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An introgressed wing pattern acts as a mating cue

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Abstract

Heliconius butterflies provide good examples of both homoploid hybrid speciation and ecological speciation. In particular, examples of adaptive introgression have been detected among the subspecies of *Heliconius timareta*, which acquired red color pattern elements from *H. melpomene.* We tested whether the introgression of red wing pattern elements into *H. timareta florencia* might also be associated with incipient reproductive isolation from its close relative, *H. timareta* subsp. nov., found in the eastern Andes. No choice experiments show a 50% reduction in mating between females of *H. t.* subsp. nov. and males of *H .t. florencia*, but not in the reciprocal direction. In choice experiments using wing models, males of *H. timareta* subsp. nov. approach and court red phenotypes less than their own, while males of *H. t. florencia* prefer models with a red phenotype. Intrinsic post-zygotic isolation was not detected in crosses between these *H. timareta* races. These results suggest that a color pattern trait gained by introgression is triggering reproductive isolation between *H. timareta* subsp. nov. and *H. t. florencia*.

Introduction

The mode and tempo of speciation remains controversial among evolutionary biologists (Mayr 1942; Turelli et al. 2001; Coyne and Orr 2004; Scopece et al. 2007). One highly debated area is the possible role of hybridization in species formation, especially among zoologists, who often see hybridization as a process that retards speciation (Dowling et al. 1997; Mallet 2007). While it is true that gene flow can impede divergence in sympatry, it is becoming recognized that hybridization might also contribute useful genetic variation, i.e. adaptive introgression (Arnold 1997; Dowling et al. 1997; Seehausen 2004; Mallet 2007; Abbott et al. 2013). Currently several examples of adaptive introgression in animal species are known and include warfarin resistance in mice, coat color in wolves, insecticide resistance in *Anopheles,* wing color pattern in *Heliconius* and several traits gained by modern humans from Neanderthals and Denisovans (Anderson et al. 2009; Green et al. 2010; Coulson et al. 2011; Song et al. 2011; Consortium 2012; Mendez et al. 2012b; Mendez et al. 2012a; Pardo-Diaz et al. 2012; Hedrick 2013; Mendez et al. 2013; Clarkson et al. 2014; Norris et al. 2015). Adaptive introgression and hybridization are processes with potential to facilitate hybrid speciation when the novel traits or parental genome reorganization promote reproductive isolation (RI) and/or adaptive divergence (Abbott et al. 2013; Seehausen et al. 2014). Hybrid swarms, for example, might contribute to speciation by founding populations with novel characteristics not seen in parents, and a number of examples are known where recently derived species show evidence for admixed genomes derived from different parental taxa (Edelist et al. 2009; Whitney et al. 2010; Czypionka et al. 2012). Nonetheless, the evidence for hybrid speciation remains controversial, and in particular, strong evidence for traits of hybrid origin contributing to RI remains elusive in most systems (Schumer et al. 2014). A simple way to test whether adaptive introgression leads to speciation in animals is by assessing its potential to generate RI in early stages of divergence. This however has been tested only a handful of times (Schumer et al. 2014; Selz et al. 2014) and, in particular, the contribution of adaptive introgression to develop novel mating preferences has only been investigated in artificial hybrids that do not occur in nature (Doherty and Gerhardt 1983; Segura et al. 2011; Selz et al. 2014).

Butterflies of the genus *Heliconius* are famous for their adaptive wing color patterns (Mallet and Jackson 1980; Jiggins 2008; Merrill et al. 2011) and provide one of the best animal examples in which hybridization is known to play a role in speciation (Mavarez et al. 2006; Melo et al. 2009). For instance, *Heliconius heurippa* is a novel non-mimetic species established as a result of hybridization, leading to both a novel wing pattern and a novel derived mating preference, constituting a case of ecological speciation where an adaptive character, acquired by hybridization, drives RI (Mavarez et al. 2006; Melo et al. 2009; Salazar et al. 2010). Additional cases of adaptive introgression in *Heliconius* include the species *H. timareta* (Consortium 2012; Pardo-Diaz et al. 2012). Phylogenetic analysis shows this taxon as sister species to *H. cydno,* a species usually displaying yellow and white wing coloration (Beltran et al. 2007; Giraldo et al. 2008). However, recent studies have uncovered several previously undescribed populations of *H. timareta* with red pattern elements (Giraldo et al. 2008; Merot et al. 2013; Nadeau et al. 2014). In the eastern Colombian Andes the endemic race *H. t. florencia*, displays an orange 'dennis-ray' wing color pattern (Giraldo et al. 2008), the most common *Heliconius* mimicry Müllerian ring in the Amazon basin (Mallet and Jackson 1980) whereas in Peru, the race *H. t. thelxinoe* shows a forewing red-banded phenotype (Merot et al. 2013). Such discoveries were unexpected because mimicry between closely related sympatric species such as *H. timareta* and *H. melpomene* had been considered unlikely (Giraldo et al. 2008; Merot et al. 2013). However, recent analysis of genomic data and genetic markers across the red color interval, have shown that red color patterns of these *H. timareta* races have been acquired through multiple adaptive introgression events from *H.*

melpomene, permitting these two species to become mimetic in the Florencia region of Colombia and in the San Martin region of Peru (Consortium 2012; Pardo-Diaz et al. 2012; Merot et al. 2013).

In the light of evidence that the red coloration of *H. t. florencia* has been acquired via hybridization, we here aim to determine whether the introgression of the rayed wing pattern from *H. melpomene* into *H. t. florencia* is associated with RI from its close relatives. We study closely related taxa in the melpomene/cydno/timareta clade found in the eastern Andes. These include the 'dennis-ray' *H. m. malleti* and an undescribed endemic taxon found near to San Vicente del Caguán (Colombia), *H. timareta* subsp. nov., for which morphological and molecular data support its identity as another subspecies of *H. timareta.* This taxon has a black background with a yellow band in the forewing, similar to nearby forms of *H. cydno* (Figure 1; Giraldo et al. *in prep*). Although this *H. t.* subsp. nov. occurs geographically close to *H. t. florencia,* a contact zone is unknown for these subspecies (Figure 1, Linares pers. obs.), mostly due to security issues in the region.

Here, we evaluated whether pre-zygotic isolation barriers have evolved between *H. t. florencia* and *H. t.* subsp. nov.. We also included *H. m. malleti* and *H. c. cordula* in these experiments, in order to determine the role of the novel *H. t. florencia* wing color pattern in RI from other parapatric and sympatric taxa from the same geographic region. In addition, these comparisons across multiple stages of divergence (from races to 'good' species) shed lights on how RI develops along the speciation continuum. This will help to understand the importance of wing color pattern acquired through adaptive introgression as a cause of speciation in the *Heliconius* butterflies.

Methods

SAMPLING AND EXPERIMENTAL POPULATIONS

During 2009 and 2010 we collected a minimum of 25 wild individuals of each *H. t. florencia* (*Tf*) and *H. m. malleti* (*Mm*) from Las Doraditas (2°41’04’’N-74°53’17’’W, Caquetá, Colombia), *H. c. cordula* (*Cc*) from San Cristobal (7°47’566”N-72°11’566’’W, Venezuela), and *H. timareta* subsp. nov. (*Tn*) from Las Morras (01°45’02” N-75°37’55”W, Caquetá, Colombia) and Guayabal (2°41’04”N-74°53’17”W, Caquetá, Colombia) (Figure 1). We used these wild individuals to establish experimental populations in outdoor insectaries of 2x3x2m^3 in La Vega (Colombia), that were provided with the host plants *Passiflora oerstedii, P. edulis, P. maliformis* and *P. ligularis* for oviposition and larvae feeding. For the adults, we provided the nectar and pollen source plants *Lantana sp., Gurania sp.* and *Psiguria sp.,* and artificial nectar solution (Merrill et al. 2011).

