


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 ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) isotope analyses were conducted to confirm the migration of *S. fonscolombii* and determine the wintering area. Stable isotope composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) 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 $\delta^2\text{H}$ values in the wings were significantly higher in immigrants (-96‰) than in residents (-134‰) and transit individuals (-124‰). High $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values in the tissue of immigrants confirmed their southerly origin.

5. Based on the species range and the global distribution of annual averages of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ 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 $\sim 36^\circ\text{N}$) to Southern Ural and the south of Western Siberia in the north ($54\text{--}55^\circ\text{N}$) 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.

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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 ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) isotopic composition in animal tissue is connected to $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of precipitation ($\delta^2\text{H}_p$ and $\delta^{18}\text{O}_p$) 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 (isoscares) 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 (Parkhar village, 37°30'N, 69°24'E, 475 m a.s.l.), the earliest record of a single juvenile specimen was made on May 16. It remains unclear whether it is a first-generation (from possibly overwintered larvae) or a second-generation (a descendant of early spring immigrants). Mass emergence occurred there in late June (from June 27). In southwestern Turkmenistan (Kara-Kala village, 38°28'N, 56°16'E, 365 m a.s.l.), newly hatched individuals were observed on June 6, and the mass emergence began on June 15. The latest emergence was observed in southern Kazakhstan (Ak Biyk village, 42°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 (Lempert, 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

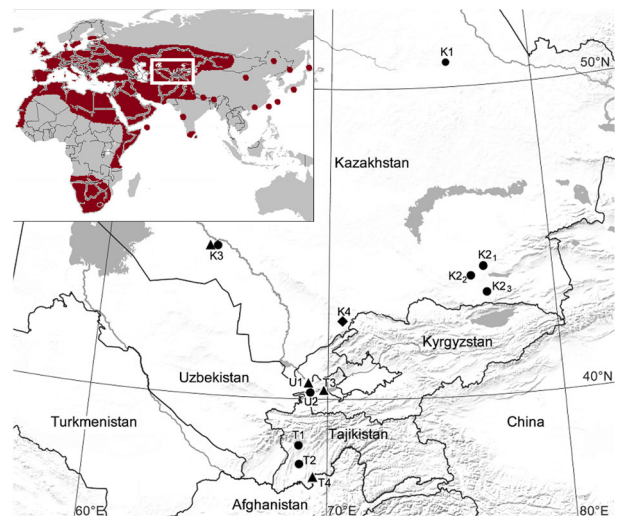


Fig 1. Sites of collection of *Sympetrum fonscolombii* for isotope analysis. The designations of localities are as in Table 1. The circle indicates immigrants, the triangle indicates residents, and the diamond indicates transit dragonflies. The inserted figure represents the range of *S. fonscolombii* (red colour). [Colour figure can be viewed at wileyonlinelibrary.com].

(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) (Weihrach & Weihrach, 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 $\delta^2\text{H}$; $^{18}\text{O}/^{16}\text{O}$ or $\delta^{18}\text{O}$) 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values in precipitation (Terzer *et al.*, 2013), we assumed immigrants would be enriched in ^2H and ^{18}O 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 isoscares should indicate the probable area of origin of migrants (Bowen, 2010). Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses were made for the tissue with different level metabolic activity (wings and legs) to clarify the geographical

