Light environment influences mating behaviours during the early stages of divergence in tropical butterflies

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ABSTRACT

Species divergence is facilitated when traits under divergent selection also act as mating cues. Fluctuations in sensory conditions can alter signal perception independently of adaptation to contrasting sensory environments, but how this fine scale variation affects behavioural isolation has received less attention, especially in terrestrial organisms. The warning patterns of *Heliconius* butterflies are under selection for aposematism and act as mating cues. Using computer vision, we extracted behavioural data from 1481 hours of video footage for 387 individuals. We show that the putative hybrid species *H. heurippa* and its close relative *H. timareta linaresi* differ in their response to divergent warning patterns, and that these differences are strengthened with increased local illuminance. Trials with live individuals reveal low-level assortative mating that are sufficiently explained by differences in visual attraction. Finally, results from hybrid butterflies are consistent with linkage between a major warning pattern gene and the corresponding behaviour, though the differences in behaviour we observe are unlikely to cause

rapid reproductive isolation as predicted under a model of hybrid trait speciation. Overall, our results highlight that the role of ecological mating cues for behavioural isolation may depend on the immediate sensory conditions during which they are displayed to conspecifics.

Keywords: *Heliconius* – Ecological Speciation – Sensory Environment – Magic traits – Hybrid Trait Speciation – Computer vision

BACKGROUND

During ecological speciation, barriers to gene flow evolve as a result of ecologically-based divergent selection [1]. These barriers are generally expected to build up gradually over time [2], with premating isolation evolving early alongside ecological differences [1,3,4]. The evolution of premating isolation is facilitated when traits under ecological selection act as mating cues (sometimes known as 'magic traits' [5,6]), as this allows divergent natural selection acting on ecological traits to be transferred to mating behaviours. However, the strength of behavioural barriers (and consequently also the strength of genetic associations between ecological and mating traits) may be influenced by the immediate, and perhaps rapidly changing, sensory conditions, but this has received relatively little empirical attention (but see [7–11]). One reason may be that robustly detecting these effects during the early stages of divergence, when they may be most relevant, presents a substantial empirical challenge.

It is well established that the sensory environment can alter signal detection and perception [12,13]. Colour perception of an object depends not only on its reflectance spectrum, but also the illumination, available light spectrum and background colouration of the environment, all of which may change within seconds and over very short distances [14]. This

52 can affect within-population preferences. For example, red colouration in male *Habronattus*
53 spiders is only an efficient mating signal if presented in broad-spectrum sunlight [15]. Beyond
54 vision, other sensory modalities can be also affected by the environment and lead to variation in
55 preferences within populations. Female swordtail fish are strongly attracted to the chemical
56 signals of conspecific males in clean water, but this preference is absent if signalling occurs in
57 polluted water [8]. Similarly, urban noise can disrupt the transmission of bird songs and interfere
58 with acoustic-based mate choice, unless singers show plasticity in song pitch [16].

59 The sensory environment may affect the evolution of reproductive isolation in two main
60 ways. First, adaptations to meet the ecological needs of different sensory environments can
61 influence female preferences, subsequently driving divergence in male signals, and leading to
62 reproductive isolation through sensory drive [17]. For example, in two closely related cichlid
63 fishes, *Pundamilia pundamilia* and *Pundamilia nyererei*, adaptation of the visual system to the
64 local environment [18,19] is associated with divergent female mate preference for male
65 colouration [18,20–24]. Second, prevailing environmental conditions may alter the efficacy of
66 signals used in mate choice [12], so that individual mating preferences may act as an important
67 reproductive barrier under some sensory conditions, but not others ('context-sensitive
68 preferences' [25]). If the strength of preference alleles depends on the sensory environment, then
69 this will influence their contribution to reproductive isolation. However, compared to the role of
70 sensory adaptation, the immediate influence of local sensory conditions on mating behaviours
71 and how this may relate to the evolution and maintenance of new species, has been less-studied.

72 By affecting context-sensitive preferences, the sensory conditions during signalling may
73 also influence the strength of genetic associations (*i.e.* linkage disequilibrium, LD) between
74 mating and ecological traits, which are typically required for speciation to proceed when gene

flow persists [26]. Specifically, when ecological traits act as mating cues, LD (between ecological and preference loci) will arise as a natural consequence of mating preferences, but this will be proportional to the strength of preference [27], which may be influenced by the sensory environment. Regardless, unless preferences are very strong, more robust coupling between mating and ecological components of reproductive isolation likely requires genetic architectures that impede recombination between relevant loci (or one-allele mechanisms, see [28]). These genetic architectures include physical linkage, which may be further strengthened by genomic rearrangements like inversions, or – in the extreme – pleiotropy, where distinct traits are controlled by the same allele [29]. To date, studies have reported physical linkage between behavioural and ecological components of reproductive isolation for a handful of animal taxa, including pea aphids [30], fish [31] and butterflies [32].

Heliconius butterflies are known for their bright warning patterns, which are often under selection for Müllerian mimicry [33]. Closely related *Heliconius* species frequently – but not always – have very different wing patterns, which additionally act as mating cues [34–40]. This contributes to assortative mating because males almost invariably prefer wing patterns resembling their own, and warning patterns in *Heliconius* are considered one of the best examples of 'magic traits' in nature [6,34,41]. Variation in these warning pattern cues is largely controlled by just four genes: *optix* [42], *cortex* [43], *WntA* [44] and *aristaless* [45]. The genetics of the corresponding shifts in visual mate preference are less well known, though recent work has implicated a handful of genes associated with neural signalling in close physical linkage to the colour pattern gene *optix* [32,46].

There is substantial evidence that colour pattern alleles have introgressed between otherwise separately evolving *Heliconius* lineages [47–53]. In particular, taxa within the

98 *heurippa-timareta* group, found in the eastern slopes of the South-American Andes, have
99 acquired red colour pattern elements from local races of the closely related species *H. melpomene*
100 via introgression of the alleles at the *optix* locus [48]. Adaptive introgression of colour pattern
101 alleles has frequently led to near perfect mimicry between local races of *H. timareta* and *H.*
102 *melpomene*; elsewhere, however, the resulting colour patterns are not shared with local
103 *Heliconius* species. In particular, *H. heurippa* has a red-yellow banded forewing pattern that is
104 not shared with other local *Heliconius* (Fig. 1A). Its close relative *H. timareta linaresi*, which is
105 assumed to represent the ancestral wing colour pattern of the *heurippa-timareta* group [54], only
106 displays a yellow band (Fig. 1A) and does not have an obvious co-mimic. These two populations
107 are geographically adjacent (Fig. 1A) and presumably share a contact zone (though it has not yet
108 been discovered). Despite their nominal status as separate species, *H. t. linaresi* and *H. heurippa*
109 likely represent an early stage of divergence; hybrids between *H. t. linaresi* and *H. heurippa* are
110 fully viable and fertile [55] and any post-mating isolation is likely limited to selection acting
111 against immigrant warning patterns.

112 The red patterns elements of *H. heurippa* are presumably maintained by strong frequency-
113 dependent selection imposed by predators [56]. However, their efficacy as intraspecific signals
114 may be influenced by how they are perceived by conspecifics under natural sensory conditions.
115 In this way, the evolution and maintenance of premating reproductive barriers may depend on
116 interactions between the signal, environment and receiver. *H. melpomene* are broadly separated
117 from *H. heurippa-timareta* (along with closely related *H. cydno*) taxa across a gradient of open to
118 closed forest and decreasing light intensity in a pattern of mosaic sympatry [57,58]. These
119 contrasting sensory environments are predicted to alter how colour patterns are perceived by
120 butterfly visual systems [59]. While *H. heurippa* and *H. t. linaresi* are not ecologically isolated in

121 this way, their forest habitats are highly heterogenous and *Heliconius* are known to settle with
122 their wings open in more brightly illuminated 'light patches' [60]. Whether changes in
123 illuminance affect male behaviours has not been investigated, but such an effect would suggest
124 that the efficacy of wing patterns as premating reproductive barriers may depend on fluctuations
125 in sensory conditions as females move through the environment.

126 To test whether warning patterns contribute to premating isolation between *H. heurippa*
127 and *H. t. linaresi*, we used a novel computer vision pipeline to extract behavioural data from
128 ~1500h of video footage, alongside choice trails to determine levels of assortative mating. Our
129 resulting data, including ~17,000 behavioural interactions for 387 *H. heurippa*, *H. t. linaresi* and
130 hybrid males, allowed us to test (i) whether the two species show differences in visual mate
131 attraction towards con- and hetero-specific female patterns and (ii) whether differences in visual
132 attraction segregate with the colour patterns, consistent with physical linkage between the
133 behavioural and known colour pattern genes. Measuring illuminance in real-time at each female
134 pattern also allowed us to ask (iii) whether these behavioural differences are influenced by
135 fluctuating local conditions.
136

137 **MATERIAL AND METHODS**

138 **(a) Butterfly collection, maintenance and crossing design.** We maintained outbred butterfly
139 stocks, estabished from wild caught individuals, at the Universidad del Rosario insectaries in La
140 Vega, Colombia (4°59' N, 74°20' W, elevation 1257 m) between January 2016 and September
141 2019. *H. timareta linaresi* was collected from Guayabal (2°41'04"N, 74°53'17"W), and *H.*
142 *heurippa* was collected from three locations, Lejanías (03°34'0"N, 74°04'20.4"W), Buenavista
143 (4°10'30"N, 73°40'41"W) and Santa María (04°53'28.2"N, 73°15'11.4"W). We used these

stocks to generate F_1 hybrids (*H. t. linaresi* mother and *H. heurippa* father) and backcross hybrids to each species (BL, backcross to *H. t. linaresi* and BH, backcross to *H. heurippa*). Details of broods can be found in Table S1. All butterflies were supplied with ~10% sugar solution in addition to *Lantana*, *Psiguria* and *Gurania spp.* as a source of pollen. Females were kept individually, with *Passiflora* plants for oviposition, whereas experimental males were kept in mixed groups. With the exception of a few individuals, eggs were collected every other day and the caterpillars were raised until eclosion in plastic cups, and fed on fresh *Passiflora* leaves.

