

Habitat fragmentation causes coevolutionary burning spots

H. De Kort^{1*}, M. Baguette^{1,2}, J.G. Prunier¹, M. Tessier³, J. Monsimet⁴, C. Turlure⁵, V.M. Stevens¹

1 Station d'Ecologie Théorique et Expérimentale (UMR 5321 SETE), National Center for Scientific Research (CNRS) and Université Toulouse III – Paul Sabatier, 2 Route du CNRS, 09200 Moulis, France.

2 Institut de Systématique, Evolution, Biodiversité (UMR 7205), Muséum National d'Histoire Naturelle, CP 50, 45 rue Buffon, 75005 Paris, France.

3 Marc Tessier, 62 chemin Del prat, 31320 Auzeville-Tolosane, France.

4 Parc Naturel Régional des Marais du Cotentin et du Bessin, 3 village Ponts d'Ouve, 50500 Carentan-les-Marais, France

5 Earth and Life Institute, Université catholique de Louvain, Croix du Sud 4, 1348 Louvain-la-Neuve, Belgium.

* Correspondence to: hanne.dekort@sete.cnrs.fr, hanne.dekort@kuleuven.be

Abstract: Habitat fragmentation increasingly threatens the services provided by natural communities and ecosystem worldwide. An understanding of the underlying eco-evolutionary processes in natural settings is lacking, yet critical to realistic and sustainable conservation. Through integrating the multivariate genetic, biotic and abiotic facets of a natural community network experiencing various degrees of habitat fragmentation, we provide unique insights into the processes underlying community functioning in real, natural conditions. The focal community network comprises a parasitic butterfly of conservation concern, and its two obligatory host species, a plant and an ant. We show that fragmentation of butterfly habitat has the potential to impair the balance between dispersal and coevolution. This process can cause coevolutionary burning spots of decreased genetic diversity and therefore of increased extinction risk. We stress that ignoring such eco-evolutionary feed-backs inherent to the very functioning of natural communities can strongly impact their persistence.

One Sentence Summary: Communities under threat of habitat fragmentation suffer increased extinction risk through coevolutionary overheating.

34 Biotic interaction networks including pollination, competition and parasitism play a key
35 role in community functioning and persistence, but global environmental changes compromise
36 their ecological and evolutionary stability (1–3). Fueled by ongoing land conversions, habitat
37 fragmentation in particular increasingly degrades ecological communities and ecosystems
38 across the globe (4, 5). Yet attempts to mitigate these impacts are hampered by a markedly
39 poor understanding of the eco-evolutionary dynamics driving biotic interaction networks under
40 threat of habitat fragmentation (6–8). Indeed, owing to technical challenges associated with
41 studying eco-evolutionary community processes in natural settings, non-experimental
42 empirical studies demonstrating how biotic interactions implicate the ability of species to
43 withstand habitat fragmentation are lacking.

44 Theoretical models and experimental work on microbial communities predict that
45 habitat fragmentation and community functioning are linked through the effects of landscape
46 connectivity on dispersal, the latter defining individual movements across the landscape (9–
47 12). According to these *in silico* and *in vitro* studies, intermediate levels of dispersal facilitate
48 species evolution and species sorting concordant with local biotic and abiotic conditions,
49 whereas low (or high) levels of dispersal can deplete (or swamp) locally adapted communities.
50 However, the exact processes determining eco-evolutionary outcomes are highly variable,
51 shaped by species' genetic architecture, their phenotypic variation, the strength and amount of
52 interactions with local biotic and abiotic variables, and the eco-evolutionary feedbacks
53 resulting from interactions between each of these components (7, 13). This complexity is
54 compromising empirical validation of theoretical predictions, urging for a more realistic
55 perspective on the impacts of altered landscape connectivity on communities and ecosystems
56 (14–16).

57 Analogously to the interwoven nature of community dynamics and adaptive evolution,
58 we propose the integration of landscape genetic disciplines in community ecology (17) to
59 address this complexity *in situ*. Ecological and evolutionary processes including drift, gene
60 flow, and abiotic and coevolutionary selection directly influence patterns of genetic variation
61 within and among communities. Coevolution in particular, i.e. the process by which two or
62 more interacting species reciprocally affect each other's evolution, may give rise to specific
63 genetic signatures when communities are spatially structured. More specifically, spatial
64 community structure can generate a geographic mosaic of coevolutionary hot spots featured by
65 strong reciprocal selection among interacting species (18–20). Where local coevolution
66 prevents successful immigration of maladapted individuals, or when it favours reduced
67 dispersal ability in isolated habitats (21), such selection mosaics can result in paralleled genetic
68 structure among interacting species (22). Assessing population genetic variation within and
69 among the different species of a community network may therefore shed light on the impact of
70 habitat fragmentation on community dynamics.

71
72 Here we present the first empirical data showing how habitat fragmentation can impact
73 the eco-evolutionary dynamics propelling communities in natural settings. We focus on a
74 community system highly appreciated for its conservation value: the threatened and specialized
75 European butterfly *Phengaris* (= *Maculinea*) *alcon alcon* (the Alcon blue, hereafter “butterfly”)
76 and its two obligatory hosts, a rare grassland plant *Gentiana pneumonanthe* (the march Gentian,
77 hereafter “plant”) and an ant of the genus *Myrmica* (here the common *M. scabrinodis*, hereafter
78 “ant”) (Fig.1A) (23, 24). Due to habitat fragmentation, the butterfly faces local extinctions
79 across its range, even where its hosts remain locally abundant (25). We focus on a mountainous
80 landscape in the French department Ariège (4890 km²), where we investigated all known
81 butterfly communities. These community sites are spatially structured into four disjoint clusters
82 (metacommunities), with among-cluster distances (>10km) exceeding the known maximum

83 dispersal distance of the butterfly (0.5km) (Fig.1B-D, Fig.S1). The communities span an
84 altitudinal gradient from 400 to 1017 m, with patch sizes varying between 275 and 17,359 m²
85 (Table S1).

86 Based on land use, plant requirements, and the biology of the butterfly, we mapped
87 butterfly dispersal probability across the landscape to obtain a relevant measure of landscape
88 connectivity for this species (Fig.1D, Table S2-3). Because habitat fragmentation deteriorates
89 landscape connectivity through reductions in the amount of habitat suitable for dispersal and
90 breeding, and through increased geographical distance among breeding sites, it typically
91 disrupts the genetic integrity of butterfly populations (26). In line with this unfavourable trend,
92 we found that decreased landscape connectivity increased the genetic distance between the
93 butterfly populations under study (Fig.S2). This finding is consistent with field observations
94 obtained during daily individual butterfly movement monitoring, showing some dispersal
95 within geographical clusters (3.55% butterflies recaptured in another site vs. 35.49%
96 recaptured on site), limited dispersal among the nearest clusters (0.51%), and no dispersal
97 among the more distant clusters (Fig. S1). By hindering butterfly movements across the
98 landscape to various extents, habitat fragmentation thus resulted in a spatial mosaic of highly
99 and poorly connected sites (Fig.1D).

100 We then explored how the genetic diversity within communities was affected by
101 landscape connectivity, local habitat size and altitude. Genetic diversity was calculated for 22
102 butterfly, 37 plant and 29 ant populations, based on high-quality single nucleotide
103 polymorphisms obtained through pooled RADseq (Fig.S3). We found a marked negative effect
104 of decreasing landscape connectivity on butterfly genetic diversity (Fig.2A, Fig.S4, Table S3),
105 implying a harmful effect of habitat fragmentation on its population dynamics. We also
106 observed that both butterfly and plant genetic diversity decreased with altitude (Fig.2B).
107 Because census population sizes did not follow an altitudinal pattern (Fig. S5), we devote this
108 result to historical post-glacial migration to higher altitudes followed by population expansion.
109 Together, landscape connectivity and altitude explained 74.23% (R^2_{adj}) of the variation in
110 butterfly genetic diversity.

