

Female asynchrony may drive disruptive sexual selection on male mating phenotypes in a *Heliconius* butterfly

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Alternative male phenotypes may be a source of novel adaptive traits and may evolve under strong sexual selection. We studied interpopulation differences in male mating behavior related to receptive female synchrony in the monandrous pupal-mating butterfly *Heliconius charitonia*. In the population in which female-receptive pupae were more synchronous, larger males were unable to monopolize mates; variance in male mating success was lower; strength of sexual selection was weak; and all males competed for access to female pupae using the same strategy (pupal mating). In the population where no more than one female was receptive at a time (extreme asynchrony), only large males competed for pupae, and among these, only the largest individuals successfully mated. Thus, variance in mating success was higher, and sexual selection within pupal maters was stronger. In this population, smaller males patrolled large areas as an alternative mating behavior. When unmated females were experimentally released, small male size was associated with higher mating success. We suggest that alternative patrolling behavior may have evolved under strong sexual selection as a consequence of high asynchrony in receptive female availability in some populations.

Key words: alternative mating behavior, disruptive selection, female synchrony, *Heliconius*, pupal mating, sexual selection. [*Behav Ecol* 21:144–152 (2010)]

Alternative male reproductive behaviors (e.g., patrolling and territoriality) expressed through behavioral, physiological, and morphological polymorphisms are common across many taxa (Shuster and Wade 1991; Ryan et al. 1992; Lank et al. 1995; Brockmann 2002; Calsbeek et al. 2002) and represent different solutions to intrasexual reproductive competition (Oliveira et al. 2008). It has been proposed that alternative phenotypes may be genetic (i.e., mating “strategies”) or environmentally (i.e., mating “tactics”) determined (Gross 1996). Genetic polymorphism (i.e., fixed strategies) requires equal mean fitness in the long term across morphs (phenotypes) for their stable coexistence, whereas for environmentally determined phenotypes (i.e., conditional tactics), equal mean fitness is not expected. Instead, alternative reproductive phenotypes seem to be determined by threshold traits influenced by quantitative trait loci (Roof 1996). Then, as in the case of other phenotypic traits, the thresholds or developmental switch points involved in tactic choice may have a genetic basis and will therefore be subject to selection and adaptive evolution (West-Eberhard 2003; Taborsky et al. 2008).

Differences in fitness between morphs depend on the genetics and heritability of each particular trait involved in the expression of alternative phenotypes. Such differences, however, also depend on diverse selective pressures such as overdominant selection (Sinervo and Zamudio 2002; Roulin 2004), frequency-dependent selection (Gross 1996; Sinervo and Svensson 2002;

Sinervo and Calsbeek 2006), or condition-dependent selection (Brockmann 2002; Kotiaho 2002). All these mechanisms have been suggested as mechanisms that allow stable coexistence of alternative behaviors (Repka and Gross 1995; Gross 1996; Alonzo and Warner 2000).

Differences in morphology have been shown to correlate with differences in male mating behavior in many different systems, but it is often not clear if morphology drives the evolution of the different behaviors (as shown in Robson and Miles 2000; Perry et al. 2004) or alternative behaviors select for different morphologies. It is clear that male morphological design can influence behavioral performance, mating abilities (e.g., flight performance), and male–male fighting interactions (Sinervo et al. 2000; Berwaerts and Van Dyck 2004; Lailvaux et al. 2004; Mendoza-Cuenca LF and De Luna E, unpublished data). Therefore, correlational selection, either natural or sexual, could favor the evolution of different phenotypes if these are linked to local mating benefits or fitness optima in the adaptive landscape (Schmitt et al. 1999; Sinervo and Svensson 2002; Sih et al. 2004; Bell 2005).

Despite considerable efforts to elucidate mating strategies, few studies address how interpopulation environmental differences might affect the evolution of alternative mating strategies, which limits our understanding of the ecological causes of selection (Sinervo and Svensson 2002). Alternative male phenotypes are predicted to evolve under strong sexual selection (Wade and Shuster 2004). Furthermore, as Emlen and Oring (1977) originally proposed, the distribution of receptive female sets the strength and the opportunity for sexual selection (sensu Shuster and Wade 2003) and thus becomes an important element in mating-system evolution.

Alternative male mating strategies may be common within the pupal-mating clade of the genus of butterflies *Heliconius* (Hernández and Benson 1998; Deinert E, personal communication). Behavioral mating polymorphism in which

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larger males usually perform the characteristic pupal-mating behavior of the clade whereas small-sized males await adult females at territories has been observed in *Heliconius sara* (Hernández and Benson 1998). In *Heliconius heurtoni*, size-based morphological differences affect mating success during competition for pupae (Deinert et al. 1994). Both sexes are long lived and occupy home ranges where they seem to establish flying routes to seek flowers, oviposition, and eclosion sites (Turner 1971; Ehrlich and Gilbert 1973; Gilbert 1991). In pupal-mating species, females are usually monandrous, and mating takes place as the females begin to eclose from the pupal case (Brown 1981; Gilbert 1991; Deinert 1997). Pupation occurs on or near the host plant. Males search out female pupae and compete among themselves for the chance to perch on female pupae and to copulate with the emerging female (Deinert et al. 1994). Although pupal mating is an extreme form of emergence site guarding (Parker 1978), its evolution may have been triggered by monandrous females becoming receptive still as pupae, generating such an intense competition among males that females would benefit from exerting what has been termed “indirect mate choice” (Wiley and Poston 1996). This idea suggests that females may set the conditions to intensify intrasexual selection among males as a cheap mechanism to select the best mate without paying the costs of direct choice.