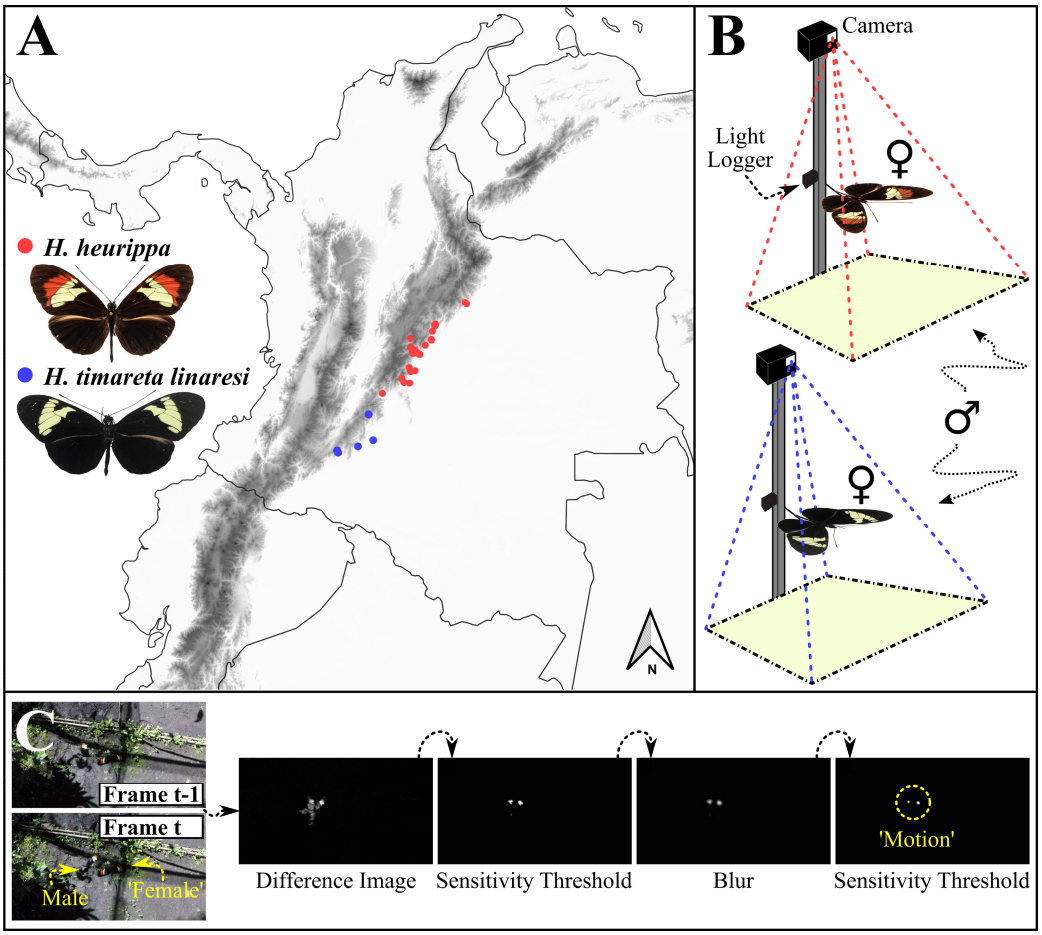

Figure 1: A) Locations at the Eastern slopes of the Colombian Andes where *H. heurippa* (red dots) and *H. timareta linaresi* (blue dots) are known to occur. B) Video recording setup. The two static mounted females were presented simultaneously to males and the area around each 'female' was surveyed with a camera. A light logger was positioned at each station, recording illuminance every second. The position of each setup in the cage was changed

every hour. C) Computer vision pipeline for *post-hoc* motion detection. A difference image is formed between each frame and its respective predecessor frame (identifying pixels with a change in value). Thresholding, blurring and again thresholding pick out significant local changes in pixels ('signals'). Frames where 'signals' were detected (as well as surrounding frames) then get extracted and combined into a new video file.

(b) Trials with mounted females. To test for differences in visual mating behaviours, we assayed males in choice trials with dead mounted *H. heurippa* and *H. t. linaresi* females presented simultaneously in a sun-exposed 4 x 4 x 2 m insectary. Virgin females were frozen on the day of eclosion with their wings spread and kept at -20°C for at least one week. They were then washed in hexane to remove residual cuticular hydrocarbons and other pheromones, and mounted onto a small piece of wire. Over the course of the experiment, we used 21 *H. heurippa* and 23 *H. t. linaresi* mounted females, and each day a randomly chosen pair of available females was used for the experiment (*i.e.* one *H. heurippa* and one *H. t. linaresi* female). Mounted females were individually attached to a horizontal wire (at ~70cm height above ground), itself attached to a plastic pipe support buried in a bucket of cement at one of six locations within the experimental cage. This location was changed every hour (hereafter 'trial'), and the new location was chosen at random (female types were never in the same location twice during the same day). Thus, our experiment included six different micro-environments with unique light conditions.

A *GoPro Hero 5 Black*™ (GoPro, San Matteo, CA) camera, equipped with 128 GB *SanDisk*™ *Extreme* SD card, was installed at each position, and about ~50cm above the respective mounted female (Fig. 1B). Resolution was set to 1920 x 1080 pixels, at a frame rate of 60 fps and a shutter speed of 1/480. The field of view was set to *Narrow*. Colour was set to *GoPro* and sharpness to *High*; ISO was set to 400; and any *auto*-setting, except for white balance, was disabled. Each camera had a *Neewer*™ UV filter attached, which reduced overexposure of the yellow bands, and was constantly charged with an *Xlayer*™ *powerbank 210033*. At same

182 height as the mounted female and ~30cm away from it, we attached a *HOBO™ UA-002-64*
183 logger to measure illuminance [*lux*] every second during the recordings (Fig. 1B). Cameras and
184 light loggers were synced to the computer's system time using *GoPro Quik*™ and *HOBOware*™
185 software, respectively, allowing us to precisely match the individual video frames and light
186 measurements.

187 Most of the virgin naive males matured in a separate communal cage before being
188 introduced into the experimental cage >4 days after eclosion, where they were tested in mixed
189 groups (median group size = 22, min = 7, max = 30). Males were numbered on the ventral side of
190 the wing and received a unique code of dots on the dorsal side of the wings, which allowed
191 subsequent identification from video footage. Each male was tested in the experimental cage over
192 the course of multiple days (median = 12 d, min = 1 d, max = 35 d). We recorded an average of
193 3.01 h (min = 0.20 h, max = 5.94 h) of material on each of the 246 recording days, with an
194 average starting time at 9.35AM (earliest = 7.48AM, latest = 2.23PM) and an average ending
195 time at 12.43PM (earliest = 9.34AM, latest = 4.23PM). Conducting this vast amount of
196 behavioural trials across different seasons and at different times allowed us to capture a variety of
197 light environments.

198

199 (**c**) **Computer vision and video analyses.** We used a custom motion-detection pipeline, which
200 *post-hoc* discarded video footage with no activity. The detection of frames with motion
201 ('signals') was based on difference imaging and consecutive steps of blurring and thresholding,
202 as implemented in the *OpenCV* library available for C++ (Fig. 1C). Not all of the frames of male
203 motion sequences made it over the threshold, so we extracted the 'signal'-frames and the
204 surrounding frames one sec before and after a signal ('chunks'). Chunks were calculated in R
205 [61], and the extraction of the frames and final reduced videos were done with the *OpenCV*

206 library in C++. Code is accessible in our GitHub repository
207 (github.com/SpeciationBehaviour/visual_preference_heurippa_linaresi). Video material was
208 processed on a HP™ ProDesk 600 G3 SFF Desktop computer (i7-7700 CPU @ 3.60GHz, 3600
209 MHz, 4 cores), faster than real time speed (runtime about 80% of video duration). All videos
210 were processed under the same threshold and blur settings. Remaining footage (with activity) was
211 curated manually at 66.6% speed using the *MPCHC™ player*. We recorded three interaction
212 behaviours: 'approach' (the male is changing its direction of flight towards the mounted female,
213 resulting in a curve or circling motion), 'courtship initiation' (sustained hovering above mounted
214 female) and 'sitting' (male sits down on mounted female). All three types of interactions were
215 combined for subsequent analyses.
216
217 **(d) Tetrad experiments with live females**. We also performed 'tetrad' trials with virgin males
218 and females to test for assortative mating between *H. heurippa* and *H. t. linaresi*. For each trial,
219 sexually mature *H. heurippa* and *H. t. linaresi* males (one of each) were allowed to acclimatize
220 for 15 mins in a 2 x 4 x 2 m insectary, at which point *H. heurippa* and *H. t. linaresi* virgin
221 females (one of each) were introduced into the same cage. Once the first mating occurred, the
222 experiment was stopped and the combination of male and female recorded. Only trials which
223 ended in mating were included in subsequent analysis.
224
225 **(e) Statistical analyses**. We measured the illuminance at both mounted females for about 83% of
226 the behavioural data points (94% for *H. t. linaresi* and *H. heurippa* males, 73% for BL hybrids,
227 88% for all other hybrids tested). Illuminance refers to the intensity of light falling onto a surface
228 and is measured in *lux*. For subsequent statistical modelling, we used the logarithm to the base 10
229 to transform *lux* measures to a measure of perceived changes in light conditions at each mounted

female (log-illuminance). We additionally scaled (and centred) each set of log-illuminance measurements, therefore making the choice of the base of the logarithm irrelevant for analyses.

All analyses were conducted in R, and raw data and an R Markdown for analysis can be found in our GitHub repository (https://github.com/SpeciationBehaviour/visual_preference_heurippa_linaresi). Posteriors will be described with a 95% equal-tailed credible interval and the mean as a point estimate. We analysed data from trials with mounted females using logistic regression with the *brms* package [64], an interface to the Bayesian software Stan [65] and implemented in R [61]. Male interactions directed towards the *H. t. linaresi* or *H. heurippa* mounted females were fitted as a binary Bernoulli-distributed response variable (*i.e.* 0 and 1, respectively). Consequently, estimates from the model can be understood as a proportion of interactions with the mounted *H. heurippa* female, with higher values indicating stronger relative attraction to the *H. heurippa* 'female' and lower values indicating stronger relative attraction to the *H. t. linaresi* 'female'. Models initially included all possible nested variations of the fully saturated model explaining effects of 1) male type, 2) log-illuminance at the *H. heurippa* 'female', and 3) log-illuminance at the *H. t. linaresi* 'female', as well as all possible interactions between 1-3 (resulting in 19 possible model structures). Individual ID and trial were fitted as random effects in all of the models, correcting for overdispersion of the data. Including trial (and in many cases also individual ID) as a random effect has often been disregarded in previous analyses (but see e.g. [66]). However, we found strong correlation among individuals' behaviour during trials (see supplementary R Markdown), likely due to the unique combination of males, mounted females and their position in the experimental cage during a given trial. The latter may have also affected sensitivity of our motion-detection software. We therefore considered it inevitable to correct for this.