MATING EXPERIMENTS

To determine the presence and strength of pre-zygotic barriers to gene flow between *H. t. florencia* and *H. t.* subsp. nov.*, H. c. cordula* and *H. m. malleti*, we used two types of experiments, no-choice mating experiments and color pattern models. We expect that as species divergence increases the strength of RI does. Thus, given the recent introgression of the ‘dennis-ray’ in *H. t. florencia* we expect that both *H. timareta* races show some indications of RI based in coloration pattern. This isolation should accentuate between the species *H. timareta* and *H. cydno,* whilst between the more divergent *H. timareta*-*H. cydno* and *H. melpomene,* isolation should be strong despite some of them display similar wing color pattern.

No-choice mating experiments

We classified the no-choice matings, including direct and reciprocal, into three categories: (i) control (same race), (ii) conspecific (same species, different race) and (iii) heterospecific (Supplementary table 1). For each combination, a virgin female was placed with a male of at least 8 days old inside an insectary for a maximum period of 8 days. The success or failure of mating was recorded either by direct observation of mating or by the presence of the spermatophore inside the female abdomen. After mating, the female was isolated in a different insectary while the male was returned to the stock. Mated males were used only once whereas unmated males were reused (Mavarez et al. 2006; Muñoz et al. 2010).

A Bayesian hierarchical model was used to estimate the probability of success for each mating type. For the full model (Supplementary table 1, Supplementary Figure 1A), we assume there is an overall rate of mating success μ for any mating class and each one of these is assumed to have a rate of mating success θ_i which comes from a distribution centered around μ. The number of times a mating success was registered y_i follows a binomial distribution with rate of success θ_i and n_i trials. For the population specific model, we assume different groups of crosses (Supplementary table 1, Supplementary Figure 1B) to have a preference p_k, drawn from the overall distribution centered around μ. In this case θ_{lk} is the rate of mating success for each type of cross l in each group of crosses k. As above, y_{lk} is the number of successes in each experiment and follows a binomial distribution with rate of success θ_{lk} and a total number of trials n_{lk}. These graphical models were implemented in the JAGS software (Plummer 2003) using the R package R2jags (Su and Yajima 2009). We used six independent Monte Carlo Markov Chains each with 20,000 collected samples and 5,000 burn-in samples. The $\hat{R}$ statistic was used to verify convergence and autocorrelation as well as to check that samples are good approximations to posterior distributions (Gelman et al. 1996). Further, we used Bayes factors (BF henceforth) (Kass and Raftery 1995) to determine whether rates of mating success are the same or different between mating types. In each case, the Savage-Dickey approximation method (Lee and Wagenmakers 2013) was used to estimate the value of the BF by comparing prior and posterior densities of the parameters (i.e. calculating the ratio between of evidence supporting the null hypothesis and that of the alternative hypothesis (H_o/H_1)). In our case, the null hypothesis is that success rate is no different between mating types (i.e. $\delta = 0.5$, where δ is the difference between the success rates of any pair of mating types) and thus, BF values below 1 support the alternative hypothesis. Finally, for comparison purposes and following previous studies in *Heliconius* (Jiggins et al. 2001b; Muñoz et al. 2010), we estimated mating preference using likelihood (supplementary likelihood analysis).

Color pattern models

Color pattern models consisted of dissected wings of dead females and were used to test the role of the color pattern preference of the males of *H. t. florencia* and *H. t.* subsp. nov.. A single male of at least 8 days old of either *H. t. florencia* or *H. t.* subsp. nov. was presented simultaneously with two female models, one being a control model (same race) and the second, the experimental model, which could be either *H. m. malleti, H. c. cordula, H. t. florencia* or *H. t.* subsp. nov. Both, the control and the experimental models were hanging from a nylon string in the center of a spherical area (60 cm diameter) and gently shaken in order to simulate real flying. We recorded the male response as approach (entered the sphere) or courtship (fluttered towards the model) (Melo et al. 2009). In total, we tested 60 males of *H. t. florencia* and 90 males of *H. t.* subsp. nov. and for each of them, we recorded a total of 20 approaches and courtships (Supplementary table 2).

In order to test the male response to the models we analyzed mate preference data using a hierarchical random effects Bayesian model for count data, which accounts for variation at both individual and population levels and has been recently implemented in ecology and evolution studies that analyze count data (Merrill et al. 2011; Lee and Wagenmakers 2013; Finkbeiner et al. 2014). In our model we estimated the rate π_j with which males of type j approached or courted experimental over control wing models, thus being the key parameter of interest. We assumed there is an overall preference μ of choosing the control wing model over the experimental in any case, and also, that each male of *H. t. florencia* and *H. t.* subsp. nov. has a preference for their control wing model type that comes from the distribution centered around μ (supplementary figure 2). It was also assumed that there is between-butterfly individual differences drawn from a distribution with mean π_j so that the *ith* butterfly on the *jth* condition has a latent preference q_{ij}. Finally, we assumed that the number of times the control type was chosen (y_{ij}) follows a binomial distribution out of a total of n_{ij} events. Beta distributions were used to model the preferences π_j and q_{ij}. The graphical model illustrating our Bayesian approach (supplementary figure 2) was implemented in JAGS (Plummer 2003) as described above. We also calculated BFs (Kass and Raftery 1995) using the Savage-Dickey approximation method (Lee and Wagenmakers 2013) to: (i) address whether males of *H. t. florencia* and *H. t.* subsp. nov. have an actual preference for their control wing pattern or if they choose a different wing pattern as frequently as their own and, (ii) address whether pairs of group mean preferences were the same or different (see BF interpretation above). Once more, for comparison purposes we estimated color pattern preference using likelihood (supplementary likelihood analysis).

ANALYSIS OF POST-MATING ISOLATION

Using the successful matings obtained in the no-choice mating experiments, we calculated hatching proportion as a measure of egg viability relative to control crosses. For this, once the female was mated we isolated her in an individual insectary with food resources and host plant for oviposition. Eggs were collected daily and larvae were reared individually. We recorded the number of eggs laid and their hatching success. The data were analyzed with the likelihood approximation implemented in BETABINO 1.1 (Jiggins et al. 2001a). Basically, a betabinomial distribution is used with count data (number of eggs) to obtain the maximum probability of observing an event (success of hatching) through different classes (category of no-choice mating). This likelihood function considers the variability within replicates of the same category and between different categories of no-choice matings. The program calculates the maximum log-likelihood under four models considering variation in the hatching proportion among classes. Likelihood ratio tests were used to differentiate among alternative models with dissimilar number of parameters (Jiggins et al. 2001a; Naisbit 2002; Salazar et al. 2005). With these experiments, we expect to observe intrinsic isolation barriers only between the more divergent taxa (*H. timareta*-*H. cydno* and *H. melpomene*).