Here, we compare male mating strategies in 2 populations of *Heliconius charitonia* in relation to the strength and opportunity for sexual selection and suggest that female temporal synchronicity may be selecting for population differences in male mating strategies.

MATERIALS AND METHODS

Study species

Heliconius charitonia (Lepidoptera: Nymphalidae) is found along forest edges and in old second-growth areas from the southern United States to northern South America (DeVries 1987; Luis-Martínez et al. 2003). Life span may be as long as 4.5 months (Cook et al. 1976; Mendoza-Cuenca L, unpublished data). Their home-range behavior is well defined, and their movement rates are low (they are “tripliners”; Gilbert 1991). They establish daily foraging routes, visiting the same adult feeding plants and larval host plants (Brown 1981; Gilbert 1991). Additionally, *Heliconius* butterflies exhibit marked fidelity to night roosts (Turner 1971; Brown 1981; Waller and Gilbert 1982; Mallet 1986), these night roosting places are stable among years (Mendoza-Cuenca L, personal observation). Females oviposit single eggs in new shoots of numerous species of *Passiflora* (Gilbert 1991). In our study sites, the main host plant is *Passiflora adenopoda*. The larvae are solitary and go through 5 instars in approximately 3 weeks. They pupate also solitarily on or near the host plant (Brown 1981). Males from the pupal-mating clade of *Heliconius* visit host plants looking for mates (Turner 1971; Ehrlich and Gilbert 1973; Brown 1981). As it is common for other pupal-mating species, females of *H. charitonia* are monandrous. Females become receptive one day prior to eclosion at which time the pupal case turns clear and the wing pattern becomes visible. Mating may take place as the female begins to eclose from the pupal case (Gilbert 1991) or later if no mating took place at this point.

Study sites

We worked with 2 natural populations of *H. charitonia* in central Veracruz, México, one near the city of Xalapa (19°30'N, 96°57'W) and the other near the town of Zimpizahua (19°27'N, 96°58'W).

Xalapa site (mean annual temperature 18.8 °C, elevation 1350 m above sea level, mean annual rainfall 1492 mm): Fieldwork was carried out in a 76 ha cloud forest remnant (although only an area of 62 ha was surveyed), along 3 consecutive years during the months in which *H. charitonia* was active (August–December 2000, June–December 2001, and July–November 2002).

Zimpizahua site (mean annual temperature 19.2 °C, elevation 1150 m above sea level, mean annual rainfall 1926 mm, 15 km from Xalapa): Fieldwork was conducted in an abandoned and thus overgrown and well-shadowed 40-ha coffee plantation with abundant natural vegetation (characteristic of cloud forest) over the time period *H. charitonia* was active in 2002 (August to October).

In both study sites, *P. adenopoda* was the oviposition host plant to *H. charitonia*. Host-plant abundance, as well as the availability of growth meristems, the only oviposition sites used by *H. charitonia*, were similar between sites (Xalapa $n = 46$ host plants in 62 ha, 0.74 plants/ha, mean = 8.08 meristems/plant, 13.02 meristems/ha; Zimpizahua $n = 33$ host plants in 40 ha, 0.82 plants/ha, mean 7.27 meristems/plant, 12.1 meristems/ha).

Survey techniques

At both sites, most (ca. 98%) of the butterflies were captured, numbered, sexed, measured (see below), released, and later “recaptured” by visual record of their mark. Sites were visited daily from 8:00 to 15:00 h over the entire reproductive period in each population. Butterflies were numbered on both sides of either the right (males) or left (females) forewing using a fine permanent white marker. For each resighting, we identified the individual and registered behavior (see below), location, time, and resource use (host plant, floral resources). These data allowed us to identify individual home ranges, resource location, and daily activity patterns of males and females, described in detail in Mendoza-Cuenca and Macías-Ordóñez (2005).

Morphological measures

We used forewing length of the left wing (WL) as the main body size index. Thorax length (TL), and abdomen depth (AD), width (AW) and length (AL) were also measured using a caliper to the nearest 0.1 mm. All body measures were performed for Xalapa 2001–2002 seasons and all but TL for the Xalapa 2000 season. Only WL was measured at the Zimpizahua site; therefore, most of the comparisons between populations were based on this trait.

