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Evidence for widespread gene flow and migration in the Globe Skimmer dragonfly *Pantala flavescens*

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Abstract. The global population structure and dispersal patterns of *Pantala flavescens* (Fabricius, 1798) are evaluated using a geographically extensive mitochondrial DNA dataset, a more limited samples of nuclear markers, wing isotopic (δ^2H) data and a literature review. No spatial or temporal haplotype structure was recovered between the samples. Isotope data suggest that most samples were immigrants at the collection locations. A literature review of migration events for the species confirms regular inter-and intra-continental migrations occur (the majority reported from Asia, Africa and Australasia), with individuals and swarms dispersing thousands of kilometers over land and oceans. Migrations coincide with prevailing winds and seasonal rains, which points to a mechanism we name the "pan-tropical *Pantala* conveyor belt", suggesting widespread gene flow is possible for an aquatic insect with excellent flying ability linked to rapid larval development.

Key words. Odonata, deuterium, haplotype, isoscape, F_{ST}, migration, ΦPT

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Introduction

The Globe Skimmer or Wandering Glider dragonfly, *Pantala flavescens* (Fabricius, 1798) (Odonata: Anisoptera: Libellulidae), is a well-known long-distance migrant. It has a circum-global distribution, and is the most widely distributed of all dragonfly species (Fraser, 1936; Russell et al., 1998). *Pantala flavescens* occurs most often in warmer climates, especially within the tropics, but also migrates seasonally into temperate zones (Borisov & Malikova, 2019; May, 2013). Its ability to disperse over great distances is demonstrated by its occurrence on offshore islands, for example on many Pacific Ocean islands (Rowe, 2004; Schmidt, 1938) including New Zealand (Corbet, 1979; Lieftinck, 1975; Rowe, 1980). *Pantala flavescens* has even reached the remote Sub-Antarctic Amsterdam Island (Devaud & Lebouvier, 2019) and is established on Rapa Nui (formerly Easter Island) (Dumont & Verschuren, 1991; Moore, 1993; Samways & Osborn, 1998). Its longest, regular, well-documented migratory route is across the western Indian Ocean (Anderson, 2009; Hobson et al., 2012a), where it has been suggested to follow seasonal (monsoonal) rains,

which allow females to take advantage of temporary pools of water for oviposition (Anderson, 2009; Corbet, 1999; Suhling et al., 2015).

The ability of *P. flavescens* to migrate such long distances is facilitated by a unique combination of morphological characteristics. These include distinct wing morphology (Alvial et al., 2019; Li et al., 2014; Moore, 1993; Sacchi & Hardersen, 2013; Suárez-Tovar & Sarmiento, 2016; Outomuro & Johansson, 2019; Zhao, 2012), of which the most obvious adaptation is their enlarged hind wing bases (a feature associated with gliding, Corbet, 1999), as well as unique thoracic musculature (Bäumler et al., 2018). Physiological features that allow individuals to migrate long distances likely involve their ability to make efficient use of lipids as fuel rather than carbohydrates (e.g., Kallapur & George, 1973); it has been suggested that dragonfly fat reserves can sustain eight hours of flapping flight. In addition, *P. flavescens* may be able to feed on insects while migrating thereby replenishing their fat stores en route (Anderson, 2009).

Given its wide distribution and extraordinary migratory abilities, there is interest in the population structure of *P. flavescens*. Studies in single countries have revealed high haplotype diversity and an expected lack of population structure (Cao et al., 2015; Hayashi & Arai, 2004; Low, 2017). Over a much wider geographical range, albeit with a small sample size, Troast et al. (2016) found that *P. flavescens* specimens from South America, North America, and Asia shared mitochondrial DNA haplotypes, suggesting high rates of gene flow and a single global panmictic population. Similarly, Alvial et al. (2017) found a lack of population structure between samples from across South and Central America using both mitochondrial and nuclear DNA markers. In contrast, a subsequent study by Alvial et al. (2019), which compared samples not only from Central and South America but also from islands in the Pacific and Indian Oceans, did find some suggestion of population structure between 'continental' and 'insular' samples using microsatellite loci, which also suggested that individuals from Rapa Nui formed a genetically distinct population. In addition, Pfeiler & Markow (2017) reanalysed published data (Low, 2017; Troast et al. 2016) and suggested that 'concluding global panmixia in *P. flavescens* may be premature.' Thus, the results from previous studies are ambiguous, with some suggesting panmixia while others suggest some degree of population structure.

Global panmixia, or random mating and uninterrupted gene flow across an entire species, appears to be uncommon in widespread species. Barriers to genetic exchange, which may be geographical, temporal or behavioural in nature, tend to lead to the evolution of genetically distinct regional populations. However, in some species dispersal or migration can lead to widespread gene flow despite the presence of obstacles (Neethling et al., 2008; Oomen et al. 2011). A commonly cited example is that of the European Eel (*Anguilla anguilla* (Linnaeus, 1758)), in which individuals from separate river basins travel to the Sargasso Sea for reproduction, leading to panmixia (Als et al., 2011; Palm et al., 2009; but see Wirth et al., 2001 for counter argument). Widespread genetic mixing has also been recorded in several other groups of organisms, including insects, birds, bats and plants (Als et al., 2001; Naro-Maciel, 2011; Neethling et al., 2008; Oomen et al., 2011; Ridgway et al., 2001; Peel et al., 2013; White et al., 2011). Nevertheless, most reports of panmixia might best be described as 'local panmixia', where random mating occurs across a population in a particular region rather than across the entire species (e.g. Peel et al., 2013; White et al., 2011). Studies on the population structure of long-range migrant insects are still rare, with the exception of locusts (*Locusta migratoria,* (Linnaeus, 1758) and *Schistocerca gregaria* Forsskål, 1775), monarch butterflies (*Danaus plexippus* (Linnaeus, 1758)), painted lady butterflies (*Vanessa cardui* (Linnaeus, 1758)), and the green darner dragonfly, *Anax junius* (Drury, 1773) (e.g., Hallworth et al., 2018).

