

1 Extensive range overlap between *Heliconius* sister species: evidence for
2 sympatric speciation in butterflies?

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Abstract

Sympatric speciation is now generally accepted as plausible, but the relative contribution that it has made to contemporary biodiversity remains to be established. We attempt to infer the frequency of sympatric speciation in *Heliconius* butterflies and allies from the geographic distributions of sister species. We compare the observed frequency geographic range overlap with expectations generated via simulations of speciation and range dynamics. Geographic ranges overlap in 8-9 cases of 21-23 heliconiine sister species pairs (depending on species concept), a much higher proportion than reported in birds and mammals. These patterns of range overlap are most consistent with simulations in which sympatric speciation is common, contributing 35%-90% of speciation events. However, a highly plausible scenario not accounted for in the simulations is allopatric or parapatric speciation followed by rapid spread of daughter species into one another's ranges. Our findings of high levels of overlap contradict a long-held tenet of biogeography that closely related animal species are usually allopatric, although we caution that the differences between *Heliconius* and other taxa may be an artefact generated by different taxonomic practices. Thus to establish whether heliconiines are exceptional will require biogeographic comparative studies for a wider range of animal taxa than have been considered to date.

Key words: Geography of speciation; butterflies; simulations, age-range correlation

Introduction

Despite a controversial history, sympatric speciation is now generally seen as

theoretically possible (Dieckmann and Doebeli 1999; Gavrillets and Waxman 2002; Gavrillets 2004) and, at least under a spatial or biogeographic definition of sympatry (Mallet et al. 2009) there are cases where it seems the most likely explanation (Sorenson et al. 2003; Barluenga et al. 2006; Savolainen et al. 2006). Consequently, much of the debate has shifted to the relative importance of different geographic modes of speciation (Jiggins 2006).

The geographical distributions of contemporary sister-species, especially the degree of range overlap, have been used to infer dominant modes of speciation at least as far back as the turn of the twentieth century (Jordan and Kellogg 1907) and variants on this approach continue to be used today (Kisel and Barraclough 2010; Papadopoulos et al. 2011). In recent years, since molecular sequence data has become available, the relationship between range overlap of sister taxa and molecular-clock based estimates of time since speciation (the age-range correlation) has been used to infer the dominant geographic mode of speciation within a clade: if most speciation was allopatric then recently diverged sister taxa should tend to be allopatric, and increasing sympatry between older taxa is attributed to post-speciation range movements. Alternatively, if most speciation was sympatric, the expectation is that recently diverged species will tend to be sympatric and sympatry between older species-pairs will be reduced (Lynch 1989; Chesson and Zink 1994; Barraclough et al. 1998; Berlocher 1998; Barraclough and Vogler 2000; Fitzpatrick and Turelli 2006; Perret et al. 2007).

Unfortunately, studies based on age-range correlations have often proven inconclusive because simulations show that a mixture of allopatric and sympatric speciation tends to produce an age-range correlation that is indistinguishable from the pattern left by a

single mode of speciation followed by extensive range movement (Barracough and Vogler 2000; Losos and Glor 2003; Fitzpatrick and Turelli 2006; Perret et al. 2007). Phillimore et al. (2008) used spatially explicit simulations of stochastic movement of the daughter species' ranges following different geographic modes of speciation to explore what geographic patterns were most informative about the geographic mode of speciation. They found that, when considered together, the proportions of sister species showing zero or complete range overlap and their degree of bimodality (i.e., the frequency of cases of zero and complete overlap) are more informative than the age range correlation about the relative frequencies of allopatric versus sympatric speciation. On applying this approach to data on the geographic distributions of sister species of birds, Phillimore et al. (2008) found that the observed patterns of range overlap were consistent with simulations in which allopatric speciation predominates and sympatric speciation contributes no more than 5% of speciation events. Here, we apply the same approach to heliconiine butterflies. We here use a spatial population genetic, or biogeographic definition of sympatric speciation (i.e. where species diverge while overlapping in geographic range). Species ranges provide little information relevant to interdemic gene flow (m), used in an alternative "demic" definition of sympatric speciation (Fitzpatrick et al. 2008; Mallet et al. 2009).

Few previous studies have tested the geography of speciation in taxa thought to be likely candidates for sympatric speciation (Berlocher 1998; Barracough and Vogler 2000; Linnen and Farrell 2010). Heliconiine butterflies (Lepidoptera: Nymphalidae: Heliconiina) fulfil a number of conditions that are thought to be conducive to sympatric speciation. First, adaptive traits (wing colour patterns involved in Müllerian mimicry) are also used in mate recognition and are frequently correlated genetically

with mate preference (Kronforst et al. 2006; Chamberlain et al. 2009; Merrill et al. 2011). It is therefore conceivable that reproductive isolation in sympatry could result from a switch in mimicry, as the impediment to divergence that recombination usually poses is circumvented (Felsenstein 1981; Gavrillets 2004). Second, heliconiines are phytophagous and highly host-specific, the majority feeding on host-plants from the family Passifloraceae. In species that exhibit host plant fidelity and mate on their hosts, reproductive isolation may arise after a host plant shift, hence it is perhaps no coincidence that many of the most compelling cases of sympatric speciation involve phytophagous insects (Bush 1969; Berlocher and Feder 2002; Drès and Mallet 2002). Heliconiines males frequently patrol host plants and monitor larvae and pupae they find there, with mating often taking place on or near the host (Mallet 1986; Estrada and Gilbert 2010). Furthermore, 42% of species in the genus *Heliconius* are known to engage in “pupal mating”, where mating sometimes occurs on or near the larval host, before females have fully emerged from their pupae (Gilbert 1991; Deinert et al. 1994). Thus, shifts in host plant use would likely generate some reduction in gene flow that could facilitate divergence and the build up of reproductive isolation. Finally, recent evidence suggests that at least three lineages, *Heliconius heurippa*, *H. timareta*, and *H. elevatus*, participated in speciation that involved hybrid transfer of colour pattern elements, an inherently sympatric process (Mavárez et al. 2006, Dasmahapatra et al. 2012).

