

 Open access • Posted Content • DOI:10.1101/2020.07.15.205435

Wing-pattern-specific effects of experience on mating behavior in *Heliconius melpomene* butterflies — [Source link](#)

[Peyton A. Rather](#), [Abigail E. Herzog](#), [David A. Ernst](#), [Erica L. Westerman](#)

Institutions: [University of Arkansas](#)

Published on: 17 Jul 2020 - [bioRxiv](#) (Cold Spring Harbor Laboratory)

Topics: [Mating preferences](#), [Heliconius melpomene](#), [Courtship](#) and [Mate choice](#)

Related papers:

- [Consistent patterns of male mate preference in the laboratory and field](#)
- [Fruit fly courtship: The female perspective.](#)
- [Biased learning affects mate choice in a butterfly](#)
- [Female mate choice is a reproductive isolating barrier in *Heliconius* butterflies](#)
- [Age-based mate choice in the monandrous fruit fly *Drosophila subobscura*](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/wing-pattern-specific-effects-of-experience-on-mating-4ad6eelz4w>

1 **Wing-pattern-specific effects of experience on mating behavior in *Heliconius***
2 ***melpomene* butterflies**

3 Peyton A. Rather*, Abigail E. Herzog*, David A. Ernst, Erica L. Westerman

4 Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701

5 *equal contributors

6

7

8 **Abstract**

9 Many animals have the ability to learn, and some taxa have shown learned mate preference. This
10 learning may be important for speciation in some species. The butterfly *Heliconius melpomene* is
11 a model system for several areas of research, including hybridization, mate selection, and
12 speciation, partially due to its widespread diversity of wing patterns. It remains unclear whether
13 these butterflies can learn to prefer certain mates and if social experience shapes realized mating
14 preferences. Here we test whether previous experience with a female influences male mate
15 preference for two different *H. melpomene* subspecies, *H. m. malleti* and *H. m. rosina*. We
16 conducted no-choice behavioral assays to determine if latency to court and whether males
17 courted (vs no courtship) differed between naïve males and males with previous exposure to a
18 young, sexually mature, virgin female. To test whether assortative courtship preference is
19 learned in *H. melpomene*, males were either paired with a female who shared their phenotype or
20 one who did not. Naïve *H. m. malleti* males courted assortatively, while naïve *H.m. rosina* males
21 did not. Experienced *H. m. malleti* males reduced their courting relative to naïve males,
22 suggesting that social experience with a sexually mature female that does not result in copulation
23 may be perceived as a negative experience. In contrast, experienced *H. m. rosina* males exhibited

24 similar courting rates to naïve *H. m. rosina* males. Our results suggest that social experience can
25 influence male mating behavior in *H. melpomene* and that behavioral plasticity may differ across
26 populations in this species.

27

28 **Keywords**

29 behavioral plasticity, mate choice, Lepidoptera, assortative mating, social learning, male choice

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47 **Introduction**

48

49 Many of the behaviors and decisions that an animal makes are affected by its observations and
50 capacity to learn. Learning can be defined as a set of processes that allows an animal to acquire,
51 store, and use information gathered from the environment (Galef and Laland, 2005). Learning in
52 animals is often complex and is likely the result of the social dynamics and settings of a species
53 (Coussi-Korbel and Frigaszy, 1995). There is a substantial amount of evidence that animals have
54 the ability to socially learn (Dukas, 1998). Some of the many behaviors that might be the result
55 of social learning include food choices, predator avoidance, and mate preferences. For example,
56 many species of fish have been observed to learn how to find food, how to recognize predators,
57 and how to assess mate quality (Brown and Laland, 2003). This breadth of learning ability,
58 however, is not limited to vertebrates (Dukas, 2008; Verzijden et al., 2012).

59

60 It is now understood that learning affects many essential activities of invertebrates, including
61 predator avoidance and social interactions (Dukas, 2008, 2010). Particularly, many insects and
62 spiders have shown the ability to learn mate preference. Studies on the wolf spider *Schizocosa*
63 *uetzi* have shown that female social experience in their penultimate juvenile period can affect
64 their mate choices as adults (Hebets, 2003). Female *Teleogryllus oceanicus* crickets modify
65 their mate preferences after hearing attractive male songs (Bailey and Zuk, 2009), and female
66 *Bicyclus anynana* butterflies learn preferences for enhanced male ornaments (Westerman et al.,
67 2012). Male *B. anynana* also learn preferences for wing pattern elements in females (Westerman
68 et al., 2014). Furthermore, work with *Drosophila melanogaster* fruit flies have shown that
69 learning to be selective leads to a higher lifetime mating success than males who court

70 indiscriminately (Dukas et al., 2006). Therefore, when it comes to mate preference and sexual
71 behavior in insects it is often beneficial to learn.

72

73 Learning can potentially increase rates of assortative mating, which can lead to speciation
74 through processes such as when young animals imprint on parents (Dukas, 2013). One such
75 example of this is how cross-fostering experiments in two subspecies of zebra finch
76 demonstrated that assortative mating is due to imprinting. Birds in this study paired with mates
77 that resembled their foster parents instead of their own phenotype (Irwin and Price, 1999). It has
78 also been shown that mate preference can be learned in mature animals, such as male guppies
79 and Syrian hamsters. These animals have demonstrated learning to discriminate against
80 heterospecific mates after courtship interactions (Verzijden et al., 2012). This type of learning
81 would help maintain speciation. With these studies in mind, we might expect that *Heliconius*
82 butterflies, or other animals with high levels of speciation, might learn to court assortatively.

