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Population ecology

Round-trip across the Sahara: Afrotropical Painted Lady butterflies recolonize the Mediterranean in early spring

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The Palearctic–African migratory circuit has been typically associated with birds. Very few insects are known to endure annual trans-Saharan circuits, but the Painted Lady butterfly (*Vanessa cardui*) is an exception. While it was demonstrated that this species massively migrates from Europe to the Afrotropics during the autumn, the existence of a reverse migration from the Afrotropics to Europe in the early spring remains hypothetical. Here, we analysed wing stable hydrogen isotope values (δ^2H) of *V. cardui* migrants collected from February to April across the circum-Mediterranean region. We assessed their region of natal origin by comparing their wing isotope signature predicted δ^2H values (isoscape). The results unambiguously demonstrate a sub-Saharan origin for many individuals, especially those collected in February, representing the first tangible evidence for a reverse northwards trans-Saharan migration in spring. This work supports the view that the Afrotropics (mostly exploited from September to February) is key in the *V. cardui* Palearctic–African population dynamics. This species relies on both temperate and tropical habitats to complete their multigenerational cycle, an unprecedented adaptation for butterflies and for most migratory insects. Such a migratory circuit has strong parallelisms with those of migratory birds.

1. Introduction

The 1500–2000 km wide Sahara Desert is a natural geographical barrier to the dispersal of most organisms, and defines two well-differentiated biogeographic regions—the Palearctic and Afrotropical. Seasonal trans-Saharan animal migration between the Palearctic and Tropical Africa has been a phenomenon almost exclusively associated with birds [1]. The capacity to travel between temperate and tropical regions allows long-distance migrants to seasonally exploit resources and favourable climates on both sides of the Sahara Desert. Although many insects disperse across climatic zones within Africa or between North Africa and the Mediterranean, few species are known to perform annual long-range trans-Saharan circuits [2].

The Painted Lady butterfly, *Vanessa cardui* is an exception, as it was recently discovered that large populations of this species migrate southward from Europe to tropical Africa during the autumn. Dedicated long-term expeditions over the sub-Saharan African region led to observations of large numbers of butterflies flying southwards and establishing to breed in the verdant African savannah at the end of the rainy season [3]. The hypothesis that most of these migrants potentially have European origins was confirmed using δ^2H isotope evidence [4]. This discovery revealed the longest single-leg migratory flight known in butterflies (greater than 4000 km), and possibly the one facing the hardest obstacles: the Mediterranean Sea and the Sahara Desert.

However, to date, the fate of the southbound autumn European migrants and that of their offspring remains unknown, which limits our understanding of *V. cardui* population dynamics. At the end of the autumn, as the African savannah rapidly dries out, the Painted Lady populations apparently disappear from the region. This observation could be explained by several hypotheses: (i) a rapid elimination of *V. cardui* migrant populations due to changing conditions (dead-end); (ii) a continued migration of *V. cardui* populations through assimilation to other migratory circuits further south in Africa; or (iii) the initiation of a reverse northward migration across the Sahara back towards Europe in spring. Here, we hypothesize that the southbound populations do not represent a migratory dead-end or are out the Europe–Afrotropics loop. Instead, we hypothesize that the offspring of these populations in the Afrotropics migrate back to the circum-Mediterranean region from February to April.

In this study, we assess natal origins for spring movements of butterflies arriving into the Mediterranean region by using stable isotope (δ^2H) data. We analyse a comprehensive sampling set from the circum-Mediterranean region (Morocco, Andalusia, Catalonia, Crete, Egypt and Israel). We test (i) whether butterflies appearing into the circum-Mediterranean region in the spring have a sub-Saharan origin and (ii) if this is an exceptional event or if it accounts for a high percentage of migrants.