We assess whether mimicry shifts and host plant shifts play a role in heliconiine speciation by examining the correlation between similarity in mimetic colour patterns / host plants of sister species and phylogenetic branch length. We interpret the intercept as indicative of overlap in colour patterns/host plants at the time of

118 speciation and the slope as indicative of gradual divergence / convergence. Sympatric
119 sister species might also be expected to be more different ecologically than allopatric
120 sister species. This is expected following sympatric speciation (where the divergence
121 can either be seen as generating reproductive isolation itself, or facilitating species
122 coexistence after speciation) and also under secondary contact (when species should
123 be ecologically divergent in order to coexist) (Hardin 1960; Coyne and Orr 2004). We
124 test for this by examining the correlation between geographic range overlap and
125 ecological similarity.

126 A third aim of this paper is to investigate the effect of the practical application of
127 different species concepts on inferences of geographic modes of speciation. Each
128 species concept places a different emphasis on geographical taxa. For instance, using
129 either morphology or molecular tools, taxa identified as species under the diagnostic
130 version of Phylogenetic Species Concept (PSC) can be identified readily in allopatry,
131 whereas biological species can be identified conclusively only via reproductive
132 isolation in sympatry. We test the effects of two versions of the biological species
133 concept (BSC), and carry out some analyses using a diagnostic version of the
134 phylogenetic species concept (PSC) as follows: (1) Under a “strict” biological species
135 concept, species are defined as groups of interbreeding populations that are
136 reproductively isolated from other such groups (Mayr 1995). Here “semi-species”
137 which hybridize relatively frequently at parapatric boundaries, or are inferred to be
138 likely to do so if they occur in complete allopatry are lumped into the same species as
139 their closest relatives; (2) Under a “relaxed” biological species concept, species are
140 characterised by substantial but not necessarily complete reproductive isolation
141 (Coyne and Orr 2004), with disjunct or parapatric semi-species considered full

species, as in current heliconiine taxonomy (Brown 1981; Lamas 2004; Rosser et al. 2012). Relaxed biological species correspond approximately to those recognised as separate genotypic clusters (Mallet 1995), for example in *H. himera* and *H. erato* hybrid zones where hybrids or intermediates may occur, but are relatively rare in a well-studied zone of parapatric overlap (Jiggins et al. 1997; Jiggins and Mallet 2000).

(3) Under the diagnostic PSC, traditional heliconiine subspecies differing in fixed, diagnostic colour pattern traits would likely be given full species status (Cracraft 1983). Due to the full phylogenetic information on these taxa being unavailable, we use geographic distribution data to investigate the likely outcome of adopting a diagnostic species concept by measuring the proportion of each subspecies range that overlaps with other subspecies (i.e. where it is polymorphic). Although we do not use molecular differences, some evidence suggests similar taxa would be diagnosed as species via molecular analysis (Quek et al. 2010).

In summary, the aims of this paper are threefold: 1) To estimate the relative contributions of different geographic modes of speciation in heliconiines. 2) To test whether shifts in color patterns and ecology are associated with speciation. 3) To investigate the influence of different species concepts on estimated relative importance of sympatric and allopatric speciation.

Methods

We compiled a database of 58,059 locality records for 70 species and 431 subspecies of heliconiines, and mapped the species and subspecies distributions using α -convex hulls to convert the point localities into vector polygons projected in a Lambert Cylindrical Equal Area projection (Edelsbrunner et al. 1983). We describe the dataset and mapping procedure in detail in Rosser et al. (2012). In the present study, we

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3 166 included an additional sister species pair of heliconiines not mapped in Rosser et al.
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5 167 (2012); *Philaethria dido* and *Philaethria ostara* cf. *diatonica*, which were mapped
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7 168 using locality records published in Constantino and Salazar (2010). We applied three
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9 169 taxonomies, corresponding to a relaxed biological species concept, a strict biological
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11 170 species concept, and a diagnostic version of the phylogenetic species concept.
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13 171 Relaxed biological species designations followed Lamas (2004) with certain
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15 172 exceptions; table S1 indicates where our taxonomy differs. Relaxed sister pairs (table
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17 173 S2) are given by Beltrán et al. (2007), with the following exceptions: i) in the absence
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19 174 of phylogenetic information, when a genus contained only two species they were
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21 175 assumed to be sisters (*Agraulis* and *Podotricha*). ii) We treated *Heliconius erato* and
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23 176 *Heliconius chestertonii* as separate sister species (Arias et al. 2008). We split
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25 177 *Heliconius demeter* into *H. demeter* and *Heliconius* sp. nov. (c.f. *eratosignis*); the two
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27 178 are sister species that overlap in N.E. Peru according to recent molecular data
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29 179 (Dasmahapatra et al. in prep.). iii) Finally, we did not use the putative sister grouping
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31 180 of *Heliconius himera* and *Heliconius hermathena* indicated by Beltrán et al. (2007)
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33 181 given phylogenetic uncertainty regarding the origins of *H. hermathena* (Jiggins et al.
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35 182 2008). Strict biological species (table S3) were defined on the basis of ability to
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37 183 overlap broadly without frequent hybridisation between sister species (Mallet et al.
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39 184 2007), with sister comparisons made by collapsing phylogenetic nodes of affected
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41 185 allopatric relaxed biological species (table S4). In order to investigate the probable
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43 186 outcome of adopting a diagnostic version of the phylogenetic species concept, for
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45 187 every polytypic species we calculated the proportional overlap of each subspecies
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47 188 with its conspecifics.
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54 189 We quantified overlap between sister species as the area of sympatry divided by the
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190 area of the smaller species range, giving an index ranging from 0-1 (Anderson and
191 Evensen 1978). To take account of the geographical incompleteness of sampling and
192 small inaccuracies in the mapping procedure, we define < 0.05 overlap as complete
193 allopatry and > 0.95 overlap as complete sympatry. Bimodality of data was quantified
194 as $(zc)/(ab)$, where z and c are the number of cases of complete and zero overlap
195 observed, and a and b are the numbers of cases of complete and zero overlap that
196 would occur if all the data were split evenly between completely and non-overlapping
197 sister pairs (Phillimore et al. 2008). The observed data were compared with values
198 generated from simulations using a two-tailed test; parameters were considered to
199 have been unlikely to give rise to the observed data if observed values fell outside the
200 2.5 and 97.5 percentiles of the simulated distribution. We then eliminated parameter
201 combinations for which any of the observed values for the three indices was unlikely
202 to arise.