To test for 'species' differences, we initially fitted models for the 'pure' males, where male type = '*H. heurippa*' or '*H. t. linaresi*'. We next tested for physical linkage between the loci underlying variation in warning pattern and relative attraction. Because differences in wing colour pattern between *H. heurippa* and *H. t. linaresi* are predominantly controlled by a single locus whose alleles control the expression of the gene *optix*, which is responsible for the red forewing bar seen in *H. heurippa,* segregation of the red bar in BL individuals allowed us to test for physical linkage (see [32,37]). This was done by fitting models for the BL males, where male type = 'red' (*Bb* genotype) or 'no red' (*bb* genotype). For the latter, we additionally fitted brood ID as a random effect. To determine which fixed and interaction terms to retain [67], we calculated the widely applicable information criterion (WAIC) and the leave-one-out information criterion (LOOIC) for each model, using the *loo* package [68], and WAIC weights using the *brms* package [64]. In all of our models, weakly informative priors (centred around the value for no preference) were set for coefficients corresponding to the different male types, which gives small prior probabilities for extreme values very close to 0 or 1. For all other coefficients, default (non-informative) priors were applied. Posteriors of the estimated marginal means (EMMs) were calculated using the *emmeans* package [69]. From this, we retrieved posteriors of contrasts and calculated the posterior probability (PP) that the EMMs differ from each other. We additionally fitted 'categorical illuminance' models to the 'pure' and the BL males, adopting the best fitting model structure determined for each dataset previously. For the 'pure' and BL males separately, we determined the median illuminance value at the *H. heurippa* 'female' (see Fig. S1) and the median illuminance value at the *H. t. linaresi* 'female' when male responses occurred. Values smaller than or equal to the median were considered 'poorly lit', and values greater than the median were considered 'brightly lit'.

For data from the tetrad experiments, we fitted observed counts of each mating outcome (type of male and female involved) as a Poisson-distributed response variable. We included the specific male-female combination of the mating outcome as a fixed factor. Transforming the resulting model estimates into proportions makes this effectively a multinomial model [70]. Non-informative default priors were applied to all of the coefficients. PP were calculated with the *brms* package [64]. We then compared the observed frequency of each mating combination to the predicted frequencies based on our measurements of visual preference from the mounted female experiment. Predictions were derived by multiplying the frequency that a male type was involved in *any* combination of mating during the tetrad experiments by its respective EMMs from the models fitted to the mounted female data. Predictions were based either on the overall EMM for each type, or on the interaction term EMMs from the categorical model. Posterior distributions for each prediction were calculated using the *binom* package under the default prior [71].

RESULTS

(a) Species comparisons: i) Divergent visual attraction behaviours in *H. timareta linaresi* and *H. heurippa* males are dependent on the light environment.

Over the course of 1.5 years, we collected 1481 hours of video footage. Using our computer vision pipeline, we reduced this to 66 hours that required human curation (*i.e.* 4.5% of the total footage recorded), during which we recorded 16,995 individual behavioural 'interactions' from 387 individual males (on average 43.9 'interactions' per male). These data allowed us to determine the effects of male type and illuminance on the relative visual attraction to the *H. heurippa* mounted 'female' (hereafter 'preference'). The best fitting model for the pure *H. heurippa* and *H. t. linaresi* males retained male type, log-illuminance at the *H. heurippa* 'female'

300 and their interaction (Table S2: model #1). Overall, our results suggest that the local light
301 environment can influence the strength of visual attraction.
302 Across the entire dataset, illuminance at the mounted *H. heurippa* female increased the
303 differences in preference between the male types, as evidenced by the interaction between male
304 type and log-illuminance at *H. heurippa* female (Fig. 2A, PP simple slope *H. heurippa* > simple
305 slope *H. t. linaresi* = 0.996). This was largely driven by an increase in log-illuminance at the *H.*
306 *heurippa* 'female' leading to a stronger conspecific preference in *H. heurippa* males (PP simple
307 slope > 0 = 0.993); there was limited support for an increase in log-illuminance at the *H.*
308 *heurippa* 'female' being associated with an increase in *H. t. linaresi* male preference for their
309 own phenotype (PP simple slope < 0 = 0.768). Overall, *H. heurippa* males showed a higher
310 proportion of interactions with the *H. heurippa* mounted females than the *H. t. linaresi* males.
311 Although supported by our Bayesian model with high credibility (posterior probability (PP) *H.*
312 *heurippa* relative visual attraction > *H. t. linaresi* relative visual attraction = 0.969), this
313 difference in preference was relatively subtle (0.07, CrI: 0.00 - 0.14) and characterised by
314 considerable within-population variation (Fig. 2B). Nevertheless, this effect nearly doubled when
315 the *H. heurippa* 'female' was in brighter light (0.13, CrI: 0.05 - 0.22; Fig 2D) and was absent
316 when the *H. heurippa* 'female' was poorly lit (0.01, CrI: -0.07 - 0.09; Fig 2C).
317

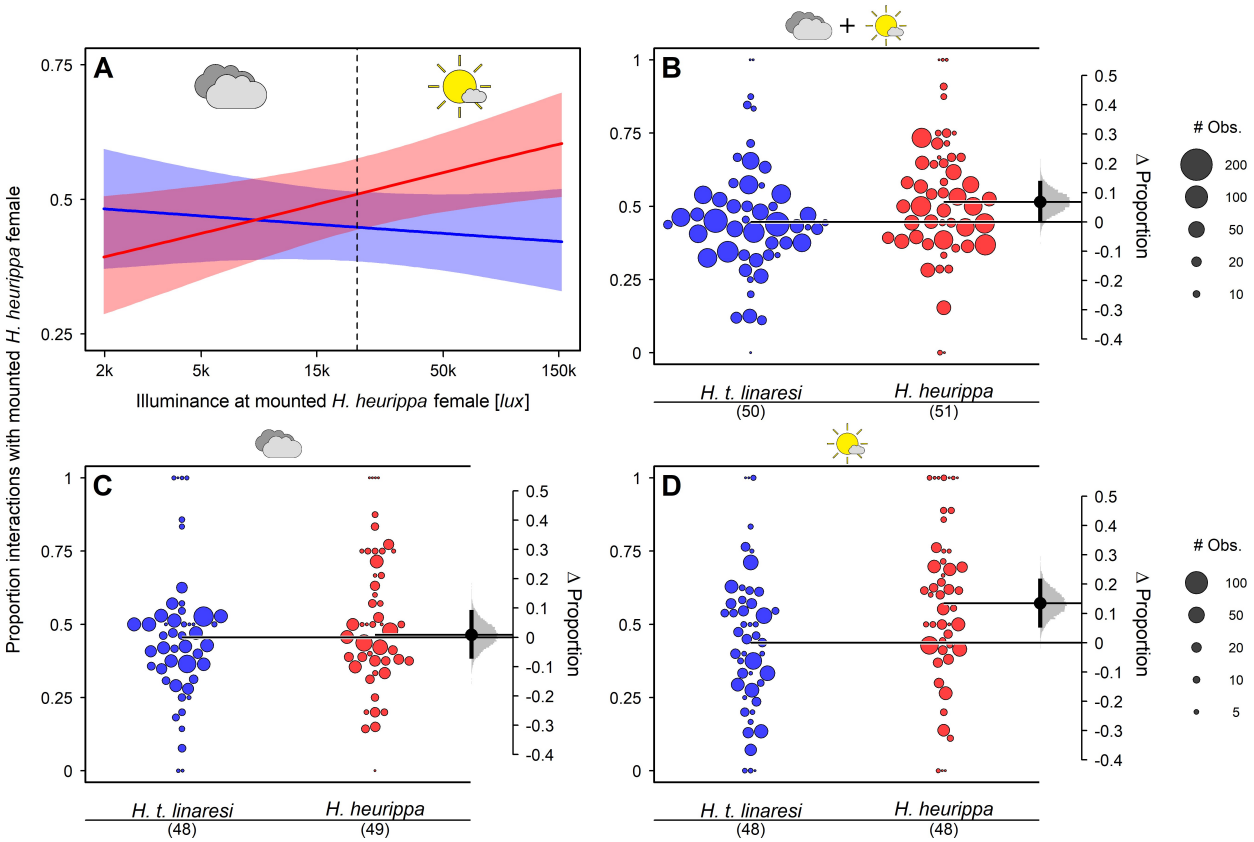

Figure 2: The effect of illuminance at the mounted *H. heurippa* female on relative attraction towards *H. heurippa* 'females' (*i.e.* the proportion of interactions with the mounted *H. heurippa* 'female' as opposed to the *H. t. linaresi* 'female'). **A**: Relative attraction towards *H. heurippa* 'females' of *H. t. linaresi* (blue) and *H. heurippa* males (red) under changing illuminance at the mounted *H. heurippa* female. Illuminance score on the x-axis was log-scaled and the coloured area around each regression line represents the 95% credible interval (CrI). Dashed vertical line represents the mean log-illuminance used as a cutoff to define the poorly and brightly lit conditions (below). Overall relative attraction towards *H. heurippa* 'females' for *H. t. linaresi* males (blue) and *H. heurippa* males (red); (**B**) across light environments; (**C**) for poorly lit *H. heurippa* 'female'; (**D**) brightly lit *H. heurippa* 'female'. Note that only preference data with the associated illuminance data were included in the underlying statistical models. B-D show the Gardner-Altman plots (see (Ho et al. 2019)), where the right-hand axis shows the difference between the two male types: horizontal lines project from the means of the posteriors for each male type (means and CrIs can be found in Table S3). Vertical thick line shows the 95% CrI for the posterior of the difference between the means, while the dot in its middle shows the mean of this posterior. In B-D, each point represents a single individual and its size is relative to the number of associated observations. The same individual males may appear in all three panels (unless they experienced only one of the light environments around the *H. heurippa* 'female' or illuminance data was not recorded during their responses, in which case they only appear in B; for individual trajectories, see Fig. S5AF). Custom swarmplot was used to distribute the dots horizontally.