111 Given the absence of a negative correlation between butterfly and host genetic diversity,
112 our results also suggest limited parasitic impact of the butterfly on its two host species. To
113 corroborate this theory, we compared ant and plant genetic diversity between sites with high
114 vs. low butterfly population density (i.e. high vs. low parasitic pressure), and sites where the
115 butterfly got extinct in the last 5 years (Table S1). The genetic diversity of the hosts did not
116 decrease with increased butterfly density (Fig.2D), indicating that the hosts are not markedly
117 impacted by butterfly population dynamics. Importantly, this also suggests that conservation
118 efforts aiming to increase connectivity for butterflies would maintain the functioning of the
119 community network without compromising the host species.

120 The strong positive correlation between butterfly and plant genetic diversity, but not
121 between butterfly and ant genetic diversity (Fig.2C, Table S4), corresponds to the shared effect
122 of altitude on their genetic diversity (Fig.2B). However, because the correlation between
123 butterfly and plant diversity is significant only where landscape connectivity is relatively low
124 (Fig.2C), genetic diversity losses in the plant (the least mobile partner of the community) may
125 have negative consequences for butterfly genetic diversity where habitat fragmentation
126 impedes butterfly gene flow between populations. To confirm the genetic dependence between
127 the butterfly and the hosts in the context of habitat fragmentation, an understanding of both the
128 abiotic and coevolutionary drivers of community dynamics is required.

129 We therefore investigated the contributions of abiotic landscape variation to butterfly
130 and host genetic structure, using multivariate landscape and community genetic approaches
131 (Table S4). To approximate the abiotic (incl. spatial) environment, we modelled latitude,
132 longitude, altitude, habitat size and landscape connectivity (for the butterfly) or geographical
133 isolation (for the hosts). We thus also accounted for all existing environmental clines that are
134 generally known to covary with these factors (e.g. temperature and precipitation). The abiotic
135 variables explained a significant proportion of the community genetic structure, varying from
136 13.72% for ants over 28.03% for plants to 32.95% for butterflies. Latitude, longitude and
137 altitude all contributed to the notable genetic structure of the butterfly and the plant (Fig.3A-
138 C, Fig.S6). The genetic structure of the ant was less pronounced, although the southern clusters
139 were strongly differentiated owing to geographical isolation and altitudinal differences
140 (Fig.3C). Together, these findings indicate an important role for spatial structure and the
141 associated abiotic clines in driving genetic differences among communities. On top of the
142 observed spatial effects, landscape connectivity influenced butterfly genetic structure (Fig.3A),
143 suggesting that habitat fragmentation interferes with its metapopulation dynamics. As expected
144 from the complexity inherent to natural communities, a large part of the genetic structure
145 remains unexplained, and could be partially due to biotic interactions which are most often
146 ignored in landscape genetic studies.

147 We thus incorporated the genetic structure of the host species as a proxy for the biotic
148 environment of the butterfly, to identify the proportion of its genetic structure covarying with
149 host genetic structure while accounting for the abiotic environment. The resulting model (Table
150 S4) provides insights in unique, independent processes driving butterfly genetic structure,
151 captured by so-called co-inertia axes, in addition to shared, interdependent processes. To assess
152 the effect of habitat fragmentation on the degree of covariation (i.e. co-inertia) between
153 butterfly and host genetic structure, we contrasted between poorly and highly connected
154 butterfly populations. Substantial variation in butterfly genetic structure can be explained by
155 host genetic structure and abiotic variation, although this covariation was only significant for
156 the poorly connected sites (covariation coefficient = 0.85^{ns} and 0.84** for high and low
157 connectivity, respectively, Table S4). Overall, most of the butterfly genetic variation co-varied
158 with the abiotic and biotic environment simultaneously (Fig.3D), indicating shared effects
159 between the abiotic variables and the host genetic structure on butterfly genetic structure. This
160 is in line with our observation that shared abiotic conditions drive the genetic structure of the
161 three species (Fig.3A-C).

162 Where landscape connectivity is high for the butterfly (Fig.3D, left panel), we observed
163 strong common contributions of abiotic variables and host genetic structure to butterfly genetic
164 structure (equivalent contributions to co-inertia axes 1-3). In addition, a limited part of the
165 butterfly genetic structure can be uniquely explained by host genetic structure, as shown by the
166 disproportional contribution of plant genetic structure to co-inertia axis 4, and of ant genetic
167 structure to axis 5. Because this parallel genetic structure between the butterfly and its two
168 hosts cannot be fully explained by abiotic variables, we suspect that reciprocal coevolutionary
169 selection between the butterfly and its two hosts has reduced effective gene flow between the
170 communities, thereby synchronizing the genetic structure of the host-parasite network. This
171 notion is concordant with previously documented coevolutionary shifts in flower phenology
172 (*G. pneumonanthe* plants) and surface chemistry (*Myrmica* ants) to escape local infestation by
173 *P. alcon* butterflies (23, 24). Where postponed plant flowering and altered ant surface
174 chemistry prevent reproduction of desynchronized butterfly immigrants, local coevolution has
175 the potential to increase the genetic distance between communities, resulting in parallel genetic
176 structure among the interacting species. Correspondingly, the genetic structure of another
177 *Myrmica* ant species has been demonstrated to be closely associated with its local surface

178 chemistry signature, evolved to counter butterfly impacts (23). We conclude that spatial
179 community structure provides opportunities for coevolution, while frequent local butterfly
180 immigration (under high landscape connectivity) maintains relatively high levels of genetic
181 diversity within metapopulations.

182 Where landscape connectivity is low (Fig.3D, right panel, Fig.4B), a more profound
183 relation between butterfly and host genetic structure was apparent. Host genetic structure
184 (plant: axes 2; ant: axis 3) and abiotic factors (axis 1) independently covaried with butterfly
185 genetic structure (Fig. 3D), likely reflecting intense coevolutionary genetic parallelism. This
186 result is consistent with the idea that habitat fragmentation can reinforce restrictions on
187 effective gene flow driven by coevolution. Alternatively, coevolution has been shown to favour
188 decreased dispersal in isolated patches as a strategy to maintain local coadaptation (21), and
189 this could also generate genetic parallelism. However, considering that even the most isolated
190 populations of this study system are potentially connected to others (Fig.1D), selection for
191 reduced dispersal seems unlikely. Because habitat fragmentation intensifies coevolutionary
192 pressures (Fig.3D, right panel), while decreasing genetic diversity (Fig.2A), our findings
193 suggest the existence of coevolutionary “burning” spots, featured by increased local butterfly
194 extinction risk. From a global viewpoint, we argue that the widespread occurrence of
195 coevolutionary interactions renders communities even more vulnerable to habitat
196 fragmentation than generally acknowledged.

197 Our study does not capture causal relationships between landscape connectivity and
198 community dynamics, but it does provide unique perspectives on the strength of *in situ* species’
199 associations under threat of habitat fragmentation. Decreased landscape connectivity due to
200 land conversion and degradation has the potential to alter the balance between dispersal and
201 coevolution while decreasing genetic diversity, resulting in locally overheated coevolution
202 featured by increased extinction risk (Fig.4). Reconnecting these communities could restore
203 coevolutionary dynamics and simultaneously improve butterfly genetic diversity and
204 population persistence rate. Interestingly, relatively dense butterfly populations with high
205 genetic diversity did not leave a noticeable mark on host genetic diversity. The gradual and
206 reciprocal accumulation of coevolutionary genetic signatures over time likely has allowed
207 coexistence without strong fluctuations in parasite and host genetic diversity. Building on this
208 finding, we emphasize the strength of community genetic studies in detecting eco-evolutionary
209 signatures that have been shaped over contemporary time periods. To shed light on the global
210 extent of our findings, it would be insightful to deploy the used integrative strategy, combining
211 pooled sequencing and multivariate genetic approaches, in other natural settings. Although a
212 pooled sequencing approach complicates the generation of genetic marker-specific inferences
213 (Fig. S3), it offers major advantages for studying *in situ* community dynamics. Its cost-
214 efficiency could allow genotyping hundreds of populations with modest research budgets, and
215 multivariate techniques subsequently allow disentangling the relative contributions of abiotic
216 and coevolutionary drivers of community dynamics.