83

84 *Heliconius* butterflies have a long lifespan compared to other species of butterflies, which allows
85 them to potentially mate multiple times (Gilbert, 1972). Therefore, the ability to learn in
86 response to mating experiences could be advantageous. Studies have shown that male mate
87 preferences evolve early in the speciation process in *Heliconius* within both intraspecific hybrid
88 mating zones and conspecific polymorphic populations (Merrill et al., 2011a). These male mate
89 preferences are based on wing color pattern cues, which are under natural selection to correspond
90 to local mimetic environments (Gray and McKinnon, 2007; Kronforst et al., 2006). *Heliconius* is
91 well known for its diversity in color patterns, and divergence in these color morphs is associated
92 with speciation and adaptive radiation (*Heliconius*-Genome-Consortium* et al., 2012).

93
94 Here we take advantage of the social butterfly species *Heliconius melpomene*, whose widespread
95 diversity of color patterns makes it an ideal model for studies on speciation and mating patterns
96 (Jiggins et al., 2004). In this species, mimetic color patterns play a key role in species
97 recognition, and mate preferences based on these patterns evolve alongside changes in wing
98 pattern (Jiggins et al., 2004). Previous studies show that mimetic coloration in this species is
99 important in choosing mates, and that these butterflies show assortative mating when choosing
100 between their own and a different, closely related species (*Heliconius cydno*) (Jiggins et al.,
101 2001). Furthermore, males often do discriminate between conspecific females with different
102 wing patterns, and do not copy the mate preferences of conspecific males who have different
103 wing patterns (Jiggins et al., 2004). However, it remains unclear whether individual *H.*
104 *melpomene* males use past social experience with sexually receptive (or non-receptive) females
105 to inform current mating decisions. The ability to learn mate preferences for intraspecific
106 variation in wing pattern may be important for the initiation of assortative mating, reproductive
107 isolation, and the speciation process.

108
109 Here we test whether experience impacts future male mate preference and courting behavior in
110 two races of *H. melpomene* using three distinct *H. melpomene* color morph phenotypes (Figure
111 1). We had three alternative hypotheses: **1)** If males learn, then we predicted that experienced
112 males would be more likely to court and have a shorter latency to court relative to naïve males.
113 This type of learning is seen in *B. anynana*, where males exposed to dorsal hindwing spot
114 number variation learn preferences for this trait (Westerman et al., 2014). **2)** If however male
115 exposure to a female is somehow a negative experience, then we predicted that experienced

116 males would court less often than naïve males. This type of learning is seen in *Drosophila* males.
117 Studies have shown that naïve male *Drosophila* court virgin females persistently, while males
118 previously exposed to an unreceptive female will then court virgin females much less vigorously
119 (Siegel and Hall, 1979). **3** If males are not able to learn, then courting was predicted to occur at
120 random in both experienced and naïve males. An example of this is seen in the butterfly *Papilio*
121 *polytes*, where a series of behavioral assays studying male preference for mimetic and non-
122 mimetic females showed that there was no difference in initial and lifetime male preference,
123 regardless of number of failed courtship attempts (Westerman et al., 2018).

124

125 **Materials and Methods**

126

127 *Study species and husbandry*

128 *Heliconius melpomene* is a widespread neotropical butterfly found in Central and South America
129 (Brower, 1994; Sheppard et al., 1985). The species is well known for its high diversity in color
130 patterns, which play an important role in speciation (Jiggins et al., 2004). These color patterns,
131 though diverse, are largely sexually monomorphic, with females and males of each morph
132 having predominantly identical wing patterns. *H. melpomene* is often used as a model organism
133 to study Müllerian mimicry and is co-mimetic with the species *Heliconius erato* (Jiggins et al.,
134 2004). The many color patterns of *H. melpomene* have arisen through convergent evolution and
135 selection for mimicry. The diversity of these patterns have allowed for the species to be used as
136 models for studies on speciation and mating patterns (Jiggins, 2017). Here we take advantage of
137 this species with different morphs that sometimes cohabitate in nature.

138

139 *H. melpomene* butterflies were either reared in a greenhouse at the University of Arkansas, or
140 were obtained as pupae from Costa Rica Entomological Supply (Alajuela Apo. 2132-4050 Costa
141 Rica.). Butterflies reared from the greenhouse colony came from a continuously breeding colony
142 kept in two, phenotypic-specific, walk-in cages (143.51 x 110.25 x 219.71 cm). Caterpillars from
143 the colony were given *Passiflora* plants *ad libitum*, and prior to pupation, plants containing
144 caterpillars were removed from the breeding cages and moved to a separate 60.96 x 60.96 x
145 142.24 cm cage until butterfly emergence from pupa. Pupae obtained from Costa Rica
146 Entomological Supply were reared on native *Passiflora* host plants in natural lighting as
147 caterpillars prior to pupation and shipping. When at the University of Arkansas, they were hung
148 in a 34.29 x 34.29 x 60.96 cm cage until emergence, and then maintained as adults in the
149 Department of Biological Sciences Greenhouse at the University of Arkansas.
150
151 Butterflies were maintained at approximately 27°C, an average relative humidity of 71.5-85%,
152 and a 13:11 light:dark cycle. The greenhouse was lit by Sun Blaze T5 high output 120-volt
153 fluorescent light fixtures (containing UV wavelengths), in addition to natural sunlight, and the
154 presence of UV light in the greenhouse was confirmed using an Ocean Optics Jaz spectrometer.
155 After emergence, each butterfly was sexed, marked with a unique number on their hind wing
156 (which does not harm the butterflies, see (Gall, 1984) for details), and then moved to 60.96 x
157 60.96 x 142.24 cm cages with food where they were kept until use in a behavioral watch. Males
158 were placed into sex- and phenotype-specific cages, so they were isolated from both females and
159 other wing patterns prior to behavioral assays. Females were placed into sex- but not phenotype-
160 specific cages, so they were familiar with the wing patterns of males they were paired with in
161 later behavioral assays. Each cage contained no more than 15 butterflies at any time and was

162 visually isolated so that butterflies could not see individuals of the opposite sex (or phenotype in
163 the case of males). Butterflies were fed BIRDS choice butterfly nectar (Birds Choice, Chilton,
164 WI, USA), which is composed of glucose, fructose, calcium salt, halide salt, and amino acids. In
165 addition, cages also contained *Passiflora* and *Lantana* plants for supplemental nectar and pollen.
166 All behavioral watches were conducted in 60.96 x 60.96 x 142.24 cm BioQuip (Rancho
167 Dominguez, CA, USA) observation cages, visually isolated from all other cages, between August
168 2017 and November 2019.