203 ***Simulations***

204 The simulation-based approach employed broadly follows Phillimore et al. (2008).
205 Here, we give an outline of the approach. Each replicate set of simulations modelled
206 speciation as many times as there are pairs of sister species. We ran 1000 replicates
207 for each possible combination of parameters and explored all possible proportions of
208 sympatric speciation events. Speciation was simulated by randomly dividing the
209 parental species' geographic range into two daughter ranges, whose positions relative
210 to one another depended on the mode of speciation being employed (allopatric,
211 parapatric or sympatric). When simulating sympatric speciation, the smaller daughter
212 range was placed randomly within the larger daughter range. For simulations of non-
213 sympatric speciation, we varied the geographic configurations of the ranges (vicariant,

peripatric and parapatric). When simulating peripatric speciation, we defined the size of the smaller range as 5% of the starting range size. Species' ranges were rectangular and the total area available to ranges was a square grid of a 100 x 100 units. Range movements were simulated by adding a random normal deviate with a mean of 0 at each time step to the vectors corresponding to the top, bottom, right and left extents of each species range. Different rates of range change were explored by varying the standard deviation of distribution from which the values were drawn, we used 0.25, 0.5, 0.75, 1, 1.5, and 2. We also examined the effect of giving the species ranges a tendency to grow by increasing the mean of the standard normal deviate to 0.1. We parameterised the duration of simulations using mitochondrial DNA divergence-based branch lengths of sister species estimated using a relaxed clock method with a multilocus sequence-based phylogeny as a relative estimate of time since speciation (Mallet et al. 2007). For each sister pair we scaled the duration of the simulations by the time to most recent common ancestor on this phylogeny. When we lacked information on branch lengths (four cases for relaxed biological species, three cases for strict biological species), we used the proximate ancestral node to set a maximum time of divergence for the sister pair, and then for each replicate assigned the branch length as a random draw from a uniform distribution between 0 and the maximum time for divergence. We used heliconiine species range sizes to set the size of the initial geographic range relative to the total area available for species ranges during simulations. We defined the area available to heliconiines as the total area occupied by the sub-tribe. We used the median range size of all heliconiine species (6.5% of the total area for relaxed biological species, 13.4% for strict biological species) and the median range size of all sister species (16% relaxed BS, 17.6% strict BS) as starting range sizes in simulations. We also ran simulations with starting ranges double these

239 sizes (because the simulations set the starting sizes of the daughter ranges by dividing
240 the initial range size in two).

241 *Age-range correlations*

242 We tested for an age-range correlation, using ordinary least squares regression with
243 geographic overlap as the dependent variable, and molecular phylogenetic branch
244 length as the predictor. Intercepts were fitted by linear regression of the arcsine-
245 transformed proportion of sympatry (Barracough and Vogler 2000). Unlike several
246 previous analysis, we included only sister species in the regression, thus avoiding the
247 problem of reconstructing ancestral ranges for comparisons within the phylogeny
248 (Stephens and Wiens 2003). Sister species without branch lengths estimates were
249 excluded from the age range correlation.

250 *Ecological divergence*

251 We classified heliconiine species wing colour patterns (Table S7) using an updated
252 and modified version of the colour pattern scheme presented by (Turner 1976; Brown
253 1981). This scheme classifies colour patterns into broad groups (e.g. black with
254 yellow forewing band and red hind-wing band). These broad colour pattern classes
255 may be further subdivided into mimicry rings, but speciation seems more likely to be
256 driven by major shifts in colour pattern rather than minor variations (Jiggins et al.
257 2001). We used Beccaloni et al. (2008) as the principal source for host plant records.
258 We excluded all records marked as dubious, and all those known/thought to have been
259 recorded from captive populations. We also excluded all records where the host plant
260 identification was marked as doubtful. If a host plant species was identified as "near"
261 to a known species, it was treated as a separate species. We measured the similarity of
262 colour patterns and host plants between sister species as x/y , where x is the number of

263 mimetic patterns or host plants shared, and y is the total number used by the sister that
 264 has fewer mimetic patterns or uses fewer host plants. We then applied two approaches
 265 to relaxed biological species to test whether shifts in ecology might be important for
 266 speciation. Firstly, we counted the number of sister species showing no overlap in
 267 colour patterns and host plants. Secondly, for the sister pairs with available molecular
 268 phylogenetic information, we used a general linear model with binomial errors to
 269 investigate the degree to which the ecological similarity of sister species is predicted
 270 by the time since that has elapsed since they shared a common ancestor. When we
 271 detected overdispersion we corrected the standard errors using a quasi-GLM model
 272 where the variance is given by $\phi\mu$, where μ is the mean and ϕ is the dispersion
 273 parameter. The slope will estimate the temporal trends in colour/host divergence or
 274 convergence and the intercept will estimate the degree of colour/host divergence at
 275 speciation (assuming (i) that species traits do not diverge so rapidly as to obscure the
 276 relationship between trait and branch length and (ii) that the mode of speciation is not
 277 mixed where some species diverge ecologically and others do not). If divergence in
 278 colour and host plant were gradual and independent of speciation, we would expect to
 279 estimate an intercept of 1 and a significant negative correlation (i.e. the most recently
 280 diverged sister species share the same colour patterns / host plants, with older sister
 281 species exhibiting decreasing similarity in these traits). Alternatively, under a
 282 punctuated model where speciation is accompanied by divergence, we would expect
 283 to estimate an intercept closer to zero (i.e. the most recently diverged sister species
 284 already have different colour patterns / host plants). To test whether the intercepts
 285 differed significantly from zero and/or one, we calculated the 95% confidence
 286 intervals of the intercept. To test whether the degree of ecological similarity is related
 287 to the degree of range overlap, overlap was included as an additional term in the

288 model.