(b) Species comparisons: ii) *H. heurippa* males mate more often with conspecific females in choice trials.

To test for assortative mating we also conducted 'tetrad' mate choice trials between *H. heurippa* and *H. t. linaresi*. During 89 tetrad trials with a total of 356 butterflies, we observed 50 con- and 39 heterospecific matings, supporting a degree of assortative mating (PP for positive assortative mating = 0.88). This trend was driven by a higher proportion of conspecific matings involving an *H. heurippa* male (0.405, CrI: 0.305 - 0.508) than heterospecific matings involving an *H. heurippa* male (0.270, CrI: 0.183 - 0.366]) (PP = 0.94). *H. t. linaresi* males did not participate more frequently in con- rather than heterospecific matings (PP = 0.43) (Fig. 3). In general, *H. heurippa* males mated more often than *H. t. linaresi* males (60 vs. 29 times). Our results closely match the predictions of the observed proportions from the mounted females experiment with brightly lit *H. heurippa* 'female' (predictions indicated as horizontal purple bars in Fig. 3), and, to some extent, those derived from all of the illuminance conditions combined (Table S4).

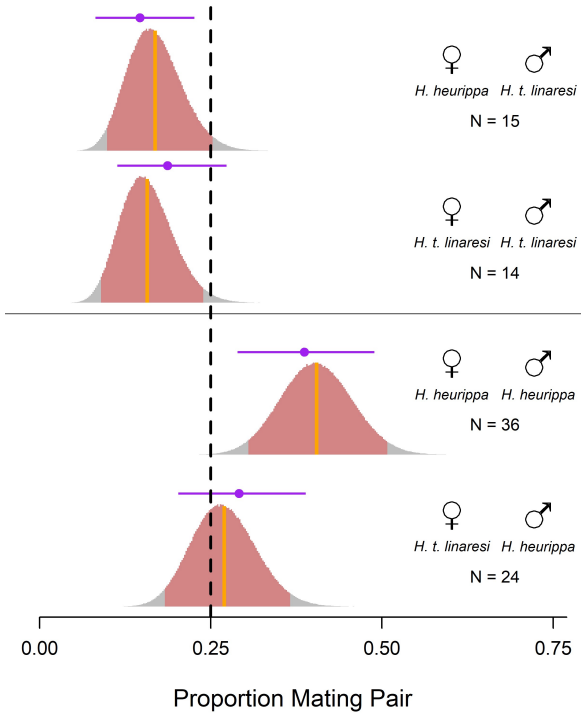

Figure 3: *H. heurippa* males show a preference for live, conspecific females in the tetrad experiments, but *H. t. linaresi* males do not. Dashed vertical line indicates expectation if neither species shows a preference to mate with its own and if both species are equally likely to engage into mating. Posterior distributions for each mating pair combination are displayed as a histogram. 95% CrI is coloured in red. Vertical orange line indicates the mean of the posterior distribution. Predictions based on the visual attraction data from the mounted females experiment with brightly lit *H. heurippa* 'female' are displayed with their 95% CrI as horizontal purple lines above the respective posterior distribution.

(c) Hybrid comparisons: Patterns of behaviour in backcross hybrids are consistent with linkage between colour and preference loci.

Estimates of preference suggest that first generation hybrid (F_1) males behave similarly as the *H. heurippa* males (Fig. S4D). This might suggest that *H. heurippa* alleles for attraction to red colour are dominant over the corresponding *H. t. linaresi* alleles; however, challenging this hypothesis, BH (backcross to *H. heurippa*) males seem to display a preference for the *H. t. linaresi* 'females' (Fig. S4E). For BL (backcross to *H. t. linaresi*) males, the fully saturated model (*i.e.* including all possible interactions) was the best fit (Table S5, model #1). Once again, the six best fitting models included the interaction between type (*i.e.* red and non-red wing pattern) and log-illuminance at the *H. heurippa* 'female' (91% of cumulative WAIC weight for an effect of this interaction term), suggesting that illuminance of the *H. heurippa* 'female' may increase existing differences in preference between the two male genotypes tested. BL males with a red band (*Bb*) were more likely to court/interact with the *H. heurippa* 'female' than BL males lacking the red band (*bb*) (Fig. S5). This difference was supported with moderately high probability by our Bayesian model (PP preference *Bb* > *bb* = 0.897). Although these represent small effects in absolute terms (0.03, CrI: -0.02 - 0.08]), this difference accounts for ~50% of the difference in means of the parental populations, consistent with linkage between colour and preference loci.

As for males from the parental populations, the difference in preference between males with the two backcross to BL genotypes (*i.e. Bb* vs. *bb*) was more pronounced when the *H.*

379 *heurippa* ‘female’ was in bright light (Fig. 4CD). There was only a small effect of the three-way
380 interaction with illuminance measures at the *H. t. linaresi* ‘female’ - the difference was slightly
381 higher when the *H. heurippa* mounted female was brightly lit (Fig. 4C, 0.11, CrI: 0.02 - 0.21), as
382 opposed to when both were brightly lit (Fig. 4D, 0.07, CrI: 0.00 - 0.13). A brightly lit *H. t.*
383 *linaresi* mounted female led to a slightly reversed pattern (*i.e. bb* > *Bb*), but only when the *H.*
384 *heurippa* mounted female was poorly lit (Fig. 4B, -0.03, CrI: -0.13 - 0.07). If both mounted
385 females where poorly lit, there was no difference in preference (Fig. 4A, 0.01, CrI: -0.05 - 0.08).
386 Across the entire dataset, illuminance at the mounted *H. heurippa* female increased the
387 differences in preference between the two BL genotypes (Fig. S2B, PP simple slope *Bb* > simple
388 slope *bb* = 0.999). While the slope for red backcross males is only slightly positive (PP = 0.859),
389 the slope for males without red is strongly negative (PP = 0.999). However, we observed a
390 stronger effect of illuminance at the *H. t. linaresi* ‘female’ than for the pure males. We found a
391 slightly negative slope for the interaction term with illuminance at the *H. t. linaresi* ‘female’ for
392 red BL males (Fig. S2D, PP = 0.722), but BL males without red showed a stronger preference for
393 the *H. heurippa* ‘female’ when the *H. t. linaresi* ‘female’ was bright (Fig. S2D, PP = 0.999).
394 Similar to the pure males, the three-way interaction indicates that a bright *H. heurippa* ‘female’
395 may more strongly induce preference for own phenotype when the *H. t. linaresi* ‘female’ is less
396 bright (Fig. S2GH and signatures in raw data Fig. S3CDGH).
397

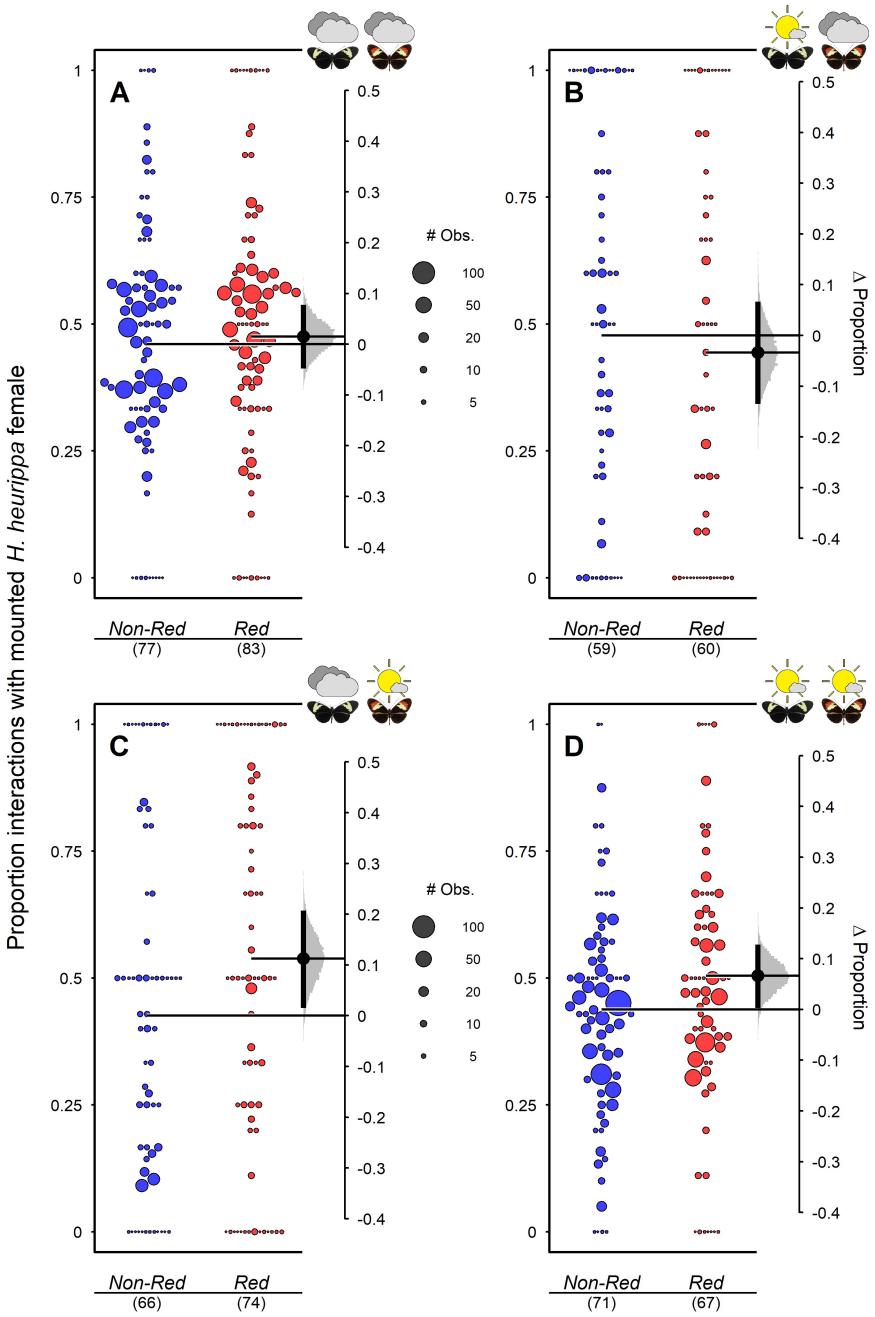

Figure 4: The effect of illuminance at the mounted *H. heurippa* female on relative attraction towards *H. heurippa* 'females' (i.e. the proportion of interactions with the mounted *H. heurippa* 'female' as opposed to the *H. t. linaresi* 'female') for BL hybrid males. Relative attraction towards *H. heurippa* 'females' for non-red (*bb*; blue dots) and red males (*Bb*; red dots): (**A**) with poorly lit *H. t. linaresi* and *H. heurippa* 'females'; (**B**) brightly lit *H. t. linaresi* and poorly lit *H. heurippa* 'females'; (**C**) poorly lit *H. t. linaresi* and brightly lit *H. heurippa* 'females'; and (**D**) brightly-lit *H. t. linaresi* and *H. heurippa* 'females'. Note that only preference data with the associated illuminance data were included in the underlying statistical model. Means and CrIs can be found in Table S3. Gardner-Altman plots are as in Figures 1B-D. For individual trajectories, see Fig. S4BC.