Results

MATING EXPERIMENTS

The Bayesian approach with hierarchical models used here allowed us to quantify uncertainty of individual and population preferences that we had not been able to estimate using likelihood methods alone that assume a single parameter to describe the preference of all individuals (supplementary likelihood analysis). However, the results obtained by both approaches were largely consistent.

Conspecific comparisons

In no-choice mating experiments we performed a total of 23 conspecific comparisons that involved both *H. timareta* races and contrasted them against 105 control comparisons (Figure 2, supplementary Table 3). In trials involving ♀ *H. t.* subsp. nov. x ♂ *H. t. florencia,* the frequency of successful mating was about half that of control matings (Figure 2, BF = 0.01384), while in trials with ♀ *H. t. florencia* x ♂ *H. t.* subsp. nov., matings occurred in the same proportion as controls (Figure 2, BF = 5.54426). Although it is clear that there is a reduction of inter-population mating, these experiments had a small sample size due to availability of specimens so it is not clear whether the asymmetric mating probability reflects a biological reality. In wing model experiments, males of *H. t. florencia* and *H. t.* subsp. nov. showed a similar preference strength in approaches and courtships (Figure 3). The males of *H. t. florencia* discriminated against the wing models of *H. t.* subsp. nov. in approaches and in courtships (Figure 3A). Similarly, when *H. t.* subsp. nov. males were exposed to wing models, they preferred their own color pattern over that of *H. t. florencia* in approaches and in courtships (Figure 3B). The model that best fits the no-choice conspecific mating experiments consisted of three parameters (Pop. Sp. 1 in supplementary table 1; pD=5.7, DIC=26.3), being better than the initial full model with a single mating probability (full in supplementary Table 1; pD=14.3, DIC=57.8). The first parameter grouped control crosses (p_1=0.863), the second parameter only included the cross ♀ *H. t. florencia* x ♂ *H. t.* subsp. nov. (p_3=0.810) and the last parameter consisted only of the cross ♀ *H. t.* subsp. nov. x ♂ *H. t. florencia* (p_2=0.644). Overall, our results seem to support that incipient mating preferences are triggering RI between *H. timareta* races.

Heterospecific comparisons

A total of 163 heterospecific comparisons were made and further compared with those of controls (supplementary Table 3). Both *H. t. florencia* and *H. t.* subsp. nov. preferred to mate with their own when tested against *H. m. malleti* (BF=$4.41\text{x}10^{-13}$ - $4.35\text{x}10^{-6}$; Figure 2). The extent of such pre-zygotic isolation is similar to that observed between *H. m. malleti* and *H. c. cordula* (BF= $1.27\text{x}10^{-5}$ - $4.09\text{x}10^{-3}$; Figure 2) and between other *H. cydno* and *H. melpomene* races studied previously (Naisbit et al. 2001). Similarly, matings between the more closely related *H. timareta* races and *H. c. cordula* were as frequent as those of their controls (BF =2.29 – 7.31, Figure 2), except for ♀ *H. t.* subsp. nov. x ♂ *H. c. cordula* whose mating was only 35.5% as likely (BF= $4\text{x}10^{-3}$ – 0.18, Figure 2). The males of *H. t. florencia* discriminated against the wing models of *H. c. cordula* in approaches and courtships but failed to differentiate models of *H. m. malleti,* that display their same wing phenotype (Figure 3A). This suggests that the presence of red wing elements, and in general the color pattern, plays a major role in mate discrimination in *H. t. florencia*. Similarly, when *H. t.* subsp. nov. males were exposed to wing models, they preferred their own color pattern over that of *H. m. malleti* and *H. c. cordula* when approaching and courting (Figure 3B) indicating that initial recognition of color pattern helps identifying possible mates and other factors likely of chemical nature, determine the success of a mating in *H. t.* subsp. nov.. For heterospecific no-choice experiments, an initial full model with a single mating probability (supplementary Table 1) was established across all trials (pD=14.3, DIC = 57.8). To test different hypotheses, the Bayesian hierarchical model was fitted in a stepwise manner by adding parameters to the initial model. When mating probabilities were estimated in a model of four parameters separating (i) control crosses, (ii) crosses involving *H. m. malleti* females, (iii) crosses with *H. timareta* females and (iv) crosses involving *H. c. cordula* females, this led to a significant improvement in the fitting of the model (pD=13.6, DIC=56.3, Pop. Sp. 2 in supplementary Table 1). This possibly reflects different mating preferences of females from different species, mostly females of *H. m. malleti* which are highly selective (p_2=0.091).

ANALYSIS OF POST-MATING ISOLATION

The likelihood model that better explained the observed hatching proportions was that of a common mean with different variances (mvvv; G_{17}=33.19; p=0.0106). None of the heterospecific crosses involving either race of *H. timareta* and/or *H. c. cordula* showed significant differences in egg hatching as compared to the control crosses (Table 2; G_9=13.6; p=0.13). F_1 hybrid males were always fertile (G_7=9.22; p=0.23; Table 2) while F_1 hybrid females showed significant reduction in their hatching proportions when compared to those of control, conspecific or heterospecific crosses (G_7=15.87; p=0.026 and G_9=18.89; p=0.0261, respectively). Interestingly, this significant reduction seems to be due only to F_1 hybrid females from the cross ♀ *H. m. malleti x* ♂ *H. t. florencia*, as when they were removed from the comparisons, the remaining F_1 females (that is, the ones resulting from the crosses ♀ *H. t. florencia x* ♂ *H. t.* subsp. nov*.,* ♀ *H. t.* subsp. nov. *x* ♂ *H. t. florencia* and ♀ *H. c. cordula x* ♂ *H. t.* subsp. nov.) did not show any signal of egg inviability (G_6=8.828; *P*=0.1835 in the comparison with control crosses and G_8=12.509; *P*=0.1299 in the comparison with heterospecific crosses). Consistently, when females from the reciprocal cross (♀ *H. t. florencia x* ♂ *H. m. melpomene*) were tested, none of the eggs they laid hatched (Table 1). These results indicate that there is no post-zygotic isolation between *H. cydno* and *H. timareta,* but there is between *H. melpomene* and *H. timareta,* consistent with previously observed crosses between other races of *H. melpomene* and *H. cydno* (Jiggins et al. 2001a; Naisbit 2002; Salazar et al. 2005).

Discussion

Recently there have been studies documenting adaptive introgression and hybrid speciation in animals (Mavarez et al. 2006; Schwarz et al. 2007; Anderson et al. 2009; Green et al. 2010;

Hermansen et al. 2011; Song et al. 2011; Consortium 2012; Pardo-Diaz et al. 2012; Hedrick 2013; Mendez et al. 2013; Clarkson et al. 2014; Lucek et al. 2014; Norris et al. 2015) however, few have experimentally shown whether introgression directly affects adaptation and/or leads to speciation when the hybrid and parents are not temporarily and/or spatially separated (Schwander et al. 2008; Melo et al. 2009; Schumer et al. 2014; Selz et al. 2014). In particular, the potential of adaptive introgression to promote RI in animal systems remains a largely unexplored question (Schumer et al. 2014).

An important requirement to address this question is having an animal system where adaptive introgression occurred recently to assess whether it is triggering RI between the forms of the newly formed polymorphic population. To our knowledge only few cases have investigated the contribution of recent hybridization to RI. Four studies showed that hybrids prefer to mate with themselves rather than with the parental species (Doherty and Gerhardt 1983; Melo et al. 2009; Segura et al. 2011; Selz et al. 2014) however, three out of those four cases namely *Anastrepha* flies, cichlid fishes and *Hyla* frogs, tested preference in F_1 artificial hybrids that do not occur in nature.