289 **Results**

290 *Range overlap of heliconiines*

291 The distribution of each smaller-ranged heliconiines species overlaps entirely (defined
292 as > 0.95) with its sister in 8 out of 23 cases under a relaxed BSC and 9 out of 21
293 cases under a strict BSC, respectively (Figures 1,2, S1). The number of non-
294 overlapping pairs (overlap < 0.05) is more strongly influenced by species concept,
295 with six sister pairs non-overlapping under a relaxed BSC and only three pairs non-
296 overlapping under a strict BSC. Accordingly, the bimodality score for overlap was
297 higher for relaxed biological species (0.36) than for strict biological species (0.25). In
298 contrast to the patterns observed for biological species, there are more allopatric
299 phylogenetic species (i.e. subspecies) (129 cases) than sympatric phylogenetic species
300 (79 cases).

301 *Simulations*

302 To quantify the relative proportions of different geographic speciation models
303 underlying the observed patterns of sister species range overlap in heliconiines we
304 compared indices calculated for observed data to those generated using simulations of
305 geographic speciation and stochastic post-speciation range dynamics. In the
306 simulations we varied the proportion of speciation events that involved sympatric
307 versus non-sympatric geographic ranges (vicariant, peripatric or parapatric), the rate
308 of stochastic post-speciation range movement and the tendency for ranges to grow.
309 Results show that when most speciation was non-sympatric, sister species with
310 completely overlapping ranges were always rare (Figs. 3A & 4A). This is because

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3 311 even extensive range movements will rarely bring allopatric or parapatric sister
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5 312 species into complete sympatry. In contrast, the number of cases of non-overlapping
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7 313 sister species varied substantially, because even small range movements can easily
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9 314 lead to some geographic overlap between sister species (Figs. 3B & 4B). In
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11 315 simulations where most speciation was sympatric, non-overlapping sister species were
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13 316 rare, because only extensive range movements are likely to make species ranges that
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15 317 arose in sympatry entirely non-overlapping (Figs. 3B & 4B). However, the number of
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17 318 cases of sympatric sister species was very variable, because even small range
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19 319 movements will often move species ranges out of complete range overlap (Figs. 3A &
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21 320 4A).
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25 321 Simulations showed that the observed numbers of overlapping sister species pairs
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27 322 were unlikely ($P < 0.05$ under a two-tailed test) to arise when sympatric speciation
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29 323 comprises less than 35% (relaxed BSC) or 43% (strict BSC) of all speciation events.
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31 324 In contrast, the observed numbers of non-overlapping pairs frequently arose in
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33 325 simulations with all proportions of sympatric speciation (although not under all
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35 326 combinations of parameters). The bimodality scores of the observed data were
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37 327 unlikely to arise in simulations where either non-sympatric or sympatric speciation
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39 328 predominated (Figs 3C, 4C), and are consistent with simulations where 13-87% of
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41 329 speciation was sympatric under a relaxed BSC, and 0-90% under a strict BSC.
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44 330 Overall, the simulations that produced combined results not significantly different to
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46 331 any of the three observed indices had frequencies of sympatric speciation between
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48 332 35%-78% (relaxed BSC) or 43%-90% (strict BSC), low to moderate range
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50 333 movements (0.25-1.5, relaxed BSC; 0.25-1, strict BSC), either zero or positive range
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52 334 growth, and where the non-sympatric speciation events were any of vicariant,
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335 parapatric or peripatric (Tables S8, S9). However, when the non-sympatric speciation
336 was vicariant or parapatric, the observed patterns were obtained only when if the
337 range growth parameter was set to zero. This is because a tendency for ranges to grow
338 frequently results in overlap between vicariant / parapatrically derived sister species.
339 All starting range sizes were able to generate the observed data, but when starting
340 range sizes covered a larger proportion of the simulation domain area, the observed
341 data were likely to arise (i.e. $P > 0.05$) under a wider range of conditions.

342 *Age-range correlations*

343 To facilitate comparisons with previous work, we conducted age-range correlations
344 for relaxed and strict biological species (Fig. S2). The intercepts of the models had
345 intermediate values (values on arcsine scale - relaxed biological species: intercept =
346 0.48 ± 0.23 (0.46 as a proportion), slope = 0.05 ± 0.03 ; strict biological species:
347 intercept = 0.8 ± 0.23 (0.72 as a proportion), slope = 0.03 ± 0.03).

348 *Colour pattern divergence*

349 We examined whether closely related species tend to share colour patterns and host
350 plants by calculating the Jaccard's similarity coefficient for pairs of relaxed biological
351 species (low scores indicate that pairs of species share few colour patterns / host
352 plants; high scores indicate that they share many). Fourteen of 23 sister species pairs
353 have no colour patterns in common (Figure 5, Table S7). We estimated a positive but
354 non-significant slope between sister species' colour pattern similarity and
355 phylogenetic branch length (figure 6A, $b = 0.22 \pm 0.14$, values on logit scale) and an
356 intercept close to zero ($= -1.83 \pm 0.99$ on the logit scale or 0.14 as a proportion). The
357 95% confidence intervals of the intercept were 0.02-0.46 (expressed as a proportion).
358 We identified no effect of sympatry on colour pattern similarity when the

contemporary geographic context of sister pairs was included as an additional predictive term in the model.