Female synchrony and availability

Every season we located the larval host plants present along female and male flying routes. We visited each plant at least twice a week and carefully inspected each for eggs, larvae, and pupae. Pupae were observed daily until the adult eclosed. Thus, for each season and population, we were able to track pupal sex ratio, development time, and the availability and synchronicity of receptive female pupae. We used the method proposed by Marsden and Evans (2004) in order to evaluate differences in synchronicity of receptive female pupae between populations. A synchrony index (SI) was calculated for each population, considering a period of 2 days of female receptivity (day of eclosion and the prior day). The Marsden SI estimates a confidence interval (CI, from 0% to 100%) of synchrony expected by chance using a null model, and compares the actual index value with such interval. Values higher than the CI denote higher synchrony than expected by chance; values under the CI denote that events are more evenly distributed in time than would be expected by chance. For the sex

determination of pupae that did not finish development, we used the genitalic scar present on the terminal abdominal segments (Scoble 1992; Deinert E, personal communication), otherwise sex was confirmed on adult emergence.

Individual male mating success

The behavior of each marked male that approached less than 10 m from a host plant was recorded according to operational definitions described in Table 1. Whenever one or more of these males visited a single pupa, we recorded and obtained for each male: 1) identity of all pupae visited, 2) the time he spent sitting on each female pupa or fighting with other males for a chance to sit on it, 3) the number of pupal cases punctured, and 4) the number of successful copulations. We calculated the widely used standardized selection differentials ("S") (Arnold and Wade 1984; Shuster and Wade 2003), in order to measure differences in male success. This is a measure of the strength of selection on the male size (mean wing size) along each phase of the mating process and to compare between both populations. We also estimated nonlinear (quadratic) selection differentials (C) for male wing size (successful vs. unsuccessful males) at the Xalapa site in order to evaluate the total effect in the variance of male wing size within a generation after adjusting for directional selection (Brodie et al. 1995). Finally, we assessed the effect of male size (wing size) and time defending pupae on mating success (one or more copulas vs. no copula) using a logistic regression.

Manipulation of female synchrony and availability

An experiment was conducted to evaluate male traits that could confer advantages in mating with flying virgin females. In October 2002, we simultaneously hung 50 (around 7-fold natural abundance, see below) nearly mature (clear pupal case) female pupae of similar size (28–30 mm) from 10 host plants (5 per plant) at the Xalapa site from among those plants

Table 1
Male behavioral categories and mating strategies

Categories	Operational definition
Visit	To fly less than 20 cm away from a pupa during more than 30 s without "sitting" on it.
Pursue	To fly over and behind another individual, less than 10 cm away
Sit	To alight on a pupa for at least 5 s
Fight	Two or more males sit simultaneously on a pupa, beating each other with their wings, seemingly trying to dislodge the opponent, also hovering seemingly trying to dislodge sitting males
Puncture	To introduce terminal segment of the abdomen into a female's pupal case.
Copulate	To insert genitalia into the female reproductive tract.
Hovering	To fly suspended over a pupa or host plant, less than 20 cm away, and for at least 10 s.
Courtship	"To pursue," flying over and behind an adult female, fluttering, and seemingly forcing it to land.
Strategies	
Prowling (pupal maters)	To constantly "visit" host plants, "hovering" larvae and female pupae, "pursuing" conspecifics of both sexes if they come closer than 1 m; sitting on pupae.
Patrolling (patrollers)	To constantly perform flight loops in the same area at least for 6 consecutive daily records in that area, pursuing conspecific males, never approaching host plants closer than 10 m

at which pupal-mating events took place the previous year. This also would test the ability of males to find pupae that they had not monitored during development. Pupae were obtained from a butterfly rearing facility we established near the study area, using local butterflies and native host plants to rear them ad libitum. Freshly emerged females were marked after eclosion and allowed to disperse. No males visited these pupae or copulated with the newly eclosed females. In order to track the mating history of these females, we intensively surveyed for them throughout the male distribution area over the following 12 days. We randomly chose feeding places, host plants, and patrolling areas and visited them daily in 30-min periods between 8:00 and 15:00 h. We recorded location and behavior of these females when found (flying, feeding, or copulating). At the end of the 12-day period, we caught and froze as many experimental females as possible ($n = 22$) for spermatophore count.

Data analysis

Coexistence of alternative mating strategies and the opportunity for sexual selection

Following Shuster and Wade (2003), the probability of invasion or coexistence by a novel mating strategy (patrolling in this case) can be estimated as the proportion of males (S) showing the alternative strategy that need to achieve matings in order for such strategy to remain in the population. The number of females required to copulate with "patrollers" (see Male Alternative Strategies below) in order for such strategy to coexist (by achieving a similar mating success, H) can also be estimated. As we know the proportion of mating (P_s) and nonmating pupal males (P_o) in each population and the number of patroller males in Xalapa, we calculated $P_{patrollers}$, $S_{patrollers}$, and $H_{patrollers}$, and thus the number of patroller males and adult females that would need to copulate in order for such strategy to coexist. We actually estimated 2 values for each of this parameters, the first one using only the number of pupal maters, and the other using both pupal maters and patrollers. This provided a value range of estimations under different assumptions. In the first one, we estimate the values needed to coexist assuming they are obtaining a mean mating success similar to that of patrollers. In the second case, the values obtained reflect what patrollers would need to invade a population starting from no mating success at all. We used only 2002 data because we only had data for both populations in that year.