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	n	Date collected	Mean $\delta^2\text{H}$ wing, ‰	$\delta^2\text{H}$ wing 95% CI, ‰	mean $\delta^2\text{Hp}$, ‰	$\delta^2\text{Hp}$ 95% CI, ‰
K1	Immigrant	Bayanaul	Kazakhstan	464	50°49'	75°45'	3	30.VI.2013	-90.6	-108.2; -73.0	-52.8	-72.2; -33.5
K2 ₁	Immigrant	Kerbulak	Kazakhstan	430	44°11'	76°55'	2	24.VI.2006	-105.6	-111.1; -100.0	-69.3	-75.3; -63.1
K2 ₂	Immigrant	Kurty	Kazakhstan	520	43°54'	76°20'	1	19.VI.2006				
K2 ₃	Immigrant	Almaty	Kazakhstan	670	43°20'	77°00'	1	21.VI.2005				
K3	Immigrant	Kyzylorda	Kazakhstan	170	44°50'	64°55'	3	29.V.1976	-85.7	-121.4; -50.1	-47.4	-86.7; -8.3
K3	Resident	Kyzylorda	Kazakhstan	170	44°50'	64°55'	9	18.VIII.1976	-116.8	-120.6; -112.9	-81.6	-85.8; -77.3
K4	Transit	Chokpak	Kazakhstan	1183	42°31'	70°36'	18	26.IX.2008	-116.9	-121.9; -112.0	-81.7	-87.2; -76.3
K4	Transit	Chokpak	Kazakhstan	1183	42°31'	70°36'	15	11.X.2009	-131.5	-139.7; -123.3	-97.8	-106.7; -88.8
T1	Immigrant	Dushanbe	Tajikistan	740	38°30'	68°48'	6	09.V.1980	-88.1	-93.1; -83.1	-50.1	-55.6; -44.6
T2	Immigrant	Krylov	Tajikistan	440	37°54'	68°51'	2	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	01.V.2014				
T3	Resident	Kayrakkum	Tajikistan	330	40°16'	69°47'	5	01.VII.1987	-129.1	-140.0; -118.1	-95.1	-107.1; -83.1
T4	Resident	Parkhar	Tajikistan	470	37°30'	69°23'	18	10.VII.1984	-147.7	-150.5; -144.9	-115.6	-118.6; -112.4
U1	Resident	Sholikor	Uzbekistan	270	40°29'	69°07'	4	04.VI.1980	-120.0	-143.3; -96.5	-85.1	-110.7; -59.3
U2	Immigrant	Bekabad	Uzbekistan	290	40°16'	69°15'	8	24.V.1980	-102.5	-111.5; -93.4	-65.9	-75.7; -55.9
U2	Immigrant	Bekabad	Uzbekistan	290	40°16'	69°15'	2	28.V.1980				

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].

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 isotopic composition hardly changing with time (Gratton & Forbes, 2006; Perkins *et al.*, 2013; Tsurikov *et al.*, 2015).

Stable isotope analysis

Samples of dragonfly wings were cleaned of surface lipids in 2 : 1 chloroform:methanol and dried for several days at 50 °C. Isotope analysis was conducted using TC/EA, EA Flash 1112, and isotope ratio mass spectrometer Thermo Delta V Plus at the Joint Usage Centre of the A.N. Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences (Moscow). Isotopic composition of hydrogen, oxygen, nitrogen, and carbon is expressed in per mil of deviation from international standards (VSMOW, VPDB, and atmospheric N), δ (‰): δX sample = ((R sample/R standard) - 1) 1000, where X is the element and R is the heavy isotope to light isotope molar ratio. Reference materials used to calibrate the mass spectrometer were: IAEA-CH-7 polyethylene, IAEA-601 benzoic acid, and IRMS certified 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 equilibration method (Wassenaar & Hobson, 2003; Hobson *et al.*, 2012b). In addition to USGS reference materials KHS (kudu horn, non-exchangeable $\delta^2\text{H}_{\text{V-SMOW}} = -35.3 \pm 1.1\text{‰}$, $\delta^{18}\text{O}_{\text{V-SMOW}} = 20.3 \pm 0.3\text{‰}$) and CBS (caribou hoof, non-exchangeable $\delta^2\text{H}_{\text{V-SMOW}} = -157.0 \pm 0.9\text{‰}$, $\delta^{18}\text{O}_{\text{V-SMOW}} = 3.8 \pm 0.3\text{‰}$), specially prepared laboratory standards were used: homogenised dragonfly wings of *Crocothemis erythraea* (Brullé, 1832) from Tajikistan (DS1, $\delta^2\text{H}$ and $\delta^{18}\text{O}$: -111.6‰ and 15.2‰, respectively), *S. sanguineum* (Müller, 1764) from Kazakhstan (DS2, $\delta^2\text{H}$ and $\delta^{18}\text{O}$: -110.1‰ and 16.2‰, respectively), *S. flaveolum* (Linnaeus, 1758) from Kazakhstan (DS3, $\delta^2\text{H}$ and $\delta^{18}\text{O}$: -110.2‰ and 15.6‰, respectively), mountain hare fur (*Lepus timidus* Linnaeus, 1758) from the Arctic Circle (Chukotka) (DS4, $\delta^2\text{H}$ and $\delta^{18}\text{O}$: -138.2‰ and 5.9‰, respectively), and human hair (DS5, $\delta^2\text{H}$ and $\delta^{18}\text{O}$: -70.8‰ and 13.0‰, respectively). The $\delta^{18}\text{O}$ values and non-exchangeable $\delta^2\text{H}$ values for five laboratory standards were obtained from IsoAnalytical Ltd (Crewe, Great Britain). Non-exchangeable $\delta^2\text{H}$ values were obtained using a three-point linear calibration after equilibration with USGS42 (human hair, non-exchangeable $\delta^2\text{H}_{\text{V-SMOW}} = -44.4 \pm 2.0\text{‰}$), USGS43 (human hair, non-exchangeable $\delta^2\text{H}_{\text{V-SMOW}} = -72.9 \pm 2.2\text{‰}$), and Eurofins 11/2/C (casein, non-exchangeable $\delta^2\text{H}_{\text{V-SMOW}} = -113.37 \pm 3.8\text{‰}$) reference materials. For the $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ 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 $\delta^2\text{H}$, 1‰ for $\delta^{18}\text{O}$, and 0.2‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