Host plant divergence

Although none of the heliconiine sister species with available data have been recorded feeding on exactly the same set of host plant species, three species use a subset of the host plants recorded for their sister. Six sister pairs show no overlap in host plants, with the remaining sister pairs showing varying degrees of overlap (Fig. 5). For the relationship between sister species' host plant similarity and phylogenetic branch length (figure 6B), we estimated a positive but non-significant slope ($=0.08 \pm 0.04$, values on logit scale) and an intermediate intercept ($=-0.6 \pm 0.52$ on the logit scale or 0.36 as a proportion). The 95% confidence intervals of the intercept were 0.16-0.6 (expressed as a proportion). The degree of sympatry was not correlated with host plant similarity when included as an additional term in the model.

Discussion

We found that 35-43% of heliconiine sister are entirely sympatric, and 52-67% have overlap > 0.5 , depending on whether a relaxed or strict biological species concept is employed. This finding is in strong contrast to birds (5% show complete overlap as defined here (Phillimore et al. 2008)) and mammals, where sympatric sister species are also rare (14-23% show overlap > 0.5 (Fitzpatrick and Turelli 2006)). The age-range correlations for heliconiines had intercepts at intermediate values, and this pattern may have arisen either via extensive range movements post speciation, or as a mixture of allopatric and sympatric speciation (Barraclough and Vogler 2000; Losos and Glor 2003). However, the high proportion of sympatric sister species we observed

in heliconiines is more informative about process; under our simulations we found that high proportions of entirely sympatric sister species are unlikely to arise without sympatric speciation making a major contribution, even if range movements had been extensive. In fact, of the scenarios covered in our simulations, the observed overlap among heliconiine sister species is most consistent with a model in which sympatric speciation is common, contributing between 35% and 90% of all speciation events. Although the inference of a high frequency of sympatric speciation seems exceptional in comparison with analyses of other taxa, genetic linkage between wing colour patterns and mate preference in heliconiines along with their phytophagous habits mean that it is not implausible in this group (Kronforst et al. 2006; Chamberlain et al. 2009; Merrill et al. 2011). In addition, recent genomic studies suggest that a number of *Heliconius* species (e.g. *Heliconius elevatus*, *H. timareta* and *H. heurippa*) may have arisen following adaptive introgression of colour pattern elements (Mavárez et al. 2006; Jiggins et al. 2008; Dasmahapatra et al. 2012). Hybrid speciation necessarily requires sympatry between the parental species and their offspring, although hybrid speciation was not explicitly modelled in our simulations. The intercepts of the regressions between wing colour pattern / host plant similarity and branch length were both significantly different from one, thus there is evidence that shifts in both traits are associated with speciation events and that the differences between sister species are not simply the product of gradual divergence over time. However, we found no association between divergence in either trait and range overlap, as might be expected if the degree of ecological divergence during speciation differed between non-sympatric and sympatric modes. A critical assumption of the simulations employed here is that they assume species

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3 406 ranges to move independently and stochastically following speciation. In reality, this
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5 407 assumption is unlikely to hold. For instance, closely related species might often be
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7 408 ecologically similar, so that competition limits sympatry (Hardin 1960). Alternatively,
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9 409 if speciation involves a shift in host plant or other ecological dimension, competition
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11 410 between sister-species would be reduced and the new species might rapidly expand its
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13 411 range in geographic sympatry. Because climatic niches tend to be conserved in sister
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15 412 species (Peterson et al. 1999), this could lead to sister species having similar
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17 413 geographic distributions. In heliconiines we cannot exclude the possibility that the
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19 414 initial stages of speciation may involve parapatric or allopatric divergence of a
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21 415 population to specialise on an alternate host plant, followed by rapid range expansion
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23 416 and geographic overlap with the sister (or parent) taxa.
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28 417 The lack of a robust densely-sampled subspecies-level phylogeny precluded
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30 418 simulations of the geography of speciation using a diagnostic phylogenetic species
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32 419 concept based on fixed colour pattern differences (Cracraft 1983). However, most
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34 420 heliconiine subspecies overlap little with consubspecifics, and so conclusions drawn
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36 421 about relative frequencies of modes of speciation from species ranges will be highly
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38 422 sensitive to the species concept applied. Differences in taxonomic practises may
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40 423 therefore explain why birds (for which the diagnostic phylogenetic species was
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42 424 originally developed) display patterns of species overlap so different from those of
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44 425 heliconiines. The rarity of sympatric subspecies also runs counter to expectations if
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46 426 speciation frequently occurs sympatrically via colour pattern shifts, as under this
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48 427 model colour pattern polymorphisms should be fairly common. Subspecies are usually
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50 428 defined as geographic variants that are relatively constant over large areas (Mallet
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52 429 2001), which will lead to some bias against classifying local morphs in polymorphic
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populations as phylogenetic “species” under a diagnostic criterion. Nonetheless, in some highly polymorphic heliconiines "weak subspecies" have been recognized, in spite of frequent local polymorphisms, for example in *Heliconius numata* (Brown 1976). In the case of *H. numata*, such polymorphisms are now known to be due multiple haplotypes whose recombination is limited by several small inversions. These inversions trap colour pattern variation in supergene allelomorphs (Joron et al. 2011).