169

170 ***Observational Experiment Time of Day Selection***

171 To determine the time of day when the butterflies were the most active, we observed butterflies
172 in colony cages for three consecutive days, between 6:00 am and 8:00 pm. Point counts were
173 conducted every thirty minutes, where behaviors (*flight, walk, flutter, abdomen lift, bask* [defined
174 by resting with wings held in open position], *rest* [defined by resting with wings held in closed
175 position], *antennae wiggle, court, and copulate*) were recorded for each cage, followed by two
176 ten-minute focal watches of one male and one female butterfly selected at random. Based on
177 observations, we determined that butterflies were most active between the hours of 10:00 am to
178 2:00 pm.

179

180 ***Behavioral Watches***

181 All behavioral watches took place between 10:00 am and 2:00 pm, the time of peak *H.*
182 *melpomene* activity in our greenhouse. Each watch consisted of a male aged ten or twelve days
183 old, and a female between three and five days old. Watches were set up based on four separate
184 treatments, N=15 per treatment per male phenotype. In this study, two male phenotypes were

185 used (*H. m. malleti* and *H. m. rosina*) and three female phenotypes were used (*H. m. malleti*, *H.*
186 *m. rosina*, and *H. m. plessini*) (Figure 1). To test whether males courted females with matching
187 wing patterns faster than they courted conspecific females with dissimilar wing patterns, we
188 tested latency to courtship and presence of courtship of naïve, 12-day-old *H.m. malleti* and *H.m.*
189 *rosina* males matched with either females of their own phenotype or females of different
190 phenotypes. To test whether previous exposure influences male latency to court, a 10-day-old
191 (naïve) male was exposed to a female (with either a similar or dissimilar phenotype) until he
192 either courted the female or 90 minutes passed without courtship. Afterward, the female was
193 removed, and the male was returned to the all-male, phenotype-specific cage. On day 12, these
194 males (i.e., experienced males) were exposed to a second female of the phenotype to which they
195 had been previously exposed. The behavior of these males was then compared to that of naïve,
196 12-day-old males exposed to similar or dissimilar wing patterned females.

197
198 On the morning of a watch, a male was placed into an observation cage approximately two to
199 three hours before the watch to acclimate to the new setting, and a female was added right before
200 the start of a watch. Once a trial began, butterflies were observed for 90 minutes or until
201 courtship took place. In the case that courtship did occur, time to court was recorded, and
202 butterflies were not allowed to copulate. Behaviors were recorded to determine if any had an
203 effect on mate preference. The number of incidents of each type of behavior (*flight*, *walk*, *flutter*,
204 *abdomen lift*, *bask* (wings open), *rest* (wings closed), *antenna wiggle*, *sitting near*, and *court*)
205 were recorded. Spectator Go BIOBSERVE (Fort Lee, NJ, U.S.A.) running on an Apple iPad was
206 used to record time to court and all behaviors of the male and female during the testing period.

207

208 *Statistical Analyses*

209 All statistical analyses were performed in JMP v. 14 (SAS Institute, Cary, NC, U.S.A.). We
210 assessed whether latency to court was influenced by male experience or female wing pattern
211 (similar or different from the male's) using a GLM with male experience and female wing
212 pattern as factors, as well as an interaction term. To assess if there was an effect of experience or
213 female wing pattern on likelihood to court we ran a nominal logistic regression model using male
214 experience and female wing pattern as factors, as well as an interaction term. Since we used two
215 different morphs for our "different female" treatments (*H.m. plesseni* and *H.m. rosina* for *H. m.*
216 *malleti* males; and *H. m. plesseni* and *H. m. malleti* for *H. m. rosina* males), we also tested
217 whether there was an effect of female phenotype on male likelihood to court in our four different
218 phenotype treatments using nominal logistic regression models. To test whether female behavior
219 during a male's first experience with a female had an effect on the observed courtship behavior
220 in later interactions with females, we analyzed all behavioral data collected on day 10 watches
221 (N=51 watches with behavioral data) and examined whether any of these behaviors were
222 predictive of male courting on day 12. To do this we ran a principal components analysis on all
223 the female behaviors and then ran logistic regression models on the first three principal
224 components.

225

226 *Ethical Note*

227 All *H. melpomene* butterflies were kept under laboratory conditions as defined by U.S.
228 Department of Agriculture, Animal and Plant Health Inspection Service permit P526P-17-00343.
229 Before being used in behavioral watches all butterflies were maintained in cages in a climate-
230 controlled setting in conditions similar to those of their native habitat, and cages were inspected

231 daily for ample food and appropriate conditions. All males and females used in a behavioral
232 watch were either euthanized by freezing for use in future analyses or added to colony breeding
233 cages where they were maintained in cages with ample food until natural death.