2. Material and methods

(a) Sampling

Vanessa cardui specimens were collected from February to April in multiple years (from 2014 to 2017) in six regions in the circum-Mediterranean at times coinciding with suspected migratory arrivals (figure 1). We recorded behavioural data at each survey site. In particular, when migratory movements were observed (i.e. specimens displaying fast directional flights, often in flocks—see electronic supplementary material, file S1), we recorded flight and wind direction and frequency of butterfly pass. Eighty seven samples were selected to represent as many localities and dates as possible, and were processed for isotope analyses (electronic supplementary material, table S1).

(b) Wind trajectories

For the sites where migratory movements were identified, backward wind trajectories were reconstructed for the sampling dates, using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) dispersion model from NOAA's Air Resources Laboratory [5]. Analyses were based on the Reanalysis database and computed on 48 h back trajectories arriving at sites at 12.00 h UTC (Coordinated Universal Time) and for three different altitudinal layers (500 m, 1000 m and 1500 m.a.g.l.), thus covering a wide altitudinal range where migrant Lepidoptera are documented to move downwind [6].

(c) Stable isotope analysis and modelling

Non-exchangeable δ^2H values from wing chitin were obtained using the comparative equilibration method [7]. All δ^2H results were reported in per mil (‰) deviations from the VSMOW-SLAP standard scale (see electronic supplementary material, file S2 for details).

To facilitate visualization of the isotopic assignment, δ^2H values were rearranged into six clusters explaining 95.3% of the variance of the dataset (figure 1). We used predictions of δ^2H variations in precipitation across the study area (electronic supplementary material, file S2) [8] and a calibration relationship between butterflies and precipitation [9] to generate a continuous-surface δ^2H model in *V. cardui* wing (or δ^2H isoscape). We compare the observed values to this model to estimate natal origins of each individual and cluster following the continuous-surface assignment approach [10].

3. Results and discussion

Spring migrants showed an important proportion (47%) of specimens with high probabilities of origin from the sub-Sahara region (mainly groups 1, 2 and 3). The remaining specimens are from groups of more ambiguous origin including the sub-Sahara, North Africa, southern Europe, the Middle East (groups 4 and 5, 47%), and from Europe (group 6, 6%). We observe that butterflies collected in February are more likely to be of sub-Saharan origin (e.g. samples from Andalusia, Morocco and Israel) than specimens collected at later dates. By contrast, butterflies from March and April showed a higher number of individuals that apparently performed shorter migrations (e.g. samples from Catalonia), or local natal origins (e.g. Crete and Israel) (figure 1). A northward wind-aided movement from Africa to Europe is supported by both observations of wind directions at ground level and HYSPLIT simulated trajectories at higher altitudinal layers, for each field site where migration was observed (figure 1 and electronic supplementary material, table S1).

The isotope data from butterflies collected in Morocco (February) suggest that migrants of sub-Saharan origin strongly contribute to replenishing *V. cardui* populations of Morocco sites (groups 2 and 3) (54%). These isotopic data are reinforced by field observations in the region of Taroudant, where butterflies were migrating northward in large numbers (27–28 February) without evidence of breeding in the region. The demonstration of a sub-Saharan origin for a substantial portion of the large populations breeding in South Morocco in March–April [6] solves the apparently paradoxical scarcity of *V. cardui* observations in the region during the winter months.

Butterflies collected in coastal Andalusia (February) after a sudden collective appearance were mostly assigned to a sub-Saharan origin (groups 2–3) (60%). A lower proportion apparently migrated from North Africa (groups 4–5) (40%), or were local (group 5). It is worth noting that February is earlier than usual for *V. cardui* arrivals to the Iberian Peninsula, where most important peaks are documented in April [6]. The isotopic data document for the first time the possibility of a direct colonization of Europe from Afrotropical grounds across the Sahara. This discovery rejects the premise that the migratory population of *V. cardui* necessarily undergoes a spring generation in the Maghreb (for Western Palaearctic migrations), although our data suggest that the majority of the population probably goes through this step.

