

# 1 Does sexual conflict contribute to the evolution of novel warning patterns?

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15

16 **Abstract.** Why warning patterns are so diverse is an enduring evolutionary puzzle. Because  
17 predators associate particular patterns with unpleasant experiences, an individual's predation  
18 risk should decrease as the local density of its warning pattern increases, promoting pattern  
19 monomorphism. Distasteful *Heliconius* butterflies are known for their diversity of warning  
20 patterns. Here, we explore whether interlocus sexual conflict can contribute to their  
21 diversification. Male *Heliconius* use warning patterns as mating cues, but mated females may  
22 suffer costs if this leads to harassment, favoring novel patterns. Using simulations, we show  
23 that drift alone is unlikely to cause pattern diversification, but that sexual conflict can assist  
24 such process. We also find that genetic architecture influences the evolution of male  
25 preferences, which track changes in warning pattern due to sexual selection. When male  
26 attraction imposes costs on females, this affects the speed at which novel pattern alleles  
27 increase. In two experiments, females laid fewer eggs with males present. However, although  
28 males in one experiment showed less interest in females with manipulated patterns, we found  
29 no evidence that female coloration mitigates sex-specific costs. Overall, male attraction to  
30 conspecific warning patterns may impose an unrecognized cost on *Heliconius* females, but  
31 further work is required to determine this experimentally.

## 32 **Introduction**

33 If selection can only exploit the best of the immediately available alternative phenotypes, how  
34 can novel ecological strategies evolve in already well-adapted organisms? This has  
35 traditionally been envisaged as the problem of peak shifts across the metaphorical ‘fitness  
36 landscape’ (Wright 1931). When the environment remains stable, in order to move from one  
37 adaptive peak (*i.e.* local optimum) to another, populations must first transverse a fitness  
38 valley, inhabited by intermediate and typically maladaptive phenotypes. To overcome this  
39 problem, genetic drift is often invoked as a means by which populations may avoid these  
40 fitness valleys (Wright 1931; Coyne and Orr 2004; Mallet 2010). However, when the traits in  
41 question are under positive frequency dependent selection, an additional complication is  
42 added: as peaks are defined by the abundance of its corresponding phenotype, new  
43 ‘unexplored’ peaks only become available once already populated by a substantial number of  
44 (initially maladapted) individuals.

45       Aposematic warning patterns, which are commonly assumed to be under strong  
46 positive frequency dependent selection, can represent considerable fitness peaks in the  
47 adaptive landscape (Mallet et al. 1990; Lindstrom et al. 2001; Borer et al. 2010; Merrill et al.  
48 2012; Chouteau et al. 2016; Gordon et al. 2021). Because predators learn to associate  
49 particular patterns with unpleasant experiences, an individual’s risk of predation should  
50 decrease as the local density of its warning pattern increases (Müller 1879; Sherratt 2008).  
51 This can lead to the convergence of warning patterns of different prey species sharing a  
52 habitat, a process coined ‘Müllerian mimicry’ (Müller 1879). However, although naively we  
53 might expect a single warning pattern to emerge, warning patterns are often very diverse  
54 within a community (Briolat et al. 2019).

55       The establishment of entirely new warning signals under positive frequency-  
56 dependent selection via predators is problematic. One possibility is that during periods of

57 relaxed selection, drift may allow new variants to rise above a threshold density until mimicry  
58 selection takes over (Mallet and Joron 1999; Sherratt 2006; Mallet 2010). Another possibility  
59 is that a model in which predators learn to avoid unpalatable prey only after sampling a fixed  
60 number is overly simplistic. For example, if predators are neophobic and generally avoid prey  
61 with unfamiliar phenotypes, novel signaling phenotypes might be favored when they are rare  
62 (Aubier and Sherratt 2015). A third possibility is that warning patterns might be  
63 multifunctional, so that their evolution is not solely governed by purifying frequency  
64 dependent processes induced by predators (Briolat et al. 2019).

65         Sex-specific selection provides a mechanism by which ecological diversity may be  
66 promoted (Bonduriansky 2011). Ecological adaptations, including strategies to exploit  
67 resources within the environment or avoid predators, are typically – though not always –  
68 shared between the sexes. Viability selection is normally expected to push ecological  
69 phenotypes towards a shared optimum. Sex-specific selection on the other hand may produce  
70 different adaptive optima for the two sexes (Andersson 1994; Arnqvist and Rowe 2005). This  
71 can result, for example, from requirements imposed on females to produce offspring or the  
72 need for males to find receptive mates (Bateman 1948). The existence of sex-specific optima  
73 can also lead to sexually antagonistic selection, and rapid evolution, even in opposition to  
74 viability selection (Arnqvist and Rowe 2005). For example, males may evolve strategies that  
75 increase their likelihood of securing mates. If these strategies impose costs on females,  
76 females may in turn evolve strategies to circumvent these male tactics, leading to further  
77 selection on males and so on. If sexually antagonistic selection involves ecologically relevant  
78 traits, this might result in peak shifts across the viability fitness landscape.

79         Since Bates (1862) first described mimicry theory, studies of *Heliconius* butterflies  
80 have made a substantial contribution to our understanding of adaptation (Merrill et al. 2015).  
81 Distasteful *Heliconius* are well known for their bright warning patterns, which are often

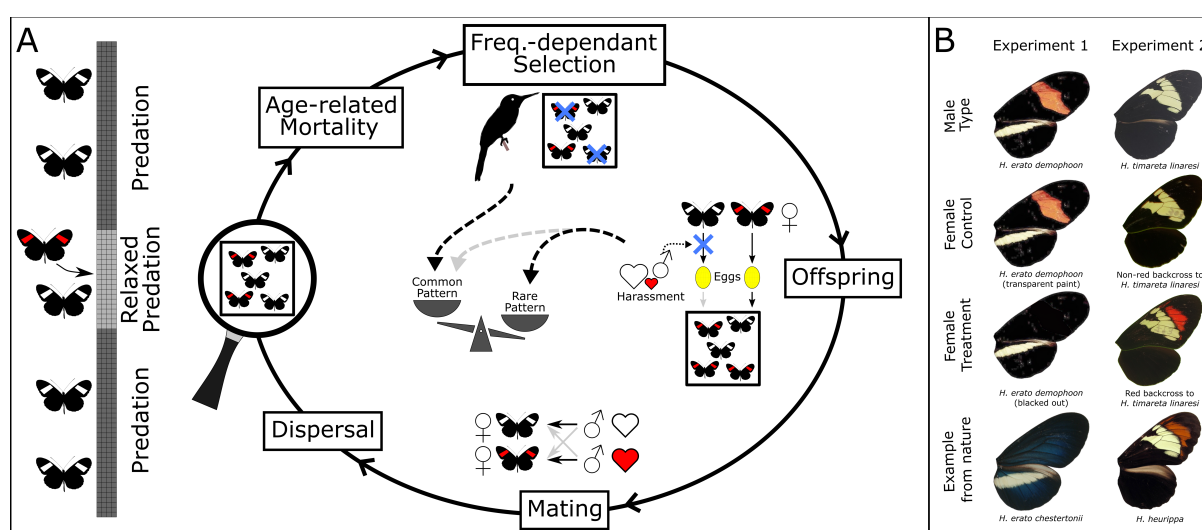
82 associated with Müllerian mimicry. These warning patterns are an important ecological  
83 adaptation in *Heliconius*, and predator-induced selection coefficients for the most common  
84 local patterns are strong (Mallet et al. 1990). Despite this, *Heliconius* butterflies exhibit a  
85 striking diversity of alternative warning patterns (Bates 1862; Merrill et al. 2015). Individual  
86 species often vary in warning pattern across their range, leading to distinct geographical color  
87 pattern types, and in some cases, such as in *H. cydno*, *H. numata* and *H. doris*,  
88 polymorphisms exist within single geographical populations. In addition, multiple warning  
89 patterns frequently coexist within a single geographical community. A given *Heliconius*  
90 species may then join many distinct mimicry rings according to local context, leading to the  
91 well-documented mosaic of warning patterns observed across the Neotropics (Brown Jr  
92 1976). Spatial variation in local predator and prey communities shape a rugged adaptive  
93 landscape, crucial to the maintenance of warning signal diversity.

