

1 **Long-distance autumn migration across the Sahara by painted lady**  
2 **butterflies: exploiting resource pulses in the tropical savannah**

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21 | references - 570, figure legends - 76)

22

23 **Abstract**

24

25 The painted lady, *Vanessa cardui*, is a migratory butterfly that performs an annual  
26 multi-generational migration between Europe and North Africa. Its seasonal appearance  
27 south of the Sahara in autumn is well known and has lead to the suggestion that it  
28 results from extremely long migratory flights by European butterflies to seasonally  
29 exploit the Sahel and the tropical savannah. However, this possibility has remained  
30 unproven. Here we analyse the isotopic composition of butterflies from seven European  
31 and seven African countries to provide new support for this hypothesis. Each individual  
32 was assigned a geographic natal origin, based on its wing stable hydrogen isotope  
33 ( $\delta^2\text{H}_w$ ) value and a predicted  $\delta^2\text{H}_w$  basemap for Europe and northern Africa. Natal  
34 assignments of autumn migrants collected south of the Sahara confirmed long distance  
35 movements (of 4000 km or more) starting in Europe. Samples from Maghreb revealed a  
36 mixed origin of migrants, with most individuals with a European origin, but others  
37 having originated in the Sahel. Therefore, autumn movements are not only directed to  
38 north-western Africa but include southward and northward flights across the Sahara.  
39 Through this remarkable behaviour, the productive but highly seasonal region south of  
40 the Sahara is incorporated into the migratory circuit of *V. cardui*.

41

42 **Keywords:** *Vanessa cardui*, insect migration, isoscapes, deuterium, Sahara, tropical  
43 savannah

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## 45 1. Introduction

46 Long-range insect migration is a timely and important topic in ecological research but  
47 one that is still in its infancy [1]. With a few exceptions [2], the small size of insects has  
48 prevented the use of exogenous markers to track their movements, which means that the  
49 most basic aspects of migration, the route itself and the distances covered, remain

50 poorly known for most species. Fortunately, this difficulty is increasingly being  
51 overcome with the use of intrinsic markers such as stable isotopes [3,4].  
52 In the Palaearctic, each year large numbers of insects undertake seasonal movements  
53 between Africa and Europe [5]. One such insect is the painted lady butterfly *Vanessa*  
54 *cardui*, which, through the succession of at least six generations, accomplishes a  
55 complete round-trip migration across most of Europe in spring and summer, and  
56 northern Africa in autumn and winter [6]. Although its general pattern of migration is  
57 known, many uncertainties regarding the distances covered by individual butterflies and  
58 the movements within Africa still exist. First, it has never been shown that the  
59 butterflies appearing south of the Sahara in autumn (sometimes in great numbers) have  
60 a European origin [6,7]. Second, although it is believed that north-western Africa  
61 (Maghreb) is colonized in autumn by European migrants, both ground-level and radar  
62 observations of northward migration in Morocco and Mauritania in October-November  
63 also point to sub-Saharan origins [6].

64

65 Here, we present new evidence on both of these questions by means of stable isotope  
66 ( $\delta^2\text{H}$ ) analysis, based on a comprehensive collection of butterflies across southern  
67 Europe, North Africa and South-Saharan Africa. Our data conclusively show that, in  
68 autumn, some European butterflies reach the tropical savannah south of the Sahara,  
69 where they are known to breed [7]. We also show that some of their offspring migrate  
70 northwards and cross the Sahara to breed in the Maghreb. These complex movements  
71 across the Sahara give a new dimension to our understanding of long-distance insect  
72 migration between Africa and Europe.

73

## 74 2. Material and methods

## 75 (a) Butterfly collection

76 We collected 334 butterflies from seven European and seven African countries around  
77 the Mediterranean, and from an extensive area south of the Sahara (figure 1). Butterflies  
78 were mainly collected in 2014 and, additionally, between 2009 and 2013 (electronic  
79 supplementary material, table S1). European samples were obtained in spring, summer  
80 and autumn (i.e. the period comprising northward and southward migrations and  
81 summer breeding), while African samples were mainly obtained from October to  
82 December (i.e. the period of colonization of North Africa and the region south of the  
83 Sahara, and the start of local emergences).

84

85 For each butterfly, wing condition was scored from 1 (fresh) to 5 (extremely worn). We  
86 assumed that categories 1 and 1.5 corresponded to recent local emergences not having  
87 undertaken migratory flights yet, and therefore these were excluded from analyses on  
88 the natal origin of potential migrants ([see electronic supplementary material, figure S1](#)  
89 [and methods, for examples of wing-wear categories and the rationale behind our](#)  
90 [assumptions](#)).