408 **DISCUSSION**

409 By collecting ~1500h of mate choice data, we have shown that the light environment can
410 influence visual mating behaviours during the early stages of divergence in *Heliconius* butterflies.
411 Although our data are characterised by considerable individual variation, we observed significant
412 differences in the visual attraction behaviours of *H. heurippa* and *H. timareta linaresi* males, and
413 this difference is stronger when the female patterns are more brightly lit. Experiments with live
414 males and females revealed a degree of assortative mating, and the differences in visual attraction
415 behaviours that we observe are sufficient to explain this. We have also shown that differences in
416 visual attraction are associated with the presence or absence of the red forewing band in
417 interspecific hybrids under bright light conditions, perhaps suggesting physical linkage between
418 the ecologically relevant colour pattern gene and those for the corresponding behaviour.

419 Studies of speciation often focus on already diverged groups, which are maintained by
420 multiple reproductive barriers [66,72], making it difficult to draw conclusions about the role of
421 the individual barriers and their ecological context. At the other extreme, barriers acting at the
422 early stages of divergence may be of small effect and, especially in the case of behavioural
423 phenotypes, require very large datasets to draw robust conclusions, which may not always be
424 feasible. Behavioural researchers increasingly use computer vision [73], and software for
425 automated tracking and individual identification is widely available (e.g. [74,75]). However,
426 applying these techniques to footage with heterogenous backgrounds, variable light environments
427 and arenas larger than the camera's field of view remains a challenge. To overcome these
428 limitations, we combined computer vision, allowing *post-hoc* motion detection, with subsequent
429 human curation. This permitted a low-cost solution, without the need for complex equipment, and

430 importantly allowed us to capture frames before and after motion is detected from many hundreds
431 of hours of video footage.

432 This large volume of data revealed that increased illuminance at the red mounted *H.*
433 *heurippa* female increased the frequency of interactions between *H. heurippa* males with *H.*
434 *heurippa* females (relative to *H. t. linaresi* females). *H. heurippa* males were no more likely than
435 *H. t. linaresi* males to interact with the *H. heurippa* female when she was poorly lit (lower 50%
436 quantile of illuminance values; Fig. 2C), but were 1.3 times (*i.e.* 0.57/0.44, Table S3) more likely
437 to interact with the *H. heurippa* female in brighter light (upper 50% quantile of illuminance
438 values; Fig. 2D). Similar effects of the light environment have been observed in aquatic
439 organisms. For example, the attractiveness of red colour during courtship is dependent on the
440 spectrum of available light, influenced by water depth and turbidity, and recognition of the con-
441 specifics may fail entirely under certain light environments (e.g. [7,10,18,23,76]). However, far
442 fewer studies have tested in behavioural experiments how changing light environments influence
443 mating preferences that contribute to population divergence in terrestrial organisms (but see
444 [15,77,78]).

445 The exact mechanisms underlying the differences in behaviour we observe under
446 contrasting lighting conditions isn't immediately clear. Insects, including papilionid butterflies
447 [79], have frequently evolved colour constancy across light environments [80,81], and although
448 this may only partially succeed [79,80], we generally expect individuals to be able to distinguish
449 *H. heurippa* and *H. t. linaresi* patterns under different brightness conditions. Alternatively, male
450 attraction to female colour patterns might to some degree be 'wavelength-specific', where
451 triggering of behaviours depends greatly on an object's emission of specific wavelengths and on
452 the intensity of those wavelengths [81]. Under this scenario, a colour cue might only trigger a

453 behaviour when presented at intense illuminance including wavelengths from the relevant part of
454 the spectrum [81–83]. The differences in illuminance we measured may correlate with spectral
455 differences; under shaded conditions, the available light may be reflected from the vegetation or
456 from outside the path of direct sunlight, and consequently lack red wavelengths (but be rich in
457 greenish or bluish light, respectively) [14,15]. Indeed, previous work modelling *Heliconius* vision
458 suggests that red patterns are more conspicuous to *Heliconius* when presented in bright sunlight
459 [59] (though whether this affects *Heliconius* behaviours has not previously been tested).

460 Regardless of the proximate mechanism, our data indicate that the prevailing light
461 conditions can influence visual mating behaviours in *Heliconius* butterflies. Unlike many other
462 examples, which largely involve anthropogenic induced changes in aquatic environments
463 [7,9,76], the effects we see here potentially act across very short time and spatial scales. This
464 reflects the forest environment where the light environment can vary rapidly across both time and
465 space. As such, male response may depend less on the broader environment (perhaps influenced
466 by forest type), than on the movement of females across smaller spatial scales (for example
467 between dimly and brightly lit patches). *Heliconius* butterflies are known to bask in the sun with
468 their wings open, particularly during the morning hours [60]. This may primarily be for
469 thermoregulation, though other species are known to display in local environments where they
470 are most conspicuous, and *Heliconius* might follow a similar strategy. *Anolis* lizards, for
471 example, are known to occupy micro-habitats in which their dewlap colour is most conspicuous
472 [84,85]. Visual modelling suggests *Heliconius* red patterns are more conspicuous to avian
473 predators when presented in bright sunlight, suggesting it may also enhance aposematism [59].
474 Whatever the ultimate reason for aggregating in sun-exposed patches, our data suggest that these
475 behaviours could enhance the strength of divergent mating preferences as a by-product.

476 Whether the differences in visual attraction behaviours that we observe translate to
477 assortative mating, and therefore contribute to reproductive isolation, is an important question.
478 Previous studies testing for assortative mating between *Heliconius* species closely related to *H.*
479 *heurippa* and *H. t. linaresi* in tetrad experiments similar to ours report very low frequencies of
480 interspecific matings [34,86,87]. This was not the case in our experiments, where trials resulting
481 in interspecific mating between *H. heurippa* and *H. t. linaresi* were not uncommon. Nevertheless,
482 the large number of trials in our study allowed us to detect assortative mating, albeit at low
483 levels, though this was much stronger for the 60 trials in which *H. heurippa* males mated (Fig. 3).
484 Unfortunately, we do not have illuminance data for these experiments, and this would be difficult
485 to measure given the movement of the females. Considering that the live females may have been
486 basking in the sun during the trials, that the overall activity of *Heliconius* is highest when it is
487 sunny [88] and that mating in *Heliconius* seems to occur much more frequently on sunny than on
488 cloudy days (M. Linares, pers. obs.), it is likely that a large proportion of the trials with mating
489 outcomes in our experiments included sun-exposed females. When accounting for the more
490 frequent involvement of *H. heurippa* males in mating, the mating rates between *H. heurippa* and
491 *H. t. linaresi* in our tetrad experiments closely match estimates derived from our experiments
492 with dead mounted females in bright light. Although we cannot rule out a role for cues acting
493 across other sensory modalities, the differences in visual attraction alone are sufficient to explain
494 the levels of assortative mating we observe.

495 Considering our data for *H. heurippa* and *H. t. linaresi* hybrids, overall, it is difficult to
496 ascertain patterns of dominance for visual attraction from our data. This is perhaps not surprising
497 given that the differences in behaviour between populations are subtle and are shaped by
498 considerable variation. However, segregation of the *optix* alleles, which control the red pattern

499 elements in *Heliconius*, in the backcross to *H. t. linaresi* did allow us to test for linkage between
500 the warning pattern cue and the corresponding behaviour. Once again, we observed illuminance-
501 induced shifts in visual attraction, so that the two types of backcross to *H. t. linaresi* males (*i.e.*
502 red, *Bb* vs. non-red, *bb*) differed in behaviour, but only under higher illuminance conditions.
503 These results are consistent with physical linkage between behavioural loci and *optix*, as has been
504 shown elsewhere – but with much greater effects – between the closely related species *H. cydno*
505 and *H. melpomene* [32]. Physical linkage will help to maintain key genetic associations (*i.e.* LD)
506 between loci underlying ecologically relevant traits and those for premating isolation, like mating
507 preferences, facilitating speciation with gene flow in general [26,28], and hybrid trait speciation
508 in particular. Although the differences between backcross to *H. t. linaresi* genotypes account for
509 ~50% of that observed in the two parental taxa, in absolute terms, the effects are unlikely to
510 permit sufficient power for a formal QTL study and caution should be exercised when
511 interpreting our results. Nevertheless, our results are consistent with a simple genetic mechanism
512 by which behavioural alleles were acquired alongside red colour pattern alleles through
513 introgression from *H. melpomene* into the '*heurippa*/*timareta*/*cydno*' lineage [89,90]. However, it
514 seems unlikely that the behavioural differences we observe here would rapidly lead to strong
515 reproductive isolation as predicted under a model of hybrid trait speciation [86,89].
516 In conclusion, the behavioural differences we report here for *H. heurippa* and *H. t.*
517 *linaresi* are similar in strength to those reported elsewhere for other *Heliconius* taxa at the early
518 stages of divergence [e.g. 38,66]. Alone these may represent only weak barriers to gene flow;
519 however, by augmenting divergent ecological selection acting on the warning pattern cue, they
520 may facilitate the accumulation of additional barriers as speciation proceeds. In addition, our
521 results suggest that the degree to which *Heliconius* warning pattern contribute to premating
522 isolation may depend on local illuminance, which can change rapidly in both time and space.