We considered mating success a good estimate of male fitness because *H. charitonia* females are monandrous (Ehrlich and Ehrlich 1978), and egg production is not limited by the resources provided by the spermatophore but by pollen feeding supply, so that there is no predicted variance in female fitness related to male mating strategy. The intensity of sexual selection was estimated as the opportunity for sexual selection (I_{mates}) within pupal maters in each population following Shuster and Wade (2003).

Statistical analyses

All analysis were done using Statistica 5.5 (Statsoft) and R 2.7.2. (R Development Core Team).

RESULTS

During 258 field days at Xalapa (78 in 2000, 105 in 2001, and 75 in 2002), we marked 544 *H. charitonia*, 264 females and 280 males (73 females and 63 males in 2000, 96 females and 112 males in 2001, and 95 females and 105 males in 2002). During 35 field days in 2002 at Zimpizahua, we marked 50 females and 99 males.

Morphological measures

Size comparisons between sexes of the same population (WL, mean \pm SD) showed that females from Xalapa (44.7 ± 2.5 mm) were larger than males (41.9 ± 2.9 mm; $t = 11.9$, $df = 542$, $P < 0.001$), but no size difference was found between males and females from Zimpizahua (females: 43.0 ± 3.1 mm; males: 42.5 ± 3.1 mm; $t = -0.946$, $df = 147$, $P = 0.345$). In all 3 years at the Xalapa site, the size difference between males and females was similar; also, female WL distribution was right skewed (Chi-Square: 18.0, df adjusted = 8, $P = 0.02$), whereas males showed a discontinuous, seemingly bimodal distribution (deviation from normality Chi-Square: 21.8, $df = 9$, $P < 0.001$, bars in Figure 3b). At Zimpizahua, wing-length distributions of both sexes were not significantly different from a normal distribution (Chi-Square = 4.65, $df = 3$, $P > 0.05$).

Male alternative strategies

During daily visits, 207 males (73.9% of 280 marked males) at the Xalapa site were sighted at least 20 times, between 3 and 15 min each time. None of those males were observed to perform the 2 different mating strategies either within or between sightings. Nearly half of the males ($n = 98$) were observed “prowling” (see Table 1), visiting host plants, seemingly looking for receptive female pupae, and all of these performed the pupal-mating behavior characteristic of *Heliconius* (“pupal maters,” Deinert 1997). The other 109 males, hereafter called “patrollers,” were never seen in areas with host plants or pupae. They patrolled the same areas across years, during the same hours of the day and following the same area boundaries, seemingly looking for flying females. Less than 20 sightings per male were accumulated for the remaining 73 (26.9%) males marked in Xalapa, however, as described above, in no case was any of these males observed using 2 strategies. From daily visits at the Zimpizahua site, all 99 marked males were observed doing all behaviors characteristic of pupal maters. Each of these males was sighted at least 12 times, and 67 (67.6%) of them were sighted at least 16 times.

Furthermore, we performed 96 focal observations on individuals of both male phenotypes at the Xalapa site (48 of each phenotype) and 32 on Zimpizahua males. Individuals were chosen randomly and followed for up to 30 min or until the individual flew out of sight. In Xalapa, 69% were observed for 30 min, 19% between 15 and 20 min, and 12% less than 15 min. In Zimpizahua, 72% were observed for 30 min, 21% between 15 and 20 min and 7% less than 15 min. None of these males in either site were observed to switch strategies during focal observations.

The combined Xalapa data showed that males that frequented host-plant areas (mean forewing length \pm SD, 43.53 ± 1.79 mm) were larger than patrollers (40.25 ± 2.32 mm, $t = 9.92$, $P < 0.001$). Discriminant analysis using the full set of morphological traits (2000–2002 seasons) clearly distinguishes the 2 behavioral phenotypes (Wilk’s Lambda = ca. 0.22, $F(12,590) = 18.6$, $P < 0.0001$) further supporting the idea that morphology is tied to behavior. Pupal maters are morphologically more similar to females (Figure 1).

The daily starting time of the reproductive behavior for males (prowling or patrolling) and females (oviposition and pupal eclosion), showed significant differences ($F = 7.7$, $df = 3$, $P < 0.001$). Post hoc tests showed that oviposition was the last behavior to start in a given day. Pupal maters apparently start looking for mates around the same time females eclose, whereas patrollers cover areas without any host plant or pupa (i.e., patrolling areas) during such time period (Figure 2).