91

## 92 (b) Stable isotope analysis and natal assignments to potential migrants

93 Non-exchangeable  $\delta^2\text{H}$  values from wing chitin were obtained using the comparative  
94 equilibration method [8]. All  $\delta^2\text{H}$  results were reported in per mil (‰) deviations from  
95 the VSMOW-SLAP standard scale (see electronic supplementary material for details).

96

97 Prior to any isotopic assignment,  $\delta^2\text{H}$  values from all samples were arranged into five  
98 groups using a k-means clustering analysis [9]. These clusters explained 92% of the  
99 variation and represented the potential groups of natal origin. The five natal areas were

100 related to eastern-central Europe (Group 1, centroid  $\delta^2\text{H} = -111$  ‰,  $n = 35$ ), western-  
101 central Europe, southern Europe and Maghreb (Group 2,  $\delta^2\text{H} = -92$  ‰,  $n = 116$ ),  
102 Maghreb and Mediterranean Islands (Group 3,  $-80$  ‰,  $n = 96$ ), western Africa (Group  
103 4,  $\delta^2\text{H} = -65$  ‰,  $n = 60$ ), and central and eastern Africa (Group 5,  $\delta^2\text{H} = -39$  ‰,  $n = 27$ )  
104 (see electronic supplementary material, figure S2).

105  
106 To assign natal origins to potential migrants, a geospatial natal assignment method was  
107 used to link butterfly wing  $\delta^2\text{H}$  values ( $\delta^2\text{H}_w$ ) to well-known spatial hydrological  
108 hydrogen isotopic distribution (isoscapes) in precipitation ( $\delta^2\text{H}_p$ ) of Europe and northern  
109 Africa [10]. The  $\delta^2\text{H}_p$  isoscape [11] was then converted to a spatially explicit butterfly  
110 wing isoscape by using a calibration relationship determined for known-origin  
111 butterflies across the western Palaearctic [12]. Probability density surfaces were  
112 obtained using the complete individual spatial probability surface (no odds ratio used).  
113 All calculations and modelling were analysed in R [13].

114

### 115 3. Results and discussion

116 Stable hydrogen isotopes confirmed that the seasonal population shift of *V. cardui*  
117 between Europe and Africa is the result of long-distance migration by successive  
118 generations (i.e. multi- or transgenerational migration; [14]). Butterflies collected in  
119 southern Europe showed a temporal decline in  $\delta^2\text{H}_w$  values, from  $-81 \pm 17$  ‰ in April-  
120 May to  $-100 \pm 19$  ‰ in July ( $r=0.49$ ,  $p<0.01$ ). This is explained by the replacement of a  
121 spring population of northward migrants having developed as larvae in North Africa, by  
122 a summer population dominated by European local emergences. Butterflies collected in  
123 Africa showed an opposite trend ( $r=0.40$ ,  $p<0.01$ ), from  $-86 \pm 9$  ‰ in early October to  
124  $-60 \pm 17$  ‰ in late November. This trend is in accordance with first arrivals of European

125 migrants in October, followed by less negative values as their offspring emerged in the  
126 following month.

127

128 Natal assignments of autumn migrants collected south of the Sahara revealed long  
129 distance movements most likely starting in southern and central Europe (natal cluster  
130 groups 2-3 mostly, 6% from group 1, 7% from group 4, figure 2a). Although the  
131 mountains in the Maghreb also appeared as a potential natal area, field observations  
132 indicate that densities in the region are very low until the arrival of European migrants  
133 in October. This means that summer breeding in Maghreb mountains is at most a local  
134 phenomenon and cannot explain the origin of most butterflies appearing south of the  
135 Sahara in autumn. Although migration between Europe and the African tropical  
136 savannah had already been suggested [6,7,15], our analyses represent the first empirical  
137 confirmation of this phenomenon. Depending on the exact origin of the butterflies, these  
138 flights could exceed 4000 km. Such flights can probably only be accomplished by  
139 taking advantage of favourable winds [6,16].

140

141 Samples from Maghreb revealed a mixed origin of migrants. Most individuals (78%)  
142 shared essentially the same European natal origins as those collected south of the Sahara  
143 (figure 2b). A smaller fraction (22%), however, appeared to originate in the Sahel (natal  
144 groups 4-5, figure 2c). Butterflies with a European origin showed a wider range of wing  
145 wear, including very worn individuals that were absent from the samples of Sahelian  
146 origin. The proportion of categories 2-3.5 versus categories 4-5 differed between these  
147 two geographical groups ( $\chi^2=4.371$ ,  $P=0.037$ ), suggesting that European butterflies  
148 comprised a mixture of early and late migratory waves, while Sahelian butterflies only  
149 corresponded to more recent waves.