In *Heliconius, H. timareta* has recently acquired wing pattern elements by hybridizing with *H. melpomene* (Consortium 2012; Pardo-Diaz et al. 2012). This gene sharing allowed the diversification of *H. timareta* across the east of the Andes by allowing it to enter mimetic rings already established between *H. melpomene* and *H. erato*. The present study shows that besides the intrinsic adaptive value of the novel mimetic and aposematic wing coloration in *H. timareta*, the introgression of this trait into this species contributes to some degree of incipient RI.

In the south east of the Colombian Andes the introgression of the 'dennis-ray' pattern from *H. m. malleti* into the ancestor of *H. timareta* led to the diversification of this species, resulting in the co-existence of the races *H. t. florencia* (‘dennis-ray’) and *H. t.* subsp. nov. that, according to our data, are developing incipient assortative mating based on presence/absence of the ‘dennis-ray’. Specifically, we observed a reduction in mating frequency in no-choice experiments between *H. timareta* races. This reduction can be due to several behavioral and ecological factors, but is likely mostly explained by the fact that females of *H. t.* subsp. nov. and males of *H. t. florencia* are less prone to mate with each other. Furthermore, males of both *H. t.* subsp. nov. and *H. t. florencia* approached and courted wing models of the other subspecies substantially less than those of their own. Thus, it seems that mating success is largely due to the males’ color pattern preference. However, females of *H. t. florencia* and males of *H. t.* subsp. nov. mated with each other despite the choosiness showed by these males in wing model experiments. This may be explained by the nature of no-choice experiments, which simulate natural situations of one to one encounters in the field, and measure reluctance but not choice. Thus, males of *H. t.* subsp. nov. prefer females of their own if they are given the choice but, when that is not the case, they are opportunistic and mate with *H. t. florencia*.

In agreement with the incipient RI detected in our experiments, we have collected hybrids between *H. t. florencia* and *H. t.* subsp. nov. in the wild (~3%; 5 out of 150 individuals sampled, that have a broader forewing band typical from *H. t.* subsp. nov. and ‘dennis-ray’ from *H. t. florencia*). However, this value is likely an underestimation. Given the dominant inheritance of the ‘dennis-ray’ phenotype, F_1 hybrids between these races will look phenotypically identical to *H. t. florencia* and can be mistakenly classified as ‘pure’. This is indeed likely, as some wild-caught *H. t. florencia* females have produced offspring with both rayed and non-rayed phenotypes (Linares pers. comm.). Finally, a comprehensive sampling across the zone of contact has not been possible due to political instability. In the light of the lack of evidence for the extent of hybridization in the wild and that the degree of RI between these morphs is likely insufficient to merit species status (and they may not necessarily diverge into good species), we prefer to think of this study as an example of what may happen during early stages of hybrid trait speciation *sensu* Jiggins et al. (2008).

On the other hand, when we examined mating behavior involving comparisons between *H. timareta* with other species, interesting observations emerged. First, matings between females of *H. t*. subsp. nov. and males of the closely related taxon *H. c. cordula* were infrequent (Figure 2), despite these two species have a very similar wing coloration (mainly differentiated by the presence of iridescence and brown hind wing forceps in *H. c. cordula;* Figure 1). This RI may be the result of both female and male choice. Males may be using iridescence as a mating cue. We observed that although males of *H. t*. subsp. nov. (non-iridescent) approach wing models of *H. c. cordula* (iridescent), they avoid courting them (Figure 3). In addition, as *Heliconius* females have odor receptors (Briscoe et al. 2013) and the males produce sex pheromones (Vanjari pers. comm.), females of *H. t*. subsp. nov. may be recognizing their conspecifics males from those of *H. c. cordula* using chemical cues, although this remains untested. However, this isolation is asymmetrical as *H. c. cordula* females mate freely with *H. t*. subsp. nov. males (Figure 2). Second, the pre-mating isolation between *H. timareta* and *H. melpomene* is strong and mediated by color and, perhaps, chemical cues. Females of *H. t.* subsp. nov. almost never mated males of *H. m. malleti* (only 1 successful cross in 30 attempts) and the reciprocal cross never occurred in our experiments (Figure 2), perhaps explained by the differences in color pattern between these species. In consequence, males of *H. t.* subsp. nov. approached and courted wing models of *H. m. malleti* in less than 30% of the trials (Figure 3). In contrast, phenotypically identical co-mimics *H. t. florencia* and *H. m. malleti* were strongly assortative, but did mate more frequently than the non-mimetic pair (less than 20%; Figure 2 and (Giraldo et al. 2008)). Furthermore, males of *H. t. florencia* approached and courted wing models of *H. m. malleti* as much as theirs (Figure 3). This suggests that recognition is likely primarily based on pheromones. There is evidence supporting this, as males of *H. t. florencia* and *H. m. malleti* are known to produce different pheromone blends (Vanjari pers. comm.). The isolation we found between *H. timareta* and *H. melpomene* is also consistent with previous studies that found that interspecific crosses between *H. cydno* (closely related to *H. timareta*) and *H. melpomene,* are highly infrequent (Jiggins et al. 2001b; Mavarez et al. 2006).

There was no egg inviability in F_1 individuals from crosses between *H. cydno* and *H. timareta* or between *H. timareta* races, while eggs laid by F_1 female hybrids between any race of *H. timareta* and *H. melpomene* always failed to hatch (Table 1), a result also observed between *H. cydno* and *H. melpomene* (Naisbit 2002). These results are consistent with the idea that the early stages of speciation are driven by divergent ecological or sexual selection, with intrinsic postzygotic isolation arising later in the speciation continuum (Seehausen et al. 2014). Here, races of the same species (*H. t.* subsp. nov. and *H. t. florencia*) show incipient mating preference, closely related species (*H. timareta – H. cydno*) have stronger assortative mating without intrinsic genetic incompatibilities and, finally, more distant species (*H. timareta/H. cydno – H. melpomene*) have developed both prezygotic and postzygotic isolation.

The presence of prezygotic isolation barriers in early stages of speciation has also has been documented between sister taxa of recent origin such as *Pundamilia* cichlids (Seehausen 2009), *Littorina* ecotypes (Conde-Padín et al. 2008; Saura et al. 2011), races of *Rhagoletis* (Powell et al. 2014)*, Ophrys* spp. orchids (Scopece et al. 2007), *Haplochromine* cichlids (Stelkens et al. 2010) and other *Heliconius* butterflies (Merrill et al. 2011). However, in none of those cases RI resulted as consequence of an introgressed trait. Additionally, our study is one of the few documenting the subsequent evolution of intrinsic postzygotic barriers in later stages of speciation (although see (Naisbit 2002; Stelkens et al. 2010; Merrill et al. 2011)).

In summary, we have confirmed that *H. timareta* is a taxon more closely related to *H. cydno*, but that the introgression of red color wing elements from *H. melpomene* has contributed to the divergence between *H. t. florencia* and *H. t.* subsp. nov. through the development of incipient assortative mating. We do not know whether this incipient mate recognition will lead to the formation of two different species but, at present, this case reflects the potential of adaptive introgression to promote and facilitate hybrid trait speciation.