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.rcac.purdue.edu/isomap/>). We created a geostatistical model of $\delta^2\text{H}$ values in precipitation ($\delta^2\text{H}_p$) as a function of elevation data (ETOPO), 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 $\delta^2\text{H}_p$ 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 $\delta^2\text{H}$ values for wings were converted into the precipitation hydrogen equivalents by applying the calibration equation ($\delta^2\text{H}_{\text{wing}} = 0.91 * \delta^2\text{H}_p - 42.54\text{‰}$; 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 $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_{\text{wing}}$ and

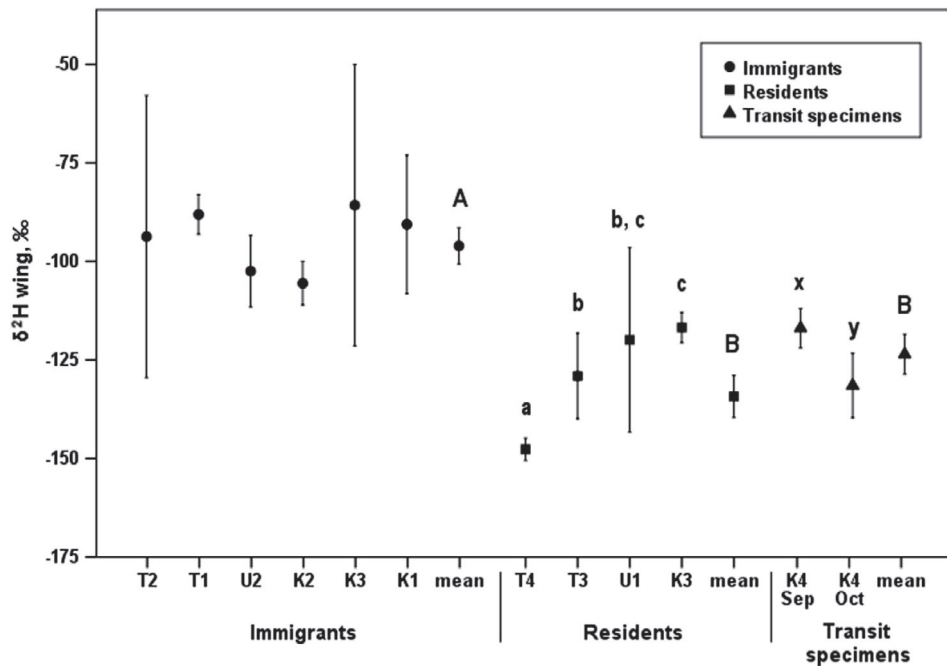


Fig 3. The $\delta^2\text{H}$ values for wings of immigrants, residents, and transit specimens of *Sympetrum fonscolombii* from different locations, and the average values for cohorts (within the cohort, the locations are aligned in order of increasing latitude). Significant differences between the three 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.