Here, we evaluate the global population structure of *P. flavescens*, using genetic data to test the hypothesis of mixing across its global range. We have compiled genetic sequences available through the NCBI GenBank online database, supplementing this with a limited number of new samples, to form the largest such dataset yet assembled, including samples from 29 countries across six continents. We estimated various population structure measures, including testing for levels of gene flow. Further, we measured stable hydrogen isotope (δ^2H) values in a small sample of *P. flavescens* wings to establish what proportion of these specimens originated in regions different from those where they were collected (Hayashi & Arai, 2004; Hobson et al., 2012a, 2012b; 2021). This allowed us to test for the degree of migratory behaviour in *P. flavescens*, on the assumption that the presence of a large number of immigrant individuals across a global range would confirm widespread migration. Finally, we compiled published observations of migratory behaviour which served to identify possible connections and pathways between and within the continents.

Materials and methods

Study organism

Pantala flavescens (Fabricius, 1798) is a member of the dragonfly family Libellulidae (Odonata: Anisoptera). The vernacular English names "globe skimmer" and "wandering glider" reflect this species' long-distance flying ability and wide distribution. Adults are red, yellow, or brown in colour, commonly observed "hawking" over open areas. Egg-laying often occurs in tandem, and females produce their eggs continuously (Koch et al., 2011; Sharma, 2017; Ware et al., 2012). Eggs are distributed widely, even being oviposited among different ponds during a single oviposition event, which is interpreted as risk-spreading to avoid loss of all offspring should temporary habitats dry out before development is completed (Schenk et al., 2004). Larvae mostly inhab-

it temporary or ephemeral lentic waters including rain-pools forming after heavy rainfalls. Offspring develop very rapidly, taking 4–6 weeks from egg hatch to adult emergence (e.g., Hawking & Ingram, 1994; Kumar, 1984; Suhling et al., 2004).

Sampling

We collected 23 specimens of *P. flavescens* from six countries during 2004 to 2015 (see Supplementary material, Table S1). In addition we downloaded genetic sequences of *P. flavescens* from NCBI GenBank (N = > 600 for COI; complete list in Supplementary material, Table S1). We included COI sequences from North America (N = 16; Canada, USA), Central and South America (N = 25; Brazil, Chile, Costa Rica, Guyana, Peru), Asia (N = 190; Cambodia, China, India, Indonesia, Japan, Korea, Malaysia, Maldives, Pakistan, Philippines, Singapore, Vietnam), Africa (N = 4; Guinea Bissau, Liberia, Senegal; unfortunately, samples from South Africa and Namibia failed during the sequencing step), Australia and the Pacific (N = 13; Rapa Nui [a territory of Chile], Fiji, French Polynesia, Hawaii, Mariana Islands, Tonga Islands). Additionally, we downloaded and ran analyses on *P. flavescens* nuclear genetic data available from NCBI (Table S1).

Genetic dataset partitions

We ran our analyses with the geographic dataset described above for the COI. Additionally, we ran the COI haplotype analyses with either (1) fewer Japanese samples or (2) excluding samples from Alvial et al. (2017, 2019).

1. Inference of population structure can be sensitive to sampling bias, (see Puechmaille, 2016). More than half of our samples came from Japan, while other regions had < 10 individuals. Thus, for additional haplotype analyses we limited the number of Japanese samples to 77 ("reduced" data set). We chose these samples to sample *P. flavescens* evenly from across the Japanese islands for which we had data. This gave us 248 *P. flavescens* sequences in total.
2. For NCBI samples from Alvial et al.'s (2017, 2019 studies (N = 40)) it was impossible to distinguish which samples were which. Hence, these were coded as "Alvial" (i.e. grouping together all of her samples from Costa Rica, Chile, Peru, Tonga, Rapa Nui, and the Maldives). Alvial et al.'s samples were included in testing our Null hypothesis that there is no global geographic structure. These samples had to be excluded from the rest of our geographic hypothesis testing scenarios as we could not infer provenance (i.e., if the samples were from the Northern or Southern, Eastern, or Western Hemisphere, see Inference of Population Structure section for more details on scenarios).

We additionally reconstructed haplotype networks for *P. flavescens* sequences available from NCBI GenBank for mitochondrial 16S and Cytochrome B, as well as nuclear 18S and Histone 3. Apart from 16S, these sequence datasets were from single countries, and so although they do provide insights into genetic diversity they shed no direct light on global population structure.

DNA Extraction/PCR Amplification

DNA was extracted from tissue of *P. flavescens* samples which had been stored in ethanol or acetone-dried. Extraction was completed using Qiagen DNeasy Blood and Tissue Kits (Maryland, USA). We sequenced the mitochondrial Cytochrome Oxidase I (COI; ~ 658 base pairs), a gene which was chosen because of a large number of existing sequences available on NCBI, allowing for this large, global dataset. Polymerase Chain Reaction (PCR) was done using the primer set of Simon et al. (1994): 1709f (TAATTGGAGGATTTGGAAATTG, T_m = 56.2°C) and 2191r (CCYGGTARAATTARAATRTARACTTC, T_m = 56.7°C). PCR reaction total volume was 25 µL, comprising 12.5 µL Taq 2X Master Mix, 5–7 µL DNA, 3.5–5.5 µL RNA-ase free water, and 1–2 µL of the forward and reverse primers.

PCR was done using an Eppendorf Thermocycler using the following parameters: initial heat step at 94.0°C held for one minute, 20 cycles of denaturation (30 s, 94.0°C), annealing (45 s, 48.0°C), and extension (45 s, 72.0°C), an additional 20 cycles of denaturation (45 s, 94.0°C), annealing (45 s, 50.0°C), and extension (45 s, 72.0°C), followed by a final extension at 72.0°C held for five minutes. PCR products were visualized via gel electrophoresis. We used commercially available Macrogen services (New York City, New York, USA) for sequencing and purification.

