





Article

Pollen metabarcoding reveals the origin and multigenerational migratory pathway of an intercontinental-scale butterfly outbreak

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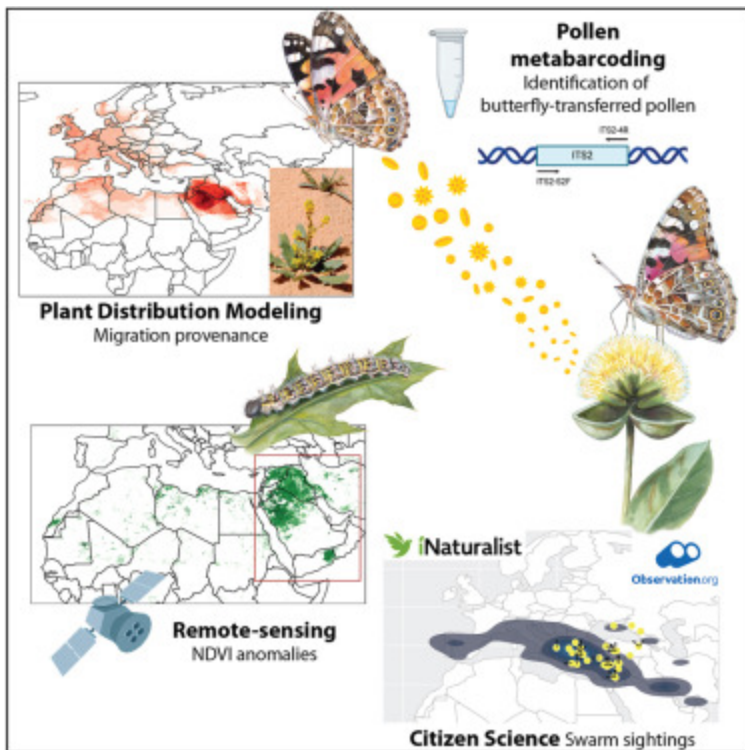
Highlights

- Citizen science reports explosions of the butterfly *Vanessa cardui* in 2019
- Metabarcoding of butterfly-transferred pollen identifies non-native plant species
- Plant distribution modeling infers butterfly migration and the outbreak's origin
- Middle Eastern outbreak impacts Europe and Africa demographics

Summary

Migratory insects may move in large numbers, even surpassing migratory vertebrates in biomass. Long-distance migratory insects complete annual cycles through multiple generations, with each generation's reproductive success linked to the resources available at different breeding grounds. Climatic anomalies in these grounds are presumed to trigger rapid population outbreaks. Here, we infer the origin and track the multigenerational path of a remarkable outbreak of painted lady (*Vanessa cardui*) butterflies that took place at an intercontinental scale in Europe, the Middle East, and Africa from March 2019 to November 2019. Using metabarcoding, we identified pollen transported by 264 butterflies captured in 10 countries over 7 months and modeled the distribution of the 398 plants detected. The analysis showed that swarms collected in Eastern Europe in early spring originated in Arabia and the Middle East, coinciding with a positive anomaly in vegetation growth in the region from November 2018 to April 2019. From there, the swarms advanced to Northern Europe during late spring, followed by an early reversal toward southwestern Europe in summer. The pollen-based evidence matched spatiotemporal abundance peaks revealed by citizen science, which also suggested an echo effect of the outbreak in West Africa during September–November. Our results show that population outbreaks in a part of species' migratory ranges may disseminate demographic effects across multiple generations in a wide geographic area. This study represents an unprecedented effort to track a continuous multigenerational insect migration on an intercontinental scale.

Graphical abstract



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Introduction

Herbivorous insect populations are inherently limited by the bottom-up pressures exerted by host plant availability.^{1,2,3} In arid and semi-arid regions experiencing strong interannual variation in precipitation, low and highly seasonal rainfall limits plant productivity. However, when unusually high rainfall occurs, a sudden rise in the quantity and quality of host plants may trigger an outbreak in insect populations by exceeding the top-down pressures exerted by natural-enemy control.^{4,5} Insect outbreak events are not fully understood mechanistically but are known to have significant impacts on ecosystem functioning, with consequences for nutrient cycles, pathogen dynamics, and plant health.^{4,5,6,7,8}

In the case of migratory insects,^{9,10,11,12} locally outbreaking populations can spread beyond their epicenter, affecting distant ecosystems.^{13,14} However, our ability to determine the full extent of the impacts of migratory insect outbreaks is hindered by logistical and technical hurdles. First, since many insect species migrate over multiple overlapping generations, studies are often limited by the difficulties of collecting observational data and samples across vast geographical areas and extended periods, which is necessary to cover each

segment of a multigenerational migratory cycle. Second, observational data alone do not inform about the complex spatiotemporal linkages of insect movement, and there are few techniques available to gather empirical connectivity data. These limitations have resulted in few attempts to link observational data with evidence of provenance across succeeding generations of insects. Studies inferring provenance, such as those using stable isotopes, typically sample only a few discrete points in time and space. While valuable to understand single-leg movements, this approach does not disclose connectivity along multigenerational migrations. Only collecting samples and provenance evidence from the entire spatiotemporal expanse of the multigenerational migratory cycle can offer a complete picture of migratory connectivity.

To address these challenges in the field of movement ecology, we studied an outbreak event of the painted lady butterfly (*Vanessa cardui*), which covers the greatest distance among insects during its annual migratory cycle.^{15,16,17,18} In 2019, huge densities of *V. cardui* were observed in various regions worldwide. A citizen science initiative allowed us to collect observational data and samples from large geographic (10 countries) and temporal (7 months) scales. To address the need for connectivity data, we developed a cost-effective method that combines information from metabarcoding of butterfly-transferred pollen grains¹⁹ and spatial analyses of plant distributions to provide evidence of provenance. This approach provided an exceptional opportunity to investigate the geographical extent and ecological implications of a wide-ranging intercontinental outbreak event for a migratory species.

Section snippets

The origin of the 2019 *Vanessa cardui* outbreak

The painted lady butterfly undergoes a cyclic multigenerational migration between tropical Africa and northern latitudes in the Palearctic in 8 to 10 generations every year.^{15,16,17,18} Consequently, each generation completes only a segment of this extensive journey. In March 2019, massive swarms of this butterfly were initially observed in parts of the Middle East and Eastern Mediterranean (EMed) (Figure 1A). Their massive presence was progressively noticed in Eastern Europe, Northern Europe,...

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Pollen grains isolated from samples of <i>Vanessa cardui</i> butterflies	This study	N/A
Chemicals, peptides, and recombinant proteins		
Beckman Coulter Agencourt AMPure XP Beads	Beckman Coulter GmbH	Cat#A63881
dNTP Mix, 10mM each	Thermo Fisher Scientific	Cat#R0192
Phusion High-Fidelity DNA-Polymerase (2U/ μ L)	Thermo Fisher Scientific	Cat#F530S
Ethanol \geq 99.5%, Ph. Eur., extra pure	Carl Roth	Cat#5054
Deionized ultrapure water, UV treated	Millipore	N/A
Optionally: 1M Tris		

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[Mass seasonal migrations of hoverflies provide extensive pollination and crop protection services](#)

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Insect Outbreaks

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