94 In addition to warning potential predators, Jocelyn Crane (1955) demonstrated that the  
95 bright warning patterns of *Heliconius* stimulate male courtship in the 1950s. Since then,  
96 numerous experiments have repeatedly shown that male *Heliconius* generally prefer ‘females’  
97 that share their own warning pattern over that of other conspecific morphs or closely related  
98 species (e.g. Jiggins et al. 2001, 2004; Kronforst et al. 2006; Merrill et al. 2011*b*, 2011*a*,  
99 2014, 2019; Sánchez et al. 2015; Hausmann et al. 2021). It seems likely that competition  
100 between males drives these genetically determined local preferences, as the ability to  
101 efficiently locate potential mates within a visually complex environment would be beneficial  
102 (Merrill et al. 2019). However, previously mated females may suffer fitness costs if these cues  
103 lead to harassment by males during oviposition or foraging. These costs would be augmented  
104 by the fact that, although individual *Heliconius* are long lived (up to 6 months), female re-  
105 mating is a rare event in most species (Walters et al. 2012). These female-specific costs could  
106 conceivably set the stage for interlocus sexual conflict, leading to an arms race between

107 warning pattern and male preferences and rapid evolution when patterns are released from  
 108 constraints imposed by aposematism (e.g. after a local reduction in predation). Ultimately,  
 109 this could be an additional factor explaining warning pattern diversification.

110 To explore how costs imposed on females by male attraction to warning patterns  
 111 might contribute to pattern diversification in *Heliconius*, we first implemented individual-  
 112 based simulations. Across a vast parameter space, we tested i) if the presence of such female  
 113 costs can favor the evolution of novel patterns as opposed to drift acting in isolation, ii) which  
 114 parameters are most relevant for such dynamics to occur, and iii) how the genetic architecture  
 115 of male attraction traits might affect the speed at which novel patterns increase in frequency.  
 116 We then performed experiments to begin to test these ideas by disrupting warning patterns of  
 117 mated *Heliconius* females with marker pens or by introgressing a novel color pattern allele  
 118 from a closely related species. We subsequently tested the hypotheses that i) males interact  
 119 less frequently with females with disrupted patterns, ii) females lay fewer eggs in the presence  
 120 of males, and that iii) this effect is less pronounced for females with experimentally disrupted  
 121 patterns.

122



123  
 124 **Figure 1: Overview of the study.** A) Visualization of the individual-based model. A novel warning pattern  
 125 allele is introduced into the center of the 96 x 4 patched arena, where predation is initially relaxed. The following

126 generations consist of five phases, shown here for a single patch. Frequency dependent selection by predators  
127 always favors the most common pattern, while harassment from males imposes selection against the most  
128 common pattern (however, if female adaptations spread faster than male adaptations, this can switch). B) Pattern  
129 phenotypes of males and females (both, ‘manipulated’ and control) used in the experiments, as well as examples  
130 from nature resembling the ‘manipulated’ patterns.

131

## 132 **Model and Methods**

### 133 **a) Individual-based simulations**

134 We formalized our verbal model and then implemented this as individual-based simulations in  
135 R (R Core Team 2019). By tracking the fate of a novel warning pattern allele, these  
136 simulations allowed us to compare how frequently novel patterns might evolve due to drift  
137 alone as opposed to when patterns are additionally involved in sexual conflict. This also  
138 allowed us to explore which parameters determine how and if sexual conflict contributes to  
139 diversification.

140

141 *Arena.* Individuals live, breed and die within a rectangular arena of 96 x 4 ‘patches’ with  
142 uniform habitat, each representing ~1km<sup>2</sup> of forest. This arena is divided between 80 ‘central  
143 patches’, and the remaining 304 ‘peripheral patches’ (see Fig. 1A). To reduce boundary  
144 effects, the arena is wrapped into a torus.

145

146 *Genetics of individual pattern and preference phenotypes.* Individuals are sexual, diploid and  
147 have discrete sexes (determined by segregation of ‘sex chromosomes’). Both warning pattern  
148 and male mating preferences are genetically determined, and all loci are assumed to be  
149 autosomal and segregate independently (Merrill et al. 2015). Many years of research have  
150 established that major color pattern elements in *Heliconius* are controlled by just a few  
151 Mendelian loci (reviewed in McMillan et al. 2020). Although a handful of genes may

152 differentiate color pattern races, here we are explicitly interested in the spread of individual  
153 color pattern alleles and so consider just a single locus. As such, individuals have a single  
154 diallelic locus determining variation in a warning pattern element (with alleles  $A$ ,  $a$ ), which is  
155 expressed in both sexes: The derived novel allele  $A$  is dominant over the ancestral allele  $a$ ,  
156 reflecting strong dominance observed at *Heliconius* color pattern loci (McMillan et al. 2020).  
157 To account for mating preferences, we assume the existence of two additive quantitative  
158 characters  $p_a$  and  $p_n$ , controlling males' attraction towards females of the ancestral and novel  
159 pattern, respectively. Both traits  $p_a$  and  $p_n$  are scaled between 0 and 1 and are each controlled  
160 by  $N$  unlinked diallelic loci with equal effects (Fig. S1). Alleles have dominance relationships  
161 so that alleles increasing attraction towards the respective pattern elements are dominant over  
162 those that reduce attraction.

163  
164 *Life cycle.* The life cycle consists of five stages per generation (see Fig. 1A): (1) age-related  
165 mortality; (2) frequency dependent selection of adults due to predation; (3) production of  
166 offspring; (4) mating; and (5) dispersal. Compared to a previous model from Duenez-Guzman  
167 *et al.* (2009), our model incorporates overlapping generations. This increases the biological  
168 realism of the model as *Heliconius* are long lived and breed throughout their adult life. Newly  
169 eclosed adults are assigned as age = 0 and their age increases by 1 each generation.

170 Individuals with age  $> 4$  are removed from the population at the beginning of each generation.

171  
172 *Predation.* Predation is modelled implicitly through a patch-specific learning threshold, where  
173 predators stop eating adult butterflies of a certain pattern once the learning score  $Q$  is reached  
174 (following Duenez-Guzman et al. 2009). The learning process for each pattern occurs by each  
175 butterfly eaten within a patch contributing 1 to the corresponding learning score for the patch.  
176 The learning score for each patch is reset every generation and no evolution in predators is

177 allowed. In the 80 ‘central’ patches of the arena, predation is initially relaxed for time  $T$ .

178

179 *Sexual conflict and reproduction.* Offspring can be produced by females either after an  
180 encounter with a male or without male encounter. We assume that during oviposition, the  
181 chance that a female encounters a male depends on the probability of encountering any  
182 particular male ( $e$ ) and the number of males within the patch ( $m$ ):

183 
$$c = 1 - (1 - e)^m \quad (i)$$

184 In a scenario of sexual conflict, during an encounter, a female may either be disturbed or not,  
185 and hence lay an egg or not. The probability that an encounter between a specific female and  
186 male leads to a disturbance by the male depends on the attraction  $R$  of this male to the female.  
187 Following Duenez-Guzman *et al.* (2009),  $R$  is dependent on the male’s preference trait  $p$  and  
188 a parameter  $\alpha$ , which is positive and measures the strength of attraction to female patterns.  
189 Large values of  $\alpha$  imply strong attraction to color and smaller values imply weaker attraction.  
190 The smaller  $\alpha$ , the less will  $R$  differ between males with different preference traits (Fig. S1).  $R$   
191 for a pair of male  $i$  with preference  $p_x$  and female  $j$  with pattern type  $x$  ( $x$  being either  $a$   
192 (ancestral) or  $n$  (novel)) is:

193 
$$R_{i,j} = \exp \left[ \alpha * (p_{i_x} - 0.5) \right] \quad (ii)$$

194 The probability that an encounter leads to a disturbance equals to  $R_{i,j}$  divided by the maximum  
195 possible value of  $R$  (where  $p = 1$ ). The total number of eggs (which directly translates to  
196 offspring),  $B$ , for a patch is Poisson-distributed with mean  $b$ .  $b$  is independent of male  
197 disturbance, and limited only by available oviposition sites and female egg laying attempts are  
198 repeated until  $B$  is reached. Gametes producing an egg are randomly hit by mutation.  
199 Mutations only occur at loci affecting attraction to patterns, at a constant rate of  $\mu$  per locus  
200 per individual per generation (no double-mutations of a locus within an individual during the  
201 same generation are allowed). Maternal and paternal alleles recombine freely.