217 Predicting how species will cope with global environmental changes is a key ambition in
218 evolutionary and ecological sciences. However, the lack of eco-evolutionary integration in
219 forecasting models may compromise their ability to predict range dynamics and to provide
220 realistic guidelines for sustainable conservation (27, 28). Based on our findings, we stress that
221 ignoring the impacts of widespread coevolutionary networks on the ability of species to cope
222 with global environmental changes can considerably affect outcomes of conservation strategies
223 and forecasting models. Moreover, whereas habitat fragmentation has the potential to alter
224 coevolutionary relations irrespective of other global environmental threats, the combined

225 impacts of multiple global environmental changes on community dynamics are expected to be
226 distressing, but have yet to be assessed.
227

228 **References and Notes:**

- 229 1. J. M. Tylianakis, R. K. Didham, J. Bascompte, D. A. Wardle, Global change and species
230 interactions in terrestrial ecosystems. *Ecol. Lett.* **11**, 1351–1363 (2008).
- 231 2. S. E. Gilman *et al.*, A framework for community interactions under climate change. *Trends Ecol.*
232 *Evol.* **25**, 325–31 (2010).
- 233 3. G. Strona *et al.*, Environmental change makes robust ecological networks fragile. *Nat. Commun.*
234 **7**, 12462 (2016).
- 235 4. N. M. Haddad *et al.*, Habitat fragmentation and its lasting impact on Earth’s ecosystems. *Sci.*
236 *Adv.* **1** (2015).
- 237 5. T. Newbold *et al.*, Global effects of land use on local terrestrial biodiversity. *Nature.* **520**, 45–50
238 (2015).
- 239 6. N. Titeux, K. Henle, J.-B. Mihoub, L. Brotons, Climate change distracts us from other threats to
240 biodiversity. *Front. Ecol. Environ.* **14**, 291–291 (2016).
- 241 7. D. Legrand *et al.*, Eco-evolutionary dynamics in fragmented landscapes. *Ecography.* **40**, 9–25
242 (2017).
- 243 8. H. Toju *et al.*, Species-rich networks and eco-evolutionary synthesis at the metacommunity level.
244 *Nat. Ecol. Evol.* **486**, 222–227 (2017)
- 245 9. M. C. Urban *et al.*, The evolutionary ecology of metacommunities. *Trends Ecol. Evol.* **23**, 311–7
246 (2008).
- 247 10. P. A. Venail *et al.*, Diversity and productivity peak at intermediate dispersal rate in evolving
248 metacommunities. *Nature.* **452**, 210–214 (2008).
- 249 11. P. Staddon, Z. Lindo, P. D. Crittenden, F. Gilbert, A. Gonzalez, Connectivity, non-random
250 extinction and ecosystem function in experimental metacommunities. *Ecol. Lett.* **13**, 543–552
251 (2010).
- 252 12. P. L. Thompson, B. Rayfield, A. Gonzalez, Loss of habitat and connectivity erodes species
253 diversity, ecosystem functioning, and stability in metacommunity networks. *Ecography.* **40**, 98–
254 108 (2017).
- 255 13. M. Baguette, S. Blanchet, D. Legrand, V. M. Stevens, C. Turlure, Individual dispersal, landscape
256 connectivity and ecological networks. *Biol. Rev.* **88**, 310–326 (2013).
- 257 14. J. B. Logue, N. Mouquet, H. Peter, H. Hillebrand, Empirical approaches to metacommunities: a
258 review and comparison with theory. *Trends Ecol. Evol.* **26**, 482–491 (2011).
- 259 15. H. Kokko *et al.*, Can Evolution Supply What Ecology Demands? *Trends Ecol. Evol.* (2017),
260 doi:10.1016/j.tree.2016.12.005.
- 261 16. J. Cote *et al.*, Evolution of dispersal strategies and dispersal syndromes in fragmented
262 landscapes. *Ecography.* **40**, 56–73 (2017).
- 263 17. B. K. Hand, W. H. Lowe, R. P. Kovach, C. C. Muhlfeld, G. Luikart, Landscape community
264 genomics: understanding eco-evolutionary processes in complex environments. *Trends Ecol.*
265 *Evol.* **30**, 161–168 (2015).
- 266 18. S. L. Nuismer, J. N. Thompson, R. Gomulkiewicz, Coevolutionary clines across selection
267 mosaics. *Evolution.* **54**, 1102–1115 (2000).
- 268 19. J. N. Thompson, B. M. Cunningham, Geographic structure and dynamics of coevolutionary
269 selection. *Nature.* **417**, 735–738 (2002).
- 270 20. P. H. Thrall *et al.*, Rapid genetic change underpins antagonistic coevolution in a natural host-
271 pathogen metapopulation. *Ecol. Lett.* **15**, 425–435 (2012).
- 272 21. D. Start, B. Gilbert, Host–parasitoid evolution in a metacommunity. *Proc. R. Soc. London B Biol.*
273 *Sci.* **283** (2016).
- 274 22. K. Räsänen, A. P. Hendry, Disentangling interactions between adaptive divergence and gene
275 flow when ecology drives diversification. *Ecol. Lett.* **11**, 624–36 (2008).
- 276 23. D. R. Nash, T. D. Als, R. Maile, G. R. Jones, J. J. Boomsma, A Mosaic of Chemical Coevolution
277 in a Large Blue Butterfly. *Science.* **319**, 88–90 (2008).
- 278 24. A. Valdés, J. Ehrlén, Caterpillar seed predators mediate shifts in selection on flowering
279 phenology in their host plant. *Ecology.* **98**, 228–238 (2017).
- 280 25. N. Mouquet *et al.*, Conserving community modules: A case study of the endangered lycaenid
281 butterfly *Maculinea alcon*. *Ecology.* **86**, 3160–3173 (2005).

- 282 26. J. A. Thomas, Butterfly communities under threat. *Science*. **353**, 2016–2018 (2016).
- 283 27. S. Lavergne, N. Mouquet, W. Thuiller, O. Ronce, Biodiversity and Climate Change: Integrating
284 Evolutionary and Ecological Responses of Species and Communities. *Annu. Rev. Ecol. Evol.*
285 *Syst.* **41**, 321–350 (2010).
- 286 28. D. H. Ikeda *et al.*, Genetically informed ecological niche models improve climate change
287 predictions. *Glob. Chang. Biol.* **23**, 165–176 (2016).
- 288 29. P. Metailié, M. Paegelow, Land Abandonment and the Spreading of the Forest in the Eastern
289 French Pyrenées in the Nineteenth to Twentieth Centuries, in Recent Dynamics of the
290 Mediterranean Vegetation and Landscape, pp. 217–236 (2015).
- 291 30. D. Galop, D. Rius, C. Cugny, F. Mazier, A History of Long-Term Human–Environment
292 Interactions in the French Pyrenees Inferred from the Pollen Data, in Continuity and Change in
293 Cultural Adaptation to Mountain Environments, pp. 19–30 (2013).
- 294 31. M. Tessier, Inventaire de l’Azuré des mouillères *Maculinea alcon* (Denis & Schiffermüller,
295 1775) (Lepidoptera Lycaenidae) en Ariège. *Bull. Soc. Linn. Bordeaux*. **43**, 205–212 (2015).
- 296 32. P. D. Etter, J. L. Preston, S. Bassham, W. A. Cresko, E. A. Johnson, Local de novo assembly of
297 RAD paired-end contigs using short sequencing reads. *PLoS One*. **6**, e18561 (2011).
- 298 33. J. Catchen, P. A. Hohenlohe, S. Bassham, A. Amores, W. A. Cresko, Stacks: an analysis tool set
299 for population genomics. *Mol. Ecol.* **22**, 3124–3140 (2013).
- 300 34. D. R. Zerbino, E. Birney, Velvet: Algorithms for de novo short read assembly using de Bruijn
301 graphs. *Genome Res.* **18**, 821–829 (2008).
- 302 35. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform.
303 *Bioinformatics*. **25**, 1754–1760 (2009).
- 304 36. H. Li *et al.*, The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. **25**, 2078–2079
305 (2009).
- 306 37. D. W. Barnett, E. K. Garrison, A. R. Quinlan, M. P. Stromberg, G. T. Marth, BamTools: a C++
307 API and toolkit for analyzing and managing BAM files. *Bioinformatics*. **27**, 1691–1692 (2011).
- 308 38. R. Kofler, R. V. Pandey, C. Schlotterer, PoPoolation2: identifying differentiation between
309 populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics*. **27**, 3435–
310 3436 (2011).
- 311 39. B. H. McRae, B. G. Dickson, G. W. Roemer, J. M. Rundall, M. Hayward, Ed., Circuitscape 4
312 User Guide. The Nature Conservancy. (2013).
- 313 40. N. Mantel, The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer*
314 *Res.* **27**, 209–220 (1967).
- 315 41. P. E. Smouse, J. C. Long, R. R. Sokal, Multiple Regression and Correlation Extensions of the
316 Mantel Test of Matrix Correspondence. *Syst. Zool.* **35**, 627–632 (1986).
- 317 42. J. Oksanen *et al.*, The vegan Package, Version 1.15-1. *Community Ecol.* (2008).
- 318 43. S. Dray, A. Dufour, J. Thioulouse, T. Jombart, S. Pavoine, Ade4: analysis of ecological data:
319 exploratory and euclidean methods in environmental sciences. R Packag. version (2009).
- 320 44. M. Nei, T. Maruyama, R. Chakraborty, The Bottleneck Effect and Genetic Variability in
321 Populations. *Evolution*. **29**, 1–10 (1975).
- 322 45. G. Luikart, J.-M. Cornuet, Empirical Evaluation of a Test for Identifying Recently Bottlenecked
323 Populations from Allele Frequency Data. *Conserv. Biol.* **12**, 228–237 (1998).
- 324 46. P. Legendre, L. Legendre, *Numerical ecology* (Elsevier, 1998).
- 325 47. S. Dray, D. Chessel, J. Thioulouse, Co-Inertia analysis and the linking of ecological data tables.
326 *Ecology*. **84**, 3078–3089 (2003).
- 327 48. A. Vanden Broeck *et al.*, Gene flow and effective population sizes of the butterfly *Maculinea*
328 *alcon* in a highly fragmented, anthropogenic landscape. *Biol. Conserv.* **209**, 89–97 (2017).
- 329