Sequences

Sequences were edited using Sequencher® (2016). These sequences, along with those from NCBI GenBank, were aligned using Clustal-X Version 2.1 (Larkin, 2007) and Mesquite Version 2.75 (Maddison & Maddison, 2017). For the COI, twenty-three de novo sequences from Africa, Australia, the Maldives, and the USA were sequenced and resulting sequences were deposited in NCBI GenBank (Accession numbers OM945847–OM945869). In total we had 25 samples from Central and South America, 16 from North America, 13 from Australasia, 190 from Asia including the Maldives, and four from Africa for the COI. For the 16S the 16 sequences were from Africa, India, Japan, Korea, Malaysia, Pakistan, and the United States of America, for the 18S the four sequences were from Japan, for the Cytochrome B the 77 sequences were from China, and for the Histone 3 the four sequences were from Japan.

Haplotype analyses

To assess variation among individuals of *P. flavescens*, we reconstructed minimum spanning networks for each gene fragment using PopArt version 1.7 (Leigh & Bryant, 2015) with the minimum spanning networks algorithm (Bandelt et al., 1999).

Inference of population structure

We also assessed population structure summary statistics using the COI genetic data. All statistics were estimated from the "reduced dataset", and required assigning individuals to a fixed number of putative geographically based hypotheses (populations), which we did in several ways. First, we estimated the number of genetic clusters/populations using K-means clustering analysis in adegenet R package 2.1.3 (Jombart, 2008; Jombart & Ahmed 2011). We selected the optimal number of clusters using the Bayesian Information Criterion (BIC); choosing the solution with the best BIC value. Discriminant Analysis of Principal Components (DAPC), as implemented in adegenet R package, was then used to assess genetic variation between identified population clusters. We also determined membership probability of each sample for each of the predicted populations; to do so we calculated the degree of genetic differentiation (F_{ST}) among predicted clusters using the hierfstat R package version 0.5-7 (Goudet, 2005), and we ran an AMOVA in the poppr package version 2.8.6 (Kamvar et al., 2014, 2015) to test whether the observed variation was higher between or within each cluster.

We estimated population genetics statistics assuming different scenarios considering possible biogeographically separated populations: (a) assuming the populations were defined based on the results from K-means clustering, (b) assuming each of the continents is a separate population, (c) assuming Northern/Southern Hemispheres are separate populations, (d) assuming Eastern/Western Hemispheres are separate populations, and (e) a null hypothesis of one panmictic population. Insects generally do not follow geo-political boundaries, but we also ran an additional scenario (f) assuming countries are separate populations. Population genetics statistics under the various scenarios were estimated in the software package R as described above. We calculated the genetic structure index, $F_{ST,}$ and other statistics (haplotype diversity, Hd; nucleotide differences, K; number of polymorphic sites, S; Tajima's D, Fu and Li's statistics, and nucleotide diversity, π) under each of these population scenarios as described above. For the single panmictic population global scenario we could not estimate F_{ST} as there was only one population.

Effective migration rates

For the COI, we used the program Migrate-N version 3.7.2 (Beerli et al., 2019) to estimate effective migration rates ($4N_eM$) and $\Theta = 4N_e\mu$ under scenarios (c) and (d). We let the migration matrix stay at default settings. We ran 10 replicate maximum likelihood Migrate-N runs of 20,000 steps per chain each with a Brownian motion model, and one long chain. We sampled every 100 steps, with a burn-in of 10,000 steps and a bounded-adaptive heating scheme with four temperatures (3 cold, 1 hot) of 1.00, 1.50, 3.00, and 10,000. To test the effect of temperature on our results, we ran an analysis with two different "hottest temperatures" and the results did not differ. We evaluated the best model using Log Bayes Factor (LBF) and highest model probability values in Migrate-N. Effective Sample Sizes (ESS) were evaluated in TRACER version 1.7.1 (Rambaut et al., 2018).

Stable Isotope Analysis

We excised wings from 29 individuals representing each of the continents of North America, Central and South America, Africa, Australia, and Asia plus the Pacific; samples were chosen from both islands and mainland areas (Table S2). All samples had been dried in acetone, which has been shown to have no impact on isotopic results (Hobson et al., 2012a; Hobson & Wassenaar, 2019). We submitted wings to the Cornell Isotope Laboratory (COIL) facility, where surface oils were removed using a 2:1 chloroform:methanol solution and air dried in a fume hood. Subsamples of wings were weighed into silver capsules, crushed, and loaded into a zero-blank carousel attached to TC/EA (Thermo, Bremen, Germany) where samples were combusted at 1350°C pyrolytically. Resultant H_2 gas was analyzed under continuous-flow isotope-ratio mass spectrometry (CFIRMS) on a Thermo Delta-V mass spectrometer using a helium carrier gas. Samples were calibrated using within-run measurements of keratin standards CBS (-197‰) and KHS (-54.1‰) according to the comparative equilibration method of (Wassenaar & Hobson, 2003). An in-house (Spectrum) commercial standard was used to correct for instrument drift. Stable-hydrogen isotope values are reported here in standard delta notation in parts per thousand (‰) deviation from the Vienna Mean Ocean Water (VSMOW) standard. Based on within-run replicate measurements of standards, we estimated measurement precision to be ~ 2‰.

Assigning *P. flavescens* to geographical origins using the stable-hydrogen isotope approach is problematic because this species is known to use ephemeral pools that may be created from highly seasonal (often monsoonal) rainfall events. Hobson et al. (2012a) modeled the origins of individuals captured on the Maldives en route to Africa using an amount-weighted mean annual precipitation model that provided the first isotopic evidence for long-distance migration in this species and pointed to origins in northern India or possibly even further north and east. That approach was based on a calibration algorithm derived from known-origin drag-

onflies in North America (Hobson et al., 2012b: Wing δ^2H = 0.91(MAD) -42.5, r^2 = 0.75, where MAD is the amount-weighted mean annual precipitation for a given site). Based on the findings of Lopez-Calderon et al. (2019), we used this relationship to evaluate whether a given dragonfly likely originated from sites of capture using the normal probability density function and with an assumed standard deviation of the residuals of the calibration algorithm to be a highly conservative 19‰ (Hobson et al., 2012b). We considered three odds ratios (2:1, 3:1, and 4:1) for each individual assignment as being a "local" or an "immigrant".