202

203 *Mating.* Mating occurs between individuals of the same patch. We assume females only mate  
204 once, and continue to lay eggs at a constant rate throughout their lives. Males are assigned to  
205 unmated females with relative probabilities equal to their attraction to patterns displayed by  
206 females (female choice is not present), which is calculated following equation (ii). The  
207 majority of females mate immediately after eclosion (and before dispersal); however, because  
208 each male can only mate 4 times per generation (mating is costly to *Heliconius* males)  
209 females may remain unmated if a patch by chance has >4 times more unmated females than  
210 males and then may be mated during subsequent generations. This imposes a very small cost  
211 on females with patterns that are less attractive to local males.

212

213 *Dispersal.* Finally, newly produced individuals disperse. We assume that each individual  
214 migrates to one of the eight neighboring patches or stays in its native patch with the same  
215 probability 1/9.

216

217 *Implementation.* To efficiently simulate the process of female egg laying and male harassment  
218 (by avoiding to individually simulate each egg laying attempt), we made use of the fact that  
219 the combined number of eggs placed by females of a patch was independent of male  
220 harassment. During each iteration and per patch,  $R$  for each possible female-male pair was  
221 computed and scaled to the maximum possible value of  $R$  (where  $p = 1$ ). The mean of these  
222 values equals to the average probability of male encounters leading to disturbance,  $d$ . The  
223 expected proportion of eggs laid after male encounter, but without disturbance,  $P$ , is hence:

224 
$$P = \frac{c*(1-d)}{c*(1-d)+1-c} \quad (iii)$$

225 The number of eggs laid after encounter with a male was drawn from a Poisson-distribution  
226 with mean  $B*P$ , and number of eggs without encounter from a Poisson-distribution with mean

227  $B*(1-P)$ . Mated females within a patch were randomly assigned to the available eggs laid  
228 without encounter. However, unattractive females were more likely to contribute to eggs laid  
229 with encounter. The relative probability of a female being assigned to those eggs equaled to  
230 the inverse of the mean attractiveness of this female to all males in the patch (as calculated  
231 following equation (ii)).

232 We ran all simulations with equal starting conditions. The arena was initially  
233 populated with 114 individuals (57 mated females and 57 males) per patch, which was a  
234 population size similar to the equilibrium population size. Ages were distributed equally over  
235 patches and sexes within a patch and ranged from 0 to 5 (as typical for the beginning of a new  
236 generation). All individuals were initially homozygous ( $aa$ ) for the recessive ancestral pattern  
237 and homozygous ( $aa$ ) for the recessive ancestral allele at loci for attraction to the novel  
238 pattern (*i.e.* minimum attraction to the novel pattern in males). Also, all individuals were  
239 homozygous ( $AA$ ) for the dominant ancestral allele at loci for attraction to the ancestral  
240 pattern (*i.e.* maximum attraction to the ancestral pattern in males). Throughout, the mutation  
241 rate  $\mu$  was set at  $10^{-5}$ .

242 At the beginning of each subsequent simulation run (*i.e.* at generation 1), a color  
243 pattern mutation was introduced into a randomly chosen individual of age 0 within one of the  
244 80 central patches, where predation was initially reduced (so that it was heterozygous  $Aa$ ).  
245 The fate of the introduced mutation was then followed until it was lost from the population, or  
246 when 2500 generations were reached. During each generation, the number of eggs laid by  
247 females within one patch,  $B$ , was drawn from a Poisson-distribution, with mean  $b = 20$ .  
248 However, for a subset of simulation runs, a bottleneck was imposed within the 80 central-  
249 most patches for the first 5 generations to increase the effects of drift, where  $b = 0.5$ .

250

251

252

253 **Table 1: Simulation parameters and their settings.**

Learning threshold ( $Q$ )	Predator absence ( $T$ )	Bottle-neck	Sex first mutant	Loci attraction ( $N$ )	Strength attraction ( $\alpha$ )	Sexual conflict	Prob. encount. ( $e$ )	
1	10	No	Female	1	1	No	0.001	
2	100	Yes	Male	5	3	Yes	0.0025	
4				10			0.005	
							0.01	
If sexual conflict present:								
$e$	0.001	0.001	0.0025	0.0025	0.005	0.005	0.01	0.01
$\alpha$	1	3	1	3	1	3	1	3
$\Delta$ eggs	+2.9%	+4.3%	+7.2%	+10.4%	+14.1%	+19.8%	+27.2%	+36.0%

254 Parameters shown at the top were systematically varied across simulation runs, with the exception of parameter  $e$   
 255 (probability of encounter between specific female and male), which was only varied if sexual conflict was  
 256 present. Below, for each combination of  $e$  and  $\alpha$  (strength of male attraction to female pattern), and assuming a  
 257 patch containing males typical for the first generation with minimum attraction to novel and maximum attraction  
 258 to ancestral pattern, the expected relative difference in number of offspring sired by a novel patterned female  
 259 versus an ancestrally patterned female is shown.

260  
 261 *Parameter space.* We systematically varied the predator learning threshold ( $Q$ ); the number of  
 262 generations that predators were absent from the central patches of the arena ( $T$ ); whether or  
 263 not a bottleneck was imposed on the central patches in the first generations; the sex of the first  
 264 mutant; the number of loci affecting each color attraction trait in males ( $N$ ); the strength of  
 265 color attraction in males ( $\alpha$ ); and whether sexual conflict was present (*i.e.* male harassment  
 266 during egg laying) or not (reference baseline). In simulation runs where sexual conflict was  
 267 present, we also varied the probability of encounter between a specific female and male ( $e$ ).

268 Additionally, we always noted the position in the arena where the first mutant occurred.  
269 Different settings for the parameters are shown in Table 1, together with the expected relative  
270 difference in number of offspring sired by a novel patterned female versus an ancestrally  
271 patterned female, as dependent on  $\alpha$  and  $e$  (assuming a patch containing males typical for the  
272 first generation with minimum attraction to novel and maximum attraction to ancestral  
273 pattern). An average of 1000 iterations were run for each of the 720 parameter combinations.  
274 576 of these parameter combinations included sexual conflict and 144 did not. Number of  
275 heterozygous and homozygous individuals at each locus in central and non-central patches  
276 were recorded during each simulated generation, and stored in a sparse matrix (Bates and  
277 Maechler 2019). Scripts to run the model are available in the supplementary R Markdown.  
278  
279 *Statistical analysis.* All analyses were conducted in *R* (R Core Team 2019). Scripts are  
280 available in the supplementary R Markdown. Numeric covariates (*i.e.*  $Q$ ,  $TN$ ,  $\alpha$ ,  $e$ ; see Table  
281 1 for annotation) were transformed into ordered factors. We tested which of the simulation  
282 parameters affects the probability of the novel warning pattern allele surviving until  
283 generation 2500 (at which point simulations were always ended) using GLMs with binomial  
284 error structure (*i.e.* success = allele remains in population, always identical with going to  
285 (near) fixation; failure = allele disappears from population). To avoid complete separation in  
286 the model, fixed effects that separated the predictor variable were not included and tested with  
287 simple binomial tests (correcting p-values with the Bonferroni method). Stepwise forward-  
288 model selection based on AIC via the *MASS* package (Venables and Ripley 2002) was used to  
289 find the model that explained the data best, maximally allowing for two-way interactions. To  
290 test for the effect of genetic architecture of male attraction traits on number of generations  
291 until the novel warning pattern went to near fixation (allele frequency = 0.95; only  
292 considering runs where the novel pattern survived), we fitted a GLM with Poisson error

293 structure with number of loci coding for each attraction trait ( $N$ ) as fixed effect. Estimated  
294 marginal means for the different predictors were extracted using the *emmeans* package (Lenth  
295 2019). The same package was used to perform Type III Anova to test for significance among  
296 fixed effects. Resulting p-values were corrected for multiple testing using the Bonferroni  
297 method.  $R^2$  values were calculated using the *dominanceanalysis* package (Bustos Navarrete  
298 and Coutinho Soares 2020).

