DIVERGENT NATURAL SELECTION AND MÜLLERIAN MIMICRY IN POLYMORPHIC *HELICONIUS CYDNO* (LEPIDOPTERA: NYMPHALIDAE)

by

DURRELL D. KAPAN

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Abstract

NATURAL SELECTION favours bright colours or bold patterns that advertise unpalatability. In a noxious polymorphic species frequency-dependent selection should lead to fixation of the common morph, because rare morphs suffer relatively higher attack rates by naive predators. This generally leads to warning colouration that is monomorphic within species and shared between species (Müllerian mimicry). However, several unpalatable species of *Heliconius* butterflies (Lepidoptera: Nymphalidae) exhibit polymorphic warning colouration within a population. One possible explanation is that divergent selection may favour different colour-pattern morphs of a single unpalatable species if each matches a different warningly coloured unpalatable Müllerian mimic species (comodels).

In this thesis I explore this hypothesis by investigating the genetic basis and fitness consequences of polymorphism for warning-colour pattern within a single species of *Heliconius* butterfly, *H. cydno.* In Western Ecuador, *H. cydno* is polymorphic for colour (yellow versus white), pattern (triangle versus band), and hind-wing band-width. I find that *H. cydno*'s colour-pattern polymorphism has a simple Mendelian genetic basis. Two alleles at a single locus with complete dominance determine colour differences (white alleles dominate over yellow). Pattern differences are slightly more complex: a single locus with three alleles (and complete dominance) or two epistatic diallelic loci can account for the variation. Relative hind-wing band-width may have a polygenic basis. Using the multi-site transplant experiment, I find that divergent selection favours transferred colour-morphs of *H. cydno* (yellow or white) that resemble their putative Müllerian comodels (*H. eleuchia* or *H. sapho* respectively). This provides unique experimental evidence for the benefit of Müllerian mimicry. Divergent selection generated by the two comodels may promote maintenance of the

colour-pattern polymorphism in *H. cydno*. In support of this hypothesis, I found the frequency of yellow *H. cydno* correlates with the density of their respective comodels (yellow *H. eleuchia* or white *H. sapho*) at different locales. *H. cydno* is also polymorphic for colour and pattern where it occurs in the local absence of comodels. Polymorphism at these sites indicates that gene-flow, reduced selection, or both helps maintain colour-pattern diversity when comodels are absent.

My research suggests that Müllerian mimicry can develop between a single species and more than one comodel taxon. In Western Ecuador, two *Heliconius* species appear to generate divergent selection favouring polymorphism in a third species *H. cydno*.

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Introduction to polymorphic Müllerian mimics

Introduction

NOXIOUS OR UNPALATABLE prey taxa often display bright colours and bold patterns to ward off visually hunting predators (Wickler 1968). Aposematic (warning) signals are subject to positive frequency-dependent or aposematic selection; in a variable species, benefits to individuals possessing a given warning signal increase as the signal becomes more common (Greenwood et. al. 1989). In other words, common morphs suffer fewer attacks (per capita) from uneducated predators. Aposematic selection, under these conditions, leads to warning-signal monomorphism within species and signal sharing between species (i.e., Müllerian mimicry, Müller 1879; Turner 1977). Müllerian mimicry between brightly coloured unpalatable insects is common in tropical environments (Wickler 1968; Owen 1971). Müllerian mimic species (known as comodels) share warning signals effectively reducing the number of predator attacks (per species) necessary to educate naive predators (Turner, Kearny, and Exton 1984). Many *Heliconius* (Lepidoptera: Nymphalidae) butterfly species inhabiting the Central and South American tropics display the same warning-colour pattern (Mallet and Gilbert 1995; Turner and Mallet 1996). An extreme example of this phenomenon is mimicry between *H. erato* and *H. melpomene* that share warning colouration in sympatry and have concordantly diverged in allopatry. In other words, each race of *H. erato* matches a race of *H. melpomene* within a local area, although races of both species differ radically between different localities in Central and South America (Sheppard et al. 1985). The only regions where more than one colour pattern exists within populations are along shared hybrid zones between different coloured races of each species (Turner 1971; Mallet 1986, 1989; Mallet et al. 1990).

In contrast, some species of unpalatable aposematic insect taxa, including several species of unpalatable *Heliconius* butterflies, display more than one warning-colour pattern sympatrically (Turner 1968a,b; Brown and Benson 1974; Linares 1996). The maintenance of polymorphism in these species is problematic because they should be unstable. Frequency-dependent selection should drive extinct those morphs with rare warning-colour patterns (Turner 1984). Several hypotheses have been advanced to account for this unusual phenomenon. Many of these distasteful polymorphic *Heliconius* species resemble other noxious warningly coloured butterflies. This leads to the hypothesis that Müllerian mimicry with more than one comodel species may promote warning-colour polymorphism. If this is true, different morphs may be subject to divergent selection to match different comodels (Brown and Benson 1974).

Heliconius cydno is a relatively common species in primary and mature second growth forests in Central and NW South America. Data from field observations and cage experiments in Costa Rica indicate that *H. cydno* are avoided by specialized insectivorous predators such as the rufous-tailed jacamar and hence are unpalatable (Chai 1986, 1990, 1996). Throughout most of its range *H. cydno* is monomorphic and occurs with one other unpalatable species of *Heliconius* butterfly (a comodel), which displays an identical warning signal (Brown 1979; Brown 1981; Linares 1996). Twelve out of the 16 described *H. cydno* races match one locally occurring *Heliconius* comodel (Brown 1979; Table 1.1). Two of the remaining four described *H. cydno* races occur in Columbia and two races occur in Western Ecuador. In the Cauca Valley of Colombia, polymorphism in *H. cydno* is thought to be transient due to habitat-induced changes in comodels during this century (Linares 1997). In Western Ecuador *H. cydno* morphs resemble one of two comodels found in sympatry (Chapter 4).

Across lowland Western Ecuador, south of 1° N latitude, *H. cydno* occurs in two colour (yellow and white) and two pattern (triangle and band) forms resulting in four different colour-pattern phenotypes (Figure 1.1). Western Ecuadorian *H. cydno* are polymorphic in the classic sense (Ford 1940); field caught females from these populations produce broods containing both colours and patterns (Chapter 2). In Western Ecuador, two of *H. cydno*'s colour-pattern morphs (yellow-triangle and white-band) appear to match two different monomorphic warningly-coloured *Heliconius* species with which they are broadly sympatric: *H. eleuchia* and *H. sapho* respectively (Figures 1.1, 4.1). One *H. cydno* population in NW Ecuador (Maquipucuna) and all populations south of 1° S along the Andean foothills in Southern Ecuador are monomorphic for yellow (Figure 1.1, 4.1). These all-yellow populations are dominated by triangle forms and are sympatric with only one comodel, yellow *H. eleuchia* (Figure 1.1, Appendix 4.1). Finally, in NW Ecuador several sites harbour dense polymorphic populations of *H. cydno* in the absence of either comodel (Figure 1.1, Table 1.1). The maintenance of polymorphism at these locales is difficult to explain, because positive frequency-dependent selection should lead to local extinction of rare morphs.

In this thesis I examine warning-colour polymorphism in *Heliconius cydno*: its genetic bases, selective consequences, and patterns in space and time. First, Chapter 2 investigates the genetic basis for *H. cydno*'s polymorphism for wing colour and pattern. Building on past *Heliconius* genetics research (Sheppard et. al 1985; Mallet 1989; Nijhout, Wray, and Gilbert 1990), I investigate the Mendelian inheritance of colour pattern using a series of crosses I performed at the University of Texas in Austin with the aid of Dr. L. Gilbert. I also look for quantitative variation in another trait potentially relevant to mimicry, that of relative hind-wing band-width of *H. cydno* (Figure 2.1).

In Chapter 3, I test whether Müllerian mimicry exists between *H. cydno* morphs and their putative comodels. The goal of this chapter is to identify whether divergent selection is a feasible mechanism promoting polymorphism. By modifying a transfer protocol developed by Mallet and Barton (1989a), I test whether divergent selection favours *H. cydno* morphs that match the most common comodel at target sites.

Divergent selection to match more than one local comodel may be responsible for *H. cydno*'s polymorphism. This is the polymorphic Müllerian mimicry hypothesis. In Chapter 4, I use observational data on *H. cydno* morph frequency at endangered rain forest sites across Western Ecuador over five years to test several predictions of the polymorphic Müllerian mimicry hypothesis.

In Chapter 5, I compare the results of my research with those studies on other species of polymorphic Müllerian mimics in *Heliconius* and other butterfly taxa. I briefly summarize polymorphic Müllerian mimicry in Western Ecuadorian *H. cydno*. I conclude by discussing new opportunities to study the evolution of complex traits using polymorphism in warningly coloured taxa.

Figure 1.1 Two colours (rows) and two patterns (columns) of *H. cydno* morphs and the two putative comodels, *H. eleuchia* (upper left) and *H. sapho* (lower right). The first column is yellow and the second column is white. The first *H. cydno* row displays the triangle phenotype (a triangular yellow or white area that continues from the post-medial forewing band into the end of the discal cell adjacent to the anterior edge of cells M3 and Cu_1 [the absence of melanic scales in the discal cell see ↓ and Figure 2.1b]). The second *H. cydno* row displays the band phenotype (band denotes the presence of melanic scales in the entire discal cell creating an unbroken post-medial band of either yellow or white across the forewing immediately outside the discal cell see ⇒). The hind-wing band-width is measured along the M3 vein (see ⇑ and Figure 2.1b).