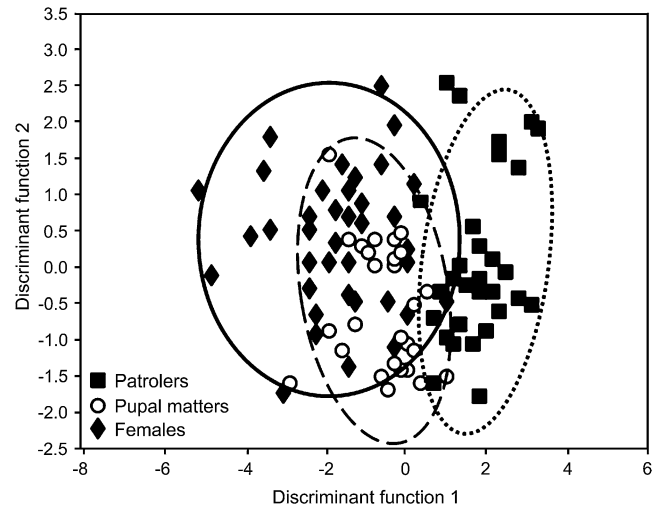


Figure 1 Discriminant analysis of morphological traits of *Heliconius charitonia*. Pupal-mating males (open circles), patrollers (filled squares), and females (filled rhombuses). Ninety-five percent confidence ellipses are shown for each sex and male morph (Xalapa population 2000–2002).

Female synchrony and availability

Because we were unable to locate all host plants at the Xalapa site until October 2000, we present 2001 and 2002 data for pupae. We found 57 pupae in Xalapa over this 2-year period and 49 pupae at Zimpizahua in 1 year. Of these, 21 females and 18 males successfully emerged at Xalapa, whereas 12 females and 10 males did so at Zimpizahua. Two or more available female pupae were never present simultaneously at Xalapa, whereas as many as 4 receptive female pupae, not separated by more than 15 m, were available at the same time at Zimpizahua (Table 2). The values of the SI (Marsden and Evans 2004) for each population showed that female pupae at Zimpizahua were more synchronized than expected by chance ($SI = 1.59\% > CI = 0.061\text{--}0.071\%$), whereas at Xalapa, they were less synchronous than expected by chance ($SI = 0.01\% < CI = 0.055\text{--}0.072\%$). The male: female-pupa ratios of each population were roughly similar (Table 2).

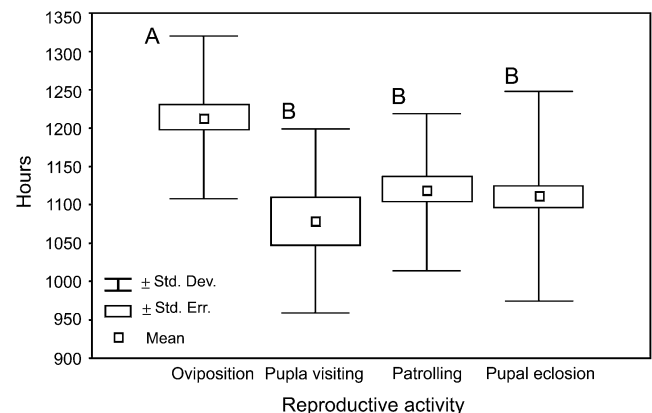


Figure 2 Starting time (mean \pm standard error) of each reproductive behavior at Xalapa site during the 2001 season. Letters indicate significant differences ($P < 0.05$) after a Tukey-Kramer post hoc tests. Oviposition $n = 71$, prowling $n = 46$, patrolling $n = 39$, and pupal eclosion $n = 15$.

Table 2
Availability and synchronicity of female pupae in both populations

	Site and year		
	Xalapa 2001	Xalapa 2002	Zimpizahua 2002
Total number of pupae	36	21	49
Eclosed female pupae	15	6	12
Eclosed male pupa	12	6	10
Receptive female pupae (mean \pm SD) when at least one was present	1.0 \pm 0.0 ($n = 15$)	1.0 \pm 0.0 ($n = 6$)	3.35 \pm 0.95 ($n = 12$)
SI (%)	0.01		1.59
Male: female pupa ratios	0.17		0.12

Individual male mating success

At the Xalapa site, each female pupa was visited throughout its development by as many as 11 (mean 6.5 ± 2.8) different males (Table 3). Males began sitting on a pupa as early as 3 days before eclosion (1.99 ± 1.00 ; Min = 0.1 day, Max = 3.86 days), and up to 5 males sat on a pupa simultaneously. In 20 of 21 observed mating events, 2 males punctured the pupal case simultaneously; although only one achieved copulation with the emerging female. Only one male copulated twice. Data did not show any advantage of arriving first because the first male to sit on a pupa copulated only in 6 of 20 events (2-tailed Binomial test for $q = 0.5$, $N = 20$, $k = 6$, $P = 0.12$). Daily visit frequency of individual males to each pupa did not predict their mating success (logistic regression, number of visits on mating success $N = 70$, $z = 0.47$, $P = 0.641$). The best predictors of pupal maters' mating success in Xalapa were the time males sat on a pupa, those that sat for longer always won (Logistic regression, time on pupa on mating success $N = 48$, $z = 2.65$, $P = 0.008$), and wing length once males started puncturing the pupal case, as the largest of the 2 always won (Logistic regression, wing length on mating success $N = 40$, $z = 2.77$, $P = 0.006$). From these results, it is clear that those males that sat for longer were the largest among those competing for each pupa.