299

### 300 **(b) Insectary experiments**

301 *Experiment 1.* To investigate female-specific costs associated with male attraction to warning  
302 patterns, we first experimentally manipulated female warning patterns with marker pens. This  
303 experiment was performed between November 2014 and August 2015 in the Smithsonian  
304 Tropical Research Institute insectaries in Gamboa, Panama (9°7'24"N, 79°42'12"W).

305 *Heliconius erato demophoon* (Fig. 1B) were collected around Gamboa and maintained in  
306 communal 2x2x2m cages (males and females separately) with ~10% sugar solution, a pollen  
307 source, and in the case of females, *Passiflora* host plants. All individuals were numbered on  
308 the ventral side of the wings. Females were assigned to a warning pattern treatment (Fig. 1B):  
309 either i) disruption of the pattern by painting over the dorsal side of the red forewing band  
310 with a black Copic™ Caio 100 marker; or one of two 'control' treatments, ii) painting over  
311 the dorsal side of the red forewing band with a colorless Copic™ Caio 0 marker (with same  
312 solvents as the black marker), or iii) handling but no marker. Visual equivalence of the two  
313 control treatments was confirmed by modelling based on the *H. erato* visual system (see Fig.  
314 S7 and supplementary methods). A few additional females experienced neither of the three  
315 pattern treatments and were excluded from all analyses, except where indicated.

316 Females were introduced individually into a 2x2x2xm experimental cage, containing a  
317 single *Passiflora biflora* host-plant (which was not re-used). Females were left to acclimatize

318 for 48 hours and all eggs laid during this period were removed. This was followed by two 48-  
319 hour experimental periods. Three *H. e. demophoon* males were then introduced into the cage  
320 during either the first or second experimental period, so that each female experienced 48  
321 hours with, and 48 hours without males. No two females experienced the same combination  
322 of males. Eggs were collected daily from the cages. For a subset of the females, we also  
323 recorded the time and duration of male-female interactions (hovering ‘courtship’ and  
324 chasing). Due to logistic necessities, total observation time differed between females (median  
325 = 3607.5, min = 929, max = 7207), largely relating to whether observations were carried out  
326 on one or both days in which females were housed with males. We excluded two females  
327 from subsequent analysis that did not lay eggs (though their inclusion does not quantitatively  
328 affect our results).

329  
330 *Experiment 2.* To further investigate female-specific costs, but to ensure biologically realistic  
331 warning patterns, our second experiment exploited the segregation of Mendelian color pattern  
332 elements in hybrids between distinct *Heliconius* populations. This was performed between  
333 July and December 2018 in the Universidad del Rosario insectaries in La Vega, Colombia. *H.*  
334 *timareta linaresi* (yellow forewing bar; Fig. 1B) were collected from Guayabal (2°41’04”N,  
335 74°53’17”W) and *H. heurippa* (yellow and red forewing bar) from Lejanías (03°34’0”N,  
336 74°04’20.4”W), Buenavista (4°10’30”N, 73°40’41”W) and Santa María (04°53’28.2”N,  
337 73°15’11.4”W) in Colombia. Outbred stocks were established and used to generate *H. t.*  
338 *linaresi* x *H. heurippa* F<sub>1</sub> and backcrosses to *H. t. linaresi* hybrids (as described in Hausmann  
339 et al. 2021). The presence of the red forewing band is controlled by a single Mendelian locus  
340 (*optix*), and segregates in the backcross to *H. t. linaresi* so that equal numbers of individuals  
341 display a (‘novel’) yellow-red forewing band phenotype or a (‘control’) yellow forewing  
342 phenotype (Fig. 1B). Sizes of the yellow and red bands was assessed using k-means clustering

343 (see Fig. S8 and supplementary methods). Shortly after eclosion, backcross females were  
344 mated to a *H. t. linarensi* male and then housed in a communal cage (2x4x2m) before the  
345 experiments. Experimental procedure was as in experiment 1 except that: 1) Insectary-reared  
346 individuals were used in all trials; 2) experimental cages contained several species of  
347 *Passiflora*, which could not be exchanged between trials; 3) only one male was introduced;  
348 and 4) eggs were only collected every 48 hours. As for experiment 1, total observation time  
349 differed between females (median = 3547, min = 1722, max = 3904).

350  
351 *Statistical analysis.* To test for the effect of warning pattern treatment on male interest, we  
352 used GLMMs with binomial error structure. The dependent variable was the proportion of  
353 seconds with courtship, and pattern treatment was fitted as fixed effect. Female ID was fitted  
354 as random effect to correct for individual variation within treatment groups, and control for  
355 repeated measures across different days. For experiment 1, the two controls (colorless marker  
356 or handled) did not differ in their effect on male interest ( $F$  ratio = 0.962,  $d.f.$  = 1,  $p$  = 0.327),  
357 and were therefore combined. For experiment 2, we fitted an additional model to data from  
358 yellow-red females, where we explained proportion of seconds with courtship by size of the  
359 red band and size of the yellow band of the female (as relative to whole wing area, to correct  
360 for female size), and with female ID as a random effect. To test for the effects of male  
361 attraction to female patterns on short-term female fecundity, we used GLMMs with Poisson  
362 errors. Here the dependent variable was the number of eggs laid over a 48h period. Male  
363 presence, female pattern and their interaction were fitted as fixed effects, and female ID was  
364 fitted as random effect. Our prediction was that any differences in short term female fecundity  
365 resulting from male attraction to specific warning patterns would be observed as a significant  
366 interaction between male presence and pattern treatment. Again, for experiment 1, the two  
367 controls (colorless marker or handled) did not differ in their effect on female fecundity ( $F$

368  $ratio = 1.071, d.f. = 1, p = 0.301$ ), nor was there a significant interaction with male presence  
369 ( $F ratio = 0.993, d.f. = 1, p = 0.319$ ), and they were therefore combined. To test for the effect  
370 of male presence on female fecundity in isolation, we fitted the same type of model structure,  
371 but only including male presence as fixed effect. For experiment 1, we additionally included  
372 females that were neither treated with a marker, nor handled (and which were excluded from  
373 all other analyses). We used the *emmeans* package (Lenth 2019) to determine the effect of the  
374 different variables (via type III Anova), and to calculate estimated marginal means (EMMs)  
375 and effect sizes (*i.e.* difference in eggs laid between experimental periods).