234

235 **Results**

236

237 *Morph-specific effect of experience on likelihood to court*

238 Experienced *H. m. malleti* males were less likely to court females than naïve *H. m. malleti* males,
239 and were more likely to court *H. m. malleti* females than either *H. m. rosina* or *H. m. plessini*
240 females (nominal logistic regression model, whole model $\chi^2=14.935$, $p=0.0019$, $AICc=75.900$,
241 $N=60$; male experience $\chi^2=8.85$, $p=0.009$; female phenotype (same or different) $\chi^2=6.85$,
242 $p=0.009$; interaction $\chi^2=0.07$, $p=0.794$) (Figure 2A). However neither experience nor female
243 wing pattern influenced *H. m. rosina* male's likelihood to court (nominal logistic regression
244 model, whole model $\chi^2=0.472$, $p=0.925$, $AICc=92.852$; $N=63$, male experience $\chi^2=0.313$,
245 $p=0.576$; female phenotype (same or different) $\chi^2=0.126$, $p=0.722$; interaction $\chi^2=0.029$,
246 $p=0.864$) (Figure 2B). The effect of prior exposure to a female on likelihood of *H.m. malleti* to
247 court two days later was independent of whether the male courted during the initial exposure
248 period (nominal logistic regression model, whole model $\chi^2=3.099$, $p=0.377$, $N=30$; courted
249 initially $\chi^2=0.182$, $p=0.670$; female phenotype $\chi^2=2.325$, $p=0.127$; interaction $\chi^2=0.181$,
250 $p=0.671$). Male courtship response to females with different wing patterns was independent of
251 the female wing pattern the male saw (*H. m. rosina* or *H. m. plesseni* for *H. m. malleti* males:
252 nominal logistic regression model, whole model $\chi^2=3.513$, $p=0.319$, $N=30$; female phenotype
253 $\chi^2=0.683$, $p=0.409$; male experience $\chi^2=2.305$, $p=0.129$; interaction $\chi^2=0.391$, $p=0.531$; *H. m.*

254 *malleti* or *H. m. plesseni* for *H. m. rosina* males: nominal logistic regression model, whole model
255 $\chi^2=0.188$, $p=0.979$, $N=32$; female phenotype $\chi^2=0.002$, $p=0.966$; male experience $\chi^2=0.387$,
256 $p=0.844$; interaction $\chi^2=0.112$, $p=0.738$).

257

258 ***No effect of experience or female wing pattern on latency to court***

259 For those males that did court during the 90 minute observation period, there was no effect of
260 experience or female wing pattern on male latency to court, for either *H.m. malleti* or *H.m.*
261 *rosina* (GLM, *H.m.malleti*, whole model F ratio=0.391, $p=0.761$, $N=23$; male experience F
262 ratio=0.375, $p=0.548$; female phenotype F ratio=0.599, $p=0.449$; interaction F ratio=0.038,
263 $p=0.848$; *H.m. rosina*, whole model F ratio=1.663, $p=0.205$, $N=25$; male experience F
264 ratio=0.200, $p=0.659$; female phenotype F ratio=3.22, $p=0.087$; interaction F ratio=1.452,
265 $p=0.242$).

266

267 ***Female Behavior Had No Effect on Future Likelihood to Court***

268 We found no effect of female behavior during first exposure on male courtship rates during
269 second exposure (Table 1 and 2).

270

271 **Discussion**

272

273 Our results show that male *H. melpomene* butterflies change their mating behavior in response to
274 a social experience. This change in behavior is lineage specific, with *H. m. malleti* males, but not
275 *H. m. rosina* males, exhibiting a reduction in likelihood to court after a social experience where
276 they interact, but do not get to copulate, with a conspecific female. This effect was independent

277 of the female's wing pattern, though it did co-occur with a lineage-specific preference for
278 assortative mating. *H. m. malleti* males courted *H. m. malleti* females more often than *H. m.*
279 *rosina* and *H. m. plesseni* females in no-choice assays, while *H. m. rosina* males courted all
280 female *H. melpomene* wing patterns equally often. Male likelihood to court was not significantly
281 influenced by female behavior, and when males did court, experienced males did not court faster
282 than naïve males.

283
284 *Heliconius* butterflies have many of the characteristics often found in species where past
285 experience informs future social behavior; thus, our finding that *H. melpomene* males modify
286 their mating behavior in response to experience, while novel, may not be unexpected. *H.*
287 *melpomene* butterflies are relatively long-lived (up to 6 months in nature) (Gilbert, 1972), highly
288 social (they roost in groups at night) (Mallet and Gilbert, 1995), and learn food sources and color
289 cues (Toure et al., 2020). They have large brains (Montgomery et al., 2016) and are both
290 physically larger, and longer lived than the butterfly *Bicyclus anynana*, which also uses past
291 experience to inform current mating behavior (Dion et al., 2020; Westerman et al., 2012;
292 Westerman et al., 2014). However, the negative effect of the pre-mating social exposure, and the
293 wing-pattern-specific response to this pre-mating social exposure, were unexpected.

294
295 We initially hypothesized that early exposure to a female would prime the males to court faster
296 and more often upon second exposure to females. This was based on previous findings in *B.*
297 *anynana*, where naïve males do not exhibit a mate preference, but males with previous social
298 experience do (Westerman et al., 2014). However, we found instead that early exposure to a
299 female was a negative experience for *H. m. malleti* males, as they tended not to court on the

300 repeat trial when exposed to a female of the same wing pattern and age as they had previously
301 seen. One possible cause for this negative response could be that males who do court on day 10
302 are pulled from the watch upon courtship and are not allowed to copulate. This type of negative
303 learning occurs in *Drosophila melanogaster* males, where previously unsuccessful males are
304 reluctant to court females who smell similarly to females who previously rejected them (Griffith
305 and Ejima, 2009; Siegel and Hall, 1979). *Heliconius* butterflies do have species-specific
306 olfactory signals which are used in mating decisions (González-Rojas et al., 2020; Mérot et al.,
307 2015), and olfactory signals have been shown to influence visual learning in *B. anynana*
308 butterflies (Westerman and Monteiro, 2013). It would be interesting to see if olfactory cues play
309 a similar role in *Heliconius*.