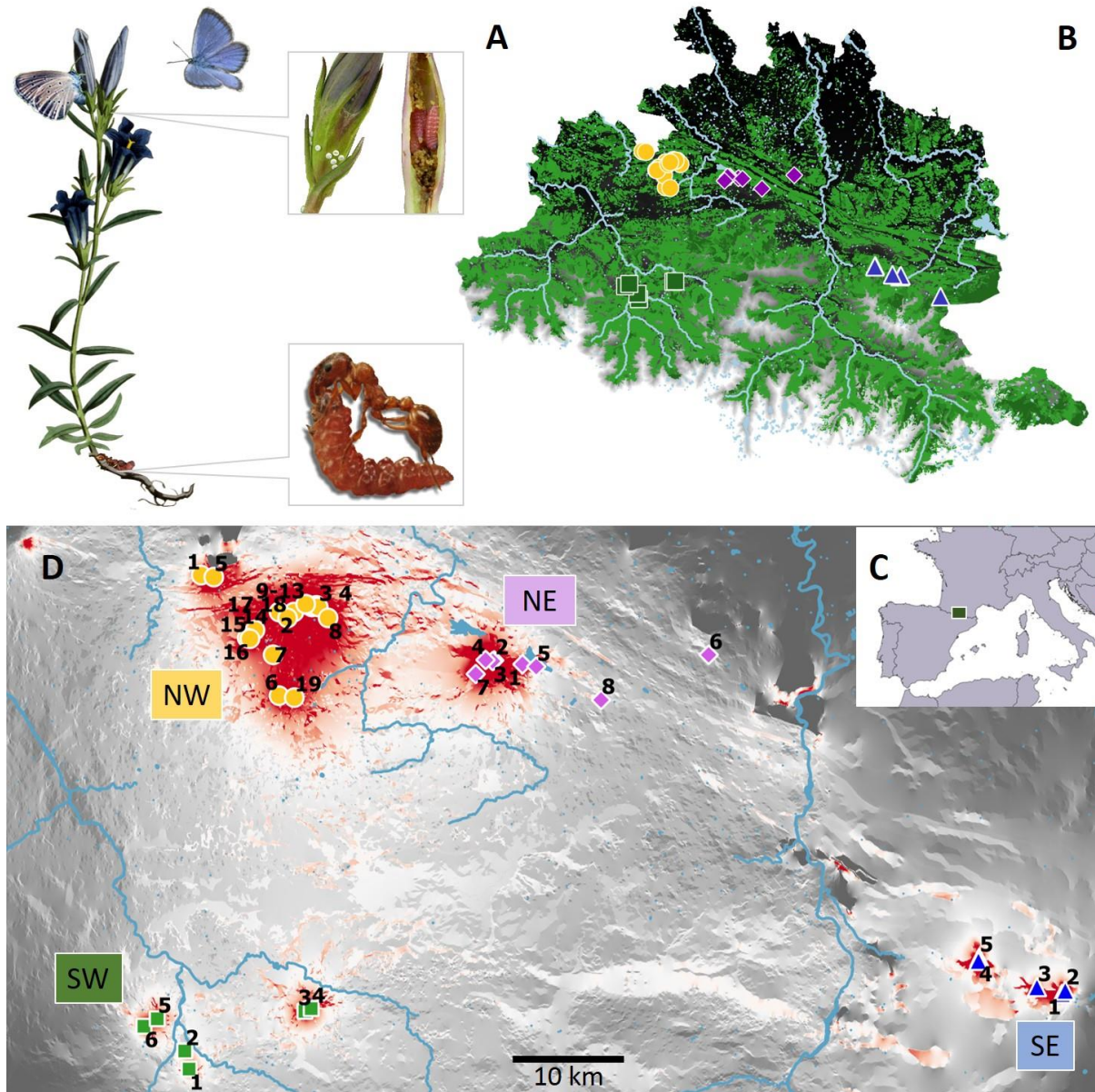
330 **Acknowledgements:**

331 Raw reads are archived at the National Center for Biotechnology Information (BioProject to
332 complete). SNP allele frequencies and flanking sequences are archived at Dryad (to complete).

333 We were pleased to obtain insightful comments on the manuscript from experts in the field of
334 community ecology and landscape genomics, including Luc De Meester, Michael Hochberg,
335 José MT Montoya, Olivier Rey, Stephanie Manel and Viktoriia Radchuk.

336 We thank Gaëlle Blanvillain, Sophie Dardenne, and many students for field assistance, and
337 Murielle Richard for lab assistance. Annie Ouin (INRA), Thomas Houet (CNRS), and the Parc
338 Naturel Régional Pyrénées Ariégeoises (Yannick Barascud and Julien Aït El Mekki) provided
339 the GIS resources used for mapping levels of habitat fragmentation. Primary financial support
340 was provided by the ANR GEMS & INDHET (ANR-13-JSV7-0010-01 and ANR-12 -BSV7-
341 0023-02). HDK, VMS, JP and MB are members of the Excellence Lab TULIP (ANR-10-
342 LABX-41).