376

## 377 **Results**

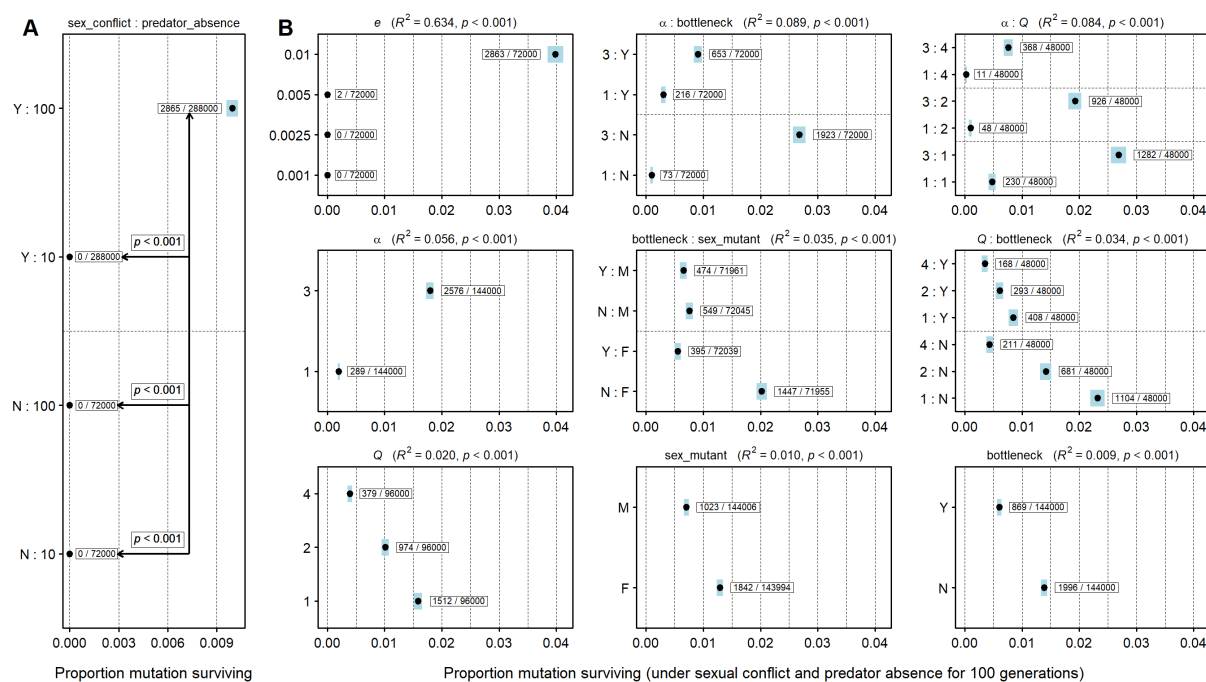
### 378 **(a) Simulation results**

#### 379 *Sexual conflict facilitates the evolution of novel warning patterns*

380 We ran simulations across 720 different parameter combinations (each replicated ~1000  
381 times). The novel pattern quickly disappeared from the population in all 144000 simulation  
382 runs where only genetic drift could contribute to the diversification of warning patterns (Fig.  
383 2A). The novel pattern allele only survived in simulations which included both, sexual  
384 conflict *and* the maximum period of relaxed predation (*i.e.*  $T = 100$  generations; Fig. 2A). The  
385 novel allele persisted in 2865 of 288000 simulations fulfilling these two criteria (*i.e.* 1%), and  
386 in each of these it also increased in frequency to near or complete fixation (Fig. S4). By far  
387 the most important additional parameter in these simulations was the probability of a male-  
388 female encounter, which explained 64% of the variance (Fig. 2B): with the notable exception  
389 of 2 simulation runs, where a value of 0.005 was sufficient, this parameter had to be at 0.01  
390 for the novel pattern allele to be retained. However, stepwise model selection also revealed  
391 that strength of male attraction to color ( $\alpha$ ), predator learning threshold ( $Q$ ), presence of a  
392 bottleneck in the central patches during the first generations ('bottleneck'), sex of the first



393 mutant ('sex'), as well as the interactions bottleneck\* $\alpha$ , bottleneck\* $Q$ , bottleneck\*sex, and  
 394  $\alpha$ \* $Q$ , also all significantly affected the retention of the novel allele (Fig. 2B and Table S2).  
 395 Overall, the most promising combination of parameter settings included sexual conflict,  $Q =$   
 396 1,  $T = 100$ , no bottleneck, female first mutant,  $N = 10$ ,  $\alpha = 3$  and  $e = 0.01$ , where the novel  
 397 allele was retained in 26% of runs (Table S1). We also found that the novel allele was more  
 398 likely to survive when the initial mutation occurred in one of the most central patches (*i.e.* far  
 399 away from active predation, Fig. S2). Indeed, the eventual fate of the novel pattern allele  
 400 seemed to be largely determined during these first generations of relaxed predation in the  
 401 central patches, as its frequency at generation 100 was closely correlated with future retention  
 402 or loss of the allele (Fig. S3).  
 403



404  
 405 **Figure 2: Presence of sexual conflict and duration of relaxed predation are the strongest predictors of**  
 406 **retention of novel pattern, followed by probability of male encounter.** A: Effect of sexual conflict ('Y' = yes,  
 407 'N' = no) and duration of predator absence from central patches on probability of novel warning pattern  
 408 retention. Proportions and 95% binomial confidence intervals are displayed for each combination, together with  
 409 p-values from binomial tests. B: Effects of simulation parameters retained during model selection (same  
 410 parameter annotation as in Table 1). R<sup>2</sup> value and p-value are shown above each panel. Interactions are indicated

411 with a ‘:’ sign above the respective panel, and combinations are separated by a ‘:’ sign at the y-axis (‘Y’ = yes,  
412 ‘N’ = no, ‘M’ = male, ‘F’ = female). Estimated marginal means (circles) and their 95% confidence intervals  
413 (blue rectangles) are shown for each predictor.

414

#### 415 *Male attraction to colors tracks warning pattern evolution*

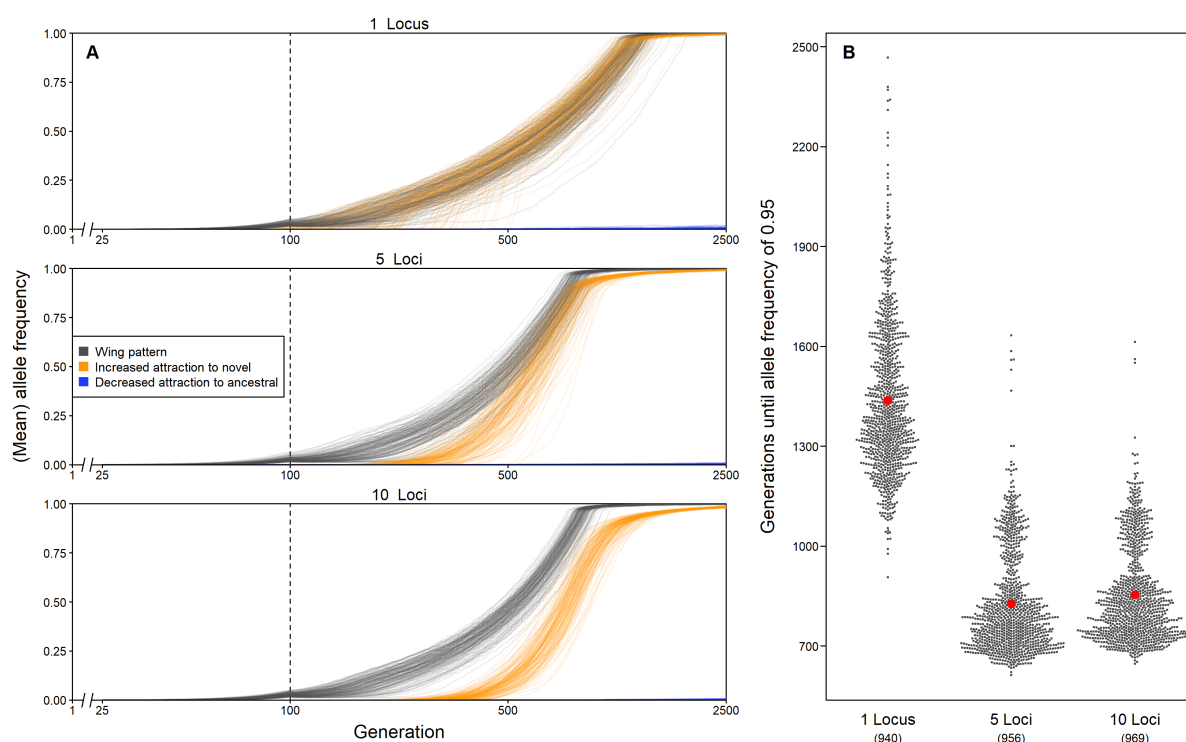
416 Overall, the mean frequency of alleles causing male attraction to the novel pattern closely  
417 tracked the frequency of the novel pattern allele, but lagged behind some generations, as  
418 typically expected during chase-away selection (Fig. 3A, see also supplementary animation).  
419 The magnitude of this lag depended on the genetic architecture of male attraction traits, with  
420 more complex architectures (*i.e.* more preference loci) generating a greater discrepancy  
421 between pattern and male attraction to this pattern (Fig. S4). In contrast, the mean frequency  
422 of alleles determining attraction to the ancestral pattern changed very little (Fig. 3A; note that  
423 male attraction to the two pattern types was controlled by independent loci): Across  
424 simulation runs, the average male was at no point more attracted to the novel than to the  
425 ancestral pattern (Fig. S5). Therefore, once the novel pattern exceeded the frequency of the  
426 ancestral pattern, both mimicry selection and selection imposed by male harassment acted in  
427 the same direction for a period of time, both favoring for the novel pattern (Fig. S5).