150

151 For a migratory insect, colonization of the Sahel and further south in autumn seems  
152 highly adaptive, as the whole region offers suitable breeding conditions coinciding with  
153 a short period of high productivity after the rainy season [7, 17]. This also explains the  
154 3500-4500 million birds migrating in autumn into this region, most of which depending  
155 on the seasonal insect populations [18].

156

157 However, strong seasonality also means that locally produced sub-Saharan generations  
158 of *V. cardui* experience rapid worsening of environmental conditions. Our data  
159 conclusively show that some butterflies migrate northwards across the Sahara, to  
160 colonize favourable areas in the Maghreb. This may seem surprising in a period when  
161 continuous southward-blowing dry winds (the so-called 'Harmattan') prevail in the  
162 Sahara [5]. However, autumn flights between western Sahel and the Maghreb also occur  
163 in other migrant insects moving down-wind (e.g. in swarms of the desert locust [19]),  
164 indicating that favourable conditions for northward migration across the Sahara still  
165 occur under some circumstances. Additional evidences come from repeated  
166 observations of northward migrations of *V. cardui* in the south of Morocco in late  
167 autumn (CS, pers. obs.).

168

169 In conclusion, our results convincingly show that autumn migration by *V. cardui* entails  
170 extremely long flights of 4000 km or more from Europe to the south of the Sahelian  
171 belt, in addition to the well-known destination in north-western Africa. Moreover, we  
172 confirm the existence of complex movements in Africa leading to the reinforcement of  
173 the autumn breeding population in the Maghreb by butterflies originating south of the

174 Sahara. This information will prove essential to model population trends in Europe in  
175 relation to the weather conditions experienced by the African populations.

176

#### 177 **Data accessibility**

178 Data supporting this article are included as part of the electronic supplementary  
179 material.

180

#### 181 **Author's contributions**

182 All authors conceived the study. C.S., G.T. and R.V. carried out the fieldwork. D.S.,  
183 K.H. and C.S. analysed the data. C.S. drafted the manuscript and all authors edited and  
184 approved the final version of the manuscript.

185

#### 186 **Competing interests**

187 We declare we have no competing interests.

188

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201

## 202 **References**

- 203 1. Chapman JW, Reynolds DR, Wilson K. 2015. Long-range seasonal migration in  
204 insects: mechanisms, evolutionary drivers and ecological consequences. *Ecol. Lett.*  
205 **18**, 287–302. (doi:10.1111/ele.12407)
- 206 2. Wikelski M, Moskowicz D, Adelman JS, Cochran J, Wilcove DS, May ML. 2006.  
207 Simple rules guide dragonfly migration. *Biol. Lett.* **2**, 325–329.  
208 (doi:10.1098/rsbl.2006.0487)
- 209 3. Flockhart DT, Wassenaar LI, Martin TG, Hobson KA, Wunder MB, Norris DR.  
210 2013. Tracking multi-generational colonization of the breeding grounds by monarch  
211 butterflies in eastern North America. *Proc. R. Soc. B* **280**, 20131087.  
212 (doi:10.1098/rspb.2013.1087)
- 213 4. Hobson KA, Anderson RC, Soto DX, Wassenaar LI. 2012. Isotopic evidence that  
214 dragonflies (*Pantala flavescens*) migrating through the Maldives come from the  
215 Northern Indian subcontinent. *PLoS One* **7(12)**, e52594.  
216 (doi:10.1371/journal.pone.0052594)
- 217 5. Pedgley DE, Reynolds DR, Tatchell GM. 1995. Long-range insect migration in  
218 relation to climate and weather: Africa and Europe. In *Insect migration: tracking*  
219 *resources through space and time* (eds VA Drake, AG Gatehouse), pp. 3–29.  
220 Cambridge: Cambridge Univ. Press.
- 221 6. Stefanescu C, Páramo F, Åkesson S, Alarcón M, Ávila A, Brereton T, Carnicer J,  
222 Cassar L, Fox R, Heliölä J, Hill JK, Hirneisen N, Kjellén N, Kühn E, Kuussaari M,  
223 Leskinen M, Liechti F, Musche M, Regan E, Reynolds D, Roy DB, Ryrholm N,