523 Without more stable differences in the sensory environment, perhaps facilitated by shifts in
524 habitat use, these fluctuations might constrain speciation. However, *Heliconius* are known to
525 aggregate and display their warning patterns in sun-exposed patches within the broader forest
526 environment, and our results suggest that this context would enhance premating isolation. Traits
527 predominantly shaped by ecology frequently act as mating cues, which by coupling divergent
528 natural selection to premating isolation can promote speciation with gene flow [6]; our results
529 highlight that this effect may depend on the immediate sensory conditions during which these
530 ecological mating cues are displayed to conspecifics.
531

532 **ACKNOWLEDGEMENTS**

533 We are very grateful to the Abondano-Almeida family for helping AEH and MF settle in
534 Colombia; Juan Sebastián Sánchez, Óscar Penagos, Isabel Leon and Lina Gabriela Melo for
535 assistance in the insectaries; Annika Neuhaus, Morgan Oberweiser, Saoirse McMahon and Lucas
536 Asis for help scoring videos; and Martin Küblbeck, Lucie Queste, Matteo Rossi, Daniel Shane
537 Wright and Stephen Montgomery for comments on the manuscript. Field collections and
538 insectary rearing were conducted under permit no. 530 issued by the Autoridad Nacional de
539 Licencias Ambientales of Colombia (ANLA). CS and CP were funded by Universidad del
540 Rosario (Grant Number: IV-FGD005). ML was partially funded by the Faculty of Natural
541 Sciences at Universidad del Rosario. AEH, MF and RMM were supported by an Emmy Noether
542 fellowship and research grant awarded to RMM by the Deutsche Forschungsgemeischaft (DFG)
543 (Grant Number: GZ: ME 4845/1-1).
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Supplementary material: Light environment influences mating behaviours during the early stages of divergence in tropical butterflies

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1) Supplementary Figures

Figure S1: ‘Poorly lit’, median and ‘brightly lit’ *H. heurippa* mounted female (left panel, from top to bottom), as seen on processed videos (time stamp and trial ID are added by the motion-detection program onto the left top corner). Median illuminance measurement around the *H. heurippa* mounted female (22’045 lux) was used as threshold for the ‘categorical’ model to define poorly and brightly lit conditions. As representatives for poorly lit and brightly lit conditions, we picked illuminance measures (specifically 6’200 and 132’268 lux) from the bottom and the top quartile of the distribution. The right panel shows the *H. t. linaresi* mounted female at the same illuminance values as a comparison. The presented frames were picked from different recording days. Each frame additionally shows a conspecific male approaching/courting the mounted female and dots used to identify individuals are visible on their wings. A change in hue of the red patch on *H. heurippa* seems to be visible under different illuminance conditions, but it should not be forgotten that 1) human vision is vastly different from butterfly vision and 2) that this is a representation produced by the GoPro camera (GoPro, San Matteo, CA), which is post-processing colour-composition of frames. [*Figure follows on next page*]

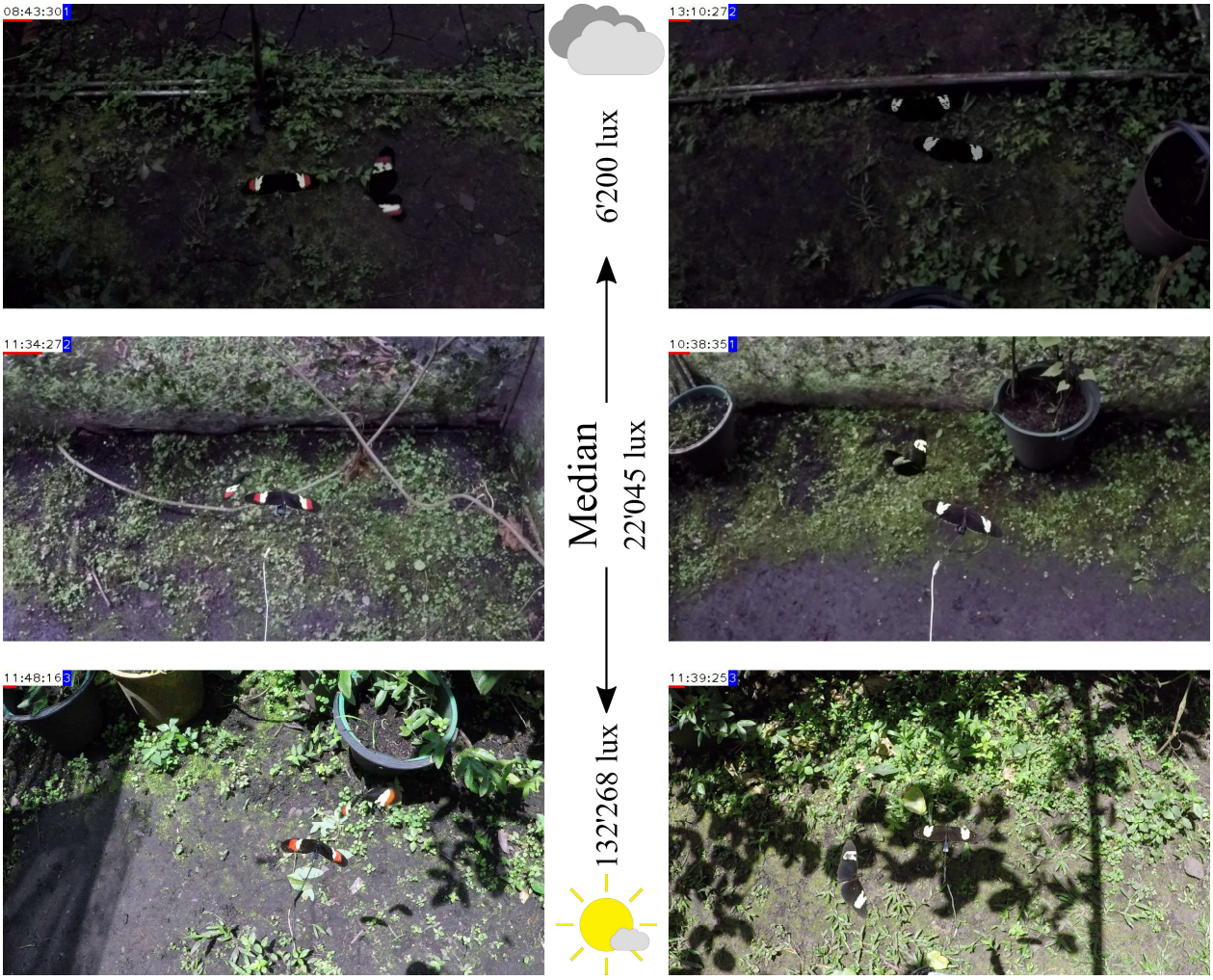


Figure S2: All interaction terms involving male type from fully saturated models (*i.e.* Table S2, model #3 for *H. heurippa* and *H. t. linaresi* males and Table S4, model #1 for backcross to *H. t. linaresi* males). **A+B**: Two-way interactions between illuminance at the *H. heurippa* mounted female and male type. Proportion of interactions with the *H. heurippa* female of *H. heurippa* (red) and *H. t. linaresi* (blue) males (A; resembles Fig. 2A, but comes from a different underlying model); and differently coloured backcross to *H. t. linaresi* males (B, red males (red), males without red (blue)). **C+D**: The same for the two-way interactions between illuminance at the *H. t. linaresi* mounted female and male type **E-H**: Three-way interactions between male type and the two illuminance measures. Male type is indicated on top of each graph. Colouration of each region in the graph depends on predicted preference for the *H. heurippa* mounted female at the given light conditions (scale depicted on right). White contour lines show Kernel densities weighted by preference score, which essentially gives the same information as the colour gradient ('peaks' are where the landscape is most red). All illuminance-axes are logarithmically scaled. Dashed lines in G and H show median illuminance measures at the respective axis, used to categorize conditions into 'poorly' and 'brightly' lit for the categorical models.

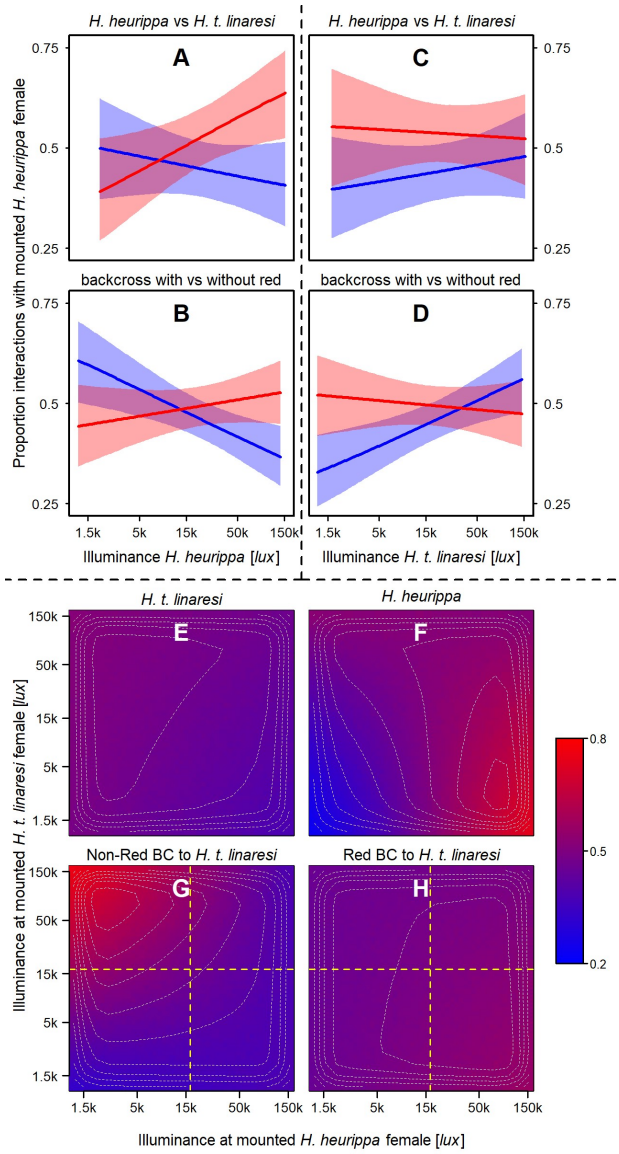


Figure S3: **A-D**: Raw data points of *H. t. linaresi*, *H. heurippa*, non-red and red backcross to *H. t. linaresi* males (from top to bottom) in a two-dimensional illuminance space. Horizontal dimension shows illuminance at the mounted *H. heurippa* female, vertical dimension shows illuminance at the mounted *H. t. linaresi* female. Blue dots indicate responses of males to the mounted *H. t. linaresi* female and red dots indicate responses to the mounted *H. heurippa* female. Kernel densities are shown for each set of data (blue lines for responses to *H. t. linaresi*, red lines for responses to *H. heurippa*). **E-H**: the two-dimensional illuminance space was then divided into 15 squares for each male type and local preferences within each square were calculated. Graphs directly relate to graphs A-D. The higher the preference for the mounted *H. heurippa* female in a square, the more red the square; the higher the preference for the *H. t. linaresi* female, the more blue (see scale on the right). Squares without response are white. These local preferences have to be interpreted in combination with the local sample sizes (as shown in left column), since they often rely on very few observations. All axes are logarithmically scaled.