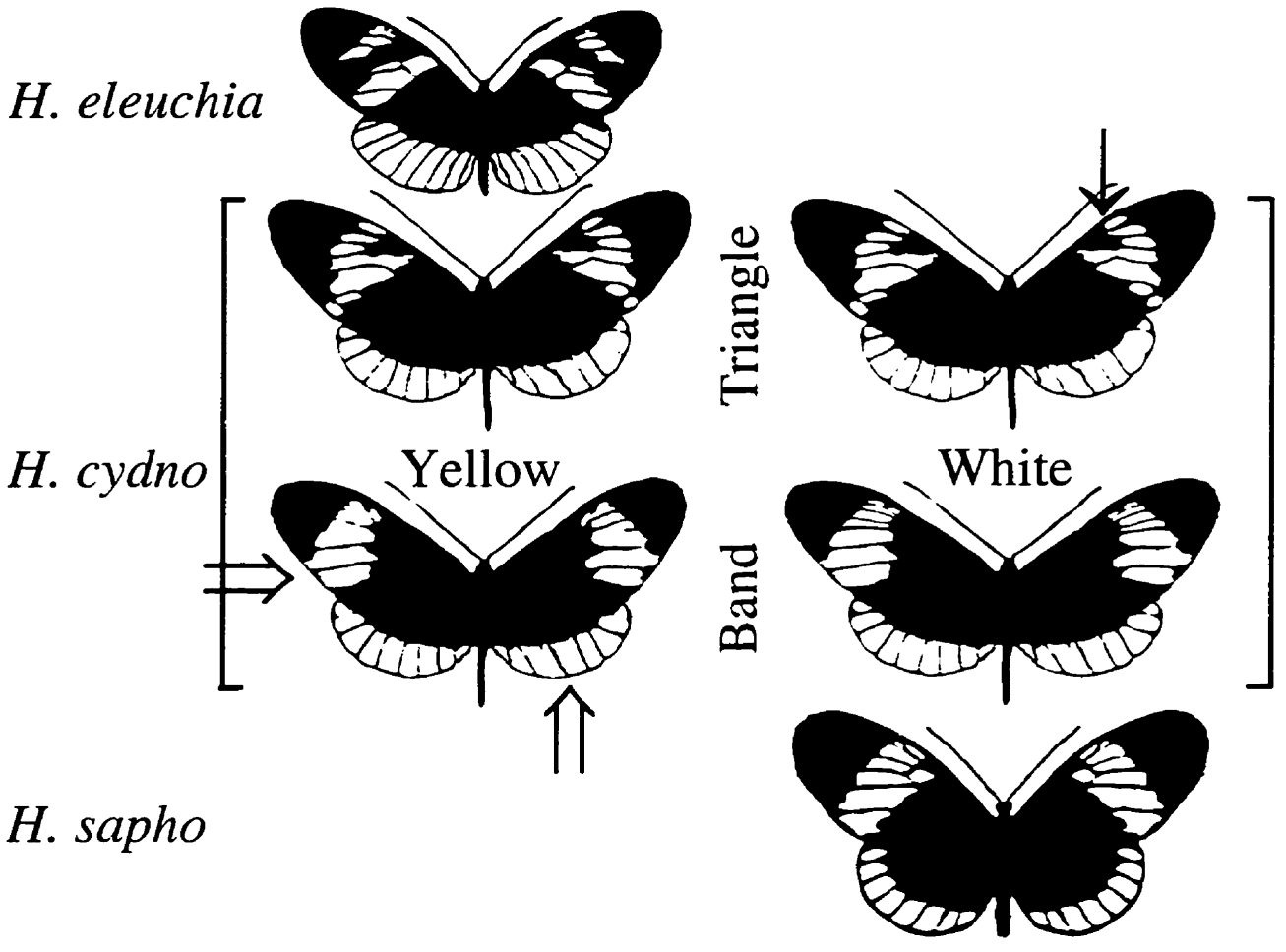

Figure 1.1 Two colours and two patterns of *H. cydno* morphs and the two putative comodels, *H. eleuchia* and *H. sapho*. The first column is yellow and the second column is white. The first *H. cydno* row displays the triangle phenotype (see ↓). The second *H. cydno* row displays the band phenotype (see ⇒). The hind-wing band-width is measured along the M3 vein (see ⇑).

Table 1.1 Races of *H. cydno* and their hypothesized comodels in Central and South America.

Comodels			*Heliconius cydno*[1]			
Genus	Species	subspecies/ race	Species	subspecies/ race	Notes	Reference
Heliconius	*sapho*	*leuce*	*cydno*	*galanthus*		Brown 1979
Heliconius	*eleuchia*	*eleuchia*	*cydno*	*chioneus*		Brown 1979
Heliconius	*hewitsonii*		*pachinus*		[1] *H. pachinus* is considered conspecific with *H. cydno* (Brower 1996, Gilbert *pers. comm.*)	Brown 1979
Heliconius	*sapho*	*sapho*	*cydno*	*cydno*		Brown 1979
Heliconius	*eleuchia*	*eleuchia*	*cydno*	*cydnides*		Brown 1979
Heliconius	*eleuchia*	*eleuchia*	*cydno*	*ssp. nov.*	may be sympatric with *H. congener ocannensis* (Brown 1979)	Brown 1979
Heliconius	*congener*	*ocannensis*	*cydno*	*cordula*	based on range maps in Brown (1979)	Brown 1979
Heliconius	*sapho*	*sapho*	*cydno*	*barinasensis*	may be sympatric with *H. congener ocannensis* (Brown 1979)	Brown 1979
Heliconius	*eleuchia*	*eleusinus*	*cydno*	*zelinde*	*H. e. eleusinus*, *H. s. chocoensis*, and *H. c. zelinde* adopt a common	Brown and Benson 1975
Heliconius	*sapho*	*chocoensis*	*cydno*	*zelinde*	color pattern in the Choco area of SW Colombia	Brown and Benson 1975
			cydno	*hermogenes*	may be involved with *weymeri/gustavi* forms	Brown 1979
Eltzunia	*humboldt*	*regalis*	*cydno*	*weymeri*	forms *weymeri*	Linares 1996
Heliconius	*erato*	*chestersonii*	*cydno*	*weymeri*	forms *gustavi*	Linares 1996
Heliconius	*congener*	*aquilionaris*	*cydno*	*ssp. nov.*		Brown 1979
Heliconius	*eleuchia*	*primularis*	*cydno*	*alithea*	Known as triangle form and is polymorphic with *haenschi*	Brown 1979
Heliconius	*sapho*	*candidus*	*cydno*	*haenschi*	Known as band form and is polymorphic with *alithea*	Brown 1979

The genetics of warning colour in polymorphic *H. cydno*

Introduction

NATURAL SELECTION favours bright warning colours in unpalatable prey (Greenwood et. al. 1989). Different unpalatable prey taxa share a common warning colour because rare variants, not recognized by insectivorous predators, are injured or killed (Brown 1972; Greenwood et. al. 1989; Chai 1990). This signal sharing, which is exhibited by a wide variety of insects, is Müllerian mimicry (Müller 1879). Resemblance between Müllerian mimic species, such as between different species of *Heliconius* butterflies (Lepidoptera: Nymphalidae), is thought to be due to the contribution of relatively few unlinked genes (Turner 1977; Sheppard et al. 1985). In contrast to the majority of Müllerian mimics, several unpalatable species of *Heliconius* butterflies exhibit distinct warning-colour polymorphisms (Brown and Benson 1974; Mallet et al. 1990; Turner 1968a). The genetic bases of these enigmatic polymorphisms are poorly known.

I investigate the genetic basis of polymorphism in an unpalatable species, *H. cydno*, from Western Ecuador. Throughout most of its range *H. cydno* co-occurs with at least one other unpalatable species of *Heliconius* butterfly (a "comodel") that displays a nearly identical warning signal (Brown 1979, 1981). Twelve out of 16 different *H. cydno* races are monomorphic and match a single unpalatable comodel species (Brown 1979). The remaining *H. cydno* races described in Brown (1979) are polymorphic in the classic sense of "the occurrence together in one habitat of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation" (Ford 1940 quoted in Ford 1953: 44). Ford includes both transient and balanced polymorphisms in this definition (Ford 1953). An example of the former is *H. cydno weymeri* in the Cauca Valley of Colombia, where one colour-pattern morph,

"*weymeri*," historically co-mimetic with a now extinct comodel species, *Elzunia humboldt regalis*, has been replaced in this century by another morph, "*gustavi*," apparently co-mimetic with the currently common *H. erato chestertonii* (Linares 1997).

A possible balanced polymorphism exists in a geographically separate population of *H. cydno* in Ecuador. Across lowland Western Ecuador, there are two colour morphs of *H. cydno* (yellow vs. white). Each colour morph has one of two common forewing patterns (triangle vs. band), making a total of four colour-pattern morphs. The pattern morphs were previously described as races, the "triangle" morph as *H. c. alithea* Hewitson and the "band" morph as *H. c. haenshii* Riffarth (Figure 1.1). However, these four morphs freely interbreed and I will refer to them only by colour and pattern. Intermediates between triangle and band are present in the field but are rare (< 4%). Two of these colour-pattern morphs of *H. cydno* apparently mimic two monomorphic *Heliconius* species with which they are broadly sympatric: the yellow-triangle morph of *H. cydno* mimics *H. eleuchia*, and the white-band morph of *H. cydno* mimics *H. sapho*, respectively (hereafter referred to as comodels, Figure 1.1). Hence mimicry may help maintain this polymorphism.

