources, and other factors, which determine the variation in δ¹³C in aquatic ecosystems (Finlay & Kendall, 2007; Seifert & Scheu, 2012).

Nitrogen isotopic signatures in S. fonscolombii dragonflies can reflect δ¹⁵N values of food resources both in ponds and in terrestrial environments. It is therefore difficult to separate the factors affecting δ¹⁵N values of dissolved nitrates and ammonium in ponds (land cover, agriculture practices, fertiliser use; Finlay & Kendall, 2007) from those affecting the spatial
variation of $\delta^{15}N$ values of terrestrial plants, which serve as the basis for food webs (Amundson et al., 2003). A higher
(on average by 2‰) $\delta^{15}N$ value for wings of immigrants,
compared to those of residents and transit dragonflies, indicates
a differential agricultural load, as well as possibly the more
southerly and arid origin of immigrants (Amundson et al., 2003).

The isotopic signature of transit individuals

If the difference in origin between immigrants and residents is
obvious, based on the consistency of the isotope data for H,
O, and partly for N, it is more difficult to determine the nature
of S. fonscolombii transit dragonflies migrating to the south
during autumn due to their proximity to residents, according
to $\delta^2$H and $\delta^{13}N$ values, as well as to immigrants, according
to $\delta^{18}O$ and $\delta^{13}C$ values (Fig. 8). Noteworthy, only transit
dragonflies demonstrate a total absence of correlation between
$\delta^2$H and $\delta^{18}O$ in wings. Incoherence in isotopic signatures of H
and O may be explained by nutrition, physiology, metabolism,
and external conditions during larval development of transit
dragonflies in ponds (Vander Zanden et al., 2016), but especially
by a comparatively low range of $\delta^{18}O$ values. For example the
absence of a correlation between $\delta^2$H and $\delta^{18}O$ in feathers of
migratory birds is associated with the role of hydration in overall
nutrition (Pekarsky et al., 2015).

In September, the average $\delta^2$H value for the wings of transit
dragonflies was noticeably higher than in October (Fig. 3).
Therefore, it may be assumed that in general, individuals
migrating in September developed in regions closest to the sites
capture during migration, whereas individuals migrating in
October developed in more northern sites, and had lower $\delta^2$H
values for their wings. This is also supported by the fact that
individuals migrating later are similar to mature individuals in
their body colouration. This suggests that they had been in the
imaginal phase for a long time, and probably flew from afar,
likely from the northernmost parts of the range. In interpreting
the area of the probable origin of transit dragonflies, we may
claim only that they might have developed north of the latitude
of the place of their capture (Chokpak Pass, 42°N). The proposed
area of origin may include Kazakhstan and the southern regions
of the Ural and Western Siberia. These are the northern limits
of the range, where the development of summer generations of S. fonscolombii has been demonstrated (Borisov, 2011, 2015;
Popova & Eremina, 2016).

Between-tissue differences in $\delta^{13}C$ and $\delta^{15}N$ values

We assumed that the differences in isotopic signatures of C
and N between wings (an inert marker of nutrition of larva)
and legs (metabolically active tissue and marker of nutrition of
imago) would be related to a change in nutrient sources
during the transition of dragonflies from an aquatic to terrestrial
existence (Seifert & Scheu, 2012). Therefore, we expected that
higher $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values would be seen in immigrants
and transit individuals that migrated from the place of origin to
habitats with different isotopic signatures in plants and potential
prey. This was confirmed in part by the difference in $\delta^{13}C$
between wings and leg tissue being more pronounced in transit
dragonflies than in residents (Fig. 9); however, immigrants were
characterised by significant variability in $\Delta\delta^{13}C$ values, with a
range of values that encompassed those for residents and transit
individuals.

Migration circuit

Considering the relative locations of the proposed natal areas
of immigrants (South-West Asia) and areas of development of
residents in Middle Asia, spring migrations of S. fonscolombii are in a general direction from the southwest to the north-
east, and autumn migrations are probably in the opposite direction.
In South-West Asia, air circulation has pronounced seasonoal
differences (monsoon circulation). The winter monsoon (November–March) is in the direction of the northeast to south-
west and contributes to the autumn migration of S. fonscolombii from Middle Asia to the southwest. The summer monsoon
(May–October), in contrast, has a northeastern direction. It is
important to note that in the spring when the monsoons change,
the effect of the Iranian branch of the Polar front is strongly
evident in South-West Asia. In the spring (March–April), the
Iranian branch of the Polar front begins to shift northward and
passes through Middle Asia (Tajikistan, 1982). This is when S.
fonscolombii migrates in northerly and northeasterly directions.

The probable distance of spring migration of S. fonscolombii
between countries of South-West Asia and the localities of
immigrants in Kazakhstan (loc. K1, K2, K3) along a direct
route maybe about 2000–4000 km. Given that migration routes
of dragonflies are generally not straightforward and are related
to the direction of prevailing winds (Corbet, 1999), as well as
the ‘guide lines’ in the landscape along which dragonflies fly
(valleys and passes in the mountains, river valleys, coastlines,
etc.), the actual migration distance may be even longer.

Thus, based on the isotopic composition of H, O, N, and C
in the wings, it maybe concluded that the migration circuit of S. fonscolombii in Asia probably covers the region from the
proposed natal areas of immigrants in South-West Asia (below
~36°N) to Southern Ural and the south of Western Siberia in the
north (54–55°N) with a maximum migration distance of more
than 4000 km.

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Author contributions

Project design: SNB; data collection: SNB; stable isotope
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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Probability surfaces of origin for Sympertrum fonscolombii cohorts from different locations based on the annual geostatistical model of δ²H values in precipitation. Individual assignments generated for the mean δ²H values of dragonfly wings converted to δ²H₂O for each collection site (mean δ²H₂O values of cohorts are shown in figures). Probable areas of origin for residents collected in K3 (a), T3 (b), T4 (c), and U1 (d) localities, immigrants collected in K1 (e), K2 (f), K3 (g), T1 (h), T2 (i), and U2 (j) localities, and transit specimens captured in September (k) and October (l) in K4 locality are depicted. Collection sites are marked in red. Grayscale depict the predicted probability (white for low likelihood ~10⁻¹⁵, black for high likelihood ~10⁻⁵) of natal origins of dragonflies. The dashed line corresponds to the 36°N parallel that limits the development of the winter generation of dragonflies in the northern part of the range. The designations of localities are as in Table 1.

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