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Migration ecology in insects: integrative approaches to trace long-distance movements of the Painted Lady butterfly *(Vanessa cardui)*

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Migratory insects may move in very large numbers, even surpassing migratory vertebrates in biomass. However, the extent of aerial flows of insects circulating around the planet, as well as their impact on ecosystems and biogeography, remain almost unstudied because of methodological challenges associated with tracking small, short-lived, organisms. In this presentation, I will show how a novel integrative approach allows reconstructing long-range insect movements, through a combination of tools on genetics, isotope ecology, ecological niche modelling, pollen metabarcoding, field ecology, and citizen science.

I will show the latest discoveries on the migrations of the Painted Lady butterfly (*Vanessa cardui*). This butterfly species is the most cosmopolitan of all butterflies, and it is known by its regular trans-Saharan migrations, that entail distances of >4000 km, similar to those of some birds. First, we track a migratory outbreak of *V. cardui* butterflies taking place at a continental scale in Europe, the Middle East, and Africa from March 2019 to November 2019. We use DNA metabarcoding to identify plants from pollen transported by the insects. From 265 butterflies collected in 14 countries over 7 months, we molecularly identify 398 plants. We develop a novel geolocation approach based on combining probability rasters from species distribution modelling of each identified plant, and thus trace back the location of the outbreak's origin and the origin of each of the subsequent generations. We show a strong representation of plants of Middle Eastern distribution in butterfly swarms collected in Eastern Europe in early spring. Swarms collected in Northern Europe in late spring were highly represented by plants of Mediterranean origin, and swarms collected in the summer in the Mediterranean likely originated in central and Northern Europe.

Second, we report the first proven transatlantic crossing by individual insects, a journey of at least 4,200 km from West Africa to South America. This discovery was possible through gathering

evidence from multiple sources, including coastal field surveys, wind trajectory modelling, phylogeography, pollen metabarcoding, and multi-isotope geolocation of natal origins. Wind trajectories were exceptionally favourable for the butterflies to disperse across the Atlantic from West Africa. Population genetic analyses clustered the butterflies collected in South America with the European-African population, ruling out the possibility that the migrants originated in America. Pollen metabarcoding showed highly represented plants endemic to the Sahelian region. Finally, a dual isotope analysis of hydrogen (δ^2H) and strontium ($^{87}Sr/^{86}Sr$) combined with a spatio-temporal niche model of suitable reproductive habitat geolocated the natal origins of the migrants to regions in Mali, Morocco, or Portugal, and thus not discarding a journey also involving a trans-Saharan crossing.

In summary, this work contributes new methodological avenues to advance our understanding of the dispersal and migration of insects. The findings here reported suggest that we may be underestimating long-range dispersal in insects, and highlight the importance of aerial highways connecting continents by trade winds. Overall, we will discuss the scale and potential implications that insect migratory movements represent for ecosystems and nature conservation worldwide.