$\delta^2\text{H}_p$ (Hobson *et al.*, 2012b) to ‘individual assignment’ tool in IsoMAP. We then correlated the individual probability surfaces of origin with the resident locations. We did not conduct likelihood assignments for a group of samples by classifying individual probability surfaces as likely versus unlikely using a 3 : 1 odds ratio (Chabot *et al.*, 2012), as these individual assignments were sufficient to verify the robustness of the method. The $\delta^2\text{H}_p$ isoscape and the probability surfaces of origin for each site were illustrated with QGIS (Quantum GIS Development Team, 2020).

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 $\Delta\delta = \delta_{\text{leg}} - \delta_{\text{wing}}$ 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, $F_{2,95} = 60.1$, $P < 0.0001$): $\delta^2\text{H}$ 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 $\delta^2\text{H}$ 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).

The $\delta^2\text{H}$ values for wings of residents, which were caught in different places, were different. There were three groups of dragonflies in this cohort (Fig. 3). Residents from the lower reaches of the Syr Darya (location K3) had significantly higher $\delta^2\text{H}$ values for wings [-116.8‰ (-120.6‰ ; -112.9‰)] than residents from the middle reaches of the Syr Darya [locations T3 and U1; $\delta^2\text{H}$ of wings: -129.1‰ (-140.0‰ ; -118.1‰) and -120.0‰ (-143.3‰ ; -96.5‰), respectively], especially those from the lower reaches of Panj River in the south-west of Tajikistan [location T4; $\delta^2\text{H}$ of wings: -147.7‰ (-150.5‰ ; -144.9‰); Figs 1 and 3]. That is $\delta^2\text{H}$ values tended to increase with a northward advance ($r^2 = 0.699$, $P < 0.0001$; Fig. 4).

Transit dragonflies that were caught at the Chokpak Pass during autumn migrations in September 2008 had higher $\delta^2\text{H}$ 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].

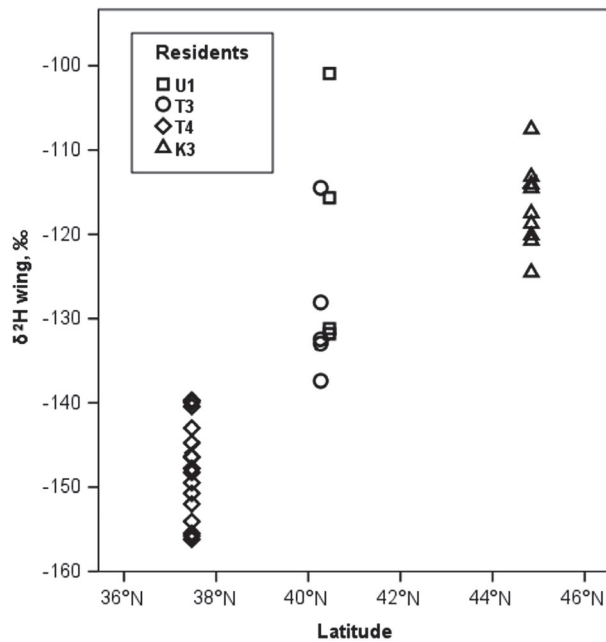


Fig 4. Dependence of the $\delta^2\text{H}$ values for wings of *Sympetrum fonscolombii* residents on the latitude of sites of capture in Middle Asia. The designations of localities are as in Table 1.