Conclusions

Naturalists have frequently observed that closely related animal species have allopatric distributions, a pattern believed so ubiquitous in vertebrates that it became known as “Jordan’s Law.” This finding was used as evidence against sympatric speciation (Jordan 1905; Jordan and Kellogg 1907; Coyne and Orr 2004). Here, we show that sister species in a group of phytophagous insects are often sympatric. In order to place this result in a broader context and establish whether it implies that Jordan’s Law can be rejected or is instead a rare exception that proves the rule will require biogeographic comparative studies to be conducted for a broader range of taxa than have been considered to date. It might be especially fruitful to study further phytophagous species-rich groups in the tropics, where levels of gene flow, parapatry, and sympatry are probably less often interrupted by climatic vicissitudes. On the basis of the results from simulations we suggest that the range overlaps observed in heliconiines are unlikely to arise without sympatric speciation. However, the observed patterns are consistent with another hypothesis: that speciation occurs mainly in allopatry/parapatry and that rapid range expansion leads to a high degree of range overlap among sister species. To bolster evidence for frequent sympatric speciation in

Heliconius it will be useful to demonstrate points along the speciation continuum, from local polymorphs to sympatric species exhibiting near complete assortative mating. Although heliconiines are typically locally monomorphic, polymorphisms do exist (Joron et al. 1999; Mallet 1999), in one case with weak assortative mating (Chamberlain et al. 2009). In addition, sympatric species with strong, but incomplete assortative mating are also known; in one population (San Cristóbal, Venezuela) of the largely sympatric species pair *H. cydno* and *H. melpomene* up to 8% of individuals are hybrids (Mavárez et al. 2006). Thus, much rests on identifying sympatric “semi-species” exhibiting intermediate levels of assortative mating.

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629 **Figure captions**

630 Figure 1. Range overlap in heliconiine butterflies. Histograms of range overlap for a)
631 phylogenetic species (=subspecies), b) relaxed biological sister species, and c) strict
632 biological sister species.

633 Figure 2. Examples of overlapping sister species (a) and non-overlapping sister
634 species (b).

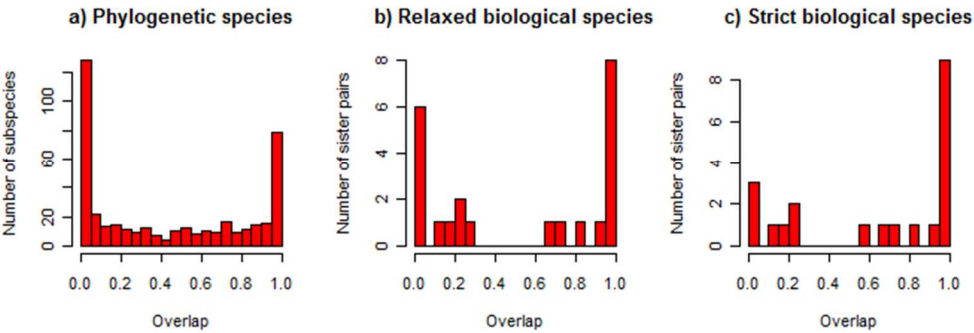
635 Figure 3. Results of simulations of relaxed biological species for selected parameters.
636 The x-axis shows the number of sympatric speciation events, the y-axis shows the
637 median number of completely overlapping species (column A), the median number of
638 non-overlapping species (column B) and the median bimodality (column C). The
639 dotted purple line shows the values observed for heliconiines. Simulations were run
640 using a medium sized range (1595), while varying the rate of range movements and
641 the tendency of ranges to grow following speciation. The geographic configuration of
642 the allopatric range (vicariant, parapatric or peripatric) is shown in the key.

643 Simulations results that were not significantly different from the observed values are
644 indicated with bold symbols.

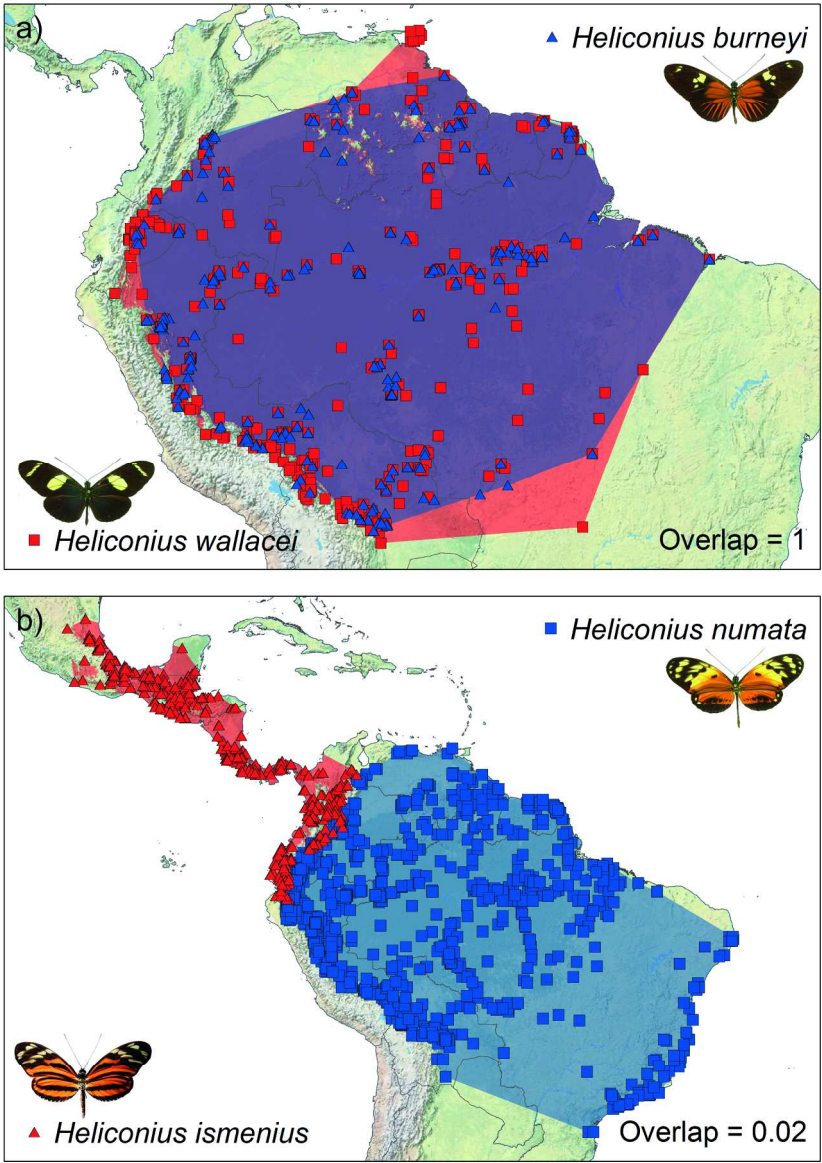
645 Figure 4. Results of simulations for 23 pairs of relaxed biological species for all
646 combinations of parameters. The x-axis shows the number of sympatric speciation
647 events, the y-axis shows the median number of completely overlapping species, the
648 median number of non-overlapping species and the median bimodality. The dotted
649 purple line shows the values observed for heliconiines. The smaller red dots show
650 simulation results that were not significantly different from the observed values, the
651 larger grey dots show simulation results that differed significantly from the observed
652 values.