The main objective of this study is to determine the number of genes responsible for *H. cydno* colour, pattern and hind-wing band-width variation. Determining the number of loci and alleles that code for *H. cydno* colour and pattern differences has three main benefits. The genetic data are inherently interesting because mimicry theory suggests the evolution of a new warning-colour pattern from an old pattern requires major mutations that provide a "rough-and-ready" resemblance to a new model (or comodel species Turner 1977, Sheppard et. al. 1985). Are different morphs of *H. cydno* due to few loci of major effect or many loci each of small effect (Mallet 1989; Linares 1996)? Are

these genes linked to form supergenes or are they unlinked? Second, understanding the genetic bases of colour and pattern inheritance allows a test of hypotheses about Müllerian mimicry's effect on the four colour-pattern morphs in the field by measuring allele frequency change and gametic correlations estimated from field counts of each butterfly morph (Mallet et. al. 1990). Finally, discerning genetic correlations between colour-pattern elements will ultimately lead to insight into how complex characters, such as mimetic warning-colour patterns, evolve.

Hypotheses

Existing *Heliconius* genetic data indicate that alleles for white are normally dominant to those for yellow (Gilbert et. al. 1988) and that these colours constitute a background on which dominant alleles (or epistatic genes) for black or red scales form patterns (Nijhout, Wray and Gilbert 1990). Based on these earlier findings I assess one hypothesis to explain colour variation and three hypotheses to explain pattern variation in Western Ecuadorian *H. cydno.*

The colour hypothesis is that differences are produced by two alleles (white [*W*] and yellow [*w*]) at a single locus, with *W* dominant and *w* recessive (Gilbert et. al. 1988). The three pattern hypotheses are as follows. First, the three pattern phenotypes are produced by two codominant alleles (*B* and *b*) at a single locus, giving the genotypes *BB* for band, *Bb* for intermediate and *bb* for triangle. Codominance of mimetic traits is common in hybrids between *Heliconius* races and species (Mallet 1989; Nijhout, Wray and Gilbert 1990). In the second hypothesis *B* is dominant to *b*, and intermediate is produced by a third allele *I*, which is dominant to *b* and recessive to *B*. The third hypothesis is that *B* is dominant to *b*, but intermediates are produced by a modifier gene with two alleles (+ or -) modifying the pattern (the + allele epistatic to the *b* allele or the - allele with no effect on *b*), giving nine possible genotypes (six of which are

shown in Table 2.2f). This is also consistent with the dominance relationships described by Gilbert and Nijhout (Gilbert et. al. 1988; Nijhout, Wray and Gilbert 1990).

Mimicry between *H. cydno* and its two comodels may favour a genetic correlation between colour, wing pattern and relative hind-wing band-width. In Western Ecuador, *H. eleuchia* has a wide hind-wing band, and *H. sapho* has a narrow hind-wing band-width (Figure 1.1). Phenotypic correlations between colour and pattern in wild populations are discussed in Chapter 4. In this chapter, I ask two questions about hind-wing band-width: 1) Is hind-wing band-width heritable? 2) Is the relative size of the hind-wing band correlated with the hypothesized genotype at the other colour and pattern loci described above?

Materials and methods

Captive rearing

I captured butterflies in Western Ecuador at Finca El Copal (0° 53' S 79° 05' W) in late August of 1993 and 1994. I packed live butterflies in new glassine envelopes and sealed them in plastic containers containing moist chemical free tissue. These containers were stored in a 12V DC auto cooler maintained at 18° C. I transferred butterflies between El Copal and Quito, Ecuador, by vehicle over one night. The following morning, I immediately flew them to Austin, Texas. Butterflies were released in rooftop greenhouse insectaries at Patterson Labs, University of Texas in Austin.

I individually marked all butterflies on the ventral hind-wing margin with a Sharpie ultra fine-point pen. Eggs from captured females were collected and reared in a growth chamber on suitable *Passiflora* host plants. This provided freshly eclosed virgin female progeny for crosses. Virgin females were numbered, scored for phenotype, and placed in greenhouses with either a yellow-triangle, white-triangle, or white-band male and monitored hourly until

mated. Females typically mate within the first 24 hours and remain *in copula* for several hours to over a day. After each female butterfly mated, I recorded the ID number and phenotype of the male butterfly. I isolated females in individual 2x2x3 meter cages and provided them with fresh plant material for oviposition. Eggs were collected daily from isolated females and reared separately in 50ml plastic cups. Larvae were provided with fresh *Passiflora* leaf material on a daily basis. Late instar larvae were reared in three to four L plastic containers to facilitate pupation. I marked and photographed each offspring and recorded its phenotype and ID number. These individuals were either returned to greenhouses to become part of the stock, retained for crossing, or frozen for later analysis.

Phenotypes

Heliconius wings consist of light and dark scaled areas (Gilbert et. al. 1988). Light areas of the fore- and hind-wing of *H. cydno* butterflies are either white or yellow. In *H. pachinus* (considered conspecific with *H. cydno* by L. E. Gilbert pers. comm.) yellow is produced when the pigment 3-hydroxykynurenine binds with a peptide or small polypeptide keeping it in an alkaline state (Gilbert et. al. 1988). White is produced when 3-hydroxykynurenine is not bound to the peptide (Gilbert et. al. 1988). Melanic scales mask underlying yellow, white or brown scales (Gilbert et. al. 1988). Melanin is usually expressed in discrete patches thought to be generated by serially repeated "pattern elements" found across the fore- and hind-wing of Nymphalid butterflies (Nijhout, Wray and Gilbert 1990). The "triangle" form *H. cydno* has a triangular white or yellow area that continues from the white or yellow post-medial forewing band into the end of the discal cell adjacent to the junction of the M3 and Cu_1 veins (see Figure 2.1b). The triangle refers to an absence of melanic scales in the end of discal cell (i.e., white or yellow scales, see Figure 2.1b). The "band" form denotes the presence of melanic scales in the entire discal cell. Visually, these melanic scales frame an

unbroken post-medial band of either white or yellow across the forewing immediately outside the discal cell. Black scales cover all the discal cell and a portion of the adjacent area between veins Cu_1 and Cu_2 (see Figure 2.1b). Intermediates between triangle and band were rare in most collections from Western Ecuador (< 4%). These individuals had partial expression of the full-band characteristic in the area of the discal cell normally expressing white or yellow scales in the triangle state (see Figure 2.1b). All pattern morphs have either white or yellow scales on the trailing edge of the hind-wing (Figure 2.1c).

I measured relative hind-wing band-width (RHWBW) as a ratio of the width of the white or yellow band (BW) on the trailing edge of the hind-wing measured along the M3 wing-vein to the total length of the M3 wing vein (BW/M3, see Figure 2.1c). Since the BW is always less than M3, I used the arcsine square-root to transform RHWBW for all statistical tests (Zar 1984). An alternative measure of the relative size of the hind-wing band would be the residuals from a regression of hind-wing band-width (BW) on M3 length (as defined above). Both measurements led to identical results, and I present results from only the former. Residuals, which depend on the total population in the regression analysis, do not facilitate between-population comparisons.

Crossing design

The crossing design was initially motivated by quantitative genetics methods (Lande 1981). I began by hand selecting the field-caught *H. cydno* population in two extreme directions using greenhouses to isolate them. After selection, I planned to perform crosses to estimate the number of loci and alleles responsible for the colour and pattern polymorphism and variation in relative hind-wing band-width. However, one selected line repeatedly went extinct so I report the results of incidental crosses made during the selection phase of the study. Both the selection phase and the crossing phase provide useful information on the nature of inheritance of warning-colour pattern

polymorphisms in *H. cydno*. Crosses of homozygous recessive and homozygous dominant genotypes at all loci (followed by subsequent F_1, F_2 crosses and back-crosses) provide the best estimate of the number of loci and alleles responsible for the colour and pattern differences (Lande 1981). If white is dominant to yellow and band is dominant to triangle (Gilbert et. al. 1988), then an optimal crossing design would begin with homozygous recessive (yellow-triangle) and homozygous dominant (white-band) individuals (Lande 1981). To create these homozygous lines I utilized two different greenhouses to select for the extreme colour-pattern morphs (yellow-triangle and white-band) thought to be co-mimetic of *H. eleuchia* and *H. sapho*, respectively.

Using the 1993 butterflies, I selected for yellow-triangle morphs by placing them alone into greenhouse number 8 and removing all white and/or band offspring that arose from the field-mated yellow-triangle females. I selected for white-band morphs from the initial 1993 butterflies by isolating them in greenhouse 6 and subsequently removing all yellow and/or triangle offspring. In the fall of 1994, I added additional El Copal field-caught butterflies to greenhouses (yellow-triangle to greenhouse 8, and white-band to greenhouse 6) and again applied selection to purify these two morphs. This was especially important in greenhouse 6 because artificial selection applied during the previous year had reduced this population to near extinction. I continued selecting for homozygous white-band butterflies during the fall 1994 and spring 1995, but I was unable to remove putative recessive colour or pattern alleles from the initial population (due to the strong selection applied which reduced the population size and may have led to inbreeding). The loss of the white-band butterflies in greenhouse 6 during the summer of 1995 prevented the planned experimental pairings. The pure yellow-triangle butterflies were not maintained after this time. During the fall of 1994 and the spring of 1995 I made incidental crosses to

explore inheritance of colour and pattern in *H. cydno*. The results of these unplanned crosses are reported here.

