SUPPLEMENTAL INFORMATION

Tracking Dragons: Stable isotopes reveal the annual cycle of a long-distance migratory insect

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SUPPLEMENTAL METHODS

*First flight temperature threshold* - Daily mean temperature obtained from PRISM Gridded Climate Data[1] on the first flight date was extracted and summarized for each observation location from 1994 – 2014 which spanned the contemporary observations.

*Habitat suitability* - We generated a habitat suitability surface to help refine stable isotope geographic assignments. The habitat suitability surface was constructed using occurrence records obtained from Global Biodiversity Information Facility ([2], n = 5099) combined with our sampling locations (n = 1188) using Maxent [3] accessed through R [4] via the dismo [5] package (see SI for more information). Duplicate locations were removed from the dataset resulting in 4226 occurrence locations. The habitat suitability model was generated using a training dataset that encompassed 80% of the occurrence records. We withheld 20% of the data to test the accuracy of predicted suitability surface. Biologically relevant variables likely to influence common green darner habitat preferences and development were included in the Maxent model. Those included mean annual temperature, minimum temperature of the coldest month, annual precipitation, precipitation of the wettest month, precipitation of the driest month and elevation. Environmental layers were obtained from WorldClim.org accessed through the raster package [6]. The modelled habitat suitability surface fit the test data well (AUC = 0.873, Fig. S5).

*Stable-isotope analysis* - Isotope analyses were performed at the Smithsonian Institution’s Stable Isotope Mass Spectrometry Laboratory in Suitland, MD, USA. Common green darner wings were washed with a 2:1 chloroform:methonal solution to remove surface oils and debris. Samples were placed in the lab 72h prior to processing to allow tissues to equilibrate to the local atmosphere [7]. A small distal portion of the wing (0.350 ± 0.001, mean ± 1 SE) was removed and packed into a silver capsule. Exuviae were prepared the same way as wings. Tissue samples were combusted in an elemental analyzer and introduced to an isotope ratio mass spectrometer via a Conflo IV interface. One in-house standard was run for every 5 unknowns to measure accuracy and precision. Analytical error was minimal (2.63 ± 0.84 ‰) based on replicate analysis of the same tissue samples (n = 110). The non-exchangeable hydrogen was determined by linear regression with calibrated in-house keratin based standards (Caribou Hoof Keratin, -197 ‰; Spectrum Keratin, -121.6 ‰; and Kudo Horn Keratin, -54.1 ‰)[7].

*Creating a wing-scape -* Locally emerged teneral and exuviae from 77 locations collected in 4 countries (Bahamas, Canada, Mexica, and United States) and 20 states were used to identify the discrimination factor between the expected amount-weighted growing-season precipitation (δ2Hp , [8] and green darner wings (*δ*H2w, Fig. S6.). The resulting dragonfly isoscape was used to determine the origin of emergence for dragonflies collected throughout the year (Fig. S4).

*Determining origin of emergence -* Emergence assignments were conducted using a spatially explicit normal probability density function [9], where the likelihood that each *δ*H2w value, y\*, originated from a given location within the surface is

where μ­b is the specific cell within the dragonfly isoscape and σb is the standard deviation (15.80 ‰) of the residuals from the discrimination equation (see above). We included the habitat suitability surface as prior information using Baye’s rule in our geographic assignments. Doing so increases the likelihood that assignments are made in areas where habitat is suitable and decreases the likelihood of assignments being made to areas that are not suitable [9]. We truncated emergence assignments for individuals captured in the spring (January – May) to locations south of the 9.17°C isocline to ensure geographic assignments were biologically plausible. The resulting likelihood surface of emergence assignment was converted into a binary surface representing likely and unlikely natal origins following a 3:1 odds ratio [10].

*Delineating populations –*We categorized capture locations using United States Department of Agriculture hardiness zones (USDA 2012; http://planthardiness.ars.usda.gov) which are delineated into thermal zones (5.55°C) based on the average annual minimum temperature. We grouped capture locations that fell within hardiness zones 5-7 and zones 11-14 to increase the sample size in the northern and southern reaches of their distribution, respectively.

*Annual cycle & larval phenology* - We combined the phenology of migration derived via stable-isotopes with temperature related larval growth[11] and emergence timing[12] to better understand the full annual cycle. We estimated the date of larval emergence based on the number of accumulated growing degree-days (base = 8.7°C[11]) since oviposition. Our estimates of oviposition were constructed from the migration phenology of adults. We assumed larva emerged when the accumulated growing degree-days surpassed 1346[11]. Degree-day accumulation was derived from daily temperature minima and maxima from 1994-2014. Surface temperature was used as a proxy for surface water temperatures. Spatial-explicit daily temperature estimates were obtained from PRISM Gridded Climate Data[1].

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SUPPLEMENTAL FIGURES

Fig. S1. Common green darner natal origins determined using stable-hydrogen isotopes from wings of adults flying in March and April in the northern reaches of their distribution (A) and September and October in the southern reaches of their distribution (B). The color ramps (A: gray to blue, B: gray to red) represent the number of emergence origin assignments to a particular region collected at sampling locations represented by the black dots. The minimum migration distance from the emergence origin to the collection site of each individual (A: n = 50, B: n = 11) is shown in the figure inset. Mean migration distance in spring (680.97 ± 178.84 km) and fall (659.46 ± 49.11 km) along with the standard error are indicated with the dotted red line and gray shaded area, respectively.

Fig. S1.