Results

Haplotypes

Our COI dataset consisted of an alignment with 686 characters. Haplotype minimum spanning analyses on all datasets suggest that most *P. flavescens* samples share a single common haplotype for COI (Figure 1, network from reduced dataset); we found one main haplotype with 24–43 additional haplotypes varying by ≤ 3 nucleotides (43 = full dataset and 24 = reduced dataset). Regardless of what level of population assignment was used (continent, Western/Eastern Hemisphere, Northern/Southern Hemisphere), we recovered the same haplotype structure. For the other gene fragments studied, one or two main haplotypes were recovered (Figure S3), with the exception of Cytochrome B, for which several haplotypes were suggested among the samples (all of which were from China). In the case of the mitochondrial marker 16S, we located 16 samples from three continents on NCBI GenBank, all of which shared a single haplotype (Figure S3). In the case of the nuclear marker H3, only four sequences were available on NCBI Genbank, three of which shared the same haplotype; the fourth sample revealed a very different haplotype, and likely represents a different species, mislabeled as *P. flavescens,* or contamination.

Population Genetics

The clustering method in the adegenet package estimated K = 1 to three populations of *P. flavescens* as most likely for COI. We used the "elbow" method to choose K = 2 and K = 3 as the population cluster values (scenario (a); Jombart & Collins, http://adegenet.r-forge.r-project.org/files/tutorial-genomics.pdf). However, as inferred clusters higher than K ≥ 2 lacked any geographic structure, we consider these values as to be difficult

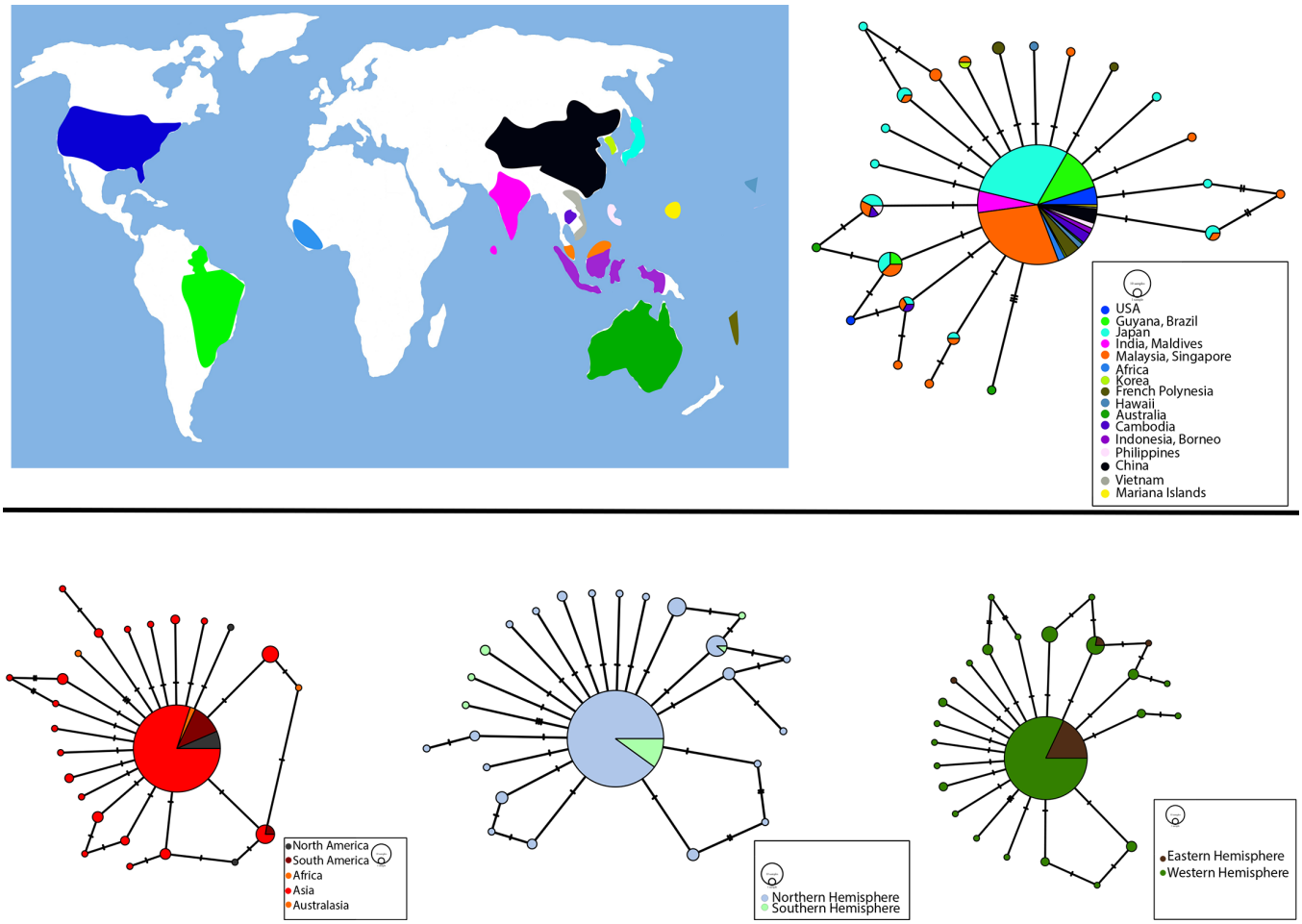

Figure 1. PopART minimum spanning network; Dataset: Alvial's samples not included, reduced number of Japanese samples. Haplotype networks coloured based on sampling country, continent, hemisphere and New/Old world.

to interpret: they do not seem to share any clear pattern that unites the individuals in these clusters (i.e., these clusters do not reflect timing of sampling, geographic region of sampling, etc.). In addition, the BIC scores did not vary greatly among the K = 1, K = 2, and K = 3 (BIC score range 300–~450) and DAPC scatterplots showed large overlap across clusters (Figure 2). We further tested whether differences between K = 2 and K = 3 could be explained by temporal bias in sampling, but as each putative K population contained samples from across sampling periods (i.e., early 2000s and early/mid 2010s), we reject this explanation for inferred patterns. Lastly, under the K = 2 and K = 3 scenarios we found low genetic structure and F_{ST} values between the populations, consistent with high rates of gene flow (most F_{ST} values were < 0.5).