At the Zimpizahua site, sitting on pupae started closer to female eclosion (Table 3). There were also differences between Zimpizahua and Xalapa in the size of males visiting each pupa (and marginally on their mean number), as well as in the size of males sitting on each pupa simultaneously. The total number of such males, however, was not different. Nevertheless, wing length failed short of significance predicting mating success of males puncturing the pupa (Logistic regression, number of visits on mating success $N = 23$, $z = 1.76$, $P = 0.079$).

Results of standardized selection differentials (SSDs; \pm 95% CI) for male wing size at different phases of the mating process

(sitting, holding, and mating) and in both populations were in accordance to the analysis of size differences. We found that selection on wing size was stronger in Xalapa if we consider 1) males that were able to sit on female pupae versus those that only visited (approached) them ($SSD_{Xalapa} = 0.42$, CI = 0.076, 0.76; $SSD_{Zimpizahua} = -0.03$, CI = -0.47, 0.41), 2) males that actually copulated versus males that only puncture the pupal case ($SSD_{Xalapa} = 1.04$, CI = 0.67, 1.12; $SSD_{Zimpizahua} = 0.83$, CI = 0.41, 1.26), 3) successful (copula obtained) versus unsuccessful males ($SSD_{Xalapa} = 1.13$, CI = 0.58, 1.49 $SSD_{Zimpizahua} = 0.78$, CI = -0.05, 1.03). While for patrollers (considering results of our female release experiment, see below), SSD values suggested sexual selection for size but in the opposite direction because we found higher and negative SSD values for patroller males that only pursued adult flying females versus those that actually mated ($SSD = -5.07$, CI = -8.77, -1.38).

Manipulation of female synchrony and availability

None of the 50 experimental pupae we hung were visited, sat on, or fought over; they were neither copulated as they eclosed nor observed flying near any host plant after eclosion. Twenty-seven of these virgin females were later observed flying in male patrolling areas, out of which 8 were pursued by at least 1 patrolling male, and 5 were seen mating with patroller males. As far as we know, this is the first record of mating for a flying female in *H. charitonia* in the wild, although it has been reported as anecdotes in culture facilities.

Our experiment showed that even relatively large patrollers, typically between 2 and 5 of them, chased adult virgin females when they entered patrolling areas. However, a logistic analysis suggests that size may be inversely related to mating success among patrollers (logistic regression, wing length on mating success $N = 20$, $z = -1.89$, $P = 0.06$). We caught and dissected 22 of these females (the 5 females observed mating were captured immediately after this observation, the remaining

Table 3
Population mating characteristics

		Populations (2002 season) (mean \pm SD)			
		Xalapa	Zimpizahua	<i>t</i>	<i>P</i>
Visiting males per pupa	<i>N</i>	6.3 \pm 2.5	7.4 \pm 5.9	1.97	0.056
	Size (mm)	43.8 \pm 1.6	42.41 \pm 3.2	2.85	0.004
Sitting males per pupa	<i>N</i>	3.4 \pm 0.9	3.8 \pm 0.8	1.18	0.25
	Size (mm)	43.5 \pm 1.5	42.4 \pm 3.2	2.45	0.015
Time sitting "prior" to female eclosion	Hours	47.8 \pm 23.9	17.1 \pm 11.2	5.1	<0.001

17 at the end of the 12-day period), and all of them contained a single spermatophore inside the bursa.

The positive value of the nonlinear selection differential ($C = 14.08$) using wing size of successful versus unsuccessful males at Xalapa suggests that disruptive selection was acting in this population, increasing the variance of this trait (Arnold and Wade 1984; Brodie et al. 1995).

Coexistence of alternative mating strategies and the opportunity for sexual selection

In the Xalapa population, 6 females were observed to mate with 5 of 60 pupal maters in 2002 ($P_{\text{pupal maters}} = 1 - (5/60) = 0.08$, $H_{\text{pupal maters}} = 6/60 = 0.10$ females/male, Table 4). Thirty-nine patrollers were recorded in the same population that year. For both strategies to coexist if they were genetically determined, the number of patrollers that would need to mate if they all mated once is approximately 3 ($0.08 \times 39 \approx 3$). Patrollers would have to mate with at least 4 females ($0.10 \times 39 \approx 4$) in order to achieve the mean mating success of pupal maters. In the same year, 12 of 99 pupal maters mated in Zimpizahua, each with only 1 female ($P_{\text{pupal maters}} = 1 - P_o = 1 - (12/99) = 0.12$; $H_{\text{pupal maters}} = 12/99 = 0.12$, Table 4). Not a single patroller was observed in Zimpizahua. The analysis pooling all males in Xalapa showed a lower value for $S_{\text{patrollers}}$ (0.05).

We also estimated a higher opportunity for sexual selection, I_{mates} , among pupal maters in Xalapa (2.86) than in Zimpizahua (1.00), and a much higher value when all Xalapa males were pooled (3.95).

DISCUSSION

Most alternative male mating behaviors seem to be threshold dependent polymorphisms that depend on the expression of a conditional trait (e.g., body size; Gross 1996). However, in some cases, alternative male mating behaviors are determined by environmental circumstances such as the spatial and temporal distribution of receptive females or the operational sex ratio in the population (Vicites et al. 2004).