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References

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Väinölä, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. Journal of Evolutionary Biology 26:229-246. doi: 210.1111/j.1420-9101.2012.02599.x.

Anderson, T. M., B. M. vonHoldt, S. I. Candille, M. Musiani, C. Greco, D. R. Stahler, D. W. Smith, B. Padhukasahasram, E. Randi, J. A. Leonard, C. D. Bustamante, E. A. Ostrander, H. Tang, R. K. Wayne, and G. S. Barsh. 2009. Molecular and evolutionary history of melanism in North American gray wolves. Science 323:1339-1343. doi:1310.1126/science.1165448.

Arnold, M. L. 1997. Natural hybridization and evolution. Oxford University Press, Oxford.

Beltran, M., C. D. Jiggins, A. V. Z. Brower, E. Bermingham, and J. Mallet. 2007. Do pollen feeding, pupal-mating and larval gregariousness have a single origin in *Heliconius* butterflies? Inferences from multilocus DNA sequence data. Biological Journal of the Linnean Society 92:221-239. doi:210.1111/j.1095-8312.2007.00830.x.

Briscoe, A. D., A. Macias-Muñoz, K. M. Kozak, J. R. Walters, F. Yuan, G. A. Jamie, S. H. Martin, K. K. Dasmahapatra, L. C. Ferguson, J. Mallet, E. Jacquin-Joly, and C. D. Jiggins. 2013. Female behaviour drives expression and evolution of gustatory receptors in butterflies. PLoS Genet 9:e1003620. doi: 1003610.1001371/journal.pgen.1003620.

Clarkson, C. S., D. Weetman, J. Essandoh, A. E. Yawson, G. Maslen, M. Manske, S. G. Field, M. Webster, T. Antão, B. MacInnis, D. Kwiatkowski, and M. J. Donnelly. 2014. Adaptive introgression between *Anopheles* sibling species eliminates a major genomic island but not reproductive isolation. Nat Commun 5:doi:10.1038/ncomms5248.

Conde-Padín, P., M. Carballo, A. Caballero, and E. Rolán-Alvarez. 2008. The relationship between hatching rate and number of embryos of the brood pouch in *Littorina saxatilis*. Journal of Sea Research 60:223-225. doi: 210.1016/j.seares.2008.1006.1003.

Consortium, T. *H.* G. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487:94-98. doi:10.1038/nature11041.

Coulson, T., D. R. MacNulty, D. R. Stahler, B. vonHoldt, R. K. Wayne, and D. W. Smith. 2011. Modeling effects of environmental change on wolf population dynamics, trait evolution, and life history. Science 334:1275-1278. doi: 1210.1126/science.1209441.

Coyne, J. A. and H. Orr. 2004. Speciation. Sinauer Associates Inc, Sunderland, MA, USA.

Czypionka, T., J. I. E. Cheng, A. Pozhitkov, and A. W. Nolte. 2012. Transcriptome changes after genome-wide admixture in invasive sculpins (*Cottus*). Molecular Ecology 21:4797-4810. doi: 4710.1111/j.1365-4294X.2012.05645.x.

Doherty, J. F. and H. C. Gerhardt. 1983. Hybrid tree frogs: vocalizations of males and selective phonotaxis of females. Science 220:1078-1080.

Dowling, T. E., Secor, and L. Carol. 1997. The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics 28:593-619. doi: 510.1146/annurev.ecolsys.1128.1141.1593.

Edelist, C., X. Raffoux, M. Falque, C. Dillmann, D. Sicard, L. H. Rieseberg, and S. Karrenberg. 2009. Differential expression of candidate salt-tolerance genes in the halophyte *Helianthus paradoxus* and its glycophyte progenitors *H. annuus* and *H. petiolaris* (Asteraceae). American Journal of Botany 96:1830-1838. doi:1810.3732/ajb.0900067.

Finkbeiner, S. D., A. D. Briscoe, and R. D. Reed. 2014. Warning signals are seductive: Relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. Evolution 68:3410-3420. doi: 3410.1111/evo.12524.

Gelman, A., X. Meng, and H. Stern. 1996. Posterior predictive assessment of model fitness via realized discrepancies. Statistica Sinica 6:733-807.

Giraldo, N., C. Salazar, C. D. Jiggins, E. Bermingham, and M. Linares. 2008. Two sisters in the same dress: *Heliconius* cryptic species. BMC Evolutionary Biology 8:doi:10.1186/1471-2148-1188-1324.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H.-Y. Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Pääbo. 2010. A draft sequence of the Neandertal genome. Science 328:710-722. doi: 710.1126/science.1188021.

Hedrick, P. W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology 22:4606–4618. doi: 4610.1111/mec.12415.

Hermansen, J. S., S. A. Sather, T. O. Elgvin, T. Borge, E. Hjelle, and G.-P. Saetre. 2011. Hybrid speciation in sparrows I: phenotypic intermediacy, genetic admixture and barriers to gene flow. Molecular Ecology 20:3812-3822. doi: 3810.1111/j.1365-3294X.2011.05183.x.

Jiggins, C. 2008. Ecological speciation in mimetic butterflies. BioScience 58:541-548. doi:510.1641/B580610.

Jiggins, C., M. Linares, R. Naisbit, E, C. Salazar, Z. Yang, and J. Mallet. 2001a. Sex-linked hybrid sterility in a butterfly. Evolution 55:1631-1638.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001b. Reproductive isolation caused by colour pattern mimicry. Nature 411:302-305. doi:310.1038/35077075.

Kass, R. and A. Raftery. 1995. Bayes factors. Journal of the American Statistical Association 90:773-795.

Lee, M. and E.-J. Wagenmakers. 2013. Bayesian cognitive modeling. Cambridge University Press, Cambridge.

Lucek, K., M. Lemoine, and O. Seehausen. 2014. Contemporary ecotypic divergence during a recent range expansion was facilitated by adaptive introgression. Journal of Evolutionary Biology 27:2233-2248. doi: 2210.1111/jeb.12475.

Mallet, J. 2007. Hybrid speciation. Nature 446:279-283. doi:210.1038/nature05706.

Mallet, J. L. B. and D. A. Jackson. 1980. The ecology and social behaviour of the Neotropical butterfly *Heliconius xanthocles* Bates in Colombia. Zoological Journal of the Linnean Society 70:1-13. doi: 10.1111/j.1096-3642.1980.tb00845.x.

Mavarez, J., C. A. Salazar, E. Bermingham, C. Salcedo, C. D. Jiggins, and M. Linares. 2006. Speciation by hybridization in *Heliconius* butterflies. Nature 441:868-871. doi:810.1038/nature04738.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

Melo, M. C., C. Salazar, C. D. Jiggins, and M. Linares. 2009. Assortative mating preferences among hybrids offers a route to hybrid speciation. Evolution 63:1660 –1665. doi: 1610.1111/j.1558-5646.2009.00633.x.

Mendez, F. L., J. C. Watkins, and M. F. Hammer. 2012a. Global genetic variation at OAS1 provides evidence of archaic admixture in Melanesian populations. Molecular Biology and Evolution 29:1513-1520. doi: 1510.1093/molbev/msr1301.

Mendez, Fernando L., Joseph C. Watkins, and Michael F. Hammer. 2012b. A haplotype at STAT2 Introgressed from Neanderthals and serves as a candidate of positive selection in Papua New Guinea. The American Journal of Human Genetics 91:265-274. doi: 210.1016/j.ajhg.2012.1006.1015.