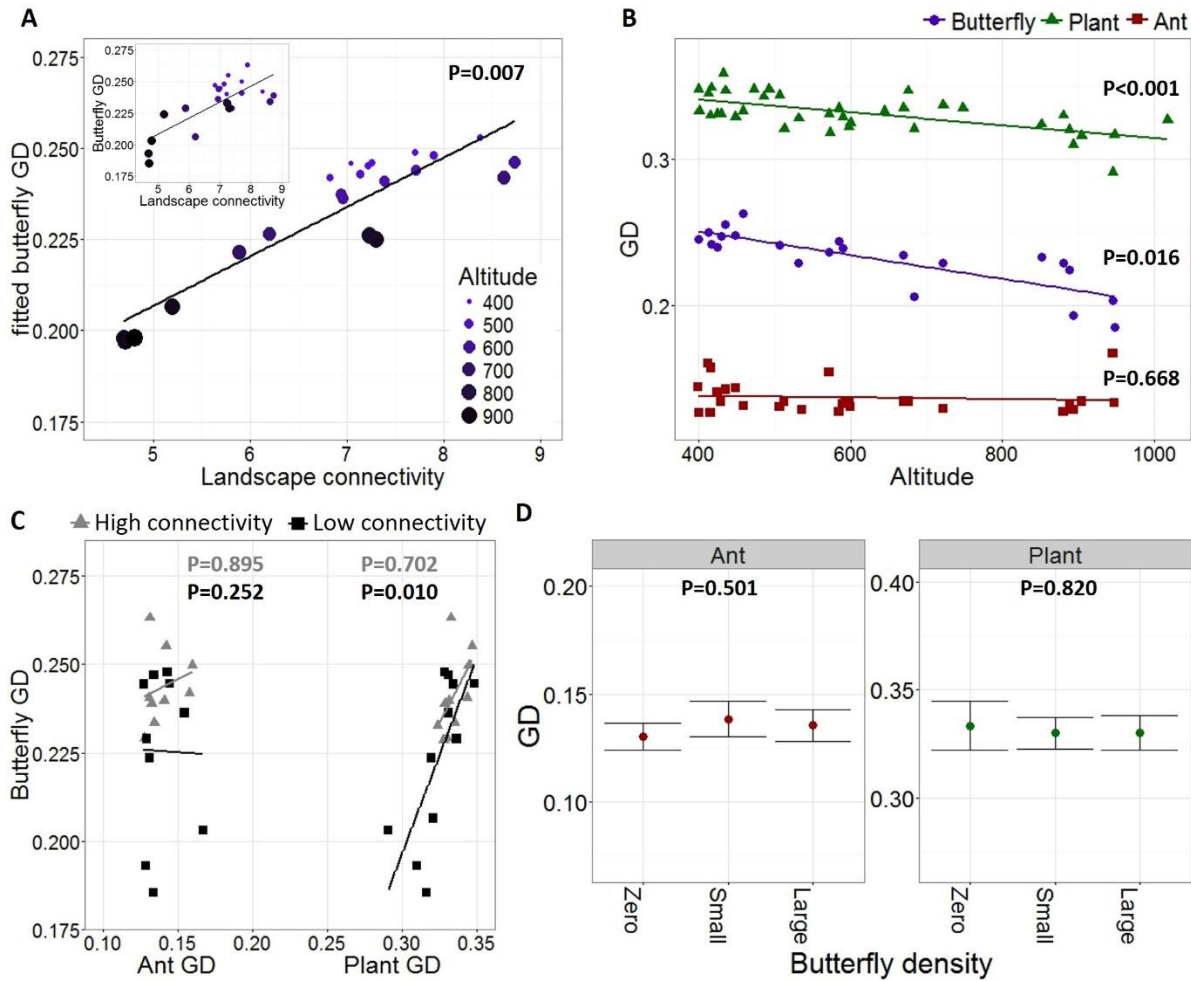
343 Hanne De Kort wrote the manuscript, assisted with sampling, and performed the lab work and
344 data analyses. Virginie Stevens and Michel Baguette provided the context of the project and
345 supervised writing, sampling and data analysis. Jérôme Prunier performed the bio-informatics,
346 and Marc Tessier located the community sites and introduced the study system. Camille
347 Turlure assisted with the analysis of the field surveys, and Jérémy Monsimet assisted with
348 sampling. All co-authors also commented on the manuscript.



349

350 **Fig. 1.** Study system. **A.** Butterfly community. After mating, females Alcon blue butterflies
351 (*Phengaris [=Maculinea] alcon*) lay their white eggs onto gentian (*Gentiana pneumonanthe*) flower
352 buds. Small caterpillars develop into the bud, at the expense of gentian's ovules. After their 3rd molt,
353 the caterpillars leave the plant and are adopted by ants (*Myrmica scabrinodis*), that rear them into their
354 underground nest in preference to their own brood. This social parasitism ends after metamorphosis.
355 For visibility, ant size was exaggerated relative to plant size. **B.** Overview of the study landscape in
356 which the samples were collected, with tree cover shown in green, and with a black to white gradient
357 reflecting altitude. Shape and colour of sampling locations correspond to their geographical cluster
358 (yellow circles: NW, purple diamonds: NE, blue triangles: SE, green squares: SW). **C.** Positioning of
359 sampling area in the French central Pyrenees (SW Europe). **D.** Landscape connectivity map of the study
360 area representing butterfly dispersal probability. Red/grey colours represent habitat favouring/impeding
361 dispersal. Rivers (blue) align with the main valleys. Geographical and demographic details about the
362 sampling sites can be found in Table S1.

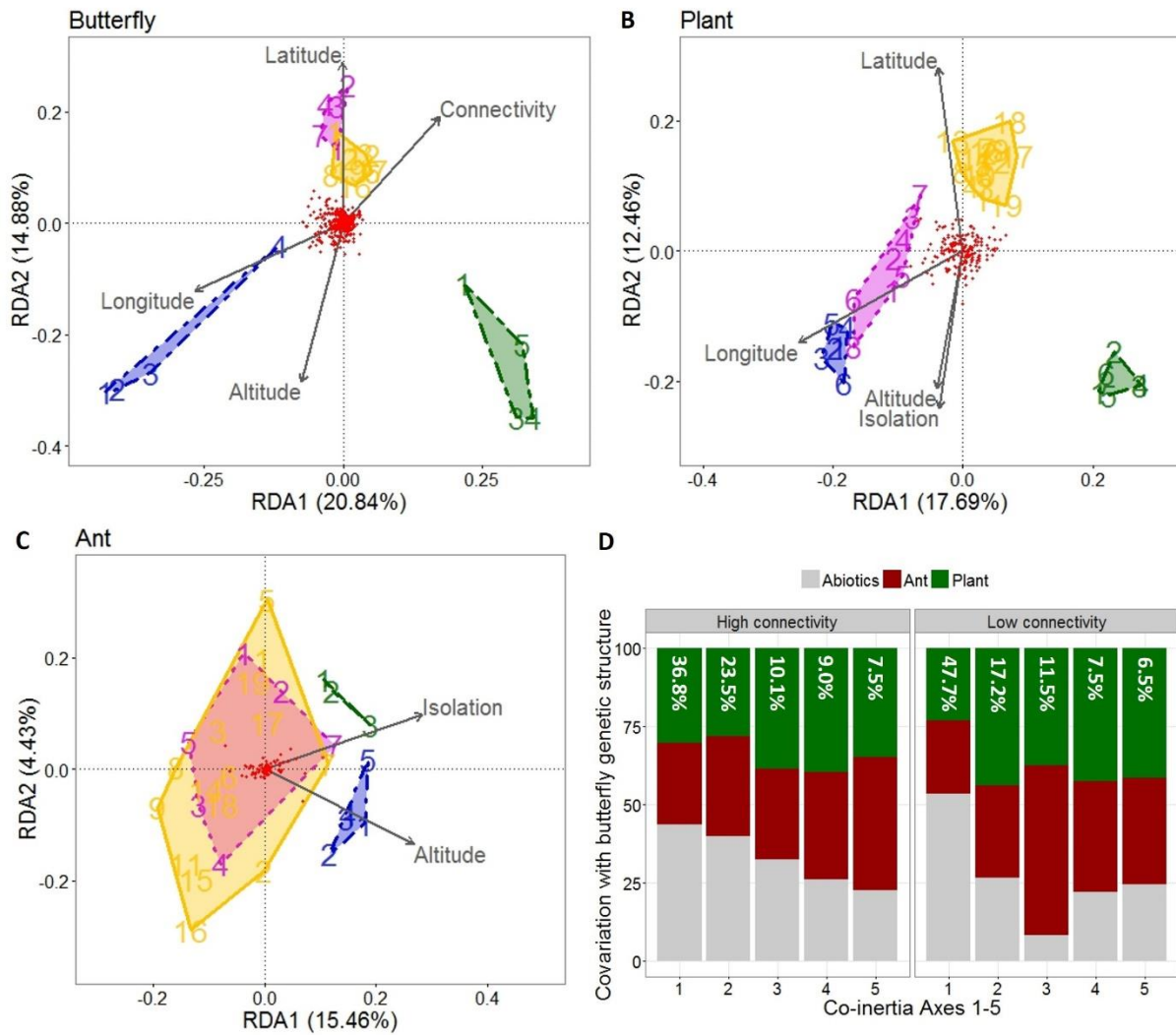
363



364

365 **Fig. 2.** Genetic diversity (GD) patterns of the butterfly community network. **A.** Correlation
 366 between landscape connectivity and fitted and original butterfly H_E values. Fitted values result from a
 367 weighted linear model with landscape resistance, altitude and patch size (Log10-transformed) as
 368 explanatory variables (Table S4). Dots get darker and larger with altitude. **B.** Correlation between
 369 altitude and genetic diversity of each species. **C.** Relation between butterfly and host genetic diversity
 370 for both high landscape connectivity (>7.2 , the median) and low landscape connectivity (<7.2). **D.**
 371 Parasitic effect of the butterfly (based on local population densities) on genetic diversity of hosts. Zero
 372 butterfly density refers to recently extinct populations.