- 224 Schmaljohann H, Settele J, Thomas CD, van Swaay C, Chapman J. 2013. Multi-  
225 generational long-distance migration in insects: studying the painted lady butterfly  
226 in the Western Palaearctic. *Ecography* **36**, 474–486. (doi:10.1111/j.1600-  
227 0587.2012.07738.x)
- 228 | 7. Talavera G, Vila R. 2016. ~~Mass migration and abundance of *Vanessa cardui* in the~~  
229 ~~subtropical African Savannah suggest and early fall colonization by European~~  
230 ~~migrants~~. *J. Lepid. Soc.* (in press)
- 231 8. Wassenaar LI, Hobson KA. 2003. Comparative equilibration and online technique  
232 for determination of non-exchangeable hydrogen of keratins for use in animal  
233 migration studies. *Isotopes Environ. Health Stud.* **39**, 211–217.  
234 (doi:10.1080/1025601031000096781)
- 235 9. García-Pérez B, Hobson KA. 2014. A multi-isotope ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) approach to  
236 establishing migratory connectivity of Barn Swallow (*Hirundo rustica*). *Ecosphere*  
237 **5**, art21. (doi:10.1890/ES13-00116.1)
- 238 10. Hobson KA, Soto DX, Paulson DR, Wassenaar LI, Matthews JH. 2012b. A  
239 dragonfly ( $\delta^2\text{H}$ ) isoscape for North America: a new tool for determining natal  
240 origins of migratory aquatic emergent insects. *Methods Ecol. Evol.* **3**, 766–772.  
241 (doi:10.1111/j.2041-210X.2012.00202.x)
- 242 11. Terzer S, Wassenaar LI, Araguás-Araguás LJ, Aggrawal PK. 2013. Global isoscapes  
243 for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in precipitation: improved prediction using regionalized climatic  
244 regression models. *Hydrol. Earth Syst. Sci. Discuss.* **10**, 7351–7393.  
245 (doi:10.5194/hess-17-1-2013)
- 246 | 12. Brattström O, Bensch S, Wassenaar LI, Hobson KA, Åkesson S. 2010.  
247 Understanding the migration ecology of European red admirals *Vanessa atalanta*

- 248 using stable hydrogen isotopes. *Ecography* **33**, 720-729. (doi: 10.1111/j.1600-  
249 0587.2009.05748.x)
- 250 ~~12-13.~~ [R CoreTeam. 2015. \*A language and environment for statistical computing\*. R](#)  
251 [Foundation for Statistical Computing.](#)
- 252 ~~13-14.~~ Chapman BB, Hulthén K, Wellenreuther M, Hansson L-A, Nilsson J-A,  
253 Brönmark C. 2014. Patterns of animal migration. In *Animal movement across scales*  
254 (eds. L-A Hansson, S Akesson), pp. 11–35. Oxford: Oxford University Press.
- 255 ~~14-15.~~ Williams CB. 1958. *Insect migration*. London: Collins.
- 256 ~~15-16.~~ Chapman JW, Nesbit RL, Burgin LE, Reynolds DR, Smith AD, Middleton DR,  
257 Hill JK. 2010. Flight orientation behaviors promote optimal migration trajectories in  
258 high-flying insects. *Science* **327**, 682–685. (doi: 10.1126/science.1182990)
- 259 ~~16-17.~~ Zwarts L, Bijlsma RG, van der Kamp J, Wymenga E. 2009. *Living on the edge:*  
260 *wetlands and birds in a changing Sahel*. Zeist, The Netherlands: KNNV Uitgeverij.
- 261 ~~17-18.~~ Newton I. 2008. *The migration ecology of birds*. London: Academic Press.
- 262 ~~18-19.~~ Symmons PM, Cressman K. 2001. *Desert locust guidelines. 1. Biology and*  
263 *behaviour*. Rome: FAO.

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267 Figure 1. Sample locations and sizes, superimposed on the isoscape of estimated  $\delta^2\text{H}_w$   
268 for the wings of painted ladies in Europe and Africa.

269

270

271 Figure 2. Assigned natal origins of painted ladies collected in autumn in the Sahel (a),  
272 and Morocco (b, c), with the corresponding number of butterflies analysed (N). Natal

273 groups 1-5 were defined with a k-means clustering analysis [\(see Material and](#)  
274 [methods\)](#). Colours depict the predicted probability (0-1) of natal origins of these  
275 [migrants](#).