In *H. charitonia*, as in many taxa including other *Heliconius* butterflies, male size may play an important role in mating success (Deinert, Longino and Gilbert 1994; Deinert 1997). At the Xalapa site, mean size of pupal maters and patrollers corresponded with the 2 modes in male size distribution (Figure 3b). Large size seems to give a performance advantage in male–male competition for access to the very limited female resource, and only the largest males visited, sat on and competed successfully for receptive female pupae. However, some patrollers were big enough to compete for female pupae, yet they did not do so (see overlapping size distributions of patrollers and pupal-maters in Figure 3b), whereas in the Zimpizahua population, even the smallest males

competed for pupae. Thus, in *H. charitonia*, size apparently does not completely determine male behavior; the mating system seems to result from site-specific differences in sexual selection influenced by variation in female availability. Presumably, various aerodynamic morphological traits other than size are highly correlated and can affect behavioral performance, foraging efficiency (Mendoza-Cuenca and Macías-Ordóñez 2005; a study on this population), mating abilities (e.g., flight performance), and mating success (Berwaerts and Van Dyck 2004; Lailvaux et al. 2004). Our discriminant analysis on the full set of morphological traits (Figure 1) suggests that shape more than size defines 2 cryptic morphs (sensu Cook and Bean 2006) with clearly distinct behavioral patterns.

In addition to the clear contrast in presence/absence of patroller males across the 2 populations, other clear difference was the synchrony of receptive female pupae (Table 2). Despite a scarcity of receptive female pupae in both populations (typical of pupal mating in *Heliconius*, Deinert 1997), our data point to interpopulation differences in receptive female pupae synchronicity (Table 2) associated with differences in the variance and mean male mating success, and in the strength and opportunity for sexual selection between populations. All this may explain the observed difference in male mating behavior. The higher temperature and annual rainfall associated with the lower elevation of Zimpizahua probably increases larval and pupal survivorship. This could increase the number of simultaneous pupae observed at this site because both populations had similar availability of host plants, similar in sizes and production of new shoots. In Xalapa, where receptive female pupae eclosion was asynchronous, fewer males could monopolize females, and only the largest males achieved copulas. This sets the conditions for invasion and coexistence of an alternative mating strategy in the population (see Shuster and Wade 2003; Table 4), small patrolling males in this case.

Although selection on pupal maters in Xalapa favors larger butterflies, selection on patrollers in this population seems to favor smaller males, thus suggesting 2 opposing selective forces acting on the evolution of mating strategies in *H. charitonia* (Figure 3). For pupal maters large body size, along a morph-specific wing shape, may confer endurance when competing for pupae. In *H. hewitsoni*, Deinert et al. (1994) found that larger males are better able to cover and defend pupae, which could also explain our results. Furthermore, larger males may store more reserves to endure long contests without food or water supply. The best “predictors” of mating success in the pupal maters of the Xalapa population were 1) the total time a male remained sitting on a pupa and 2) wing length once males started puncturing the pupal case. On the other hand, patrollers competed for adult virgin females by performing

Table 4
Coexistence of patrollers and opportunity for sexual selection

	Zimpizahua Pupal maters ($n = 99$) (observed)	Xalapa		
		All males ($n = 99$) (observed)	Pupal maters ($n = 60$) (observed)	Patrollers ($n = 39$) (estimated)
Successful males	12	5	5	2–3
Unsuccessful males	87	94	55	36–37
Copulated females	12	6	6	2–3
P_o	0.88	0.95	0.92	0.92–0.95
Mean mating success H ($H_{\text{patrollers}}$ to coexist)	0.121	0.058	0.1	0.06–0.10
$S_{\text{patrollers}}$ to coexist	0.12	0.05	0.08	
I_{mates}	1	3.95	2.86	—

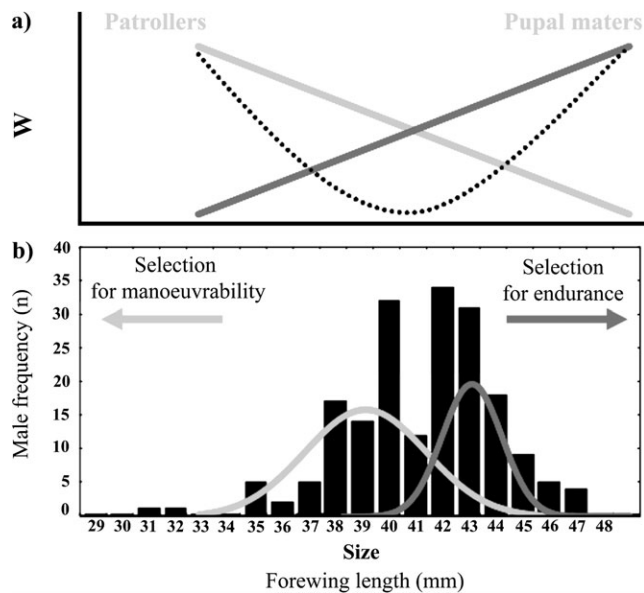


Figure 3

Alternative male mating strategies of *Heliconius charitonias*, patrollers versus pupal maters in 599 the Xalapa population: (a) Hypothetical fitness lines of males performing each mating strategy, the black dotted curve indicates the expected fitness function, estimated as mating probabilities. (b) Disruptive sexual selection acting on males of *H. charitonias*, male forewing length distribution using data of all 3 years is shown in bars. The size distribution of each strategy is shown using fitted curves. The 42-mm size class between the 2 highest frequencies (41 and 43 mm) was consistently underrepresented in all 3 years.