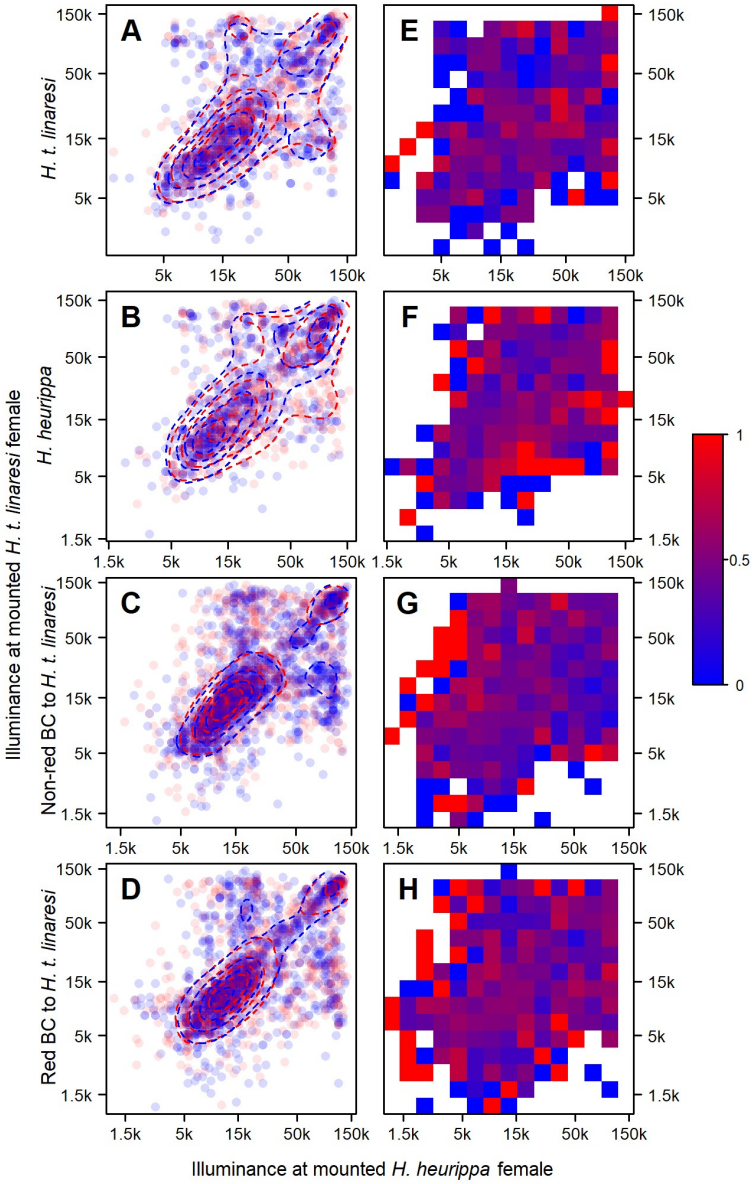


Figure S4: Individual trajectories from categorical models. Visual preferences of pure males and hybrid males are displayed by type. Within each type, data is split depending on illuminance around the *H. heurippa* mounted female, into poorly and brightly lit. Whenever an individual appeared under both light conditions, it is connected by a reaction norm line in this plot. Transparency of reaction norm lines within each panel is scaled by the maximum number of observations available for one individual within this panel. For *H. t. linaresi*, *H. heurippa* and BL males, posterior means for each EMM are calculated from the same underlying categorical models as used for Fig. 2 and Fig. 4, respectively. For F_1 and BH males, a categorical model was fit including male type, the illuminance category around the *H. heurippa* 'female' (determined again by the median measurement) and their interaction. EMMs were then extracted using the same logic as for the other types. The difference between two groups (in this case between poorly and brightly lit conditions within each type) is shown by the same Gardner-Altman plot type as used for Fig. 2 and Fig. 4. Small red and blue line located at the right side of each panel show estimators for *H. t. linaresi* and *H. heurippa* males from Fig. 2B as a reference. [*Figure follows on next page*]

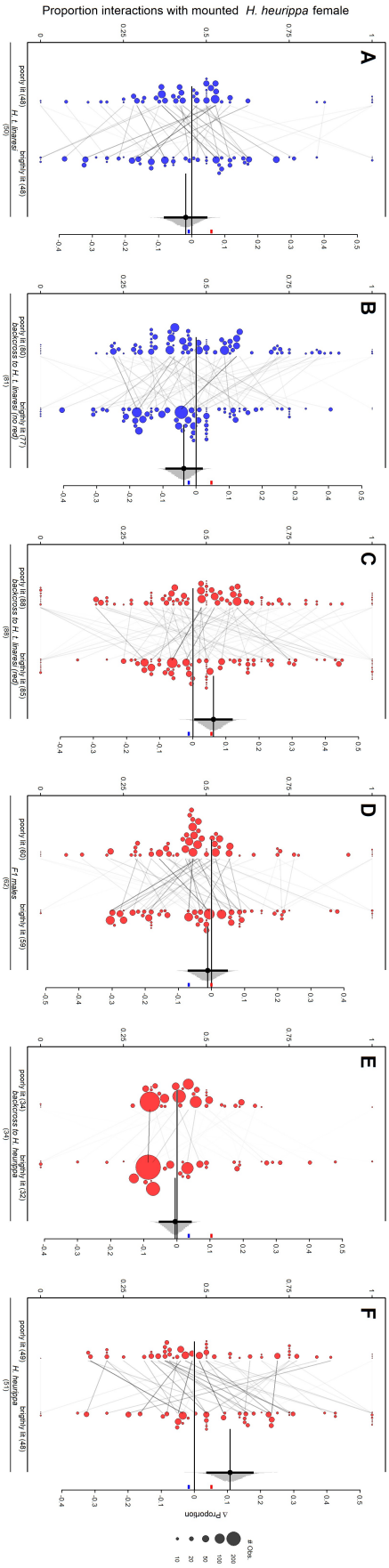
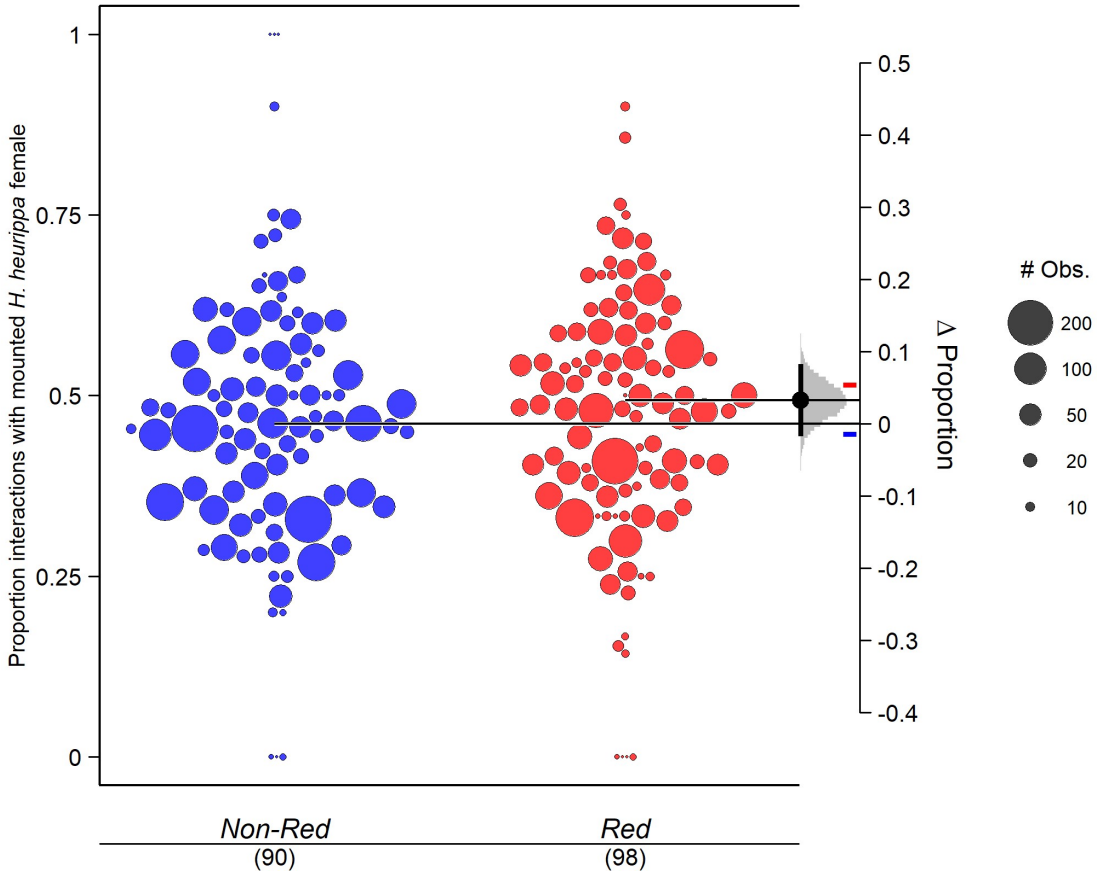

Proportion interactions with mounted H. heurippa female
A
poorly lit (48)
H. f. linaresi
(35)
brightly lit (48)
B
poorly lit (80)
backcross to H. f. linaresi (no red)
(61)
brightly lit (77)
C
poorly lit (88)
backcross to H. f. linaresi (red)
(66)
brightly lit (85)
D
poorly lit (60)
F1 males
(62)
brightly lit (59)
E
poorly lit (34)
backcross to H. heurippa
(34)
brightly lit (32)
F
poorly lit (49)
H. heurippa
(31)
brightly lit (49)
Δ Proportion
Obs.
10
20
50
100
200

Figure S5: Relative attraction towards *H. heurippa* 'females' (i.e. the proportion of interactions with the mounted *H. heurippa* 'female' as opposed to the *H. t. linaresi* 'female') for non-red (*bb*) BL males (blue dots) and red (*Bb*) BL males (red dots). Data and estimators are across all light environments. Note that only preference data with associated illuminance data were included in the underlying statistical models (a few of the raw data points displayed here therefore were not considered by the underlying model). The difference between the two groups is shown with the same logic as used for Fig. 2 and Fig. 4. Small red and blue line located at the right side of each panel show estimators for *H. t. linaresi* and *H. heurippa* males from Fig. 2B as a reference.