428

#### 429 *Genetic architecture of male attraction influences the speed at which novel pattern alleles* 430 *spread in simulations with sexual conflict*

431 Although the number of loci encoding attraction phenotypes was not retained in our model  
432 testing for survival of the novel pattern allele, it did have a strong effect on how fast the novel  
433 pattern allele’s frequency increased in the population, in cases where it persisted (Fig. 3B and  
434 S6). With just one locus determining attraction to each pattern, simulation runs required on  
435 average ~600 generations longer to reach a frequency of 0.95 of the novel pattern allele  
436 compared to simulation runs with 5 loci (z-test:  $z = 394.812$ ,  $p < 0.001$ ) or 10 loci (z-test:  $z =$

437 375.969,  $p < 0.001$ ). Surprisingly, simulation runs with 5 or with 10 loci did not differ much  
438 from each other in this respect (and the trend was even slightly reversed, z-test:  $z = -20.558$ ,  $p$   
439  $< 0.001$ ). These differences were observed across all possible combinations of the other  
440 simulation parameters (see supplementary R Markdown).  
441



442  
443 **Figure 3: Male attraction tracks changes in warning pattern and its genetic architecture affects the speed**  
444 **at which novel patterns spread.** A: Allele frequencies over generation time for novel alleles at the warning  
445 pattern locus (gray), loci for male attraction to the novel pattern (orange; novel alleles increase attraction) and  
446 loci for male attraction to the ancestral pattern (blue; novel alleles reduce attraction). Means were calculated  
447 whenever multiple loci were involved. Panels are split by different genetic architectures of male attraction traits  
448 (1, 5 or 10 loci). Results from only one evolutionary scenario are shown (sexual conflict present;  $Q = 2$ ;  $T = 100$ ;  
449 no bottleneck; sex first mutant = female;  $\alpha = 3$ ;  $e = 0.01$ ) and only including those where the novel pattern  
450 persisted. x-axis is log-scaled, and frequencies at generations  $< 25$  are not displayed (as they are invisibly small).  
451 Vertical line at 100 generations indicates end of relaxed predation. B: Speed at which the novel pattern allele  
452 reaches a frequency of 0.95, as dependent on number of loci for male attraction traits (including data across all  
453 other simulation parameters). Estimated marginal means are shown in red (CIs are invisibly narrow).  
454

455 **(b) Empirical results**

456 *Warning patterns influence male harassment of mated H. erato females*

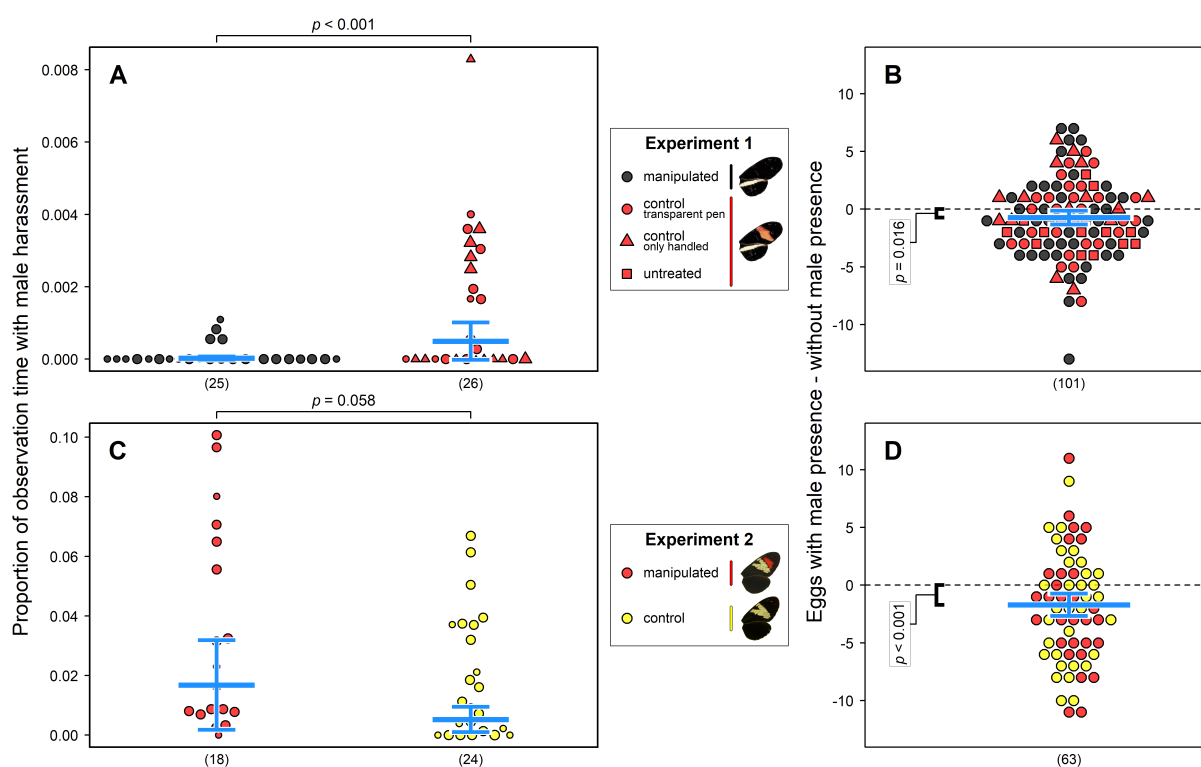
457 In experiment 1, we carried out observations for 58 individual females, including 30  
458 experimental butterflies with disrupted warning patterns, and 28 butterflies subjected to one  
459 of the two control treatments (14 of each). After removing outliers (five experimental and  
460 four control individuals, see Fig. S9 and supplementary methods), females with intact  
461 warning patterns were harassed more often than those with disrupted patterns (Fig. 4A,  $F$   
462  $ratio = 13.34$ ,  $d.f. = 1$ ,  $p < 0.001$ ; inclusion of the outliers did not qualitatively affect the  
463 results: Fig. S9A,  $F ratio = 6.24$ ,  $d.f. = 1$ ,  $p = 0.013$ ). In experiment 2, we carried out  
464 observations for 45 individual females, including 20 ‘experimental’ butterflies with yellow-  
465 red forewing pattern, and 25 ‘control’ butterflies with yellow forewing pattern. Overall, males  
466 were much more responsive than in experiment 1. Surprisingly, including all individuals,  
467 females with ‘experimental’ pattern were harassed more often than those with ‘control’  
468 pattern (Fig. S9B,  $F ratio = 4.54$ ,  $d.f. = 1$ ,  $p = 0.033$ ), though this trend was no longer  
469 significant when outliers (two ‘experimental’ and one ‘control’ individual) were removed  
470 (Fig. 4C,  $F ratio = 3.61$ ,  $d.f. = 1$ ,  $p = 0.058$ ). However, for yellow-red females with  
471 measurements of the band sizes available (12 of the 18 from Fig. 4C), we found that male  
472 harassment decreased as the size of the red band increased (Fig. 10A, z-test for slope  $\neq 0$ :  $z =$   
473  $2.566$ ,  $p = 0.010$ ), whereas the size of the yellow band had no effect (Fig. 10B, z-test:  $z =$   
474  $0.116$ ,  $p = 0.908$ ).