Newly eclosed females were crossed to white-triangle, white-band or yellow-triangle males by temporarily placing these females in greenhouses 4 (mixed population), 6 or 8, respectively. Periodic observations of these females were made. Matings are hard to miss because males clasp the female for several hours to one day. All matings were recorded and males that were observed clasping females were marked with a dot of coloured indelible ink to facilitate subsequent identification, recapture, numbering, and measurement. Females that did not mate in 24 to 48 hours were removed and excluded from the analysis. Parental genotypes from these crosses were inferred according to the assumptions of each genetic hypothesis being tested. First, dominance relationships were confirmed by the ease with which pure phenotypes were achieved by selection in greenhouse 8 (yellow-triangle) and difficulty in greenhouse 6 (white-band). Second, obvious segregation for colour or pattern in broods resulting from parents who shared the same colour (white) or the same pattern (band) confirmed dominance assignments. For example, the two white parents of brood #7 produced both white and yellow offspring (Table 2.1). This is consistent with white being dominant to yellow. Both parents must have been heterozygous for colour.

I assessed the likelihood of a given hypothesis by analyzing broods from parents whose genotypes are inferred from families with mixed offspring; if genotypes cannot be inferred the cross was excluded. This method is potentially biased, because small broods resulting from crosses that would normally produce offspring of mixed colour and pattern may fail to do so (Weir 1990). Maintaining a constant family size could eliminate this bias by allowing adjustment of expected offspring ratios (Weir 1990). However, this was not possible owing to small sample size.

Statistical Tests of Genetic Models

I compared frequencies of offspring phenotypes from crosses to expected offspring frequencies predicted by the different hypotheses for colour and pattern inheritance (Table 2.2). Expected frequencies for the one-locus two-allele hypothesis for colour are found in Table 2.2a. Those for pattern are found in Table 2.2b-f. Three hypothesized heterozygous one-locus three-allele genotypes (*BI*, *Bb*, *Ib*) and the one homozygous recessive genotype (*bb*) can produce nine possible mating combinations (those consistent with the brood results are shown in Table 2.2e). The two-locus modifier hypothesis predicts the ratios shown in Table 2.2f.

I use *G*-tests to assess the goodness of fit of offspring frequencies to particular hypotheses. The *G*-test statistic is equivalent to twice the difference between the *ln*-likelihood of the data and the expected offspring counts (Zar 1984). The *G*-test statistic's additivity property allows data from different broods that bear on the same hypothesis to be combined or partitioned (Edwards 1992; Mallet 1989). First, I combined broods whose parent's inferred genotypes had the same expected offspring ratio. The *G*-statistic for the combined broods is then compared to a Chi-square distribution with degrees of freedom equal to the number of phenotype classes (columns) minus one within each inferred genotype (rows in Table 2.2). Each genetic hypothesis also predicts different ratios for each different inferred parental genotype. Thus, an overall test of the hypothesis is equivalent to the sum of the individual (row) *G*-statistics with the sum of the degrees of freedom from each test statistic minus 1 (e.g., the first two rows in Table 2.2a; Mallet 1989). One degree of freedom was lost for crosses resulting in all recessive offspring (either colour or pattern) because models compared here share the same predictions with respect to all recessive offspring. Any strong departures from the overall hypothesis would show up in the sum *G*-statistic (Edwards 1992). Contingency tests such as the *G*-test can be sensitive to

low expected cell counts but are more robust than χ^2 tests (Zar 1984). Corrections for small sample sizes were not used because this destroys the additivity of the test statistic, and low cell counts are ameliorated in a combined test (Mallet 1989). Zero cell counts in any one row give no information and thus are not included in calculating the *ln*-likelihoods; however, the lack of observations where zeros are expected lends powerful support a given genetic hypothesis (compare expected ratios with data in Table 2.2). Mallet (1989) also simulated small sample size 2x2 contingency tables and found a minimum sample size of 11 gave a nominal significance of $P = .02$ - $.09$ for $G_1 = 3.84$, $P < .05$.

Given these problems and the preliminary nature of these data, I use these methods to discuss the hypotheses for which the data are consistent and highlight how future research can help distinguish between the remaining alternatives.

Heritability and genetic correlations of relative hind-wing band-width

Heritability estimates were calculated from mid-parent and mean family values of relative hind-wing band-width. To assess genetic correlations between RHWBW and colour-pattern genes, I arranged genotypes inferred from crossing results (see results below) on a linear scale from fewer to more alleles coding for yellow and triangle patterns. This allows a preliminary test for an association between hind-wing band-width and the genotypes at the colour-pattern loci. Colour was assumed to be one-locus with two alleles (*W*, *w*), and genotypes of parents or offspring were coded as -1 for the *W* allele and +1 for the *w* allele. For pattern alleles I assumed the 1-locus three-allele model because it better allowed assignment of genotypes. Pattern alleles were coded as -1 for *B* and +1 for *b*. Intermediate alleles were coded as -.5 instead of zero because they contribute partially to a "banded" phenotype. Summing both colour and pattern scores

gives a potential range for extreme genotypes of -4 to +4. In some cases the second allele at the colour or pattern locus could not be inferred due to dominance and was therefore not scored. This resulted in a reduced observed range of genotype scores (-2.5 to +4) potentially causing errors in the predictor variable and violating the assumptions of linear regression. Therefore, to test for a relationship between the mean family "genotypic value" and the transformed RHWBW, I used a Spearman's rank correlation.

Results

Selection and dominance

Some wild-caught yellow-triangle females produced white and/or band offspring. However, selection to purify yellow-triangle was completed in a single step. After field-mated females died or re-mated within the greenhouse and their freshly eclosed white or band offspring were removed, no further white or band butterflies were produced in greenhouse 8. This was true of both cohorts taken from the field (1993 and 1994). Their yellow-triangle offspring, when paired with other yellow-triangle lab descendents, never produced white or band offspring (D. D. Kapan and L. E. Gilbert, pers. obs.). Conversely, white-band butterflies crossed to other white-band butterflies often produced yellow and triangle offspring for over eight generations in two separate years after all yellow and triangle butterflies and their progeny were removed (D. D. Kapan and L. E. Gilbert, pers. obs.). This was true of both cohorts of white-band butterflies brought in from the field (August 1993 and August 1994) and their white-band descendants. These selection results indicate that: 1) field-caught yellow-triangle females had mated with white and/or band males in the field; 2) that field-caught white-band butterflies include heterozygotes that carry yellow and triangle alleles; and 3) it is easy to select for yellow-triangle (pure population after one generation) but not for white-band (colour and pattern dominant), which we were unable to purify over a two-year period (possibly due to inbreeding).

Therefore, I conclude that the source population was polymorphic for both colour and pattern and that alleles for yellow and triangle are recessive to those for white and band patterns.

The dominance inferred from the selection phase of the study is also consistent with preliminary cross data. Of a total of 16 crosses, no triangle-triangle crosses produced band phenotypes and no yellow-yellow crosses produced white phenotypes. The strength of the latter result is compromised by small sample size (only one yellow-yellow cross; see Table 2.1). Conversely, band-band crosses often produced triangle offspring, and white-white crosses often produced yellow offspring (Tables 2.1 & 2.2). The ease of selecting for all yellow-triangle morphs in greenhouse 8 and the remainder of the colour segregation across broods strongly imply that yellow is recessive to white.

Colour

I tested the goodness-of-fit of the one-locus two-allele hypothesis for colour with six relevant crosses (Table 2.1). Crosses involving only white parents (broods 2, 3 and 7) produced both white and yellow butterflies in approximately a 3:1 ratio when broods are summed (Table 2.2a, $G_1 = 0.14$, $P = 0.71$). Crosses with only one white and one yellow parent (broods 6 and 15) produced offspring in an approximately 1:1 ratio (Table 2.2a, $G_1 = 0.40$, $P = 0.53$). Finally, one yellow by yellow cross produced 27 all-yellow offspring (brood 16). The cumulative value of the *G*-statistic for the informative broods of the one-locus two-allele colour hypothesis is low ($G_2 = 0.54$, $P = 0.76$), indicating that the single-locus two-allele hypothesis for colour is consistent with the data.

Pattern

Two codominant alleles at a single locus is the simplest hypothesis to account for the range of pattern phenotypes (band, intermediate and triangle). Under this hypothesis all parents can be assigned an unambiguous genotype based on

their phenotype; either *BB* (band), *Bb* (intermediate) or *bb* (triangle). However, the data categorically reject this hypothesis because crosses between two-banded parents (brood 1) produced some triangle offspring; crosses between band and triangle parents (broods 2 to 5) produced all three phenotypes; and one cross (brood 6) between an intermediate parent and a band parent produced some triangle offspring. All of these results are impossible under the co-dominance hypothesis thus ruling it out (Table 2.2b).

Crosses involving only band or triangle parents and offspring are consistent with two-alleles at a single locus with *B* completely dominant to *b* (Table 2.2c). Because intermediates are recessive to band patterns, we can lump intermediate with triangle offspring (15 broods) to create a more powerful test. Under this test, the data do not contradict the expected offspring ratios for one-locus two-alleles *B* and "*Ib*" (see Table 2.2d, $G_2 = 0.68$, $P = 0.71$).