Mendez, F. L., J. C. Watkins, and M. F. Hammer. 2013. Neandertal origin of genetic variation at the cluster of OAS immunity genes. Molecular Biology and Evolution 30:798-801. doi: 710.1093/molbev/mst1004.

Merot, C., J. Mavarez, A. Evin, K. K. Dasmahapatra, J. Mallet, G. Lamas, and M. Joron. 2013. Genetic differentiation without mimicry shift in a pair of hybridizing *Heliconius* species (Lepidoptera: Nymphalidae). Biological Journal of the Linnean Society 109:830–847. doi: 810.1111/bij.12091.

Merrill, R. M., Z. Gompert, L. M. Dembeck, M. R. Kronforst, W. O. McMillan, and C. D. Jiggins. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. Evolution 65:1489-1500. doi: 1410.1111/j.1558-5646.2010.01216.x.

Muñoz, A. G., C. Salazar, J. Castaño, C. D. Jiggins, and M. Linares. 2010. Multiple sources of reproductive isolation in a bimodal butterfly hybrid zone. Journal of Evolutionary Biology 23:1312-1320.

Nadeau, N., M. Ruiz, P. Salazar, B. Counterman, J. A. Medina, H. Ortiz-Zuazaga, A. Morrison, W. O. McMillan, C. D. Jiggins, and R. Papa. 2014. Population genomics of parallel hybrid zones in the mimetic butterflies, *H. melpomene* and *H. erato*. Genome Research:doi: 10.1101/gr.169292.169113.

Naisbit, R., E., Jiggins, C, D., Linares, M., Salazar, C., Mallet, J. 2002. Hybrid sterility, Haldane's rule and speciation in *Heliconius cydno* and *H. melpomene*. Genetics 161:1517-1526.

Naisbit, R. E., C. D. Jiggins, and J. Mallet. 2001. Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. Proceedings of the Royal Society B: Biological Sciences 268:1849-1854. doi: 1810.1098/rspb.2001.1753.

Norris, L. C., B. J. Main, Y. Lee, T. C. Collier, A. Fofana, A. J. Cornel, and G. C. Lanzaro. 2015. Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. Proceedings of the National Academy of Sciences 112:815-820. doi:810.1073/pnas.1418892112.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, O. W. McMillan, and C. D. Jiggins. 2012. Adaptive introgression across species boundaries in *Heliconius* butterflies. PLoS Genet 8:e1002752. doi:1002710.1001371/journal.pgen.1002752.

Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling *in* F. L. Hornik, and A. Zeileis, ed. Proceedings of the 3rd international workshop on distributed statistical computing, Vienna, Austria.

Powell, T. H. Q., A. A. Forbes, G. R. Hood, and J. L. Feder. 2014. Ecological adaptation and reproductive isolation in sympatry: genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*. Molecular Ecology 23:688-704. doi: 610.1111/mec.12635.

Salazar, C., S. W. Baxter, C. Pardo-Diaz, G. Wu, A. Surridge, M. Linares, E. Bermingham, and C. D. Jiggins. 2010. Genetic evidence for hybrid trait speciation in *Heliconius* butterflies. PLoS Genet 6:e1000930. doi:1000910.1001371/journal.pgen.1000930.

Salazar, C. A., C. D. Jiggins, C. F. Arias, A. Tobler, E. Bermingham, and M. Linares. 2005. Hybrid incompatibility is consistent with a hybrid origin of *Heliconius heurippa* Hewitson from its close relatives, *Heliconius cydno* Doubleday and *Heliconius melpomene* Linnaeus. Journal of Evolutionary Biology 18:247-256. doi: 210.1111/j.1420-9101.2004.00839.x.

Saura, M., M. Martínez-Fernández, M. J. Rivas, A. Caballero, and E. Rolán-Alvarez. 2011. Lack of early laboratory postzygotic reproductive isolation between two ecotypes of *Littorina saxatilis* (Mollusca, Gastropoda) showing strong premating sexual isolation. Hydrobiologia 675:13-18. doi: 10.1007/s10750-10011-10788-z.

Schumer, M., G. Rosenthal, and P. Andolfatto. 2014. How common is homoploid hybrid speciation? Evolution 68:1553–1560. doi: 1510.1111/evo.12399.

Schwander, T., S. S. Suni, S. H. Cahan, and L. Keller. 2008. Mechanisms of reproductive isolation between an ant species of hybrid origin and one of its parents. Evolution 62:1635-1643. doi: 1610.1111/j.1558-5646.2008.00387.x.

Schwarz, D., K. D. Shoemaker, N. L. Botteri, and B. A. McPheron. 2007. A novel preference for an invasive plant as a mechanism for animal hybrid speciation. Evolution 61:245-256. doi: 210.1111/j.1558-5646.2007.00027.x.

Scopece, G., A. Musacchio, A. Widmer, S. Cozzolino, and J. True. 2007. Patterns of reproductive isolation in Mediterranean deceptive orchids. Evolution 61:2623-2642. doi: 2610.1111/j.1558-5646.2007.00231.x.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology & Evolution 19:198-207. doi:110.1016/j.tree.2004.1001.1003.

Seehausen, O. 2009. Progressive levels of trait divergence along a ‘speciation transect’ in the Lake Victoria cichlid fish *Pundamilia*. Pp. 155–176 *in* R. K. Butlin, J. Bridle, and D. Schluter, eds. Speciation and Patterns of Diversity. Cambridge University Press, Cambridge.

Seehausen, O., R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, P. A. Hohenlohe, C. L. Peichel, G.-P. Saetre, C. Bank, A. Brannstrom, A. Brelsford, C. S. Clarkson, F. Eroukhmanoff, J. L. Feder, M. C. Fischer, A. D. Foote, P. Franchini, C. D. Jiggins, F. C. Jones, A. K. Lindholm, K. Lucek, M. E. Maan, D. A. Marques, S. H. Martin, B. Matthews, J. I. Meier, M. Most, M. W. Nachman, E. Nonaka, D. J. Rennison, J. Schwarzer, E. T. Watson, A. M. Westram, and A. Widmer. 2014. Genomics and the origin of species. Nat Rev Genet 15:176-192. doi: 110.1038/nrg3644.

Segura, D. F., M. T. Vera, J. Rull, V. Wornoayporn, A. Islam, and A. S. Robinson. 2011. Assortative mating among *Anastrepha fraterculus* (Diptera: Tephritidae) hybrids as a possible route to radiation of the fraterculus cryptic species complex. Biological Journal of the Linnean Society 102:346-354. doi: 310.1111/j.1095-8312.2010.01590.x.

Selz, O. M., R. Thommen, M. E. Maan, and O. Seehausen. 2014. Behavioural isolation may facilitate homoploid hybrid speciation in cichlid fish. Journal of Evolutionary Biology 27:275-289. doi: 210.1111/jeb.12287.

Song, Y., S. Endepols, N. Klemann, D. Richter, F.-R. Matuschka, C.-H. Shih, M. W. Nachman, and M. H. Kohn. 2011. Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. Current Biology 21:1296-1301. doi:1210.1016/j.cub.2011.1206.1043.

Stelkens, R. B., K. A. Young, and O. Seehausen. 2010. The accumulation of reproductive incompatibilities in African cichlid fish. Evolution 64:617-633. doi: 610.1111/j.1558-5646.2009.00849.x.