Likelihood-based assignment of geographic origins

To validate the geographic assignment method based on the $\delta^2\text{H}_p$ 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ values for wings were found between dragonfly cohorts ($F_{2,95} = 77.10$, $P < 0.0001$). The $\delta^{18}\text{O}$ 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 $\delta^{13}\text{C}$ values for wings of immigrants and transit dragonflies [−23.2‰ (−24.7‰; −21.7‰) and −23.8‰ (−24.7‰;

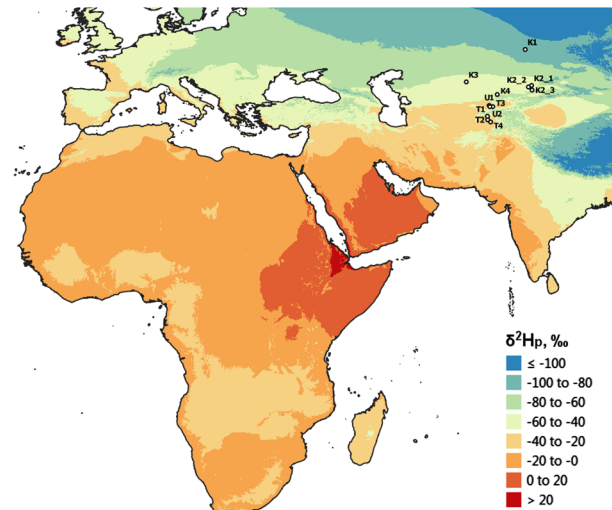


Fig 5. Predicted annual precipitation $\delta^2\text{H}$ isoscape for the *Sympetrum fonscolombii* dragonfly range. [Colour figure can be viewed at wileyonlinelibrary.com].

−22.9‰), respectively] were significantly lower than the $\delta^{13}\text{C}$ 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

Immigrants had wing $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the wing and leg tissue

The isotopic signatures of wings and legs of dragonflies were highly correlated ($\delta^{13}\text{C}$: $r^2 = 0.929$, $P < 0.0001$; $\delta^{15}\text{N}$: $r^2 = 0.925$, $P < 0.0001$). The difference in $\delta^{13}\text{C}$ values between wings and legs of immigrants was on average small but varied significantly [mean $\Delta\delta^{13}\text{C}$: 0.1‰ (−0.4‰; 0.6‰)]; the range of $\Delta\delta^{13}\text{C}$ 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 $\Delta\delta^{13}\text{C}$ values [−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 $\delta^{15}\text{N}$ between wings and legs, the three dragonfly cohorts were similar [$\Delta\delta^{15}\text{N}$: −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].

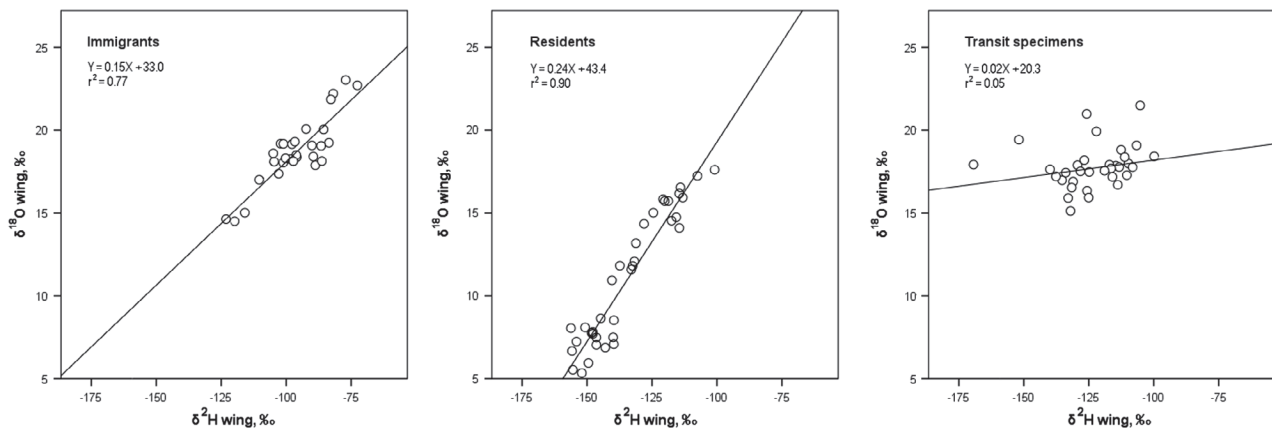


Fig 6. The relationship between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values for dragonfly wings of different cohorts of *Sympetrum fonscolombii*.