310

311 Although males did not learn to prefer certain phenotypes, avoidance learning from a negative
312 experience could be beneficial to these males. *D. melanogaster* males have demonstrated
313 learning to reduce courting females of the species *Drosophila simulans*, as these females
314 typically reject mating attempts by male *D. melanogaster* (Dukas, 2004). Heterospecific
315 courting can be costly for males because they could be wasting time and energy courting females
316 that are likely to reject them (Dukas, 2009). This suggests that learning to avoid unreceptive
317 females, or learning mate preference in general, may be beneficial relative to indiscriminately
318 courting females. Negative learning in *H. m. malleti* may therefore lead to higher lifetime mating
319 success, particularly if females who reject them once are likely to reject them again, and re-
320 encounter rates are high due to nighttime social roosting.

321

322 It is worth noting that a characteristic of the social experience we used, the removal of a male as
323 soon as he initiated courtship, resembles a component of the experimental design historically
324 used in *Heliconius* butterfly mate choice trials. In many studies, males are allowed to approach a
325 female and initiate courtship, and then they are physically removed from the female before given
326 an opportunity to copulate (Chamberlain et al., 2009; Kronforst et al., 2006; Merrill et al., 2019;
327 Merrill et al., 2011b). These males are then tested repeatedly, and past experience is often not
328 accounted for when male preference is assessed, assuming that past experience does not inform
329 present courting decisions. This assumption is partially based on a previous study showing that
330 exposure to conspecific females with different wing patterns does not induce a preference for
331 those wing patterns (Jiggins et al., 2004). However, this earlier study did not test for a negative
332 effect of exposure on male preference or courtship behavior. Our results support the previous
333 finding that prior exposure does not induce a positive preference, but suggest it instead may be a
334 negative experience, at least for *H. m. malleti*. This wing-pattern specific response to experience
335 should be seen as a cautionary tale for future comparative research with *Heliconius* butterflies, as
336 repeated trials may be experienced differently by different lineages, which could confound
337 interpretation of results. It also highlights the importance of checking for both positive and
338 negative valence when testing the presence of learning.

339

340 The presence of both a lineage-specific response to prior experience and a lineage-specific
341 presence of assortative courtship suggest that *H. m. malleti* and *H. m. rosina* may experience
342 different mating-related selective pressures. Maintaining the capacity to learn can be
343 energetically costly, and is often associated with fitness trade-offs, such as reduced fecundity
344 (Kotrschal et al., 2013; Snell-Rood et al., 2011), reduced lifespan (Burger et al., 2008; Kotrschal

345 et al., 2019), or extended development time (Kolss and Kawecki, 2008). Though conspecifics, *H.*
346 *m. malleti* and *H. m. rosina* rarely co-occur in nature (Brower, 1996). The stronger innate
347 preference and response to prior social experience exhibited by *H. m. malleti* suggest that *H. m.*
348 *malleti* butterflies may, on average, co-occur with a more diverse *Heliconius* butterfly
349 community than *H. m. rosina* butterflies, as is suggested by previously published range maps
350 (Brower, 1996; Sheppard et al., 1985). This could lead to the maintenance of male assortative
351 courtship and response to prior social experience in *H. m. malleti*, as limiting courtship efforts to
352 those most likely to be successful would be energetically adaptive in an environment with many
353 unreceptive females (Dukas et al., 2006). Assortative courtship and response to prior social
354 experience may not be as strong in *H. m. rosina* as a result of either differences in generational
355 exposure to polymorphic conspecifics, or differences in female receptivity. If most *H.*
356 *melpomene* females are receptive to *H. m. rosina* males, independent of female wing pattern,
357 there would be little pressure for males to maintain an assortative preference among conspecific
358 wing patterns, or to maintain the ability to use past social experience to inform current courting
359 behavior.

360

361 *H. m. malleti*'s response to prior experience could be associated with its assortative preference or
362 a high social learning capacity. If *H. m. malleti* males have a stronger innate assortative
363 preference than *H. m. rosina* males, it is possible that the initial exposure to a female creates a
364 stronger negative memory for *H. m. malleti* males than for *H. m. rosina* males. Aversive signals
365 are easier than appetitive signals for *D. melanogaster* to learn, and this is hypothesized to be due
366 to the type of response to the initial cue (Schwaerzel et al., 2003). Alternatively, *H. m. malleti*
367 might have a higher learning capacity (social or otherwise) than *H. m. rosina*. *Heliconius cydno*

368 and *Heliconius melpomene* have different sized brain neuropils associated with sensory
369 processing (Montgomery et al., 2020); it would be interesting to see if similar neuropil variation,
370 and associated variation in learning, occurs in *H. m. malleti* and *H. m. rosina*. Future research
371 should examine the neural responses of *H. m. malleti* and *H. m. rosina* males to an early social
372 experience, as well as their relative flower color and nectaring location learning abilities and
373 neural anatomy, to determine the mechanisms underlying their observed difference in response
374 to prior social experience.

375

376 **Conclusion**

377

378 Here we show that male *H. melpomene* butterflies use past social experience to inform current
379 mating behavior. This response is lineage (wing pattern) specific, and coincides with lineage-
380 specific differences in male assortative preference. Our findings strongly suggest that there are
381 lineage-specific selective forces acting on cognitive function in *Heliconius* butterflies. Future
382 research should explore the effect of cognition on speciation in this speciose group.