F_{ST} values between different biogeographical scenarios varied, ranging from below 0.05 in scenario (b) (between Asia and Africa, Asia and Australasia, North America and Asia) indicating little population differentiation, to 0.55 between Africa and Australasia (Table 1) suggesting greater population differentiation. However, with only four samples from Africa, all of which were from West Africa, this result should be treated with caution. For scenario (c), F_{ST} was 0.007; for scenario (d) F_{ST} was 0.004. In short, for the geographical scenarios (b)–(d) our dataset suggests high gene flow and no genetic structure between putative populations.

Effective migration rates

Migrate-N found widespread gene flow in COI. Migrate-N analytical results suggested that individuals migrating (in either direction) from Western Hemisphere to Eastern Hemisphere was the best fit with ln(Prob(D|Model)) = -4158.48 compared to the model for individuals migrating (in either direction) from the Northern/Southern Hemisphere (Northern/Southern Hemisphere ln(Prob(D|Model)) = -6480.57). Within the Eastern/Western Hemisphere analysis, movement in both directions had normal distributions of migration rates, M, although relative variance around the means varied. Mean migration rate for the Western Hemisphere-Eastern Hemisphere direction was 478.2, and the mean migration rate in the Eastern Hemisphere

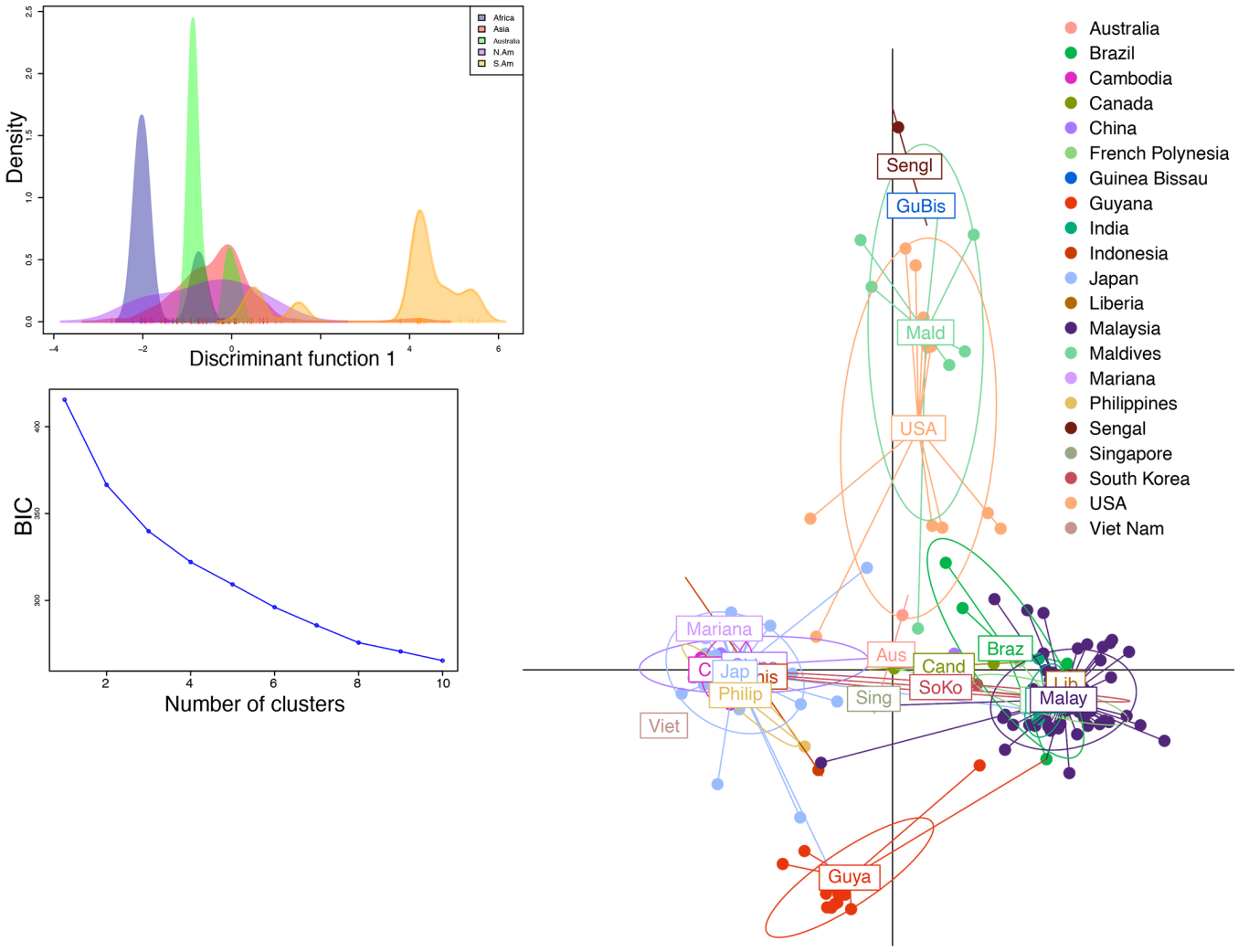

Figure 2. Density and cluster plots for defined populations; (A) density plot assuming continents are populations; (B) BIC values for the putative number of K clusters, (C) cluster plot assuming countries are populations; In each cluster plot and density plot putative populations are colour-coded.

Table 1. Pairwise F_{ST} values under various population scenarios: (a) the K-means clustering analysis populations estimated in adegenet R package, (b) assuming continents are separate populations.

Scenario (a)	**K=2**		**K=3**		
Population F_{ST}	**2**	**1**	**3**	**2**	**1**
3	–	–	0.0	0.217	0.158
2	0	0.168	0.217	0	0.277
1	0.168	0	0.158	0.277	0
Within population variation	58.983		56.462		
Among population variation	41.017		43.538		

Scenario (b)					
Population F_{ST} (b)	**Africa**	**Asia**	**Australasia**	**N America**	**S America**
Africa (N=4)	0	0.029	0.551	0.197	0.223
Asia (N=190)	0.029	0	0.002	0.031	0.062
Australasia (N=13)	0.551	0.002	0	0.158	0.111
North America (N=16)	0.197	0.031	0.158	0	0.131
South America (N=25)	0.223	0.062	0.111	0.131	0
Within population variation	95.889				
Among population variation	4.111				

Table 2. Population genetics statistics based on different population scenarios, with K: nucleotide differences, π: nucleotide diversity, S: # of polymorphic sites. $p \leq 0.05$ in bold.