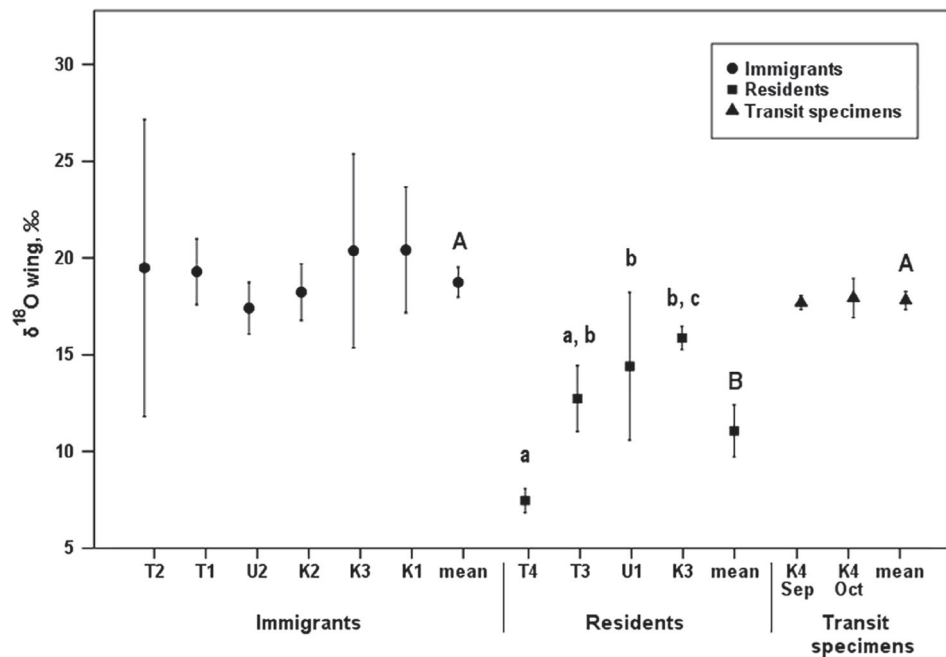


Fig 7. The $\delta^{18}\text{O}$ values for wings of three cohorts of *Sympetrum fonscolombii* dragonflies from different locations and the average $\delta^{18}\text{O}$ values for the cohorts (within the cohort, the locations are aligned in order of increasing latitude). Significant differences between 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 $\delta^2\text{H}$ in precipitation ($\delta^2\text{H}_p$) 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 ^2H 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 $\delta^2\text{H}_p$ and $\delta^{18}\text{O}_p$ values.