Two more hypotheses are the one-locus three-allele model, where the third allele is intermediate, and the two-locus two-allele model where the second locus is a modifier of the first. Table 2.2e shows inferred genotypes and offspring counts for the one-locus three-allele hypothesis involving 15 broods. The inferred genotypes predict segregation patterns similar to those found, although sample size is small in two of the three offspring categories. Predictions of the single locus three-allele hypothesis are indistinguishable from the two-locus modifier hypothesis (Table 2.2f). Additional crosses between individuals heterozygous for the *I* (or +) allele and recessive triangle individuals could distinguish between the single-locus three-allele and two-locus modifier hypothesis with sufficient family size. It is important to note that variable penetrance of the band characteristic could be heritable. Thus, the third allele or modifier could be due to heritable variation in penetrance (Mallet 1989)

Because the gene-frequencies in the collected population are unknown and family size is not constant, it is impossible to calculate the probability that crosses excluded from this analysis (such as the first two crosses in Table 2.2a) were from heterozygous parents (because parental genotypes could not be assigned).

Heritability of hind-wing band-width

In addition to colour and pattern, the relative size of the white or yellow hind-wing band (as defined in methods) is the last mimetically relevant trait analyzed. Quantile-quantile plots of RHWBW and its component traits (M3 and BW length) indicate that variation in this trait is approximately normally distributed (data not shown). Of the 16 broods presented in Table 2.1 I had measurements of the size of the hind-wing band for both parents and at least one offspring of only 10 families. Figure 2.2 shows the results of the mid-parent offspring regression for the relative size of the hind-wing band-width. This relationship is suggestive of additive genetic variability for this trait amongst the study population ($h^2 = 0.67$, one-tailed $P = 0.069$; see Table 2.3). The heritability values from the mother offspring regression and the father offspring regression are similar but also non-significant (see Table 2.3).

Association between hind-wing band-width and colour-pattern loci

The two different comodels have different relative hind-wing band-widths. The all-yellow species *H. eleuchia* has a wide hind-wing band (average ratio = 0.77 ± 0.001 SE), whereas white *H. sapho* has a narrow hind-wing band (average ratio = 0.39 ± .0001 SE; see Chapter 4). Mimicry between these comodels and extreme *H. cydno* morphs leads to the prediction that hind-wing band-width of yellow-triangle butterflies (co-mimetic of *H. eleuchia*) should be greater than the hind-wing band-width for white-band (co-mimetic of *H. sapho*). Is hind-wing band-

width relatively greater when associated with genes coding for yellow and triangle patterns?

The relationship between mean RHWBW and mean genotype scores for all 16 families is significant (Figure 2.3, Spearman's $\rho = 0.56$, $Z = 2.17$, $P = 0.015$). This indicates a genetic correlation between RHWBW and colour pattern in the predicted direction. Repeated analysis with colour and pattern scored separately revealed most of the correlation was between RHWBW and allelic status at the colour locus (Spearman's $\rho = 0.59$, $Z = 2.28$, $P = 0.011$) whereas the pattern locus is not significantly correlated with RHWBW (Spearman's $\rho = 0.35$, $Z = 1.35$, $P = 0.089$).

Discussion

The main mimetic resemblance between *H. cydno* morphs and the two comodels (*H. eleuchia* and *H. sapho*) appear to be due to two genes of large effect. Colour variation is due to a single-locus with two alleles, where one allele (*W*) is completely dominant to the other. The largest phenotypic differences between band and triangle patterns are also generated by variation at a single locus with two major alleles (*B*, *b*) with one allele (*B*) completely dominant. Intermediate pattern phenotypes were either due to a third allele segregating at the pattern locus or a modifier allele with epistatic effects on the recessive pattern allele. The genetic basis of colour and pattern polymorphism in *H. cydno* is similar to the genetic basis for colour and pattern differences between other races or species of *Heliconius* (Turner 1977; Sheppard et. al. 1985; Gilbert et. al. 1988; Mallet 1989; Nijhout, Wray and Gilbert 1990; Linares 1996). This consistency with earlier crossing data suggests that problems with small family size, inferring parental genotypes from segregation in families, and combining data across broods did not obscure the simple Mendelian basis of inheritance of colour and pattern.

However, despite consistency with more extensive crosses between races and species of *Heliconius* butterflies the hypotheses supported by my data need to be tested further on Western Ecuadorian *H. cydno*. In the future, I plan to distinguish between the one-locus three-allele hypothesis and the two-locus modifier hypothesis by carrying out crosses of intermediates versus triangle butterflies with increased brood sizes for several generations. Crosses with increased brood size and increased range of relative hind-wing band-width will also help improve heritability estimates. More complex modifier hypotheses for the inheritance of pattern are possible (see Linares 1996), but they do not merit consideration in the present study given the paucity of the data. The present data also do not allow a test of independent assortment of colour and pattern genes.

Modifiers

Modifier loci are also known from other traits in *H. cydno* (Linares 1996). Intermediates between triangle and band phenotypes may be caused by alleles with similar effects to a gene described in a study of *H. cydno weymeri* forms *weymeri* and *gustavi* inhabiting the Cauca Valley near Cali in Colombia (Linares 1996). In his study, Linares found a white spot present in the distal area of the discal cell of the *weymeri* form. This white spot appeared to be controlled by a single locus (*DC*) with two alleles and incomplete dominance. Linares found an intermediate sized white spot in heterozygotes, whereas homozygotes (DC_1DC_1 genotypes) had no white spot. Homozygotes (DC_2DC_2) had a larger white spot, characteristic of the *weymeri* form. Linares also found a "major mimicry locus" *L* whose two allelomorphs code for differences in the fore- and hind-wings of *weymeri* and *gustavi* forms. *L* is epistatic to the *DC* locus. The *gustavi* forms (L^GL^G) have forewings that are nearly completely covered with dark scales. The phenotypic effects of (L^GL^G) provide a good resemblance to *gustavi*'s putative comodel *H. erato chestertonii*. This resemblance is caused by the effects of the L^G

allele that converts several white areas of the forewing to dark and appears to partially mask the effect of the DC_2 allele. In my study, the *B* allele appears to be able to mask intermediate variants. If a modifier locus similar to *DC* generated the intermediate morphs of *H. cydno* found in this study, epistasis between the two alleles (*B* masking +) may be very similar to that between L^G and DC_2. One difference between L^G and *B* is that L^G appears to only partially mask homozygotes for DC_2DC_2, whereas *B* appears to totally mask intermediate patterns generated by the putative + allele. A second difference between L^G and *B* is that the phenotypic effects of *B* are limited to a small portion of the discal cell, whereas the effect of L^G is more widespread including the entire forewing (see Linares 1996 for illustrations). Additional research is necessary to determine the genetic bases for Ecuadorian *H. cydno* forewing variation.

Quantitative variation in hind-wing band-width?

In my study, all butterflies possess white or yellow trailing hind-wing bands. This character is not found in *weymeri* or *gustavi* forms found in the Cauca Valley. However, it is present in a third Colombian subspecies *H. c. cydnides* whose comodel (*H. eleuchia eleuchia*) also has a light trailing edge hind-wing band (Brown 1979; Linares 1996). Linares (1996) found a single locus (*Sb*) with three alleles controlling submarginal differences between *weymeri*, *gustavi* and *cydnides*. (The latter form has allele Sb_3 coding for the absence of melanic scales on the hind-wing marginal area.) In Western Ecuador, *H. cydno* appears to have a gene with similar action to the Sb_3 allele. However, continuous variation in the width of the marginal band relative to size of the hind-wing suggests either a polygenic basis for this character or a high degree of environmental variance. Genetic variance in this character could be due to alternate allelomorphs at a similar locus to *Sb* or an epistatic modifier locus controlling the amount or position of the melanic scales demarcating the edge of the hind-wing marginal band. A final possibility is a pleiotropic effect of the colour-pattern variation itself on hind-

wing band-width. This latter possibility should not be quickly ruled out, at least for a pleiotropic effect of pattern, because widening hind-wing bands and generating the triangle both involve a loss of melanin, whereas narrowing the hind-wing band and generating the band involve an increase in melanin.

The relationship between RHWBW and the average genotype for colour of offspring from laboratory crosses could be due to linkage between colour and band-width, an epistatic interaction between loci for colour and RHWBW or disequilibrium between these loci. Although the present data do not distinguish between these possibilities, Dr. Gilbert simultaneously selected both yellow-triangle and white-band populations for colour, pattern and, when possible, hind-wing band-width. In addition, the laboratory butterflies were derived from the comodel free El Copal population where no correlation between RHWBW and colour-pattern was found (Chapter 4). Thus it is likely that selection *in the lab* built up disequilibrium in the crosses.

Although a genetic basis for similar variation in the relative position of wing-pattern elements in *Heliconius* has been found by L. Gilbert (e.g., the *Cs* locus; see Nijhout, Wray and Gilbert 1990), relative hind-wing band-width in Western Ecuador *H. cydno* may potentially be influenced by several genes of small effect. If this were true, it would contrast with almost all existing *Heliconius* genetics to date, which generally show mimetic characters have a simple Mendelian basis (Sheppard et. al. 1985; Mallet 1989; but see Linares 1996). Additional research to determine the nature of genetic variation and influence of environmental variation on hind-wing band-width and correlations between band-with and colour-pattern characters would be very fruitful.