Population	**Tajima's D**	**K**	**π**	**S**	**Hd variance**	**Fu and Li's D**	**Fu and Li's F**	**Fst**
Western Hemisphere (N=41)	-1.37923 *p*>0.10	0.300	0.001	3	0.2360.009	-1.50777 *p*>0.10	-1.70431 *p*>0.10	0.004
Eastern Hemisphere (N=207)	-2.18914 ***p*<0.01**	0.943	0.006	24	0.5730.002	-3.24440 ***p*<0.05**	-3.38651 ***p*<0.02**	
Northern Hemisphere (N=228)	-2.16518 ***p*<0.01**	0.494	0.003	20	0.5810.002	-3.69480 ***p*<0.02**	-3.69542 ***p*<0.02**	0.007
Southern Hemisphere (N=20)	-1.79423 0.10>*p*>0.05	0.251	0.002	3	0.3280.009	-1.64715 *p*>0.10	-1.97431 0.10>*p*>0.05	
All data	-2.35627 ***p*<0.01**	0.452	0.003	22	0.3480.002	-4.45510 ***p*<0.02**	-4.34133 ***p*<0.02**	

to Western Hemisphere direction was 40.0; the mean migration rate in the North-South direction was 311.1, and the mean migration rate in the South-North direction was 19.5. In short, the results of this analysis suggest more migration going from west to east and north to south than in the opposite directions.

Stable Isotope Analyses

Our isotope data indicated a broad span of isotopic origins for our samples, ranging from xeric to mesic habitats and different geographic origins (Figure S1). Most individuals had lower wing δ^2H values than predicted considering mean amount-weighted local precipitation δ^2H (Figure S1), implying more temperate regions of origin. In only three cases was the individual wing δ^2H value higher than expected from the local collection site. Using an odds ratio of 2:1 or 3:1 resulted in assignment of at least 20 out of 29 individuals as immigrants vs. locally produced (Table 3). This changed only slightly with a more conservative 4:1 odds ratio (19 out of 29). Note that these estimates represent minimum numbers of immigrants; specimens with δ^2H values consistent with local origin could have originated from a different area with a similar local precipitation δ^2H.

Table 3. Stable isotope (δ^2H) results of wing chitin of individual *Pantala flavescens* taken from subsamples of collections across at the sites as indicated. Also indicated are the assignment to immigrant (im) or local (loc) based on given odds ratios as described in the Methods.

ID	Location	Latitude	Longitude	Elevation (m)	Wing δ^2H (‰)	Precip. δ^2H (‰)	Predicted wing δ^2H (‰)	Odds Ratio 2:1	3:1	4:1
1	India	13.5662	78.499	0	-96.0	-58.7	-58.01	im	im	im
2	China	30.4919	119.615	1506	-121.7	-87.0	-108.06	loc	loc	loc
3	China	30.4919	119.615	1506	-94.4	-57.0	-108.06	loc	loc	loc
4	Japan	35.8896	139.652	12	-145.5	-113.1	-93.50	im	im	im
5	Japan	38.5587	140.85	208	-138.4	-105.3	-97.14	im	im	im
6	Australia	-18.9	145.77	0	-141.9	-109.2	-55.28	im	im	im
7	Australia	-18.9	145.77	0	-120.1	-85.2	-55.28	im	im	im
8	Australia	-18.9	145.77	0	-70.6	-30.8	-55.28	loc	loc	loc
9	Australia	-18.9	145.701	0	-83.2	-44.7	-55.28	im	im	im
10	Senegal	14.6433	-12.3363	23	-81.0	-42.3	-67.11	loc	loc	loc
11	Senegal	14.6433	-12.3363	23	-100.0	-63.1	-67.11	im	im	im
12	Senegal	13.9726	-15.0079	55	-107.5	-71.4	-68.93	im	im	im
13	Guinea-Bissau	11.683	-14.7977	14	-92.7	-55.1	-69.84	im	im	loc
14	Cruise ship	18.0294	64.1041	0	-89.7	-51.8	-58.01	im	im	im
18	Guyana	6.4833	-58.2167	0	-126.6	-92.4	-49.82	im	im	im
19	Guyana	6.4833	-58.2167	0	-108.5	-72.5	-49.82	im	im	im
20	Hawaii	21.6403	-158.063	5	-76.7	-37.5	-48.91	im	im	im
21	Hawaii	21.6403	-158.063	5	-62.8	-22.3	-48.91	loc	loc	loc
22	Hawaii	21.6403	-158.063	5	-65.5	-25.2	-48.91	loc	loc	loc
23	Hawaii	21.6403	-158.063	5	-77.1	-38	-48.91	im	im	im
24	Peru	-7.449	-73.9403	295	-88.6	-50.6	-76.21	loc	loc	loc
25	Peru	-9.8933	-76.32	1981	-96.7	-59.5	-108.06	loc	loc	loc
26	Peru	-9.8933	-76.32	1981	-89.9	-52.0	-108.06	loc	loc	loc
27	Brazil	-12.196	-38.9414	250	-137.3	-104.1	-48.00	im	im	im
28	Costa Rica	8.6143	-83.5411	304	-115.5	-80.2	-85.31	im	im	im
29	USA	35.96	-83.924	260	-130.9	-97.1	-73.48	im	im	im
30	USA	30.4642	-84.5953	43	-105.3	-69.0	-58.92	im	im	im
31	USA	30.3356	-97.9112	175	-89.2	-51.3	-61.65	im	im	im
32	USA	39.8683	-75.1877	4	-116.3	-81.1	-78.94	im	im	im

Discussion

Our genetic data suggest a lack of population structure across the global range of *P. flavescens,* which can be attributed to widespread migration. This interpretation is supported by our isotope data and by our literature review. These findings suggest the existence of a mechanism we call the pantropical *Pantala* conveyor belt.