383

384 **Acknowledgements**

385 We would like to thank Alexis Okoro for assistance with data processing, and Sushant Potdar,
386 Grace Hirzel, Matt Murphy, Dylan Meyer, and Tim Sullivan for assistance with butterfly
387 husbandry. This research was funded by the University of Arkansas.

388

389

390

391 **Data Accessibility Statement**

392 Analyses reported in this article can be reproduced using the data provided by Rather et al.,
393 (XXX).

394

395

396 **References**

397

398 Bailey NW, Zuk M, 2009. Field crickets change mating preferences using remembered social
399 information. *Biological Letters* 5:449-451. doi: 10.1098/rsbl.2009.0112.

400 Brower AVZ, 1994. Rapid morphological radiation and convergence among races of the
401 butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution.
402 *Proceedings of the National Academy of Sciences of the United States of America*
403 91:6491-6495.

404 Brower AVZ, 1996. Parallel race formation and the evolution of mimicry in *Heliconius*
405 butterflies: A phylogenetic hypothesis from mitochondrial DNA sequences *Evolution*
406 50:195-221.

407 Brown C, Laland KN, 2003. Social learning in fishes: a review *Fish and Fisheries* 4:280-288.
408 doi: 10.1046/j.1467-2979.2003.00122.x.

409 Burger JMS, Munjong K, Pont J, Kawecki TJ, 2008. Learning ability and longevity: A
410 symmetrical evolutionary trade-off in *Drosophila*. *Evolution* 62:1294-1304. doi:
411 10.1111/j.1558-5646.2008.00376.x.

412 Chamberlain NL, Hill RI, Kapan DD, Gilbert LE, Kronforst MR, 2009. Polymorphic butterfly
413 reveals the missing link in ecological speciation. *Science* 326:847-850.

414 Coussi-Korbel S, Fragaszy DM, 1995. On the relation between social dynamics and social
415 learning. *Animal Behaviour* 50:1441-1453.

416 Dion E, Pui LX, Weber K, Monteiro A, 2020. Early-exposure to new sex pheromone blends
417 alters mate preference in female butterflies and in their offspring. *Nature*
418 *Communications* 11. doi: 10.1038/s41467-019-13801-2.

419 Dukas R, 1998. *Cognitive Ecology: The evolutionary ecology of information processing and*
420 *decision making*. The University of Chicago Press.

421 Dukas R, 2004. Male fruit flies learn to avoid interspecific courtship. *Behavioral Ecology*
422 15:695-698. doi: 10.1093/beheco/arh068.

423 Dukas R, 2008. Evolutionary Biology of Insect Learning. *Annual Review of Entomology*
424 53:145-160. doi: 10.1146/annurev.ento.53.103106.093343.

425 Dukas R, 2009. Dynamics of learning in the context of courtship in *Drosophila persimilis* and *D.*
426 *pseudoobscura*. *Animal Behaviour* 77:253-259.

427 Dukas R, 2010. Social learning in insects. *Encyclopedia of Animal Behavior* 17:176-179.

428 Dukas R, 2013. Effects of learning on evolution: robustness, innovation and speciation. *Animal*
429 *Behaviour* 85:1023-1030. doi: 10.1016/j.anbehav.2012.030.

430 Dukas R, Clark CW, Abbott K, 2006. Courtship strategies of male insects: when is learning
431 advantageous? *Animal Behaviour* 72:1395-1404.

- 432 Galef BG, Laland KN, 2005. Social learning in animals: Empirical studies and theoretical
433 models. *BioScience* 55:489-499.
- 434 Gall LF, 1984. The effects of capturing and marking on subsequent activity in *Boloria*
435 *acrocynema* (Lepidoptera: Nymphalidae), with a comparison of different numerical
436 models that estimate population size. *Biological Conservation* 28:139-154.
- 437 Gilbert LE, 1972. Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings*
438 *of the National Academy of Sciences of the United States of America* 69:1403-1407.
- 439 González-Rojas MF, Darragh K, Robles J, Linares M, Schulz S, McMillan WO, Jiggins C,
440 Pardo-Diaz C, Salazar C, 2020. Chemical signals act as the main reproductive barrier
441 between sister and mimetic *Heliconius* butterflies. *Proceedings of the Royal Society of*
442 *London B* 287:20200587. doi: 10.1098/rspb.2020.0587.
- 443 Gray SM, McKinnon JS, 2007. Linking color polymorphism maintenance and speciation. *Trends*
444 *in Ecology & Evolution* 22:71-79. doi: 10.1016/j.tree.10.005.
- 445 Griffith LC, Ejima A, 2009. Courtship learning in *Drosophila melanogaster*: Diverse plasticity
446 of a reproductive behavior. *Learning & Memory*.
- 447 Hebets EA, 2003. Subadult experience influences adult mate choice in an arthropod: Exposed
448 female wolf spiders prefer males of a familiar phenotype. *Proceedings of the National*
449 *Academy of Sciences of the United States of America* 100:13390-13395.
- 450 *Heliconius*-Genome-Consortium*, *Dasmahaptra KK, Walters JR, Briscoe AD, Davey JW,
451 Whibley A, Nadeau NJ, Zimin AV, Hughes DST, Ferguson LC, Martin SH, Salazar C,
452 Lewis JJ, Aldler S, Ahn S, Baker DA, Baxter SW, Chamberlain NL, Chuahan R,
453 Counterman BA, Dalmay T, Gilbert LE, Gordon K, Heckel DG, Hines HM, Hoff KJ,
454 Holland PWH, Jacquin-Joly E, Jiggins FM, Jones RT, Kapan DD, Kersey P, Lamas G,
455 Lawson D, Mapleson D, Maroja LS, Martin A, Moxon S, Plamer WJ, Papa R,
456 Papanicolaou A, Pauchet Y, Ray DA, Rosser N, Salzberg SL, Supple MA, Surridge A,
457 Tenger-Trolander A, Vogel H, Wilkinson PA, Wilson D, Yorke JA, Yuan F, Balmuth
458 AL, Eland C, Gharbi K, Thomson M, Gibbs RA, Han Y, Jayaseelan JC, Kovar C,
459 Mathew T, Muzny DM, Onger F, Pu L, Qu J, Thornton RL, Worley KC, Wu Y, Linares
460 M, Blaxter ML, French-Constant RH, Joron M, Kronforst MR, Mullen SP, Reed RD,
461 Scherer SE, Richards S, Mallet J, McMillan WO, Jiggins CD, 2012. Butterfly genome
462 reveals promiscuous exchange of mimicry adaptations among species. *Nature* 487:94-98.
463 doi: 10.1038/nature11041.
- 464 Irwin DE, Price T, 1999. Sexual imprinting, learning and speciation. *Heredity* 82:347-354.
- 465 Jiggins C, 2017. The ecology and evolution of *Heliconius* butterflies. Oxford: Oxford University
466 Press
- 467 Jiggins CD, Estrada C, Rodrigues A, 2004. Mimicry and the evolution of premating isolation in
468 *Heliconius melpomene* Linnaeus. *Journal of Evolutionary Biology* 17:680-691. doi:
469 10.1111/j.1420-9101.2004.00675.x.
- 470 Jiggins CD, Naisbit RE, Coe RL, Mallet J, 2001. Reproductive isolation caused by colour pattern
471 mimicry *Nature* 411:302-305.
- 472 Kolss M, Kawecki TJ, 2008. Reduced learning ability as a consequence of evolutionary
473 adaptation to nutritional stress in *Drosophila melanogaster*. *Ecological Entomology*
474 33:583-588.
- 475 Kotschal A, Corral-Lopez A, Kolm N, 2019. Large brains, short life: selection on brain size
476 impacts intrinsic lifespan. *Biology Letters* 15:20190137. doi: 10.1098/rsbl.20190137.