2) Supplementary Tables

Table S1: Information on different experimental hybrid lines. Left column shows F_1 broods from which lines originated. For the third line, we summarized two F_1 crosses and their subsequent crosses into one hybrid line. These two F_1 crosses had the same *H. heurippa* father, but a different *H. t. linaresi* mother. Individual counts in brackets show how many males of each brood were tested in the experiment. 32 other F_1 males from 7 broods were tested in the experiment and are not mentioned here, as their brood was not involved in generating backcrosses (13 individuals/5 broods of those being from a cross between *H. t. linaresi* mother and *H. heurippa* father and 19 individuals/2 broods being from the reciprocal F_1 cross with *H. heurippa* mother and *H. t. linaresi* father). *H. heurippa* is abbreviated as *heu* and *H. t. linaresi* as *lin* in the table.

F_1 brood	**Backcross to *lin* with male F_1**	**Backcross to *lin* with female F_1**	**Backcross to *heu* with male F_1**
mother: *lin*, father: *heu*	mother: *lin*, father: F_1	mother: F_1, father: *lin*	mother: *heu*, father: F_1
C18_002 (12 indiv.)	7 broods (45 indiv.)	2 broods (9 indiv.)	2 broods (20 indiv.)
C18_020 (6 indiv.)	3 broods (83 indiv.)	1 brood (15 indiv.)	1 brood (9 indiv.)
C18_034 & C18_036 (half-sib.) **(13 indiv.)**	4 broods (18 indiv.)	2 broods (10 indiv.)	3 broods (6 indiv.)
C19_014 (-)	1 brood (8 indiv.)	-	-

Table S2: 19 possible fixed-effect structures for the pure males of *H.* heurippa and *H. t.* linaresi (fixed effect 'type'). 'I_H' and 'I_L' stand for log-illuminance measures at the *H.* heurippa and the *H. t.* linaresi mounted females, respectively. A '*' sign in a model formula indicates that all involved variables as well as all possible interactions between them are included. All models include trial and male ID as random effects. Each model structure has a LOOIC and WAIC value, which mostly agree. Based on the WAIC value, differences between best model and other models are calculated (ΔWAIC) as well as a model-weight score.

#	Fixed Effects Term	LOOIC	WAIC	ΔWAIC	$Weight_{WAIC}$
1	~ type * I_H	3773.40	3768.52	0.00	0.29
2	~ type * I_H + I_L	3774.63	3769.68	1.16	0.16
3	~ type * I_H * I_L	3775.20	3770.08	1.56	0.13
4	~ type * I_H + I_H * I_L	3775.99	3771.04	2.52	0.08
5	~ type * I_H + type * I_L	3776.53	3771.52	3.01	0.06
6	~ type * I_H + type * I_L + I_H * I_L	3776.62	3771.56	3.04	0.06
7	~ type	3777.82	3773.07	4.55	0.03
8	~ type + I_L	3778.10	3773.20	4.68	0.03
9	~ type + I_H	3778.11	3773.33	4.81	0.03
10	~ I_L	3778.50	3773.62	5.10	0.02
11	~ type * I_L	3778.83	3773.95	5.43	0.02
12	~ 1	3779.24	3774.44	5.92	0.02
13	~ I_H	3779.45	3774.58	6.06	0.01
14	~ type + I_H + I_L	3779.75	3774.87	6.35	0.01
15	~ I_H + I_L	3780.58	3775.58	7.06	0.01
16	~ type + I_H * I_L	3780.77	3775.80	7.28	0.01
17	~ type * I_L + I_H	3780.82	3775.99	7.47	0.01
18	~ type * I_L + I_H * I_L	3781.61	3776.60	8.09	0.01
19	~ I_H * I_L	3781.85	3776.78	8.26	0.00

Table S3: Posterior means and 95% equal-tailed credible intervals for male types from Fig. 2, Fig. S5 and Fig. 4. Minus ("-") in the second column stands for a poorly lit mounted female, a plus ("+") for a brightly lit one.

Male type	Light environment	Mean	Lower	Upper
H. t. linaresi (left in Fig. 2B)	Across all	0.447	0.383	0.513
H. heurippa (right in Fig. 2B)	Across all	0.515	0.449	0.582
H. t. linaresi (left in Fig. 2C)	*H. heurippa* -	0.464	0.391	0.540
H. heurippa (right in Fig. 2C)	*H. heurippa* -	0.456	0.385	0.527
H. t. linaresi (left in Fig. 2D)	*H. heurippa* +	0.437	0.362	0.514
H. heurippa (right in Fig. 2D)	*H. heurippa* +	0.572	0.493	0.650
BL without red (left in Fig. S5)	Across all	0.461	0.414	0.508
BL with red (right in Fig. S5)	Across all	0.493	0.444	0.543
BL without red (left in Fig. 4A)	*H. heurippa* - / *H. t. linaresi* -	0.460	0.406	0.515
BL with red (right in Fig. 4A)	*H. heurippa* - / *H. t. linaresi* -	0.475	0.419	0.531
BL without red (left in Fig. 4B)	*H. heurippa* - / *H. t. linaresi* +	0.477	0.401	0.554
BL with red (right in Fig. 4B)	*H. heurippa* - / *H. t. linaresi* +	0.443	0.359	0.528
BL without red (left in Fig. 4C)	*H. heurippa* + / *H. t. linaresi* -	0.426	0.350	0.505
BL with red (right in Fig. 4C)	*H. heurippa* + / *H. t. linaresi* -	0.538	0.461	0.616
BL without red (left in Fig. 4D)	*H. heurippa* + / *H. t. linaresi* +	0.438	0.384	0.491
BL with red (right in Fig. 4D)	*H. heurippa* + / *H. t. linaresi* +	0.504	0.446	0.563

Table S4: Posterior means and 95% equal-tailed credible intervals for estimates of proportions of each mating outcome from tetrad experiments. First row is based on empirically measured data, the other rows are stochastic predictions based on mounted female data from all light environments, from a poorly lit *H. heurippa* mounted female and a brightly lit one, respectively.

Estimates based on	***H. heurippa*** ♀ ***H. t. linaresi*** ♂	***H. t. linaresi*** ♀ ***H. t. linaresi*** ♂	***H. heurippa*** ♀ ***H. heurippa*** ♂	***H. t. linaresi*** ♀ ***H. heurippa*** ♂
tetrad experiment	0.168 [0.099; 0.253]	0.157 [0.09; 0.239]	0.405 [0.305; 0.508]	0.27 [0.183; 0.366]
mounted females: across all	0.149 [0.084; 0.23]	0.184 [0.111; 0.27]	0.349 [0.255; 0.45]	0.329 [0.236; 0.429]
mounted females: poorly lit *H. heurippa*	0.152 [0.086; 0.233]	0.181 [0.109; 0.266]	0.315 [0.224; 0.414]	0.363 [0.267; 0.465]
mounted females: brightly lit *H. heurippa*	0.146 [0.082; 0.226]	0.187 [0.114; 0.273]	0.387 [0.289; 0.489]	0.291 [0.203; 0.389]

Table S5: 19 possible fixed-effect structures for the BL males (fixed effect 'type' refers here to wing colour phenotype of hybrids). 'I_H' and 'I_L' stand for log-illuminance measures at the *H. heurippa* and the *H. t. linaresi* mounted females, respectively. A '*' sign in a model formula indicates that all involved variables as well as all possible interactions between them are included. All models include trial, male ID and brood as random effects. Each model structure has a LOOIC and WAIC value, which mostly agree. Based on the WAIC value, differences between best model and other models are calculated (ΔWAIC) as well as a model-weight score.

#	Fixed Effects Term	LOOIC	WAIC	ΔWAIC	$Weight_{WAIC}$
1	~ type * I_H * I_L	6815.06	6811.69	0.00	0.38
2	~ type * I_H + type * I_L + I_H * I_L	6815.98	6812.60	0.91	0.24
3	~ type * I_H + type * I_L	6817.15	6813.84	2.15	0.13
4	~ type * I_H + I_H * I_L	6817.82	6814.43	2.74	0.10
5	~ type * I_H	6820.15	6816.86	5.17	0.03
6	~ type * I_H + I_L	6820.78	6817.48	5.79	0.02
7	~ type + I_H * I_L	6821.33	6817.96	6.27	0.02
8	~ I_H * I_L	6822.03	6818.67	6.98	0.01
9	~ type	6821.94	6818.68	6.99	0.01
10	~ type * I_L + I_H * I_L	6822.28	6818.95	7.27	0.01
11	~ 1	6822.46	6819.18	7.50	0.01
12	~ type + I_H	6822.96	6819.70	8.01	0.01
13	~ type + I_L	6823.18	6819.94	8.26	0.01
14	~ I_H	6823.50	6820.19	8.50	0.01
15	~ I_L	6823.80	6820.52	8.84	0.00
16	~ type + I_H + I_L	6824.36	6821.03	9.35	0.00
17	~ type * I_L	6825.21	6821.92	10.23	0.00
18	~ I_H + I_L	6825.20	6821.93	10.24	0.00
19	~ type * I_L + I_H	6825.55	6822.23	10.54	0.00