Supergenes, linkage and mimicry

Fitness in Müllerian mimics is positively frequency dependent (Benson 1972; Mallet and Barton 1989a, Chapter 3). As a result, *Heliconius* butterflies and other

Müllerian mimics are usually monomorphic within species and share warning colouration between species. Throughout most of its range different races of *H. cydno* follow this rule: monomorphic populations match a single unpalatable comodel species (Brown 1979; Brown 1981; Table 1.1). Resemblance of a Müllerian mimic to a comodel species typically involves several unlinked genes (Turner 1977). Supergenes, linked blocks of alleles coding for near complete resemblance to more than one comodel species, are usually not found (Turner 1977). Long-term polymorphism (such as found in Batesian mimicry) is thought to be necessary for the development of supergenes, through selection for modifiers that are tightly linked with a major mimicry locus (but see Charlesworth and Charlesworth 1975). However, some Müllerian mimics have supergenes. The classic difference between the "Postman" and "Dennis-rayed" patterned *H. erato* and *H. melpomene* races may be due one supergene (the D^R/r locus, Mallet 1989). Linares identified the *L* locus in Colombian *H. cydno* as another potential supergene. In both of these cases, supergenes were identified by crossing different races of *Heliconius* that either abut at a hybrid zone (Mallet 1989) or are formed by transient polymorphisms brought about by temporal change in comodels generated by habitat modification (Linares 1997).

Polymorphism maintained by simultaneous mimicry of more than one comodel may persist for periods long enough to allow the accumulation of modifiers to build supergenes (Turner 1977; Sheppard 1963). Does *H. cydno* from Western Ecuador possess supergenes? The B/b locus could represent such a gene. Pattern differences between band and triangle phenotypes may be the result of a larger linked group of genes. Intermediates could be due to rare crossing over between component loci (see Mallet 1989), however, this scenario is not likely because the phenotype frequency of intermediates in the field is too high (~ 4%, see Chapter 4). The effect of the B/b locus is limited to a small area of the forewing and could be accounted for by the action of a single pattern element as

described by Nijhout, Wray and Gilbert (1990). Hind-wing band-width is very likely associated with an individual's genotype at the colour-pattern loci. Lab data support a link between colour and hind-wing band-width whereas field data from sites with two comodels show a phenotypic correlation between pattern and hind-wing band-width. Thus RHWBW is not consistently associated with colour or pattern (Chapter 4). Finally, field surveys of phenotype frequencies at El Copal fit Hardy-Weinberg proportions in six out of seven sampling periods (Chapter 4). This indicates that the major colour and pattern alleles assort relatively independently (see Chapter 4). In conclusion, although *H. cydno* in Western Ecuador appears to be in a situation conducive to the evolution of supergenes, apparently none have been formed.

Strong linkage between colour and pattern is apparently absent despite the long-term persistence of *H. cydno* polymorphism in Western Ecuador. This persistence should provide an opportunity for the evolution of supergenes that better match each different comodel (Chapter 4). Several possible genetic or selective constraints may be responsible for the lack of linkage between colour and pattern genes in Western Ecuadorian *H. cydno*. First, *Heliconius* species have a high haploid chromosomes number ($\geq$ 19, Suomalainen et. al. 1972, Brown 1981), thus colour and pattern loci likely exist on separate chromosomes. Second, temporal and spatial variation in the predominant comodel may favour different morphs at different times or places such that the order of morph fitness is not always identical. This would destroy linkage (Chapter 4). Third, even in the presence of both comodels, *W_B_* genotypes (white-band phenotypes thought to be mimetic of *H. sapho*) and *wwbb* genotypes (yellow-triangle phenotypes thought to be mimetic of *H. eleuchia*) may not necessarily be superior over *W_bb* genotypes (white-triangle phenotypes). *WW* homozygotes may be as fit when expressed with at least one *B* allele (white-band) as with two *bb* alleles (white-triangle) when the white comodel *H. sapho* is common (as may

be the case at Tinalandia, Chapter 4). The opposite may be true when *H. eleuchia* is common; all-yellow homozygotes (*ww*) may only be fit when expressed with bb homozygotes (yellow-triangle butterflies, see Chapter 3). Another possibility is that *W_bb* individuals (white-triangle) have a general-purpose phenotype which, depending on the environment, gains limited protection from the presence of *H. eleuchia* or *H. sapho*, but gains general protection from being the most abundant morph of *H. cydno* at polymorphic sites (Chapter 4; but see Chapter 3). Finally, selection against colour-pattern mismatches is not predicted in areas where *H. cydno* is common, but not sympatric with any comodel species (such as the El Copal source site, see Chapter 4). In the absence of comodels, each morph (if sufficiently common) may lead to predator generalization resulting in weak or no detectable selection against morphs with the lowest frequency (Chapter 4). This would lead to a breakdown of linkage. This appears to be the case with the lack of phenotypic correlation between RHWBW and pattern genes at El Copal (Chapter 4).

Dominance

The *B* allele shows a clear dominance relationship to the *I* (or +) and the *b* allele consistent with Nijhout, Wray, and Gilbert's (1990) findings that in seven out of the 10 loci they studied the addition of red, brown, or black scales (increase in a pattern-determining activity) was dominant to yellow or white (lack of or decrease in pattern-determining activity). The two exceptions to this dominance rule in their study, are codominant alleles at one locus *Fs* in *H. cydno*, and another two loci (*D* and *R* in *H. melpomene*), that have red dominant to black respectively. Allelic codominance at many loci is often revealed by crossing parapatric races (Mallet 1989) or species of *Heliconius* (L. E. Gilbert, unpublished data). Dominance in these Müllerian mimicry systems is thought to be due to the evolution of dominance modifiers (Turner 1977; Mayo and Burger 1995), which improves the resemblance of a given race to the local comodels because bird

predators remove intermediate or "fuzzy" patterned individuals (Mallet 1989). Codominance occurs more often in crosses between races and species where the alleles are foreign to the genetic background. In *H. cydno* complete dominance at the *B/b* locus may be due to sustained polymorphism over time allowing the opportunity for dominance modifiers to evolve and eventually become linked to a major pattern locus. More work on Western Ecuadorian *H. cydno* is necessary to determine if this has occurred.

Conclusions

Preliminary findings indicate that the genetic basis for colour morph differences in *H. cydno* is simple, one locus with two alleles. Pattern morphs also segregate at one locus with two major alleles and either a rarer "intermediate" allele or the presence of a separate epistatic modifier locus. Possible heritability in the continuous hind-wing band-width suggests polygenic basis or a simple genetic basis with environmental noise. Polymorphisms, such as those found in *H. cydno*, provide interesting model systems to study the genetics of traits that have potentially evolved under strong selection.

Figure Legend

Figure 2.1 Two colours (rows) and three patterns (columns) of *H. cydno* phenotypes (A.). Intermediate patterns have an intermediate level of melanin on scales in the distal portion of the discal cell (see methods for further information). In the "triangle" form the light gray area indicated by ↓ is either white or yellow (B.). Intermediates have a smaller white area in the center of the gray area indicated by ↓. In the "band" form the gray area indicated by ↓ is filled with melanic scales. Visually, this frames an unbroken post-medial band of either white or yellow across the forewing immediately outside the discal cell. The relative hind-wing band-width is the ratio of BW to M3 as indicated by the gray arrows (C.; see methods for description).

Figure 2.2 Mid-parent offspring regression of the relative size of *H. cydno* hind-wing band-width in 10 families with measurements from both parents and offspring. The offspring family mean for each cross was regressed against mid-parent value of RHWBW. Line represents a linear regression of the two variables (see methods for further information).

Figure 2.3 Relative size of *H. cydno* hind-wing band-width as a function of genotype in offspring. The mean sibling hind-wing band-width ratio for each family is plotted against the mean sibling genotype value for each family. The value of the genotype was determined by summing the following alleles at the hypothesized colour loci: *W* (White) = -1, *w* (Yellow) = +1, and pattern loci *B* (Band) = -1, *I* (Intermediate) = -.5, and *b* (Triangle) = +1. When the genotype at a given allele was unknown, nothing was added or subtracted from the totals. Owing to the masking effects of dominant alleles, "genotypes" of individuals with values < 0 were usually not fully known. Line represents a linear regression of RWHBW on genotype value (although a Spearman's rank correlation was used in statistical comparisons, see methods for further information).