acrobatic flight. Small size apparently gives a mating advantage, perhaps due to higher maneuverability also associated to a morph-specific wing shape (Crompton et al. 2003; Mendoza-Cuenca and Macías-Ordóñez 2005). A fitness function predicted for the Xalapa population (Figure 3a), together with the positive value of the nonlinear selection differential for this population, would suggest disruptive sexual selection acting on male size, and as in other cases when selection acts in favor of extreme phenotypes, it could favor the evolution of a developmental switch that achieves 2 discrete phenotypes with no or few intermediates (Emlen and Nijhout 2000). A similar bimodal distribution occurs at least in another pupal-mating butterfly, *Heliconius hortense* (Mendoza-Cuenca L, unpublished data), which may suggest that if medium-sized males were too small to compete for female pupae and too large to compete for adult flying females, disruptive selection could be caused by a nonlinear costs and benefits function in pupal-mating species (Gadgil 1972). This would result in 2 adaptive peaks in the population associated with each male mating strategy. The fact that some males develop as patrollers even though they are large enough to be pupal maters suggests at least some genetic basis behind this alternative behavioral polymorphism, probably behind a condition threshold during development influencing which morph will develop.

Asynchrony in female availability seems to increase male-male competition and promote male patrolling behavior, or at least it seems to allow its coexistence with pupal maters. Different behavioral phenotypes could be migrating from different populations under different selective pressures and coexist only for a fraction of the year. This has been suggested for *Heliconius numata* (Joron et al. 1999), where polymorphic adults coexist given their high mobility although the color polymorphism may be the result of site-specific pressures in

different areas. However, the presence of an entirely (or mostly) patrolling population has not been documented in pupal-mating *Heliconius* species. The genetic bases, if any, of the alternative male mating behavior and associated morphology of *H. charitonias* are unknown, a fact that prevented a complete evolutionary analysis. However, this is not required for the measurement of selection on phenotypic characters (Arnold and Wade 1984). The size overlap of pupal maters and patrollers does not suggest either a threshold equal-fitness switch point nor a status-dependent decisions (as expected by conditional tactics); rather it suggests an alternative male mating strategy regulated by male mating behavior and somehow related to male morphology. However, whether the male behavioral dimorphism is due to phenotypic plasticity or to genetic polymorphism, does not compromise the evolutionary nor the ecological implications of our results.

The existence of an alternative mating strategy only in Xalapa may be explained by the low mean mating success within pupal maters because only a handful of males mate at all. Following Shuster and Wade (2003) and using our 2002 data, we estimated that considering all males or only the pupal maters, the percentage of mating success that patrollers would need to coexist in the Xalapa population is smaller than the required to invade Zimpizahua (Table 4). This means that even in the most conservative case, if only 2 of the 39 patrollers observed in Xalapa in 2002 mated with flying females, such a strategy would remain in the Xalapa population. Our experimental introduction of females confirmed that natural mating events involving patrollers may have gone undetected by us. We only observed 5 of at least 22 copulas that occurred during the experiment based on spermatophore counts, even though we were specifically seeking them out, whereas pupal-mating events were easily monitored once all the host plants in the area had been located. West-Eberhard (2003), however, has suggested that purely genetic determination of alternative morphs may be exceptionally rare; thus, their coexistence may not even require arguments of equilibrium or equal fitness. Our observation of adult females mating in *H. charitonias*, in addition to observations of adult courtships in other pupal-mating species such as *Heliconius erato* (Klein A, personal communication) and *H. hortense* (Mendoza-Cuenca L, unpublished data), suggests that alternative mating strategies may be common in *Heliconius* pupal-mating species.

We could expect to find a great variety of environmental conditions influencing female pupae availability over the geographic range of *H. charitonias*. The genetic basis of pupal mating and alternative male mating behaviors across populations in this and other *Heliconius* species offer a promising line of research. The links between environmental variables (e.g., female synchrony) and mating strategies in conspecific populations have seldom been studied, and even when they have, a clear association has seldom been found (Jones 2002). One interesting prediction derived from our hypothesis of disruptive selection by female asynchrony is that alternative mating strategies will be more common in *Heliconius* species with solitary larvae (e.g., *H. charitonias*), than in more gregarious species (e.g., *Heliconius sapho*) because females would pupate closer in space and time. Interpopulation differences such as the ones described in this paper are probably common and offer the best opportunities for such exploration.

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