475

476 *Male presence reduces the number of eggs laid by females*

477 There was considerable variation in the number of eggs laid, both, between experimental  
478 periods (*i.e.* males present and males absent) for individual females, as well as between  
479 females (Fig. 4B+D). Nonetheless, in both experiments, females laid fewer eggs in the

480 presence of males (experiment 1, including females without warning pattern treatment:  $F$   
 481  $ratio = 5.83$ ,  $d.f. = 1$ ,  $p = 0.016$  (Fig. 4B); experiment 2:  $F ratio = 12.49$ ,  $d.f. = 1$ ,  $p < 0.001$   
 482 (Fig. 4D)). Over a two-day period, females in the presence of males laid  $\sim 0.73$  [CI: 0.14-1.32]  
 483 fewer eggs in experiment 1 (a reduction of 13%; GLMM estimate without male = 5.45, with  
 484 male = 4.72) and 1.70 [CI: 0.74-2.66] fewer eggs in experiment 2 (a reduction of 19%;  
 485 GLMM estimate without male = 8.86, with male = 7.15). However, we found no evidence of  
 486 an interaction between the presence of males and warning pattern treatment on the number of  
 487 eggs laid (experiment 1:  $F ratio = 1.110$ ,  $d.f. = 1$ ,  $p = 0.291$ ; experiment 2:  $F ratio = 0.005$ ,  
 488  $d.f. = 1$ ,  $p = 0.943$ ). Hatching success, measured for a subset of females in experiment 2, was  
 489 also unaffected by male presence (Fig. S11).  
 490



491  
 492 **Figure 4: Males harass mated females with intact warning patterns more often in experiment 1. Females**  
 493 **lay fewer eggs in the presence of males in both experiments.** Panels A+B correspond to experiment 1 (*H.*  
 494 *erato*), panels C+D to experiment 2 (*H. timareta*). Warning pattern treatment is indicated by dot color and shape.  
 495 A+C: Proportion of total observation time males spent harassing females with different patterns. Area of dots is

496 relative to total number of observed seconds. Estimated marginal means and their confidence intervals (CIs) are  
497 shown in blue. B+D: Difference between eggs laid with and without male presence. Values below 0 indicate that  
498 male presence reduces fecundity. Effect sizes and their confidence intervals (CIs) are shown in blue.

499

## 500 **Discussion**

501 The warning patterns of *Heliconius* butterflies have become a textbook example of natural  
502 selection (e.g. Barton *et al.*, 2007; Futuyama & Kirkpatrick, 2017), but the origins of their  
503 considerable diversity remain problematic. We explored whether sex-specific selection might  
504 contribute to the evolution of novel warning patterns. Using individual-based simulations, we  
505 have shown that drift alone is unlikely to account for the spread of novel warning pattern  
506 alleles. Instead our simulations suggest that harassment of previously mated females by males  
507 attracted to their bright warning patterns – in association with periods of initially relaxed  
508 predation – could facilitate the spread of novel pattern alleles. We also showed that genetic  
509 architecture of male attraction traits can influence how quickly a novel pattern may spread  
510 through the population, as it determines how fast males adapt their corresponding mating  
511 preferences. Data from insectary experiments also provide some support that sexual conflict  
512 can facilitate the spread of novel patterns: The presence of males reduced short-term female  
513 fecundity, highlighting that unwanted male attention may well favor the evolution of female  
514 ‘defensive’ traits in *Heliconius*. However, we found no evidence in our experiments that  
515 novel warning patterns mitigate female-specific costs.

516 In our simulations, we considered a large parameter space to investigate which  
517 variables may facilitate the spread of novel warning pattern alleles. Notably, in all 144000  
518 simulations without sexual conflict, the novel allele was rapidly lost from the population,  
519 regardless of other parameter values. In contrast, among the remaining 576000 simulations  
520 where sexual conflict was present, the novel allele was retained in ~0.5% of runs. Despite the  
521 striking diversity of warning patterns in *Heliconius*, the spread of a novel pattern allele is

522 presumably a rare event, so even this apparently modest increase could make a substantial  
523 difference across 12 million years (Kozak et al. 2015) of *Heliconius* diversification. Overall,  
524 our simulations suggest that drift acting in isolation may be unlikely to facilitate the evolution  
525 of novel warning patterns, and that sex-specific selection may be an important, previously  
526 unrecognized factor.

527         Alongside a potential role for sex-specific selection, our simulations also suggest that  
528 increased periods of relaxed predation and high probabilities of encounter between mated  
529 females and males are important factors determining the fate of novel pattern alleles. Periods  
530 of relaxed predation have frequently been invoked as a necessary prerequisite for the spread  
531 of novel warning patterns in aposematic butterflies (Mallet and Joron 1999; Mallet 2010).  
532 High population density (leading to increased probabilities of encounter) has also been shown  
533 theoretically to drive diversification through interlocus sexual conflict (Gavrilets 2000).  
534 Notably, in 2 of our simulations runs, the novel pattern persisted with a moderate encounter  
535 probability between females and males. This suggests that even at lower encounter rates,  
536 sexual conflict can contribute to the evolution of novel warnings patterns. Of course, the  
537 interpretation of our simulations must depend on how likely its parameters reflect reality. The  
538 benefits that we introduced to females with novel patterns in our simulations (during relaxed  
539 predation; see Table 1) are broadly comparable to the higher fecundity experienced by  
540 females in the absence of males in our empirical data. However, although a great deal is now  
541 known of *Heliconius* biology (Merrill et al. 2015; Jiggins 2017), making them an excellent  
542 subject for exploration with individual based simulations, it is important to note that we still  
543 know little about their predators, both in terms of population densities or learning functions  
544 (Jiggins 2017). Similarly, although *Heliconius* can exist in high densities, this – to our  
545 knowledge – has not been systematically assessed.

546 In evolutionary arms-races, adaptations in one party are contested by counter-  
547 adaptations in the other (Arnqvist and Rowe 2005). The genetic architecture underlying these  
548 adaptations can play a crucial role during these dynamics, as it may affect how fast one party  
549 can distance itself, or conversely catch up, to the other party. Our simulations show that male  
550 preferences rapidly track changes in female wing pattern cue, and that very simple genetic  
551 architectures speed up this process. This in turn decreases the speed at which the novel pattern  
552 allele spreads across the population, as female benefits from novel patterns are reduced when  
553 male interest in this pattern increases. However, this does not affect whether or not the novel  
554 pattern eventually reaches high frequencies in our simulations. The final fate of the novel  
555 pattern allele in our simulations seems to be largely determined in the first generations after  
556 its occurrence, during which the novel pattern is at very low frequency in the population, and  
557 hence, there is not yet strong selection for increased attraction to this pattern.

558 We modelled attraction of males to novel or ancestral patterns with two independent  
559 traits, controlled by different sets of loci (following Duenez-Guzman et al. 2009). Alleles that  
560 reduce attraction to the ancestral pattern did not markedly increase in frequency in any of our  
561 simulations. Males in our simulations suffered few costs from being attracted to females. This  
562 seems reasonable considering the sparsity of receptive *Heliconius* females in the wild,  
563 securing access to which possibly outweighs costs associated with attraction to the ‘wrong’  
564 female (Estrada and Jiggins 2008). In our simulations, this meant that a reversal in harassment  
565 probability of differently colored females never occurred (*i.e.* the novel pattern was never  
566 harassed more than the ancestral pattern). If both attraction traits were controlled by the same  
567 loci (*i.e.* an increase in attraction to the novel pattern requires a decrease in attraction to the  
568 ancestral pattern), this might lead to such reversals and possibly increase the probability for  
569 color polymorphisms to occur. This would be an interesting question for further empirical and  
570 theoretical work, but is beyond the scope of the current study.



571 Both our experiments support the hypothesis that male presence is costly for mated  
572 females, at least in the short term, as shown by a reduction of eggs laid. Although we cannot  
573 rule out the possibility that competition between females and males over food resources, as  
574 opposed to male harassment, accounts for this reduction in laying rate, we consider this  
575 unlikely. In our experiments, butterflies were provided with multiple flowers and feeders  
576 (similar to cages housing much larger number of butterflies, where average laying rates per  
577 butterfly are within the same range), and it seems unlikely that this was a limiting resource.  
578 Although it has not previously been shown experimentally, *Heliconius* researchers frequently  
579 keep mated females separate from males to increase egg yield (independent of overall  
580 butterfly density). We therefore consider it more likely that male harassment interrupting  
581 females during egg laying, or during foraging or scouting for host plants, explains our data. In  
582 support of this view, the reduction in the eggs laid was more pronounced in experiment 2, in  
583 which we also observed much higher levels of male-female interactions (despite having only  
584 a single male with each female).