373



374

375

376

377

378

379

380

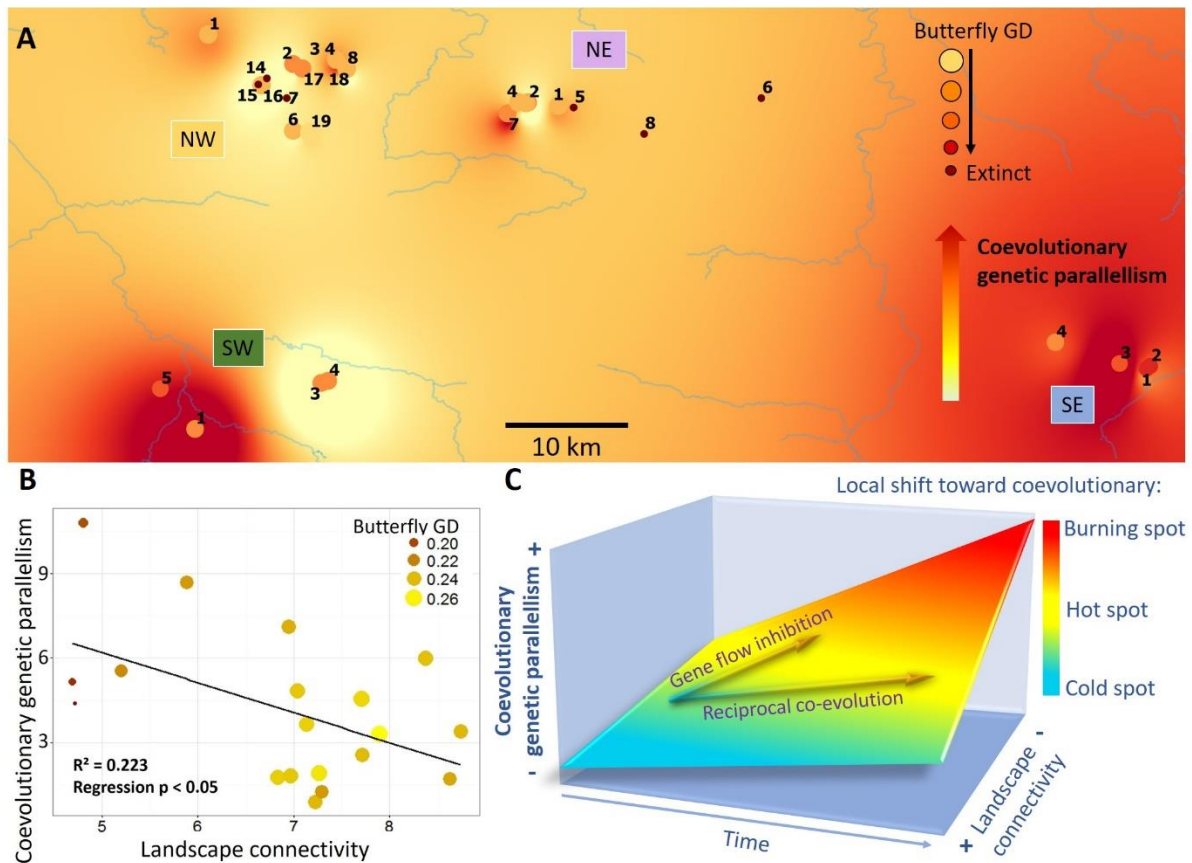
381

382

383

Fig. 3. Role of environmental factors contributing to the genetic structure of the butterfly community network. **A-C.** Triplots representing the genetic structure of butterfly, plant and ant, respectively, and the abiotic factors significantly contributing to this structure (Table S4). Colours correspond to spatial structure in Fig. 1D. **D.** Contribution of host genetic structure and abiotic factors to the genetic structure of Alcon populations. The first five axes of the co-inertia analyses explained a total of 86.89% and 90.34% of the covariation between the Alcon genetic matrix and the environmental matrix (altitude, longitude, latitude, landscape connectivity, ant and plant genetics), for the highly and poorly connected populations, respectively (Table S4). Contributions of each axis to the total coinertia are shown in white at the top of the bar plot.

384



385

386 **Fig. 4.** Coevolutionary dynamics over space and time. **A.** Map showing the degree of
 387 coevolutionary genetic parallelism between the butterfly and its two hosts. The degree of genetic
 388 parallelism likely due to coevolution was derived from a coinertia analysis, through extracting the axis
 389 that was mainly represented by host genetic structure (>90%) rather than by abiotic variation (Table
 390 S5). Size and colour of site symbols reflects butterfly genetic diversity. Burning spots are featured by a
 391 combination of low genetic diversity and high coevolutionary genetic parallelism. The extinctions in
 392 the NW cluster are due to recently abandoned grazing management. **B.** Relation between coevolutionary
 393 genetic parallelism and landscape connectivity. **C.** Graphical representation of a landscape varying in
 394 the degree of connectivity. Communities in overly connected landscapes experience a gene flow
 395 overload, resulting in sub-optimal conditions for coevolution (blue, cold spots). The absence of
 396 coevolution is mirrored by a lack of coevolutionary genetic parallelism among species. Intermediate
 397 landscape connectivity facilitates reciprocal coevolution, giving rise to coevolutionary hot spots
 398 (yellow), featured by species that show genetic similarities due to coevolution. Highly fragmented
 399 landscapes (poor landscape connectivity) compromise coevolutionary dynamics through selection for
 400 decreased dispersal and/or constraints on effective gene flow, finally resulting in coevolutionary
 401 burning spots of increased extinction risk.

402 **Supplementary Materials:**

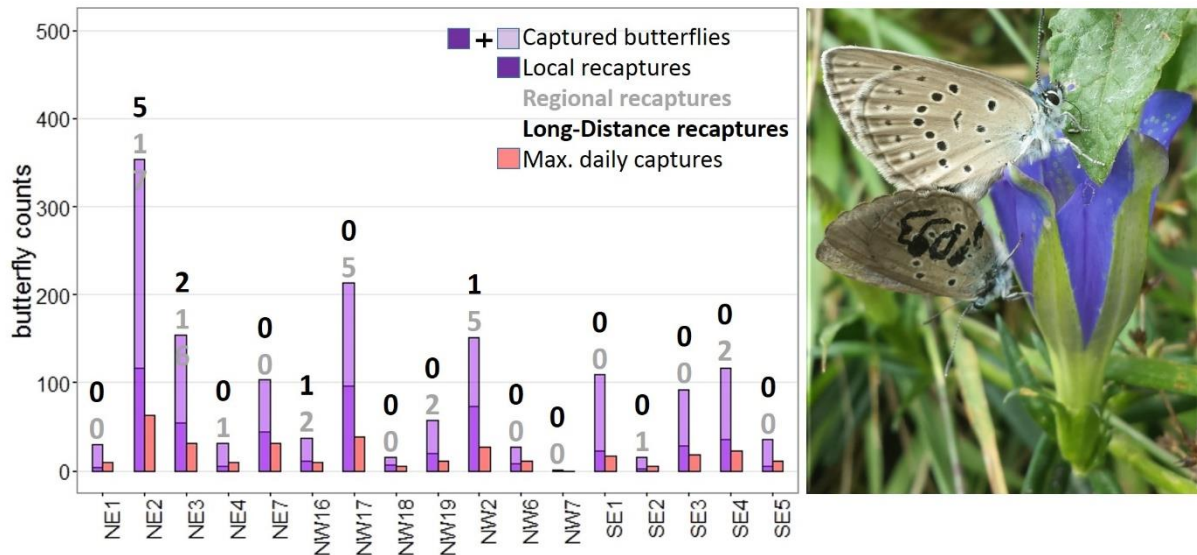
403 Material and Methods

404 Figures S1-S6

405 Tables S1-S5

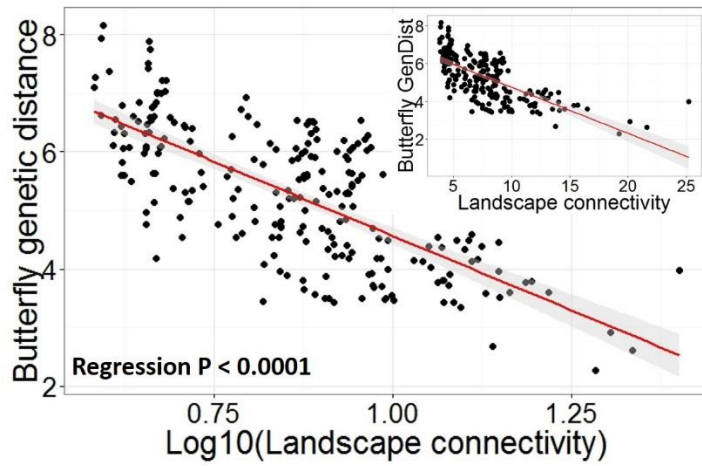
406 References (29-48)