Here, we have compiled the largest *P. flavescens* haplotype dataset to date, including for the first time individuals from each of the five continents where this species is usually found. Our results demonstrate genetic homogeneity across all geographic regions, suggesting either a recent shared common ancestry or widespread dispersal. With this globally sampled dataset we find not only a lack of genetic structuring among regions, but also high levels of gene flow, and this suggests some level of panmixia. First and foremost, one main haplotype is shared among most individuals collected across five continents for COI and all individuals collected across three continents for 16S. Secondly, F_{ST} values (i.e. estimates of genetic structure within and between populations) for COI suggest little population differentiation and high levels of gene flow between Northern/Southern Hemispheres, Eastern/Western Hemispheres, and most continents (Table 2); country dataset results were not biologically meaningful, and are likely unreliable given small sample sizes for some countries (cf Excoffier, 2007). Thirdly, from our stable isotope analysis it is clear that a majority of the individuals sampled were immigrants to their locations of capture, further supporting widespread mixing of *P. flavescens*.

Nevertheless, incomplete lineage sorting, or recent shared common ancestry, may also be associated with such low F_{ST} values. In support of this possibility, Tajima's D values (which estimate deviation from neutral expectations) were negative for all areas for COI, suggesting population expansion. These values were statistically

significant in the Eastern and Northern hemispheres, suggesting less polymorphism than expected under neutral mutation and genetic drift, which in turn implies that the population is not in equilibrium. This provides alternative hypotheses to high gene flow, namely that the population may have experienced a genetic bottleneck or may be experiencing purifying selection, either of which would also explain the apparent lack of any genetic structure in *P. flavescens* across its range.

K-means clustering (a means of partitioning observations into clusters, which in this case partitions individuals into putative populations) suggests that there may be several populations, although none of the "populations" identified can be easily interpreted in terms of a biological pattern. For example, the clusters contained individuals from several countries, continents, and hemispheres, across different sampling years, with no apparent pattern. Similarly, we did not find any statistical support for putative populations defined on the basis of various geographic scenarios. F_{ST} values were lowest when grouping individuals as Northern/Southern Hemisphere or Eastern/Western Hemisphere populations. While there is greater apparent genetic structure among some continents, this may be biased by unequal sampling across regions.

Migrate-N (a program which models migration using genetic datasets) suggests that of two tested scenarios, east-west migration is more probable compared to north-south migration; this can be interpreted as more common, suggesting the east-west direction is a more common migration route than north-south migration which makes sense given *Pantala flavescens*' use of intertropical convergence zone winds. F_{ST} values for COI recovered between *P. flavescens* groups were low (North-South hemisphere scenario; East-West hemisphere scenario), which also suggests high gene flow among hemispheres (see also Table 2 for additional population genetics statistics). The F_{ST} values were similar to those of the highly migratory *Anax junius* (microsatellite F_{ST} = 0.02-0.08, Matthews, 2007), and lower than those of somewhat migratory *Ischnura hastata* (Say, 1839) (ND1 F_{ST} = 0.105, Lorenzo-Carballa et al., 2010), or *Cordulegaster sarracenia* Abbott & Hibbitts, 2011 (microsatellite F_{ST} = 0.423, Abbott et al., 2018). It is possible that by using a more rapidly evolving marker, such as SNPs or microsatellites, additional structuring between areas might be revealed. Alvial et al. (2019), however, showed very small differences in Hd, π, and number of haplotypes whether COI or microsatellites were used for *P. flavescens*, although with microsatellites some structure was found between what they characterised as "continental" and "insular" regions. Their continental samples were limited to Central and South America. Microsatellites may evolve more rapidly than COI (e.g., McDonald & Potts, 1997), and thus if *P. flavescens* colonized the Americas, most likely from Africa, in the relatively recent past, it is possible that such population structure might be apparent in microsatellite data (2019) but not in COI data (as demonstrated here).

We also note that among the individuals studied by Alvial et al. (2019) there was high relatedness only among their Rapa Nui samples. This could indicate that those samples shared parents, and as a result any interpretation of population structure should be treated with caution as these are unreliable. Alvial et al.'s (2019) samples from Rapa Nui also suggested signatures of a recent genetic bottleneck; we do not have samples from Rapa Nui, but when their samples were included in analyses with our samples we found no evidence of genetic structure in COI. If all the samples Alvial et al. (2019) collected during one trip from Rapa Nui were of closely related individuals, that could explain the perhaps artificially high level of structure between Rapa Nui and the Americas. Future work should include collection of a time series of *Pantala* from Rapa Nui to determine whether Alvial et al.'s (2019) results can be confirmed with samples with lower relatedness. Indeed, future work should examine more nuclear data to evaluate what biogeographical patterns are reflected in the nuclear genome of *P. flavescens*; there can be genetic discordance between gene fragments due to phenomena such as incomplete lineage sorting and introgression, while patterns of events such as hybridization and isolation can lead to biogeographical discordance (Toews & Brelsford, 2012). In addition to augmenting knowledge of Rapa Nui, future efforts should also aim to increase genetic sampling across the continent of Africa. More generally, we encourage further study with more and larger samples, and a range of genetic markers. We note in particular that for COI (for which at least 71 haplotypes have been identified) very much larger sample sizes than used here would be desirable, and also that the study of faster-evolving genetic markers than COI may be needed to fully elucidate the population structure of *P. flavescens*.

Our stable isotope data support the general perception that *P. flavescens* is a highly vagile species. The majority of individuals investigated here were classed as immigrants, which means we collected individuals from areas other than their natal regions regardless of where or when we sampled. Stable isotope analysis has previously shown that *P. flavescens* collected in Maldives were all immigrants (Hobson et al., 2012a), while those collected in Japan were mostly immigrants (Hobson et al., 2021). The fact that we observed only small numbers of potentially local individuals in our samples confirms that most adults disperse soon after emergence (e.g. Corbet, 1964; Kumar, 1972). Increased isotope sampling in areas like the Andes, where we had only potentially local individuals, and particularly on isolated islands (coupled with paternity studies) could assist with establishing whether there are exceptions to the patterns suggested by our results here, (i.e. whether there are any resident non-migratory populations in other parts of the range of *P. flavescens*). However, even in Hawaii, two out of four sampled individuals were classed as immigrants.