653 Figure 5. Heliconiine phylogeny based on nuclear and mitochondrial DNA loci
654 (Beltrán et al. 2007), but with mitochondrial DNA divergence-based branch lengths,
655 adapted from (Mallet et al. 2007), with sister pairs of relaxed biological species in
656 black. Photos show example phenotypes for sister pairs; the upper species in each pair
657 is shown in the photo to the left and the lower species in the photo to the right. The
658 proportion of the smaller range that overlaps the larger range is shown in black in the
659 pie chart. Host plant use shows the number of host plant species used by the upper
660 species in black and the number used by the lower species in grey, with the number of
661 host plant species shared by the sisters in stripes. Nodes in the phylogeny marked with
662 an asterisk indicate sister species pairs for which branch length data were not
663 available for one of the sisters. These species pairs were not included in regressions of
664 ecological traits against branch lengths.

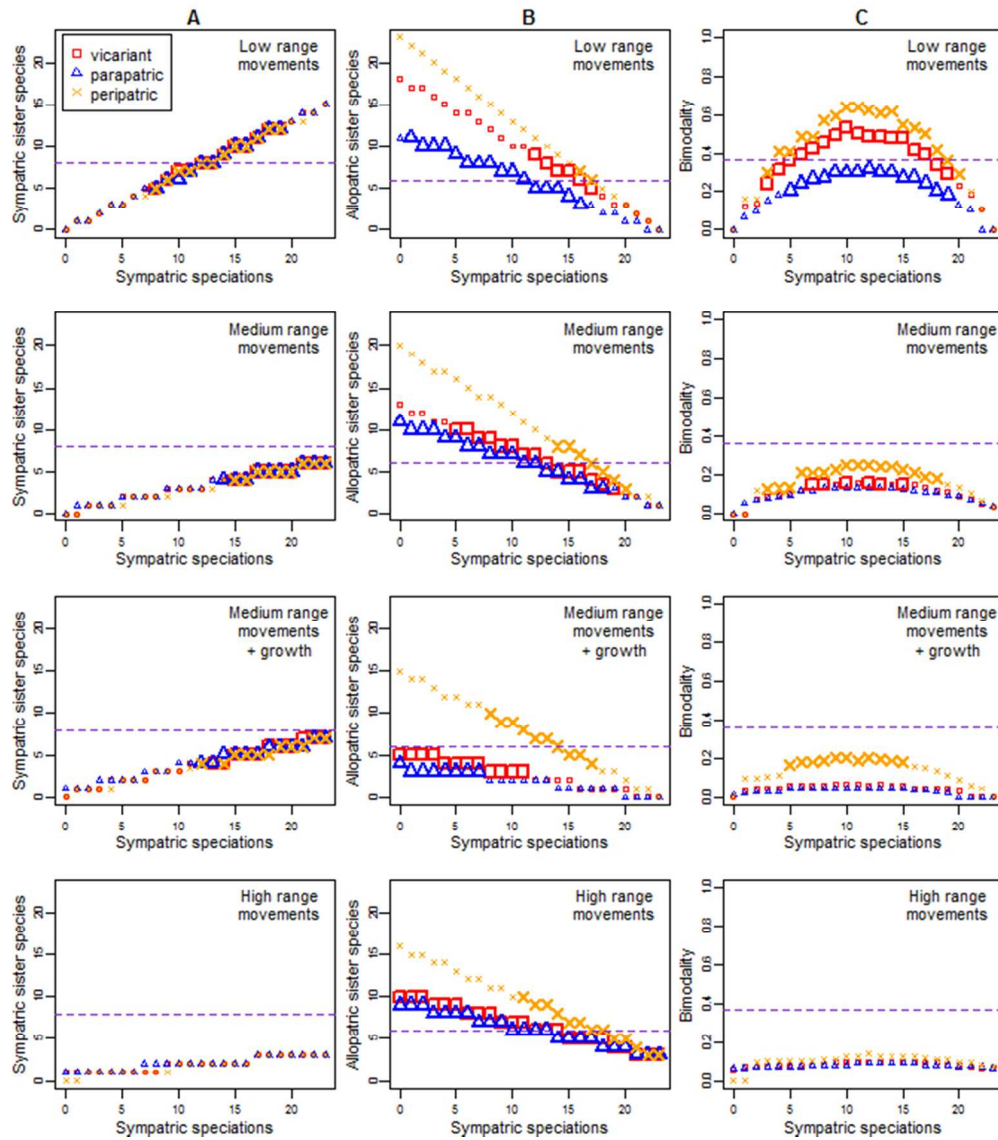
665 Figure 6 –Sister species’ wing colour pattern (A) and host plant (B) similarity plotted
666 against phylogenetic branch length.