407



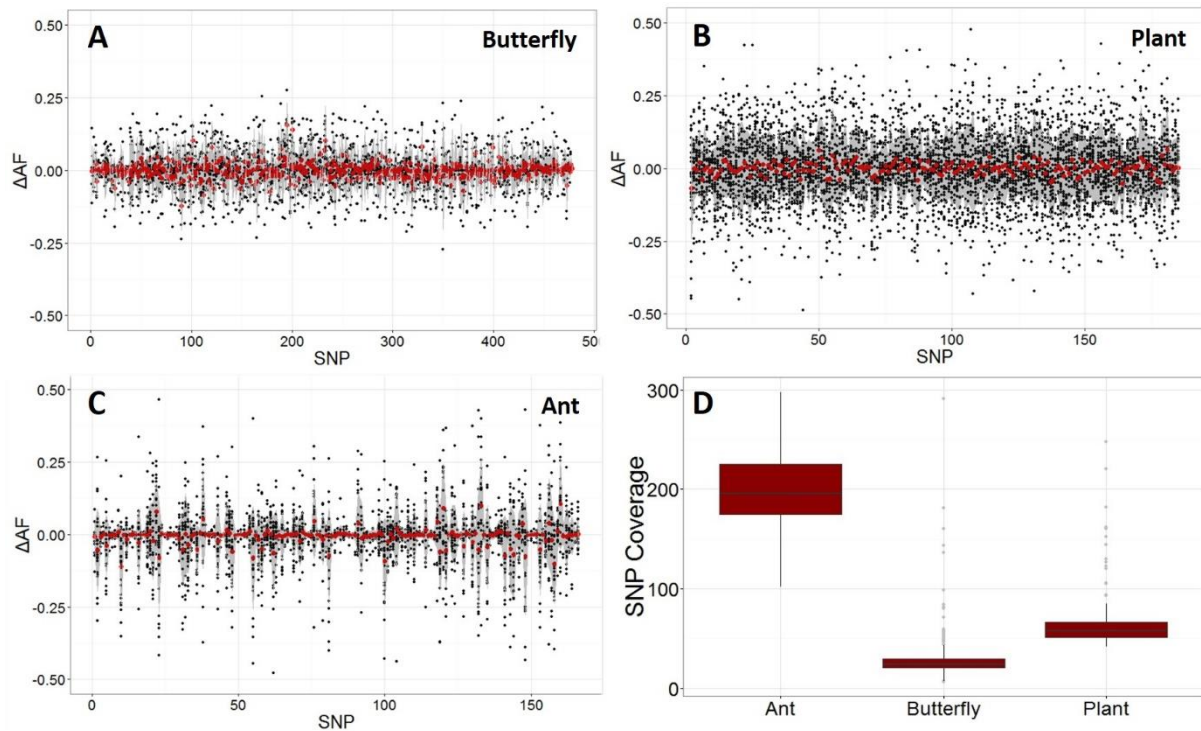
408

409 **Fig. S1.** Summary of individual movement monitoring of the NE, NW and SE cluster (summer of
410 2014), showing number of (i) captured butterflies that were subsequently marked (captured
411 butterflies), (ii) butterflies that were recaptured on site (local recaptures), (iii) butterflies that were
412 recaptured and originated from another site within the same geographical cluster (regional recaptures),
413 butterflies that were recaptured and originated from another site of another geographical group
414 (long-distance recaptures). Long-distance recaptures only occurred between the NW and the NE
415 metapopulation. These are the first field observations of *Maculinea alcon* dispersal exceeding 0.5 km
416 (49). In addition, the number of captures on the best day (day with most captures) is shown. A
417 photograph taken during individual movement monitoring shows a marked butterfly mating with an
418 unmarked butterfly (marked later that day).
419



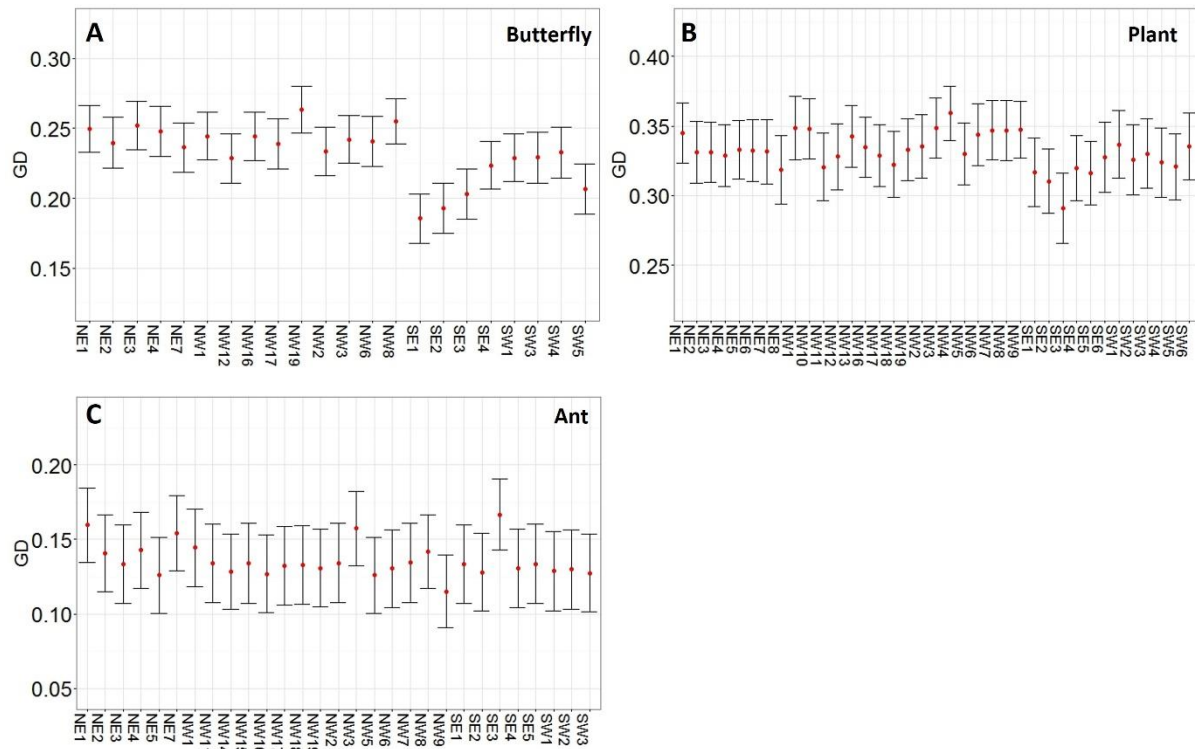
420

421 **Fig. S2.** Effect of landscape connectivity on butterfly genetic distance. The scatterplot shows
422 pairwise landscape connectivity values vs. genetic distance. Regression p-value < 10^{-16} for bot log10-
423 transformed and original pairwise connectivity distances. Mantel test results can be found in Table S3.