- 477 Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajicksek S, Brännström I, Immler S,
478 Maklakov AA, Kolm N, 2013. Artificial selection on relative brain size in the guppy
479 reveals costs and benefits of evolving a larger brain *Current Biology* 23:168-171. doi:
480 10.1016/j.cub.2012.058.
- 481 Kronforst MR, Young LG, Kapan DD, McNeely C, O'Neill RJ, Gilbert LE, 2006. Linkage of
482 butterfly mate preference and wing color preference cue at the genomic location of
483 wingless. *Proceedings of the National Academy of Sciences of the United States of*
484 *America* 103:6575-6580.
- 485 Mallet J, Gilbert LE, 1995. Why are there so many mimicry rings? Correlations between habitat,
486 behaviour and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean*
487 *Society* 55:159-180.
- 488 Mérot C, Frérot B, Leppik E, Joron M, 2015. Beyond magic traits: Multimodal mating cues in
489 *Heliconius* butterflies. *Evolution* 69:2891-2904. doi: 10.1111/evo.12789.
- 490 Merrill RM, Gompert Z, Dembeck LM, Kronforst MR, McMillan WO, Jiggins CD, 2011a. Mate
491 preference across the speciation continuum in a clade of mimetic butterflies. *Evolution*
492 65:1489-1500. doi: 10.1111/j.1558-5646.2010.01216.x.
- 493 Merrill RM, Rastas P, Martin SH, Melo MC, Barker S, Davey JW, McMillan WO, Jiggins CD,
494 2019. Genetic dissection of assortative mating behavior. *PLoS Biology* 17:e2005902. doi:
495 10.1371/journal.pbio.2005902.
- 496 Merrill RM, Van Schooten B, Scott JA, Jiggins CD, 2011b. Pervasive genetic associations
497 between traits causing reproductive isolation in *Heliconius* butterflies. *Proceedings of the*
498 *Royal Society of London B* 278:511-518. doi: 10.1098/rspb.2010.1493.
- 499 Montgomery SH, Merrill RM, Ott SR, 2016. Brain composition in *Heliconius* butterflies,
500 posteclosion growth and experience-dependent neuropil plasticity. *The Journal of*
501 *Comparative Neurology* 524:1747-1769. doi: 10.1002/cne.23993.
- 502 Montgomery SH, Rossi M, McMillan WO, Merrill RM, 2020. Neural divergence and hybrid
503 disruption between ecologically isolated *Heliconius* butterflies. *bioRxiv*. doi:
504 10.1101/2020.07.01.182337.
- 505 Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M, 2003.
506 Dopamine and octopamine differentiate between aversive and appetitive olfactory
507 memories in *Drosophila*. *Journal of Neuroscience* 23:10495-10502.
- 508 Sheppard PM, Turner JRG, Brown KS, Benson WW, Singer MC, 1985. Genetics and the
509 evolution of Mullerian mimicry in *Heliconius* butterflies. *Philosophical Transactions of*
510 *the Royal Society B* 308:433-610.
- 511 Siegel RW, Hall JC, 1979. Conditioned responses in courtship behavior of normal and mutant
512 *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of*
513 *America* 76:565-578.
- 514 Snell-Rood EC, Davidowitz G, Papaj DR, 2011. Reproductive tradeoffs of learning in a
515 butterfly. *Behavioral Ecology* 22:291-302. doi: 10.1093/beheco/arq169.
- 516 Toure MW, Young FJ, McMillan WO, Montgomery SH, 2020. *Heliconiini* butterflies can learn
517 time-dependent reward associations. *bioRxiv*. doi: 10.1101/2020.06.06.135459.
- 518 Verzijden MN, ten Cate C, Servedio MR, Kozak GM, Boughman JW, Svensson EI, 2012. The
519 impact of learning on sexual selection. *Trends in Ecology and Evolution* 27:511-519. doi:
520 10.1016/j.tree.2012.05.007.