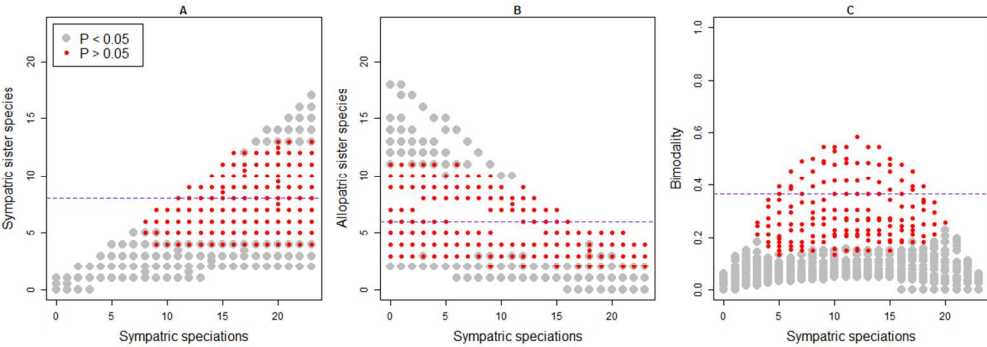
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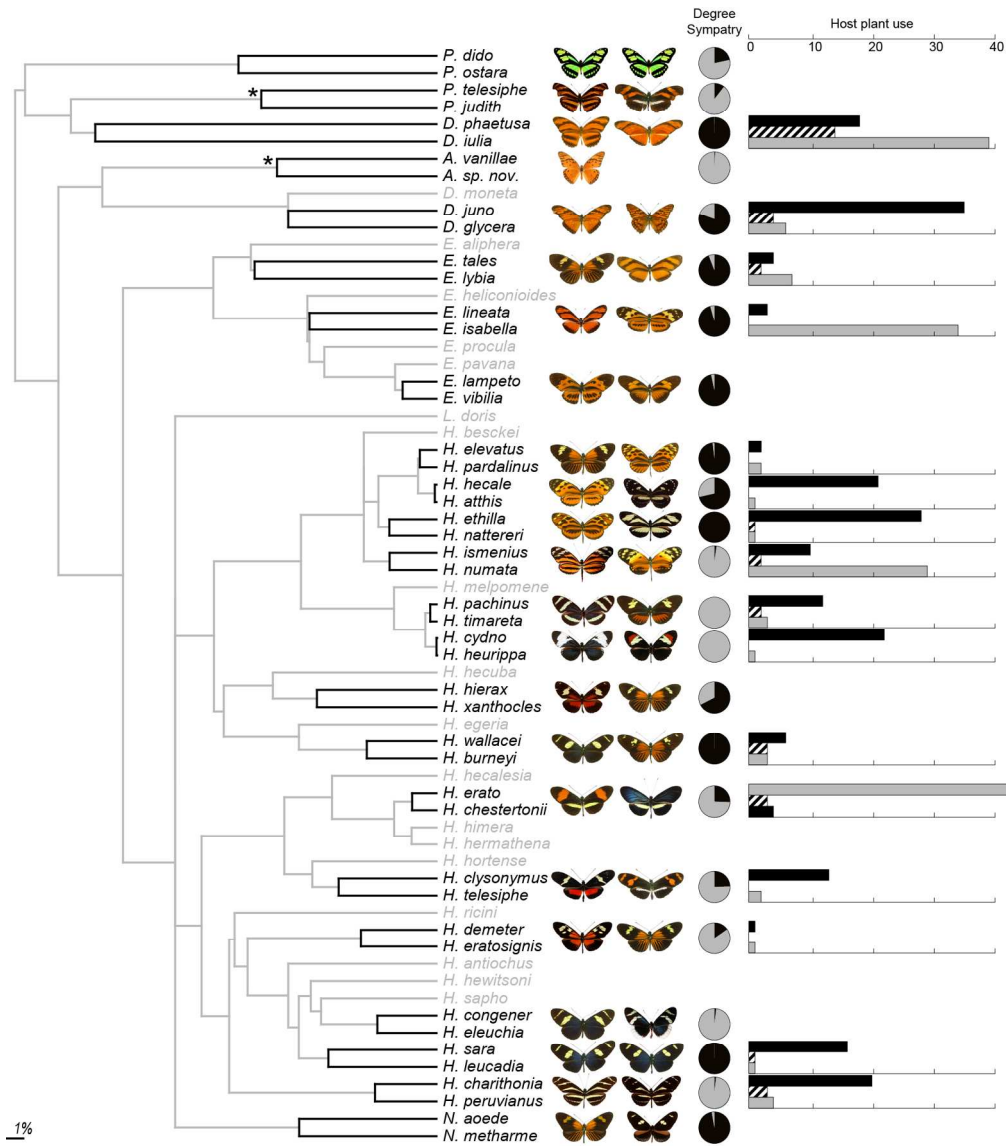
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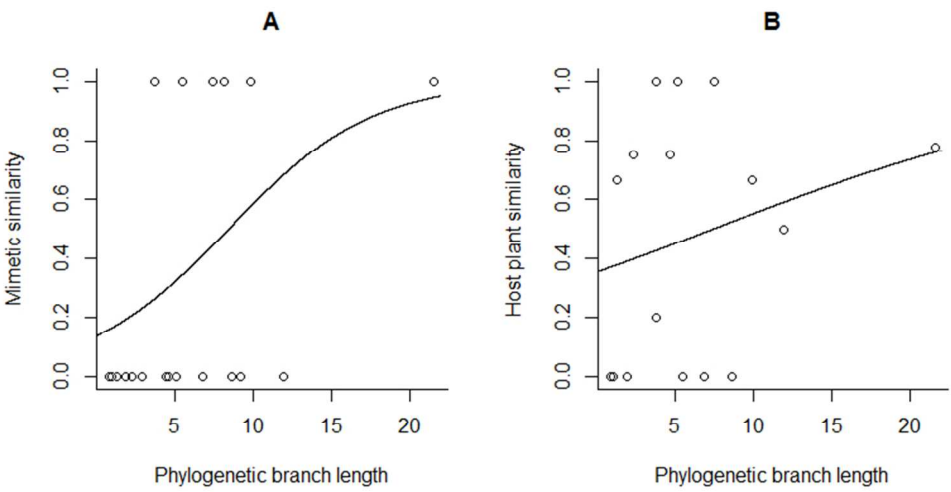
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