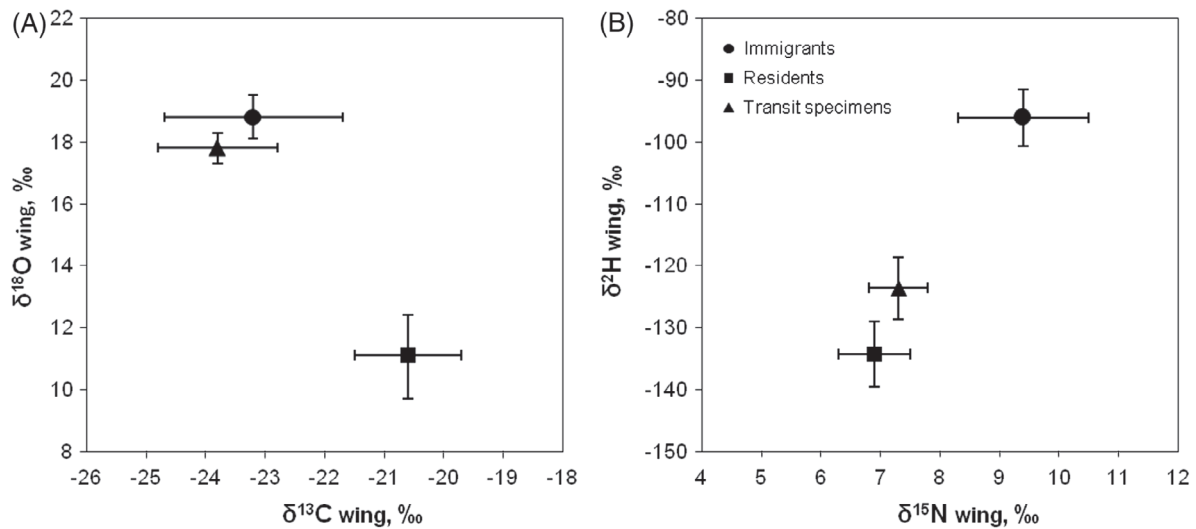


Fig 8. Multi-isotope signatures of *Sympetrum fonscolombii* cohorts: $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ (a), $\delta^2\text{H}$, and $\delta^{15}\text{N}$ (b) values for wings. Means and 95% confidence intervals are shown.

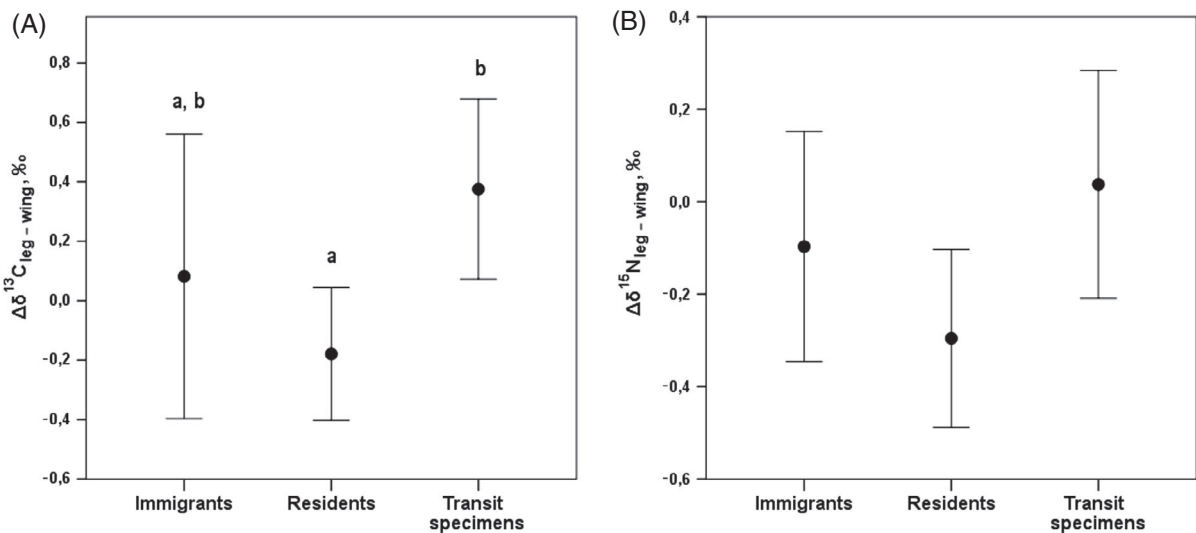


Fig 9. The difference in $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) values between leg tissue and wings of cohorts of *Sympetrum fonscolombii* dragonflies. Significant differences are indicated by different letters (unequal n Tukey HSD, $P < 0.05$). Means and 95% confidence intervals are shown.

Here, high mountain systems are co-located with vast plains. According to the altitudinal pattern, values for $\delta^2\text{H}_p$ and $\delta^{18}\text{O}_p$ 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 $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_p$ and $\delta^{18}\text{O}_p$ 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values requires future examination.