Samples collected in coastal Catalonia (April) were flying in a northwest direction and directly arriving from the sea. These specimens were assigned to groups 3, 4 and 5. These groups all indicate high probabilities of origin from North Africa, suggesting flying distances no shorter than 500 km across the open sea. Backward wind trajectories support a high-altitude trans-Mediterranean flight potentially starting

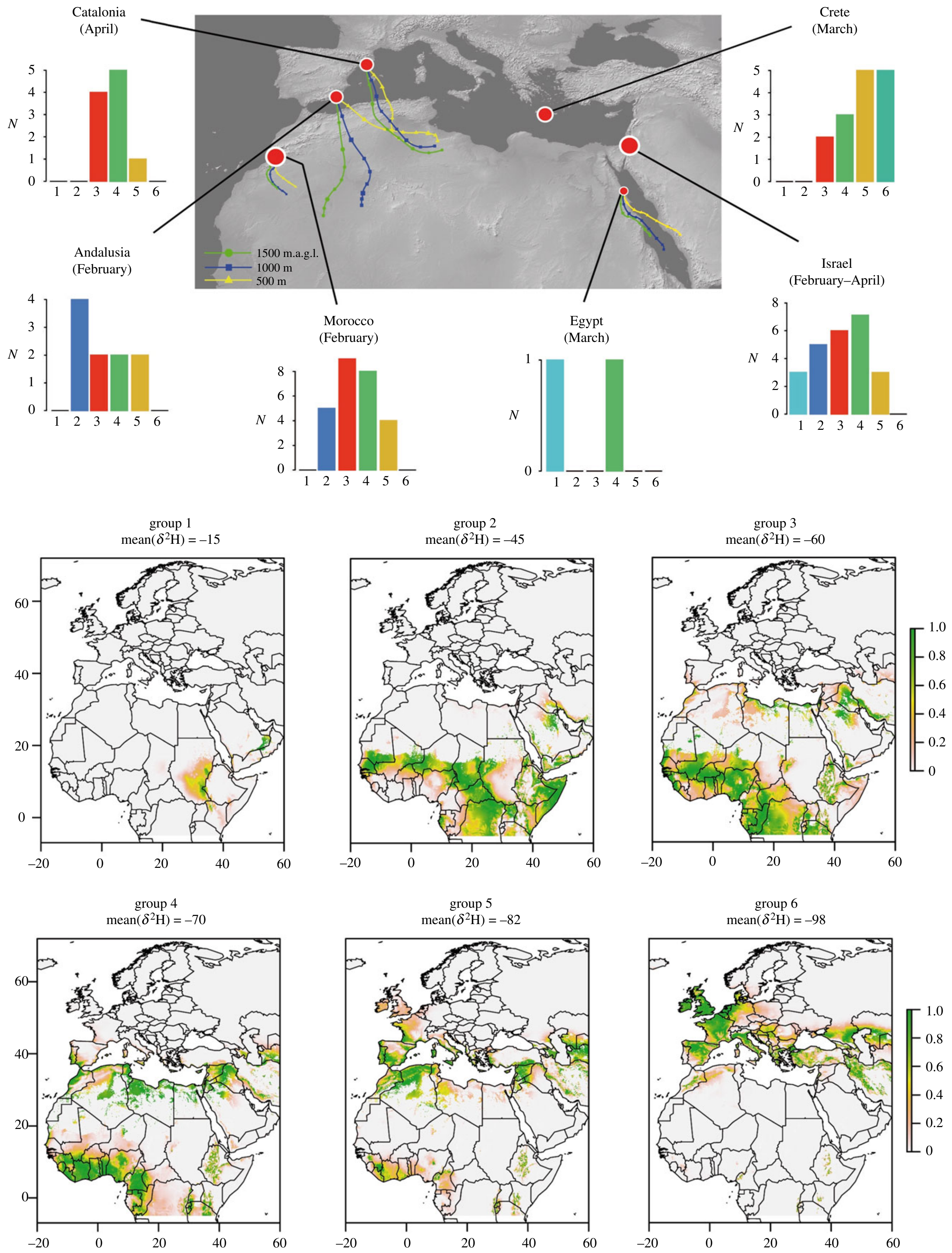

Figure 1. Natal origins of *V. cardui* estimated from δ^2H stable isotopes. Sampling sites are indicated in the upper map by red points, with size proportional to the number of individuals analysed. Bar graphs indicate a number of individual assignments to the six natal groups. Colours in the lower maps depict the predicted probability (0–1) of natal origins and mean δ^2H values are indicated. 48 h backward wind trajectories at three altitudes (500 m, 1000 m and 1500 m.a.g.l.) are shown for the specific sites and dates where peaks of migration were observed.