Su, Y. and M. Yajima. 2009. R2jags: A Package for Running jags from R. R.

Turelli, M., N. H. Barton, and J. A. Coyne. 2001. Theory and speciation. Trends in Ecology & Evolution 16:330-343.

Whitney, K., R. Randell, and L. Rieseberg. 2010. Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. New Phytol 187:230-239. doi: 210.1111/j.1469-8137.2010.03234.x.

Figure legends

Figure 1. Geographic distribution and phenotypes of *H. timareta* across South America. The species *H. m. malleti* and *H. c. cordula* are also depicted. The distributions of taxa are estimated from locality data compiled by Neil Rosser and Claire Merot (Rosser et al. 2012; Merot et al. 2013). Circles represent possible (but not confirmed) contact zones. Background map image was downloaded from ETOPO (Amante and Eakins 2009).

Figure 2. Mating frequency in no-choice mating experiments with virgin adult females. *Tn: H. timareta* subsp. nov; *Tf: H. timareta florencia; Cc: H. cydno cordula; Mm: H. m. malleti*. Cross type is specified as *female x male*. Error bars represent 95% credible interval of the posterior distribution.

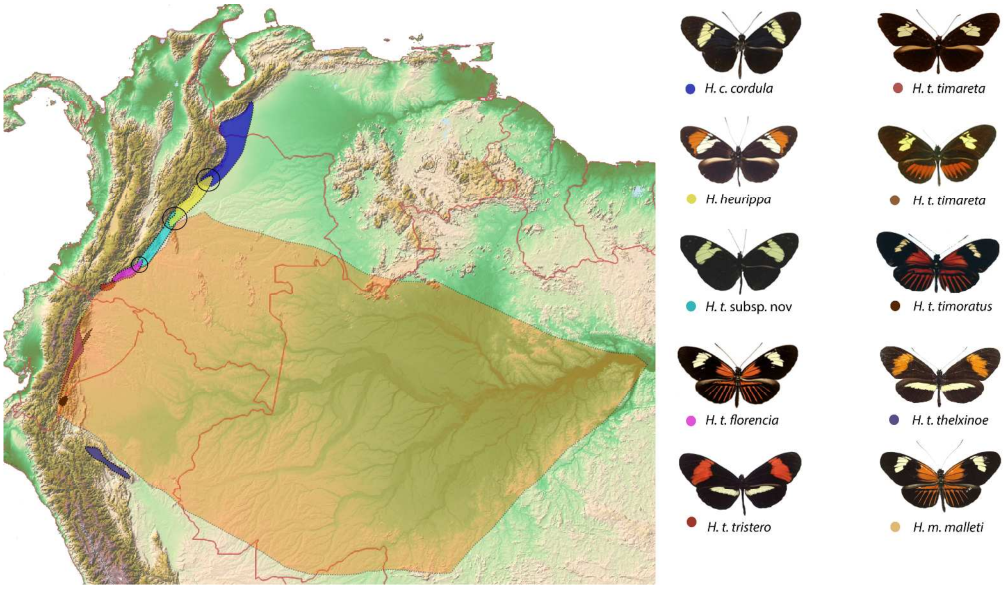
Figure 3. Violin plots showing the entire posterior distribution of the approaches and courtships of males of (A) *H. t. florencia* and (B) *H. timareta* subsp. nov. (depicted at the top of each panel) to female wing models of *H. m. malleti, H. c. cordula, H. t.* subsp. nov. and/or *H. t. florencia* (bottom of each panel). The y-axis corresponds to the preference towards the experimental model π_j. Values above 0.5 suggest preference for the own pattern while those below 0.5 suggest preference for the experimental model.

Table 1. Proportion of viable eggs in control (same races), conspecific (same species, different race), heterospecific and F_1 crosses

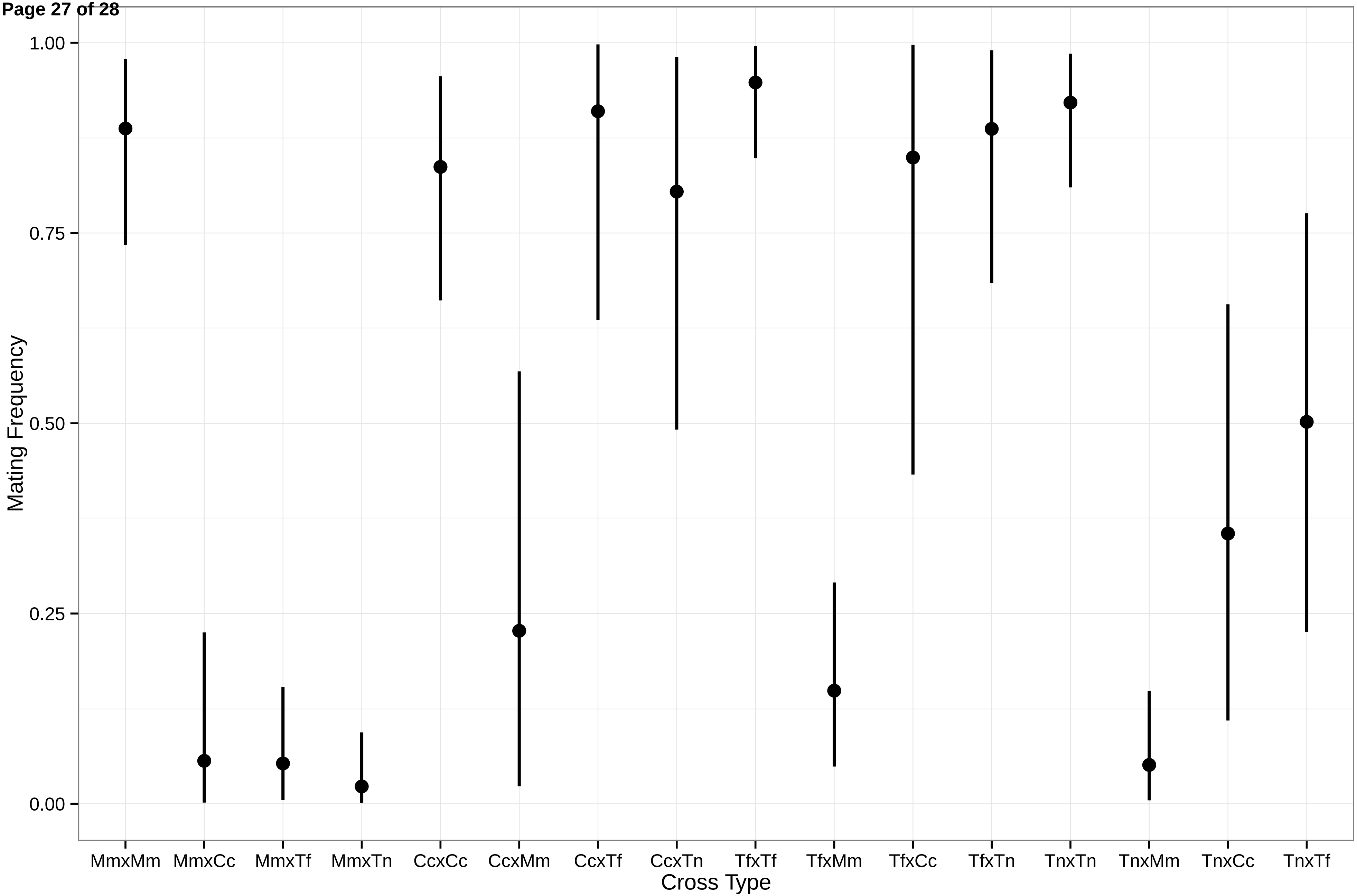
Cross type (♀ genotype x ♂ genotype)				No. of broods	No. of eggs	Proportion of viable eggs	SE	Variance	SE
Control	*Tn*	*x*	*Tn*	4	288	0.63	0.07	0.01	0.01
	Tf	*x*	*Tf*	4	111	0.76	0.07	0.15	0.01
	Cc	*x*	*Cc*	29	1377	0.64	0.05	0.06	0.01
	Mm	*x*	*Mm*	4	103	0.60	0.13	0.05	0.04
Conspecific	*Tf*	*x*	*Tn*	3	210	0.70	0.03	0.0001	--
	Tn	*x*	*Tf*	4	192	0.55	0.05	0.003	0.006
Heterospecific	*Cc*	*x*	*Tn*	3	236	0.53	0.04	0.003	0.005
	Tn	*x*	*Cc*	3	138	0.61	0.04	0.0001	--
	Tn	*x*	*Mm*	5	485	0.51	0.06	0.01	0.01
	Mm	*x*	*Tf*	2	83	0.43	0.18	0.06	0.04
	Mm	*x*	*Tn*	0	--	--	--	--	--
F_1	*[Tf x Tn]*	*x*	*Tn/Tf*	5	390	0.53	0.02	0.0001	--
	[Tn x Tf]	*x*	*Tn/Tf*	6	375	0.60	0.06	0.01	0.01
	Tf	*x*	*[Tf x Tn]*	1	43	0.64	0.07	0.0001	--
	Tn	*x*	*[Tn x Tf]*	4	261	0.55	0.03	0.0001	0.003