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

In the case of residents, the trend in the increase of the $\delta^2\text{H}$ 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 $\delta^2\text{H}$ of meteoric waters in the lowlands;

the northern areas usually have lower $\delta^2\text{H}_p$ values (Bowen *et al.*, 2005; Terzer *et al.*, 2013). We associate this trend with the fact that the closer the mountains are to the sites of dragonfly capture, the lower are the $\delta^2\text{H}$ values for dragonflies wings (Figs 1 and 4). In our case, the southernmost site of residents (T4, 37°N; the average $\delta^2\text{H}$ 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 $\delta^2\text{H}$ 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 their 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 $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_p$ value (Terzer *et al.*, 2013). Equally important for the likelihood-based assignment of geographic origins is that the calibration equation linking $\delta^2\text{H}_{\text{wing}}$ and $\delta^2\text{H}_p$ 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 $\delta^2\text{H}_{\text{wing}}/\delta^2\text{H}_p$ calibration equation for amphibiont 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 $\delta^2\text{H}$ 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 $\delta^2\text{H}$ 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 $\sim 36^\circ\text{N}$.

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 $\delta^2\text{H}$ value for the wings of these dragonflies were $-117 \pm 16\text{‰}$ with a high variation ($n = 49$, range from -151 to -83‰). The significant overlap of $\delta^2\text{H}$ 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 a much 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 $^2\text{H}_p$ and $^{18}\text{O}_p$ (Bowen, 2010). Therefore, the patterns of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in *S. fonscolombii* wings was found for immigrants and residents, although it was absent in transit individuals (Fig. 6). The $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}_p$ 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 its different spatial origin. Residents had higher $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ in aquatic ecosystems (Finlay & Kendall, 2007; Seifert & Scheu, 2012).

Nitrogen isotopic signatures in *S. fonscolombii* dragonflies can reflect $\delta^{15}\text{N}$ values of food resources both in ponds and in terrestrial environments. It is therefore difficult to separate the factors affecting $\delta^{15}\text{N}$ values of dissolved nitrates and ammonium in ponds (land cover, agriculture practices, fertiliser use; Finlay & Kendall, 2007) from those affecting the spatial

variation of $\delta^{15}\text{N}$ values of terrestrial plants, which serve as the basis for food webs (Amundson *et al.*, 2003). A higher (on average by 2‰) $\delta^{15}\text{N}$ 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 $\delta^2\text{H}$ and $\delta^{15}\text{N}$ values, as well as to immigrants, according to $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values (Fig. 8). Noteworthy, only transit dragonflies demonstrate a total absence of correlation between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$ values. For example the absence of a correlation between $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in feathers of migratory birds is associated with the role of hydration in overall nutrition (Pekarsky *et al.*, 2015).

In September, the average $\delta^2\text{H}$ 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 of capture during migration, whereas individuals migrating in October developed in more northern sites, and had lower $\delta^2\text{H}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$

between wings and leg tissue being more pronounced in transit dragonflies than in residents (Fig. 9); however, immigrants were characterised by significant variability in $\Delta\delta^{13}\text{C}$ 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 northeast, and autumn migrations are probably in the opposite direction. In South-West Asia, air circulation has pronounced seasonal differences (monsoon circulation). The winter monsoon (November–March) is in the direction of the northeast to southwest 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 may be 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 analysis: ASB, AGZ, IKI, AVT; data analysis: SNB, IKI, AVT;

paper writing: SNB, IKI, AVT. The authors have declared no conflict of interest.

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 *Sympetrum fonscolombii* cohorts from different locations based on the annual geostatistical model of $\delta^2\text{H}$ values in precipitation. Individual assignments generated for the mean $\delta^2\text{H}$ values of dragonfly wings converted to $\delta^2\text{H}_p$ for each collection site (mean $\delta^2\text{H}_p$ 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 $\sim 10^{-15}$, black for high likelihood $\sim 10^{-5}$) 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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