in Algeria. Remarkably, four specimens from group 3 (40%) had high probabilities of having sub-Saharan origins, suggesting much longer migratory path.

Samples in Crete (late March) were collected close to the coast, but apparently not in migration. These specimens were dominantly from regional or local origin (groups 5–6) (67%)

with a minor portion likely from northern Libya or Egypt (groups 3–4) (33%). Sub-Saharan natal origins cannot be discarded (especially for the two specimens assigned to group 3), but are probably more frequent earlier in the year. The dominance of local population is consistent with field observations of a few breeding sites in the southern part of the island. It is possible that *V. cardui* can marginally breed and survive the winter in Crete (as well as in other parts in the Mediterranean coastal regions), however, we did not notice empty larval silk nests in host plants (a good indicator of past larval growth).

Israel showed the widest range of isotope signals, probably in part due to the larger temporal range of sample collection (February–April) across multiple years (2014–2016) and sites. In total, 67% of the specimens were assigned to groups 3, 4 and 5, which show a complex pattern of likelihoods in the north Arabian Peninsula, as well as in sub-Saharan regions (e.g. Ethiopian highlands). This broad range of origin might reflect the actual geographical origin of *V. cardui* in Israel (and the Mediterranean Middle East) as a confluence zone for migrations from Africa, Arabia and, probably, Iran. A total of 33% of the specimens, all collected between February and March, were assigned to groups 1 and 2. These groups indicate a sub-Saharan or south-Arabian natal origin for these Israel specimens, thus involving long-distance migrations (greater than 2000 km), with a timing coinciding with trans-Saharan flights observed in Andalusia and Morocco. By contrast, specimens collected in February (particularly in 2016) were from all five population groups present in Israel. This is consistent with observations of simultaneous large breeding occurrence and migratory movements in 2016 [11], associated with exceptional El Niño rainfall in the Middle East.

Lastly, two specimens flying northward were collected from the coastal Red Sea in Egypt (late March) and were assigned to groups 1 and 4. Sub-Saharan origins in the Sudanese river systems and the Ethiopian highlands seem to be the more plausible options, even for group 4, which also includes potential natal areas north of the collecting site.

The discovery that the circum-Mediterranean region is colonized from the sub-Sahara in early spring has general implications for the spatio-temporal model of *V. cardui* migration. It suggests that the bulk of the population is likely located in the Afrotropics and not in the Maghreb during the winter months (from December to February). These populations flying into the temperate zone are most probably the offspring of the vast colonizations of the sub-Sahara during the autumn [3], which circulate within the Afrotropics until reversing migrations. A return movement has also been observed to occur marginally in western Africa during the autumn [4]. This scenario closes the loop for the Palearctic–African migratory system of *V. cardui* and shows that the annual distance travelled by the successive generations may reach about 12 000 km, including the crossing of the Sahara Desert twice. We conclude that a dead-end scenario for the late autumn colonization of the Afrotropics can be discarded. While some specimens might integrate within alternative migration routes within Africa, a large portion of the population migrates back towards Europe, remaining within the Africa–Europe migration circuit.

Data accessibility. The data have been uploaded as electronic supplementary material.

Authors' contributions. G.T. and R.V. conceived the study and drafted the manuscript. G.T., R.V., D.B. and M.G.-P. carried out fieldwork. C.B. and G.T. analysed data. All authors edited and approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We declare we have no competing interests.

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