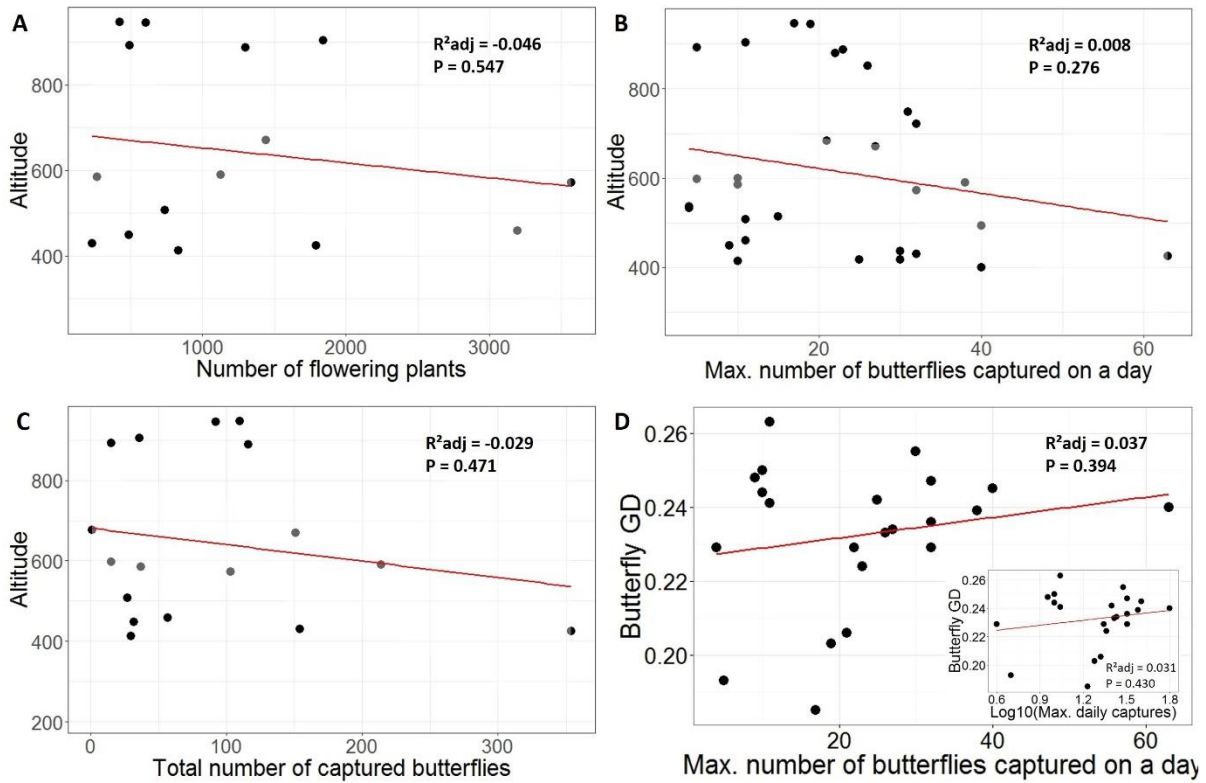
424

425 **Fig. S3.** Reliability (A-C) and coverage (D) of the SNPs used for all analyses. To assess the
426 reliability of the SNP allele frequency (AF) estimates, a total of 6 (butterfly), 30 (plant) and 23 pool
427 replicates (anta) were available, and SNP AFs were compared across pool replicates by calculating the
428 difference in AF (ΔAF) for each SNP in each pair of pool replicates (black dots). Average ΔAF values
429 per SNP are represented by red dots. ΔAF varied substantially, yet randomly, across SNPs and pools,
430 but average ΔAF was <0.01 for each species, rendering the AF estimates suitable for multivariate
431 genetic comparisons among populations and species. A detailed account of the pooled RADseq
432 procedure that resulted in these SNPs is provided in the Material & Methods section.



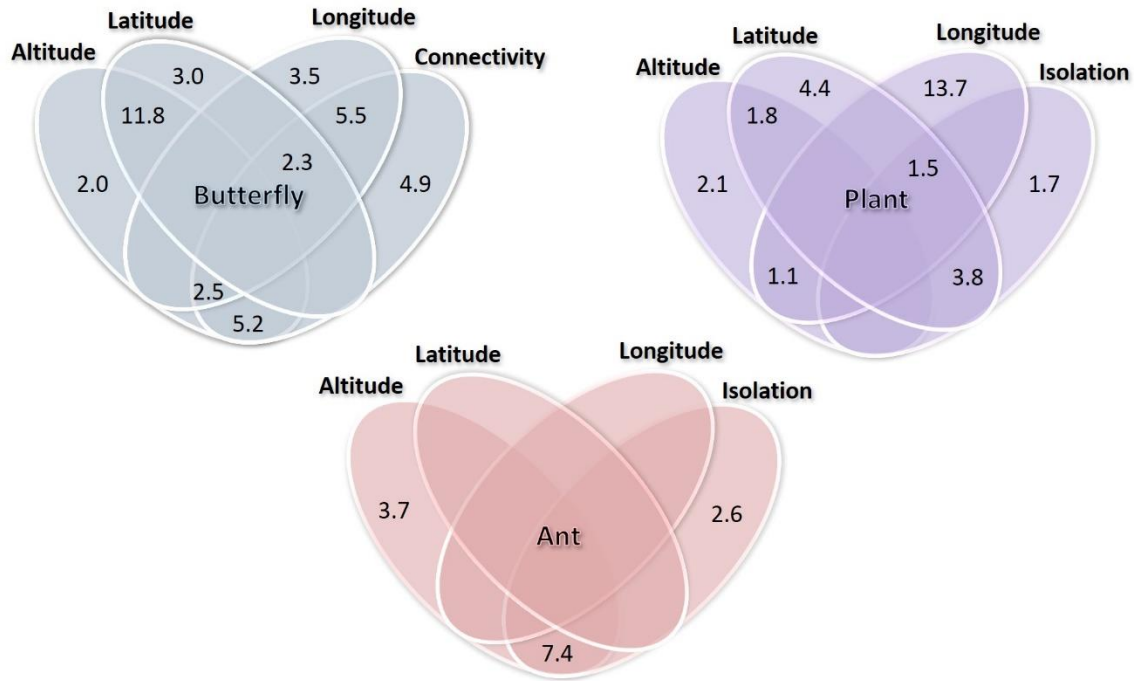
433

434 **Fig. S4.** Genetic diversity (mean expected heterozygosity, Table S1) of all successfully
435 genotyped butterfly (A), plant (B) and ant (C) populations.



436

437 **Fig. S5. Field summary statistics.** A. Absence of a relation between plant population size
438 (approximated by flowering plant counts) and altitude. B-C. Absence of a relation between butterfly
439 population sizes, based on individual movement monitoring, and altitude. Field methodology is
440 provided in Table S1. Statistics are based on linear regression. D. Absence of a relation between
441 butterfly population size and butterfly GD.



442

443 **Fig. S6.** Venn diagram showing unique and common abiotic contributions (%) to butterfly,
444 plant and ant genetic structure. Values were obtained through redundancy analyses with variation
445 partitioning (see Table S4). Only significant contributions are shown.

446 **Table S1.** Spatial, genetic and demographic population characteristics.

447 **Table S2.** Assignment of butterfly dispersal costs to (i) land use map categories, (ii) plant
448 presence, and (iii) geological structures that have been found to be associated with plant
449 presence. Higher dispersal costs correspond to lower landscape connectivity. The combination of
450 cost assignments that best explained the genetic distance among butterfly populations (based on
451 partial Mantel tests) is clarified in Table S3. These final dispersal costs were used to calculate
452 pairwise and average landscape connectivity.

453 **Table S3.** Table of connectivity scenarios with partial Mantel test results. Connectivity
454 scenarios reflect combinations of dispersal costs assigned to land use, plant presence and geology
455 (=likely plant presence).

456 **Table S4.** Statistical modelling underlying the p-values provided in the text. Models are ranked
457 according to the location of corresponding results in the manuscript. Butterfly habitat size was Log10-
458 transformed to meet model assumptions. To assess the effect of pool size on regression model
459 robustness, regression models were weighted by pool size and compared to unweighted models.
460 Weighing by pool size did not affect model parameters.

461 **Table S5.** Overall coinertia analysis used to calculate degree of coevolutionary genetic parallelism
462 between the butterfly and its two hosts.