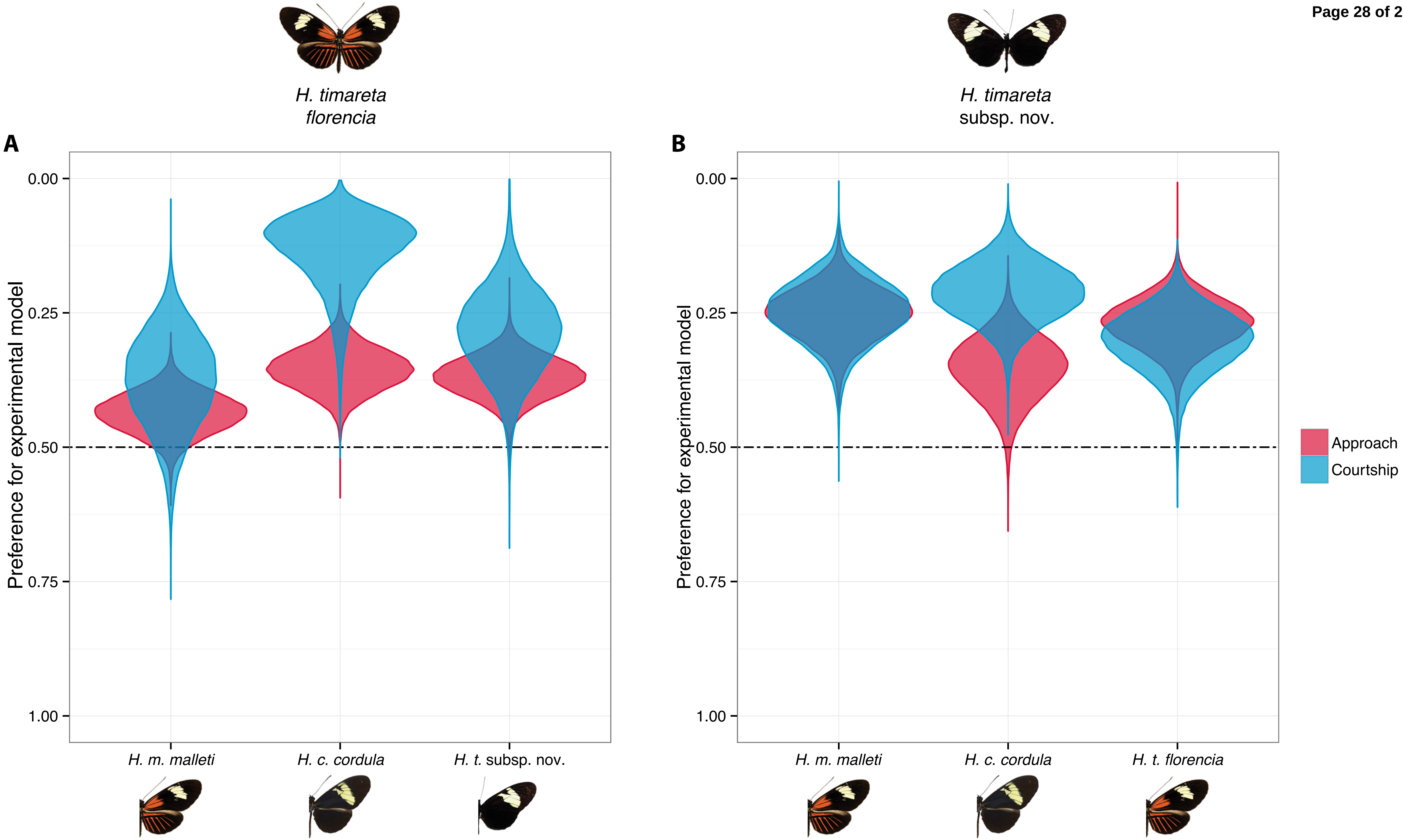
[Cc x Tn]	*x*	*Cc/Tn*	4	331	0.59	0.02	0.0001	0.002
[Mm x Tf]	*x*	*Mm*	2	70	0.28	0.05	0.0001	--
[Tf x Mm]	*x*	*Tf*	2	80	0	--	--	--
[Tn x Mm]	*x*	*Mm/Tn*	12	816	0	--	--	--
Mm	*x*	*[Mm x Tf]*	2	106	0.64	0.20	0.10	0.06
Tn/Mm	*x*	*[Tn x Mm]*	8	970	0.41	0.05	0.02	0.01

Crosses are specified as female genotype x male genotype. The symbol (/) means or.



Geographic distribution and phenotypes of H. timareta across South America. The species H. m. malleti and H. c. cordula are also depicted. The distributions of taxa are estimated from locality data compiled by Neil Rosser and Claire Merot (Rosser et al. 2012; Merot et al. 2013). Circles represent possible (but not confirmed) contact zones. Background map image was downloaded from ETOPO (Amante and Eakins 2009).
301x175mm (300 x 300 DPI)


Mating Frequency
1.00
0.75
0.50
0.25
0.00
MmxMm
MmxCc
MmxTf
MmxTn
CcxCc
CcxMm
CcxTf
CcxTn
TfxTf
TfxMm
TfxCc
TfxTn
TnxTn
TnxMm
TnxCc
TnxTf
Cross Type


H. timareta florencia
H. timareta subsp. nov.
A
B
Preference for experimental model
0.00
0.25
0.50
0.75
1.00
H. m. malleti
H. c. cordula
H. t. subsp. nov.
H. t. florencia
Approach
Courtship