Finally, a literature review confirmed large-scale movements of *P. flavescens* between locations where it is commonly found (text S1, Table S3; Figure S2). There are several possible pathways of population exchange, often as large swarms, between continents and even across large stretches of open ocean (text S1). Most of these observations were made in association with particular wind patterns such as the Inter-Tropical Convergence Zone (ITCZ) rain fronts, but also associated with typhoons and cyclones. Noted dispersal events imply that individuals are able to cover distances of several thousand kilometers even over oceans and seas, e.g., Anderson, 2009; Drake et al., 1995; Sparrow et al., 2020). This supports the idea that migration and dispersal may be wind-driven or wind-supported. We assume that seasonal appearances at off-shore islands can provide clues for reconstructing cross-ocean migration routes (cf. Anderson, 2009). One such migration route has been described by Anderson (2009) crossing the Indian Ocean between India and Africa via the Maldives and Seychelles Islands, and there is additional evidence for cross-Indian Ocean travels (text S1); Hobson et al. (2021) also describe oceanic crossing by individuals collected on Japanese islands. As many Pacific islands are also visited by *P. flavescens,* there is support for the idea of intercontinental pathways via the Pacific Ocean. Whether *P. flavescens* can regularly cross the eastern Pacific barrier remains unclear. The most likely route connecting the Eastern and Western Hemispheres seems to be via the central Atlantic Ocean from West Africa, where individuals would be supported by trade winds blowing towards northern South America and the Caribbean. This dispersal path has been demonstrated for two other migratory Odonata of African origin, *Anax ephippiger* (Burmeister, 1839) and *Tramea basilaris* (Palisot de Beauvois, 1807) (Lambret et al., 2013; Hedlund et al., 2020; Meurgey & Poiron, 2012) as well as the desert locust *Schistocerca gregaria* (Forsskål, 1775) (Lovejoy et al., 2006). Seasonal winds may also assist the movement of swarms of *P. flavescens* as far away from the tropics as about 52°N in East Asia (Borisov, 2012; Cao et al., 2015), North America (May, 2013) and, recently, also in parts of Europe (Buczyński et al., 2014); in these high latitude regions they probably do not survive the winter (Borisov, 2012; Borisov & Malikova, 2019; May, 2013). In Asia and Africa, movements seem to be mainly associated with the seasonal shifts of the ITCZ and the associated rain-bearing winds, while reasons for massive overshoot of the ITCZ in North America are not yet fully understood (May, 2013). In the Southern Hemisphere most larger landmasses except for Antarctica and southern Patagonia regularly receive migratory swarms of *Pantala* (although *P. flavescens* has been found at least as far south as 37°S in Argentina; del Palacio et al., 2017). Our conclusion, based on observations compiled from literature (supplementary material), is that the extraordinary dispersal abilities of *Pantala flavescens* coupled with seasonal wind patterns, support the observed global pattern of gene flow and migration.

Physiological and morphological features that may allow individuals to efficiently migrate long distances likely involve their ability to efficiently use lipids as fuel rather than carbohydrates (e.g., Kallapur & George, 1973; it has been suggested that dragonfly fat reserves can sustain eight hours of flapping flight), as well as distinct wing morphology (e.g., Alvial et al., 2019; Moore, 1993; Sacchi & Hardersen, 2013; Suárez-Tovar & Sarmiento, 2016; Outomuro & Johansson, 2019; Zhao et al., 2012), and unique thoracic musculature (Bäumler et al., 2018). Further, a major precondition (or trait) for being an obligate dragonfly migrant may be the ability to develop successfully in ephemeral freshwater habitats, which are usually formed after seasonal rains (Corbet, 1999; Suhling et al., 2019). This seasonality in breeding habitat is directly linked to the propensity of *P. flavescens* to follow the ITCZ.

Conclusions

Using COI data from samples taken across most of *P. flavescens'* range, we found high gene flow and a lack of genetic structure, with one main haplotype shared globally. This result is supported by isotope data which suggest frequent long-distance migration. We also compiled extensive observational evidence from the literature which supports these findings. We conclude that there is full genetic exchange among all continents, i.e., a globally panmictic population of *P. flavescens* seems possible, even if very isolated islands may be excluded from regular migration. The mechanism supporting panmixia is regular long-range migration within and between continents, even across oceans. Trans-oceanic migration sustained by trade winds and additional storm events explain the ability of individuals to reach distant islands and to travel between continents, while active flight and movement of seasonal rains in relation to the ITCZ can explain patterns observed over continents. All evidence points at a mechanism we call the pantropical *Pantala* conveyor belt, a mechanism which makes it possible for an insect with excellent flying ability and rapid development to achieve some level of global panmixia.

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Author contributions

JLW, GS, DT, KAH, and FS conceived the ideas; JLW, CMM, DT, KAH, GS, FS, and HJ collected the data; JLW, MKK, FS, and KAH analysed the data; JLW, GS, and FS led the writing; all authors contributed to writing of the manuscript and figure & table preparation.

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Supplementary material

Supplementary Figure S1. Patterns of wing isotopes (a) world map generated from Google Earth (Map data ©2020 Google, earth.google.com/web/, United States) with wing isotope ranges indicated for subsample of 32 specimens, map generated with Google Earth (b) scatterplot of wing isotope values versus predicted wing isotope values, the dashed line depicts the 1:1 situation, the shaded area the confidence interval.

Supplementary Figure S2. Map from Google Maps (Map data ©2020 Google, earth.google.com/web/, United States) generated in Excel, compiling data from Tables 3 and S3. Intertropical convergence zone (ITCZ) displayed as a line. TOM = transoceanic migration, CMS = coastal migratory swarms, TMS = terrestrial migrating swarms, LDD = long distance dispersal events to formerly uninhabited areas.

Supplementary Figure S3. PopART minimum spanning network; Datasets: Cytochrome B (N = 77), Histone 3 (N = 4), 16S (N = 16), and 18S (N = 4). Haplotype networks coloured based on sampling country unless only one country of origin for the samples, in which case the networks are coloured black.