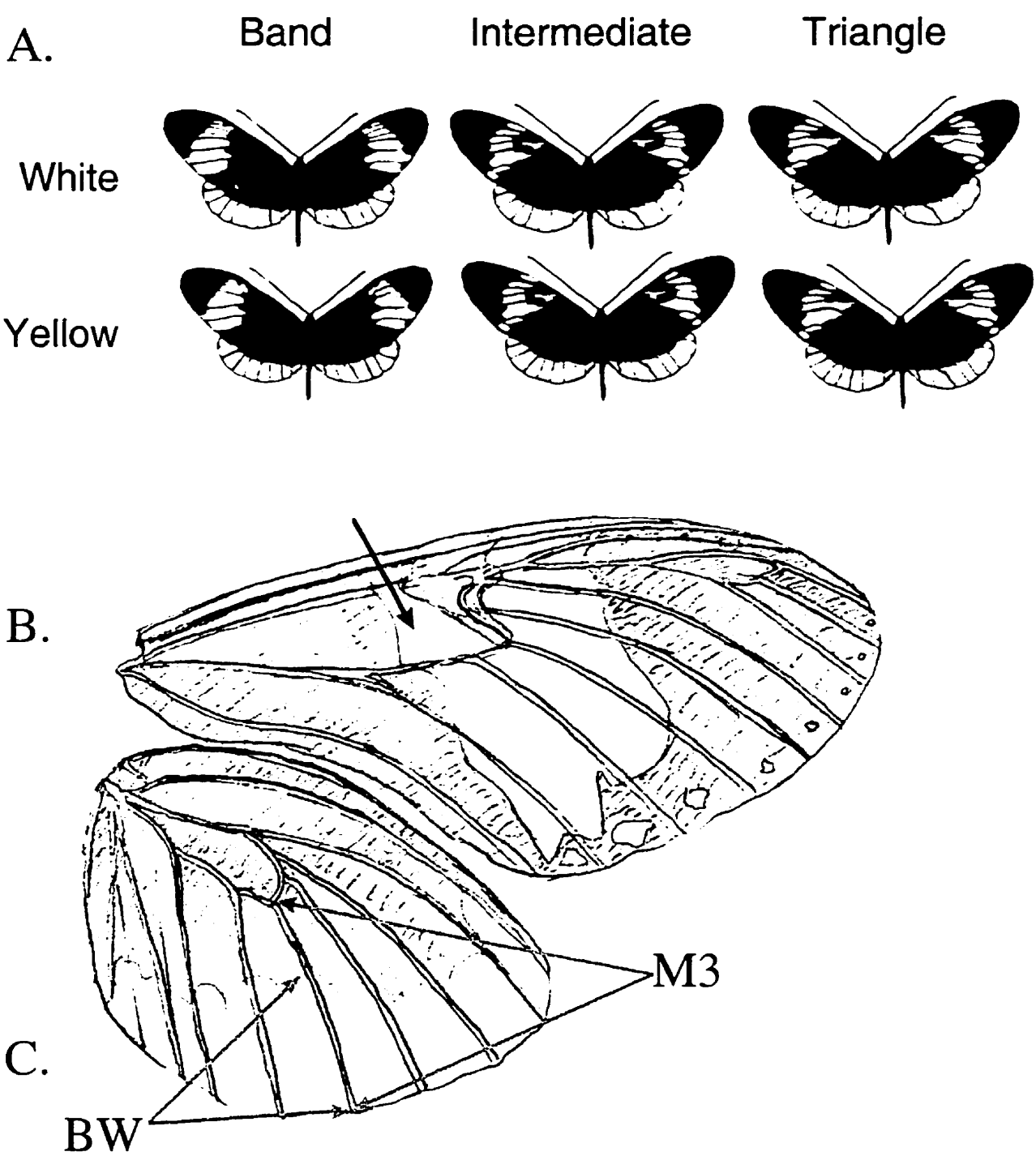

Figure 2.1 Two colours (rows) and three patterns (columns) of *H. cydno* phenotypes (A.). Intermediate patterns have an intermediate level of melanin on scales in the distal portion of the discal cell (see methods for further information). In the "triangle" form the light gray area indicated by ↓ is either white or yellow (B.). Intermediates have a smaller white area in the center of the gray area indicated by ↓. In the "band" form the gray area indicated by ↓ is filled with melanic scales. Visually, this frames an unbroken post-medial band of either white or yellow across the forewing immediately outside the discal cell. The relative hind-wing band-width is the ratio of BW to M3 as indicated by the gray arrows (C.; see methods for description).

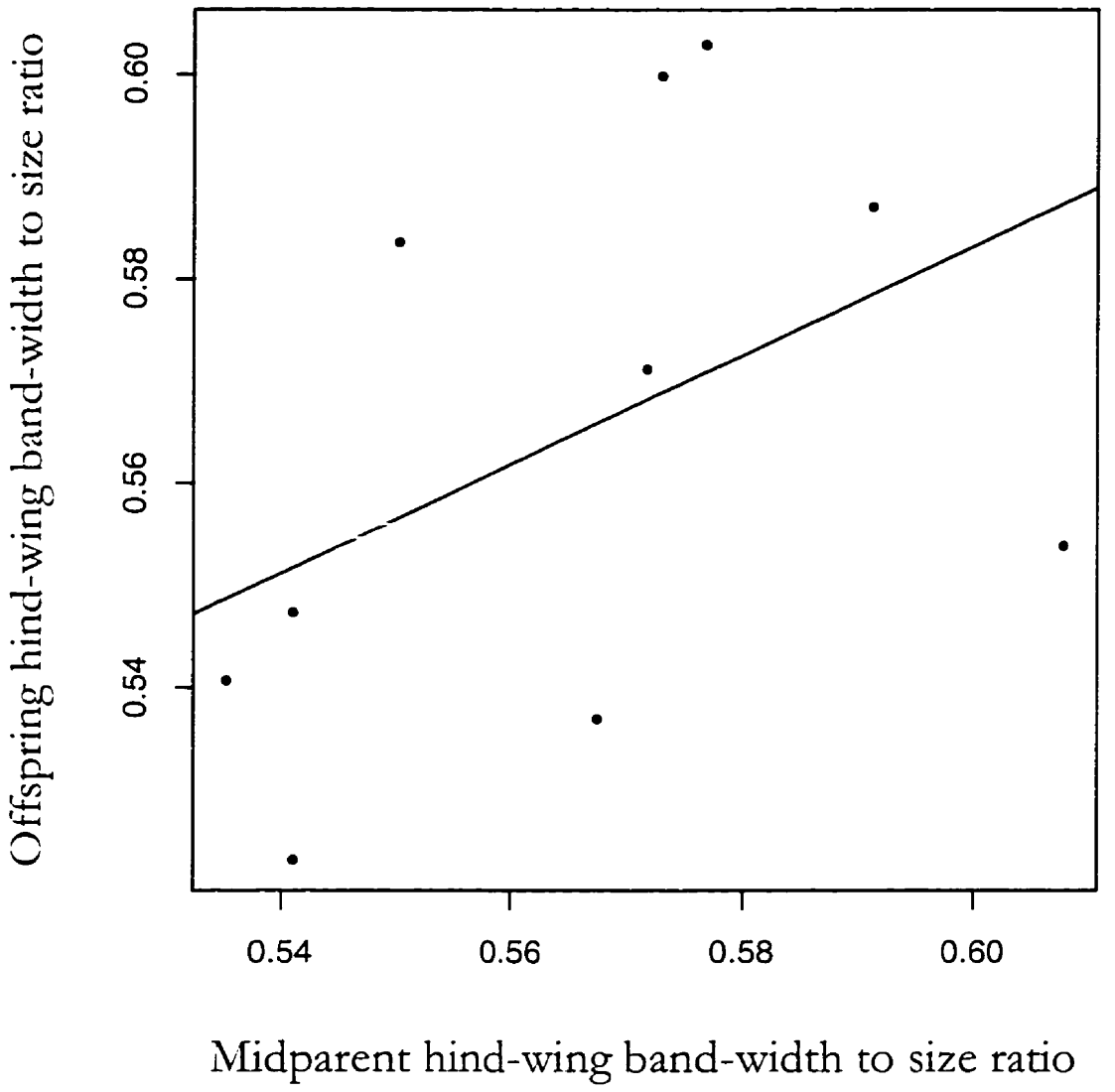

Figure 2.2 Mid-parent offspring regression of the relative size of *H. cydno* hind-wing band-width in 10 families with measurements of both parents and offspring.