585 *H. erato* and *H. timareta* females respectively laid on average 13% and 19% fewer  
586 eggs when males were present. Although this may seem a relatively small reduction, female  
587 *Heliconius* only lay a few eggs per day, and if consistent across a female's reproductive life  
588 (up to several months), this would represent a significant reduction in fitness. Notably, this is  
589 comparable to per locus estimates of selection acting on warning pattern due to predation  
590 (albeit at the lower end; e.g. per locus  $s = 0.13-0.40$  in *H. erato* and *H. melpomene*, Mallet et  
591 al. 1990). Once again, extrapolation of our results to natural populations must be treated with  
592 some caution. In particular, the activity and density of individuals in our insectary enclosures  
593 might not reflect the situation in the wild. For example, in wild *Heliconius* males often show  
594 much higher activity than captive individuals (potentially leading to high rates of encounter  
595 between females and males). Local abundance of *Heliconius* in the wild *can* be very high, and

596 interactions frequent, but there is considerable variation in density between sites (Merrill,  
597 pers. obs.). As such, it is unclear whether the densities in our insectary experiments reflect  
598 those in natural populations and this remains an important caveat. Nevertheless, given the  
599 large effective population sizes of many *Heliconius* species, even a relatively small fitness  
600 cost resulting from male harassment could have significant effects on the evolution of  
601 associated traits (like warning pattern).

602         Although our experiments reveal that male presence can reduce short-term female  
603 fecundity, they provide only limited evidence for a key prediction of our hypothesis: That  
604 novel warning patterns should mitigate costs resulting from male harassment. *H. erato*  
605 females with disrupted patterns did receive less attention from males; however, there was no  
606 effect of pattern treatment on short term female fecundity (*i.e.* number of eggs laid with males  
607 present). In our experiments with *H. timareta*, we found no evidence that novel patterns  
608 reduce male harassment (indeed, there is some evidence that males are more interested in  
609 females with the red band) or that warning pattern affects the number of eggs laid with males  
610 present.

611         Despite this, it is *perhaps* premature to rule out a role of sexual conflict as a factor  
612 contributing to the evolution of novel warning patterns. The increased interest of males  
613 directed towards females with intact patterns observed in our first experiment, mirrors  
614 previous experiments testing male attraction to population-specific warning patterns in *H.*  
615 *erato* (e.g. Muñoz et al. 2010; Finkbeiner et al. 2014; Merrill et al. 2014). Indeed, our warning  
616 pattern manipulation of *H. erato demophoon* creates a similar phenotype to *H. e. chesteronii*  
617 (see Fig. 1B), which Muñoz *et al.* (2010) have shown to be less attractive to neighboring, red-  
618 banded populations of *H. erato*. Why the differences in interactions we observed do not  
619 translate into differences in female short-term fecundity is not immediately clear. One  
620 possibility is that anti-aphrodisiac pheromones, transferred from males to females during

621 mating (Gilbert 1976), which have also been hypothesized to reduce male harassment  
622 (Estrada et al. 2011), may mitigate any detectable costs associated with initial male interest.  
623 Another possibility is that our experiments simply lack power, especially considering the  
624 large variation in the number of eggs laid.

625         The results of our experiments with *H. timareta* may simply reflect the lack of strong  
626 differences in visual attraction. In particular, mate choice experiments with *H. t. linaresi*  
627 males – which were run concurrently with experiments reported here – revealed that males  
628 show only very weak preferences for the conspecific yellow pattern over a yellow-red pattern,  
629 as used in the present study (Hausmann et al. 2021). Recently, it has also become apparent  
630 that the composite color patterns of *H. heurippa* may not be the target of differences in male  
631 attraction between different species of *Heliconius* (Mavárez et al. 2021). We initially chose to  
632 study these taxa because introgressing a red band into *H. t. linaresi* recreates a *H. heurippa*-  
633 like pattern (see Fig. 1B), and is thought to reflect the evolutionary history of this putative  
634 hybrid species. We consider the initial acquisition of color pattern alleles through  
635 hybridization and introgression an especially likely scenario affecting the kinds of dynamics  
636 we describe here. A stronger effect of warning pattern in mitigating fecundity loss due to male  
637 presence may be observed if repeating our experiments with other *Heliconius* species that are  
638 known to show stronger color based mate choice, such as *H. melpomene* (Merrill et al. 2019).  
639 Further experiments across a broader range of populations would be important to robustly test  
640 a role for sexual conflict in driving warning pattern divergence in *Heliconius*.

641         The rise of a new variant at an ecologically relevant locus due to interlocus sexual  
642 conflict is a challenging concept, especially if it is at the same time constrained by positive  
643 frequency dependent natural selection (e.g. Sherratt 2008; Briolat et al. 2019). As the new  
644 variant will be less advantageous for one sex than the other (in our example, males with novel  
645 warning pattern suffer higher costs than females), an additional component of (intra)locus

646 sexual conflict is introduced, where male and female adaptations at the same locus are in  
647 conflict (Schenkel et al. 2018). Such combined effects can hinder, but possibly also induce  
648 antagonistic coevolution between males and females (Pennell and Morrow 2013; Pennell et  
649 al. 2016). A role for sex-specific selection driving divergence in primarily ecological traits  
650 has been suggested previously (Bonduriansky 2011). In poison frogs, it seems that sexual  
651 selection due to female preferences for bright colors has contributed to inter-population  
652 differences in warning signals (Maan and Cummings 2009). Similarly, evidence suggests that  
653 male harassment drives phenotypic diversity in wing color in damselflies (Svensson et al.  
654 2005). Although there seems to be little evidence that these damselfly color morphs are  
655 ‘ecologically relevant’ (*i.e.* affecting an individual’s survival due to interaction with  
656 heterospecific individuals, or the abiotic environment), diversity appears to enhance  
657 population performance more generally by reducing overall fitness costs to females from  
658 sexual conflict (Takahashi et al. 2014). Among *Papilio* butterflies, which are often female-  
659 limited Batesian mimics, non-mimetic ‘male-like’ females might exist to avoid unwanted  
660 attention of males. In *P. dardanus*, males do indeed prefer to approach mimetic over non-  
661 mimetic (male-like) females (Cook et al. 1994). Our study contributes to this body of work by  
662 explicitly testing for fitness effects resulting from sexual conflict relating to an ecological  
663 trait.

664  
665 In conclusion, our theoretical results show that drift alone might be unlikely to drive  
666 diversification of warning patterns. However, once these patterns are additionally involved in  
667 mate choice, and the two sexes have different reproductive strategies (e.g. males are adapted  
668 to mate as often as they can, while females are not), sexual conflict can arise and contribute to  
669 warning pattern diversification. The speed at which novel patterns increase in frequency will  
670 then depend on the genetic architecture underlying adaptations in the sexes arising from this

671 arms-race. Our empirical results show that females can indeed suffer fitness costs from  
672 unwanted male attention, but this was not mitigated by females displaying unusual patterns.  
673 The failure of detecting such interaction could be caused by a number of factors, the most  
674 likely of which is that color based preferences of males in the taxa we used are not strong  
675 enough to see a clear effect. Future work may repeat these experiments with taxa that are  
676 known to show stronger color based mate choice.

677  
678 Data accessibility. Supplementary methods and results, data and analysis scripts (in form of  
679 an R Markdown document) are included as electronic supplementary material.

680

681 Acknowledgements:

682 We are grateful to the Ministerio del Ambiente and the Autoridad Nacional de Licencias  
683 Ambientales (ANLA, permit 530 awarded to the Universidad del Rosario) for permission to  
684 collect butterflies in Panama and Colombia, respectively. We are very grateful to the  
685 Abondano-Almeida family for being a great support to AEH and MF in Colombia; Juan  
686 Sebastián Sánchez, Óscar Penagos, Isabel Leon and Rachel Crisp for assistance in the  
687 insectaries. RMM is indebted to Chris Jiggins for valuable discussions. SN was funded by a  
688 British Ecological Society Small Research Grant awarded to RMM, and RMM was  
689 additionally supported by a Junior Research Fellowship from King's College Cambridge.  
690 AEH, MF and C-YK were funded by an Emmy Noether fellowship and research grant  
691 awarded to RMM by the Deutsche Forschungsgemeinschaft (DFG) (Grant Number: GZ: ME  
692 4845/1-1).

693

694 Author contributions: RMM, AEH and MF designed the research. AEH and RMM wrote the  
695 simulation model. AEH, MF, SN and RMM collected the behavioral data. AEH and MF

696 analyzed the behavioral data and C-YK analyzed the wing spectral data. RMM, ML, WOM,  
697 CP-D, and CS and contributed experimental animals and facilities in addition to academic  
698 supervision. AEH, MF and RMM wrote the paper with contributions from all authors.

699

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