- 521 Westerman E, Hodgins-Davis A, Dinwiddie A, Monteiro A, 2012. Biased learning affects mate
522 choice in a butterfly. *Proceedings of the National Academy of Sciences of the United*
523 *States of America* 109:10948-10953. doi: 10.1073/pnas.1118378109.
- 524 Westerman E, Monteiro A, 2013. Odour influences whether females learn to prefer or to avoid
525 wing patterns of male butterflies. *Animal Behaviour* 86:1139-1145. doi:
526 10.1016/j.anbehav.2013.09.002.
- 527 Westerman EL, Chirathivat N, Schyling E, Monteiro A, 2014. Mate preference for a
528 phenotypically plastic trait is learned, and may facilitate preference-phenotype matching.
529 *Evolution* 68:1661-1670.
- 530 Westerman EL, Letchinger R, Tenger-Trolander A, Massardo D, Palmer D, Kronforst MR, 2018.
531 Does male preference play a role in maintaining female limited polymorphism in a
532 Batesian mimetic butterfly? *Behavioural Processes* 150:47-58. doi:
533 10.1016/j.beproc.2018.02.014.

534
535
536
537
538
539
540

541 **Figure Legends**

542

543 **Figure 1.** *Heliconius melpomene* phenotypes. A) *H. m. malletti*, B) *H. m. rosina*, and C) *H. m.*
544 *plesseni*.

545

546 **Figure 2.** Lineage-specific effect of experience on male courtship. A) *H. m. malletti* reduce
547 courtship after exposure to, but not copulation with, a female (N=60, male experience $\chi^2=8.85$,
548 $p=0.009$; female phenotype (same or different) $\chi^2=6.85$, $p=0.009$; interaction $\chi^2=0.07$, $p=0.794$).
549 B) *H. m. rosina* do not (N=63, male experience $\chi^2=0.313$, $p=0.576$; female phenotype (same or
550 different) $\chi^2=0.126$, $p=0.722$; interaction $\chi^2=0.029$, $p=0.864$).

551

552

553

554 **Tables and Table Legends**

555

556 **Table 1.** Loadings for principle components from PCA for female behavior during the training

557 period for day 10 males.

558

559

560

	PC 1	PC 2	PC 3
Flutter Count (Ct)	0.513	0.091	0.046
Fly Ct	0.432	-0.047	-0.134
Walk Ct	0.441	-0.171	-0.010
Lift Abdomen Ct	0.061	0.216	0.817
Bask Ct	0.292	0.023	0.023
Court Ct	-0.032	0.548	-0.516
Ant Wiggle Ct	0.235	-0.416	-0.160
Resting Ct	0.19372	0.035	0.096
Sitting Near Ct	0.414	0.662	0.013
% Variance Explained	34.712	14.446	11.563
% Total Variance Explained	34.712	49.158	60.721

561

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582

583

584 **Table 2.** Female behavior during early exposure did not influence male likelihood of courting in
585 later female encounters. Test statistics and p-values from logistic regression models using
586 composite behavioral variables PC1, PC2, PC3. N=51.

	PC 1	PC 2	PC 3
χ^2	0.046	0.013	1.460
P-value	0.830	0.907	0.226

587

588

589

590

591

592

593

594

595

596

597

598

599

600

601

602

603

604

605

606

607

608

609

610

611 **Figures**

612

613 **Figure 1.**

614



615

616

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631

632

633

634

635

636

637

638

639

640

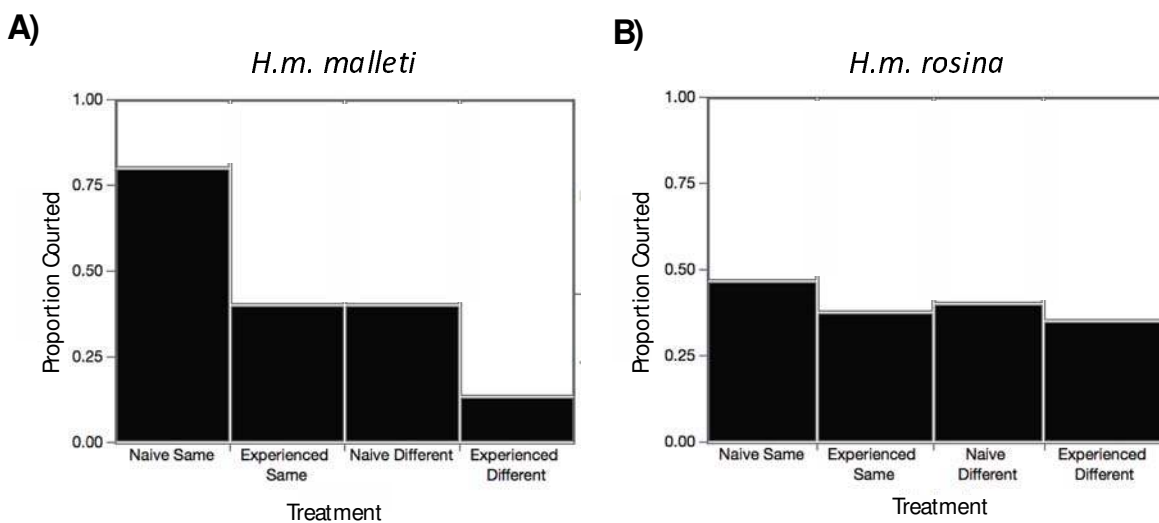
641

642

643

644
645
646
647

Figure 2.



648
649