Fig. S2. Minimum migration distance between natal origin and collection site of adult common green darners flying in the northern reaches of their distribution. Adults collected in March and April were pooled to increase sample size. Individuals captured late in the season, August and September were also combined to increase sample size. The number of adults captured in each month are shown inside the each column. Figure graphic was drawn by John Muir Laws and was used with permission.

Fig. S2.



Fig. S3. Migratory status of common green darners (*Anax junius*) captured at the northern (dark gray polygon) and southern (light gray polygon) terminus of their distribution. The proportion of migratory individuals (light-blue) and locally emerged (gray) individuals sampled are shown for each month in the northern (top right) and southern (bottom right) portion of their distribution. The dotted lines and shaded polygons represent the predicted proportion and 95% confidence interval within each month, respectively. Predictions were made using a loess smoother.

Fig. S3.



Fig. S4. Predicted common green darner (*Anax junius*) nymph development (black lines) within hardiness zones 7-11 assuming eggs are laid (black point) on the first day in each month flying adults are observed (gray boxes). Nymphs are able to eclose after accumulated growing degree days above a base of 8.7 °C reach 1346 days [10] (dotted horizontal line). Nymphs enter a state of diapause when growth is flat.

Fig. S4.



Fig. S5. Predicted habitat suitability of Common Green Darners. Habitat suitability (low suitability = 0, high suitability = 1) of darners was derived using Maxent from occurrence records. The area under the curve (AUC) was high indicating the predicted habitat suitability fit the data well.

Fig. S5



Fig. S6. Discrimination relationship between stable hydrogen isotope signatures found in amount-weighted growing-season precipitation (δ2Hprecipitation) and those found in local, recently emerged common green darner adults (tenerals) and exuviae (δ2Hwing). This discrimination relationship was used to create a common green darner isoscape for use in assigning the natal origins to adult common green darners captured throughout their range.

Fig. S6



SUPPLEMENTAL TABLES

Table S1. Common green darner (*Anax junius*) survey data of first flight observations. Survey locations are indicated by the state of observation followed by the region or county where the surveys took place. If only the state is listed, surveys were conducted statewide with the exception of Maryland (MD). Surveys were conducted at the Patuxent Wildlife Research Center, in Prince George's County, Maryland. The earliest observation date (First Flight Date) represents the earliest date where an adult was observed flying in the region. The geographic coordinates of the geometric center of the state, county or location of sampling and the period covered by surveys (Years; if reported) are reported. The mean elevation of the state, county or location of sampling was determined from the SRTM 90m digital elevation model (m above sea level) accessed through the raster package[6] in program R.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Survey Location | First Flight Date | Ordinal Day | Latitude | Longitude | Years  Surveyed | Elevation  m. asl |
| GA-RV1 | 23-Feb | 54 | 34.453 | -85.015 | - | 263.831 |
| GA-CP1 | 6-Mar | 62 | 32.062 | -83.008 | - | 84.146 |
| IN2 | 10-Mar | 69 | 39.920 | -86.282 | 1900-1940 | 228.571 |
| NC-M3 | 10-Mar | 69 | 35.611 | -82.785 | 1994-2014 | 923.248 |
| NJ4 | 13-Mar | 72 | 40.110 | -74.656 | 2002-2006 | 73.708 |
| NC-PD3 | 15-Mar | 74 | 35.384 | -80.776 | 1994-2014 | 228.481 |
| OK5 | 20-Mar | 79 | 36.551 | -96.255 | 2003-2007 | 265.179 |
| GA-PD1 | 22-Mar | 81 | 33.614 | -83.781 | - | 260.448 |
| MD6 | 24-Mar | 83 | 39.033 | -76.809 | 1991-1995 | 62 |
| NC-CP3 | 25-Mar | 84 | 34.544 | -78.750 | 1994-2014 | 39.684 |
| OH7 | 5-Apr | 95 | 40.415 | -82.709 | 1896-1999 | 282.393 |
| WV8 | 5-Apr | 95 | 38.643 | -80.614 | 1995-2010 | 510.573 |
| CT9 | 14-Apr | 104 | 41.575 | -72.738 | 1999-2007 | 147.661 |
| NY10 | 15-Apr | 105 | 42.921 | -75.597 | 2005-2009 | 342.287 |
| GA-BR1 | 17-Apr | 107 | 34.818 | -84.150 | - | 614.035 |
| VT11 | 19-Apr | 109 | 44.075 | -72.663 | - | 371.329 |
| ME12 | 6-May | 126 | 45.274 | -69.203 | 1999-2004 | 233.79 |

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Table S2. Sample size of common green darner (*Anax junius*) adults whose natal origins were assigned using stable-hydrogen isotopes captured in the hardiness zones (Hardiness Zone) used to summarize populations during each calendar month (Month).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hardiness Zone | Month | | | | | | | | | | | |
| Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
| 7 | 0 | 0 | 5 | 45 | 53 | 74 | 52 | 10 | 7 | 3 | 0 | 0 |
| 8 | 0 | 0 | 2 | 14 | 45 | 65 | 53 | 16 | 3 | 1 | 0 | 0 |
| 9 | 0 | 0 | 1 | 12 | 22 | 20 | 67 | 8 | 0 | 1 | 0 | 0 |
| 10 | 0 | 2 | 14 | 23 | 27 | 12 | 17 | 4 | 3 | 1 | 0 | 0 |
| 11 | 8 | 10 | 45 | 27 | 13 | 11 | 13 | 10 | 8 | 3 | 7 | 7 |