276

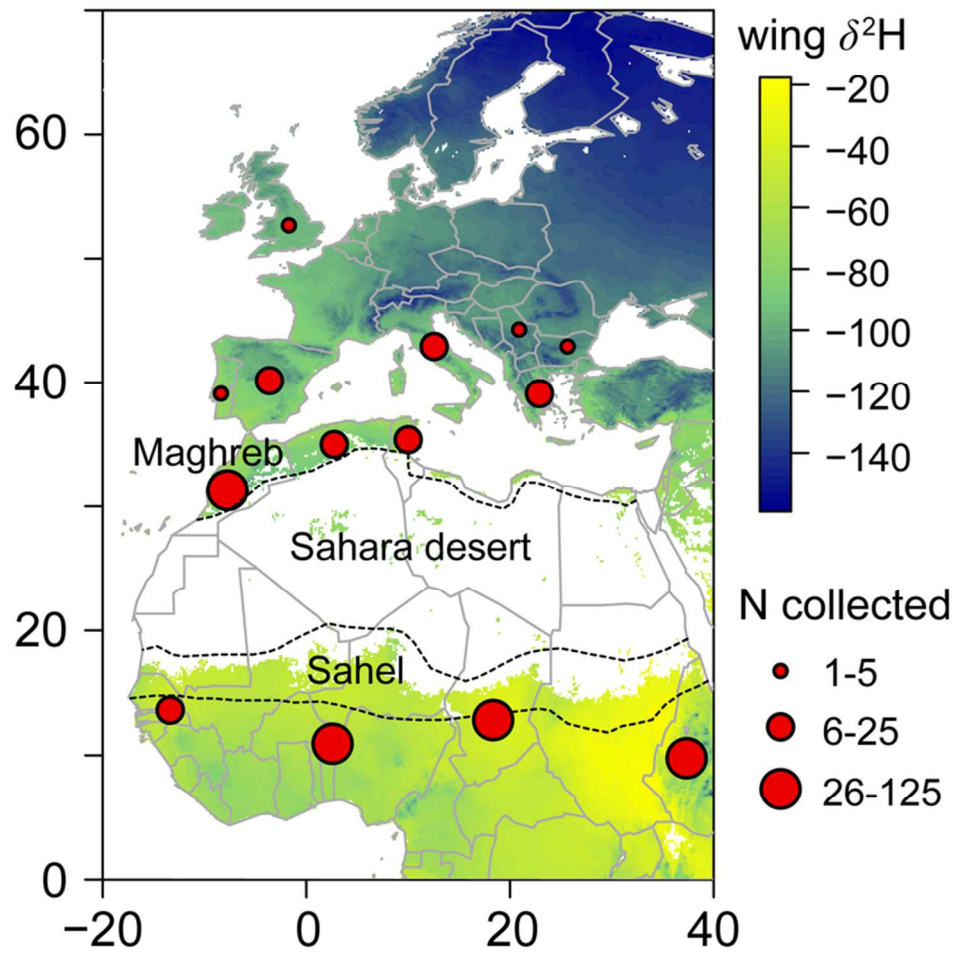


Figure 1. Sample locations and sizes, superimposed on the isoscape of estimated  $\delta^2\text{H}_{\text{w}}$  for the wings of painted ladies in Europe and Africa.

75x77mm (300 x 300 DPI)

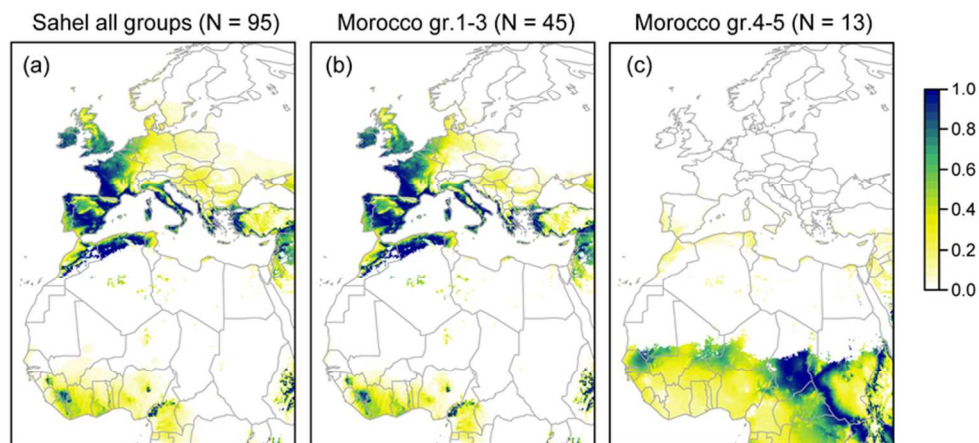


Figure 2. Assigned natal origins of painted ladies collected in autumn in the Sahel (a), and Morocco (b, c), with the corresponding number of butterflies analysed (N). Natal groups 1-5 were defined with a k-means clustering analysis (see Material and methods). Colours depict the predicted probability (0-1) of natal origins of these migrants.

73x34mm (300 x 300 DPI)