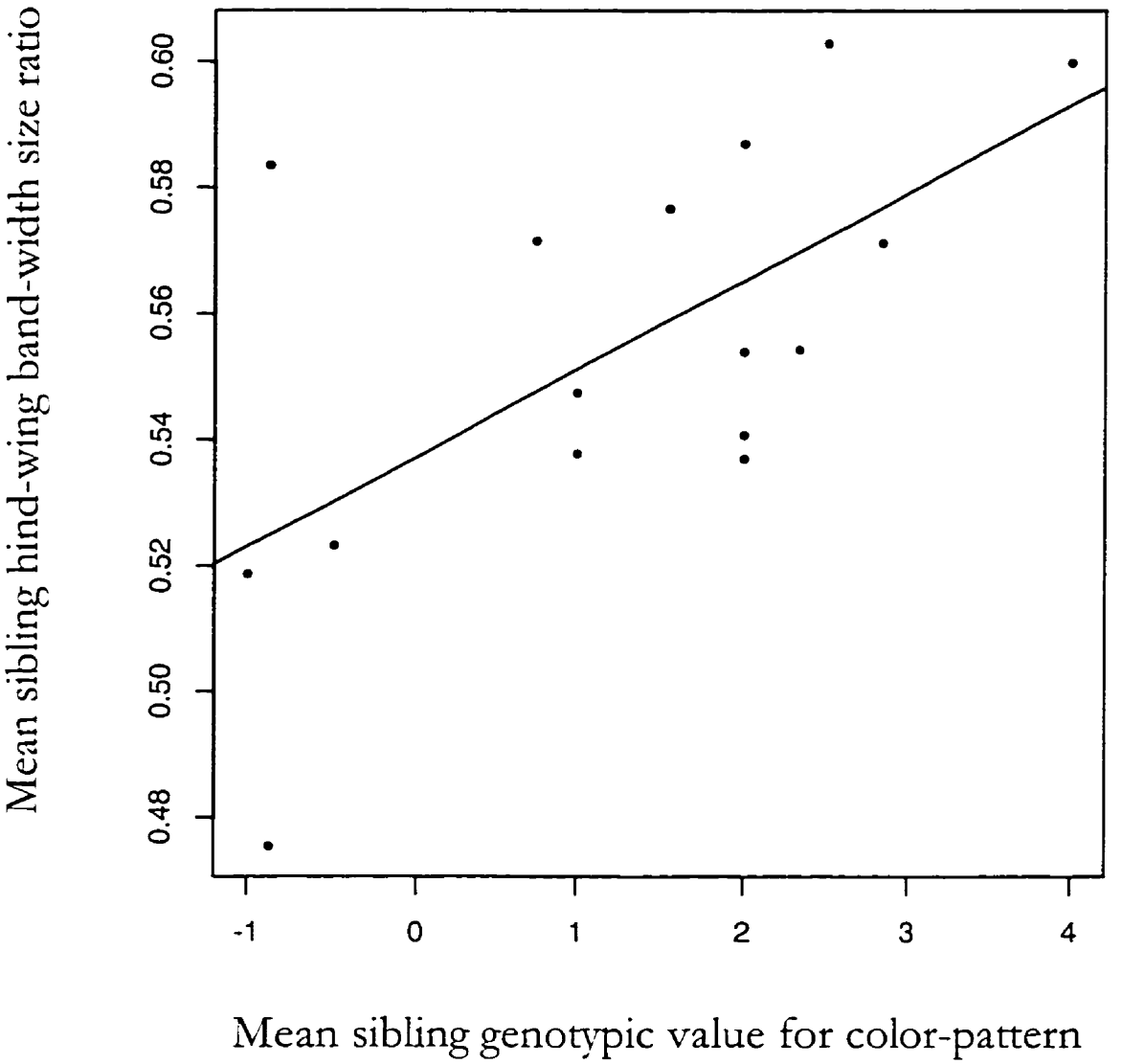

Figure 2.3 Relative size of *H. cydno* hind-wing band-width as a function of genotype in offspring.

Table 2.1 Phenotypes of parents and their offspring.

Phenotypes and identity of the parents					Offspring Phenotypes											
					White						Yellow					
		Female		Male	Band		Intermediate		Triangle		Band		Intermediate		Triangle	
Brood number	Female	Code	Male	Code	F	M	F	M	F	M	F	M	F	M	F	M
1	White-band	R72	White-band	R70	1	4			1	2						
2	White-band	94	White-triangle	102			1					1				
3	White-triangle	n20	White-band	X[1]					1		1					1
4	White-triangle	n39	White-band	132	2											
5	White-triangle	R 96	White-band	R38	1	2		1								
6	Yellow-intermediate	n35	White-band	76		1					1			1	3	
7	White-triangle	121	White-triangle	102					6	7					1	2
8	White-triangle	R1	White-triangle	R26					5	7						
9	White-triangle	R 103	White-triangle	R102					3	1						
10	White-triangle	R 97	White-triangle	R82						1						
11	White-triangle	R42	Yellow triangle	R14					14	14						
12	White-triangle	R50	Yellow triangle	R 91					9	6						
13	White-triangle	R 98	Yellow triangle	R80					1	3						
14	White-triangle	.85	Yellow triangle	R80					1	1						
15	White-triangle	R 145	Yellow triangle	R 203					1	2						1
16	Yellow-triangle	R88	Yellow triangle	R 99											18	9

[1] This male was not marked while mating.

Table 2.2 a-c. Comparisons of alternative hypotheses for inheritance using counts of offspring phenotypes for particular inferred parental genotypes.[1]

	Hypothesis		Inferred	Offspring Phenotype				
	loci/alleles	dominance / epistasis	genotypes of cross[2]	White	Yellow		Expected ratio	brood number[3]
a.	1 / (W,w)	complete	Ww*Ww	15	6		3:1	2,3,7
			Ww*ww	4	6		1:1	6,15
			ww*ww	0	27		0:1	16
				Band	Intermediate	Triangle		
b.	1 / (B,b)	codominant	BB*BB	5	0	3	1:0:0	1
			BB*bb	7	2	2	0:1:0	3
			BB*Bb	2	1	3	1:1:0	6
			bb*bb	0	0	113	0:0:1	7,8,9,10,11,12,13,14, 15,16
				Band	Intermediate	Triangle		
c.	1 / (B,b)	complete	Bb*Bb	5	0	3	3:0:1	1
			Bb*bb	1	0	2	1:0:1	3
			bb*bb	0	0	113	0:0:1	7,8,9,10,11,12,13,14, 15,16

[1] Totals are less than table 1 since some parents cannot be assigned unambiguous genotypes for a particular hypothesis.

[2] Genotypes are those hypothesized depending on genetic model being contemplated.

[3] Brood numbers as in table 1.

(Table continued following page).

Table 2.2 d-f (continued). Comparisons of alternative hypotheses for inheritance.[1]

Hypothesis			Inferred	Offspring Phenotype				
	loci/alleles	dominance / epistasis	genotypes of cross[2]	Band	(Intermediate & Triangle)		Expected ratio	brood number[3]
d.[4]	1 / (B,(lb))	complete	B(lb)*B(lb)	5	3		3:1	1
			B(lb)*(lb)(lb)	7	8		1:1	2,3,5,6
			(lb)(lb)*(lb)(lb)	0	113		0:1	7,8,9,10,11,12,13,14,15,16
				Band	Intermediate	Triangle		
e.[5]	1 / (B,I,b)	complete	Bb*Bb	5	0	3	3:0:1	1
			Bb*bI	2	1	3	2:1:1	6
			Bb*bb	1	0	2	1:0:1	3
			BI*bb	4	2	0	1:1:0	2,5
			bb*bb	0	0	113	0:0:1	7,8,9,10,11,12,13,14,15,16
				Band	Intermediate	Triangle		
f.[6]	2 / (B,b; +,-)	complete with modifier	Bb,- -*Bb, - -	5	0	3	3:0:1	1
			Bb,- -*bb, - + or Bb,+ -*bb, - +	2	1	3	2:1:1 or 4:3:1	6
			Bb,- -*bb, - -	1	0	2	1:0:1	3
			Bb,+ +*bb, - - or Bb,+ -*bb, - -	4	2	0	1:1:0 or 2:1:1	2,5
			bb,- -*bb, - -	0	0	113	0:0:1	7,8,9,10,11,12,13,14,15,16

[1, 2, 3.] See footnotes previous page.

[4.] All intermediates analysed as if they were triangles denoted (lb).

[5.] Intermediates considered a third class of alleles recessive to B and dominant to b.

[6.] Intermediates due to at least one + allele at an epistatic modifier locus affects of which are masked by B and not by b.

Table 2.3 Heritability estimates of relative hind-wing band-width from parent-offspring regressions.[1]

Parents	b[2] (heritability)[2]	Standard Error	N[3]	P[4]
Mother	0.45	0.35	13	0.116
Father	0.28	0.47	10	0.286
Mid-parent	0.67	0.41	10	0.069

[1] Falconer and Mackay 1996.

[2] Twice the regression coefficient for single-parent offspring regressions and equal to the regression coefficient for mid-parent offspring regressions.

[3] Number of families in comparison.

[4] P-value for 1-tailed hypothesis of positive regression slope.

The benefit of Müllerian mimicry: divergent natural selection in polymorphic *Heliconius cydno*

Abstract

Polymorphism in one unpalatable species could be favoured by divergent selection to match two or more other unpalatable species (known as comodels) that possess different warning-colour patterns. In this chapter I test whether the mechanism of divergent selection is feasible by transferring different colour-pattern morphs of the polymorphic species *H. cydno* to sites dominated by one of two hypothesized comodels (the yellow species *H. eleuchia* or the white species *H. sapho*). Paired experimental and control *H. cydno* were released at four sites in Western Ecuador. Control butterflies' colour matched locally dominant comodels, whereas experimental butterflies differed from dominant comodels. White *H. cydno* experimentals (two out of three replicates) and yellow experimentals in a fourth replicate had shorter life expectancies than controls. Control butterflies survived approximately three times longer than experimental butterflies. Survival differences (measured as initial and subsequent disappearance rates) were in the predicted direction in seven of eight comparisons. In low density treatments, control butterflies, whose colour matched local comodels, survived significantly longer than experimental butterflies. In one high-density replicate, only initial differences in disappearance between control and experimental butterflies were observed. This appears to be the first direct experimental evidence of the benefit of Müllerian mimicry: positive frequency-dependent selection favours *H. cydno* morphs that match numerically dominant comodel species.

Introduction

Bright colours and bold patterns are utilized by unpalatable or noxious species to ward off visually hunting predators (Wickler 1968). These warning signals are thought to be subject to aposematic (positive frequency-dependent) selection: benefits of possessing a given warning signal increase as the signal becomes more common (Greenwood et. al. 1989). Thus aposematic selection is predicted to lead to warning-signal monomorphism within species (Turner, Kearny, and Exton 1984) and signal sharing between species (Turner 1977; Sheppard et. al. 1985) known as Müllerian mimicry (Müller 1879).

There is a large body of comparative evidence for aposematic selection and Müllerian mimicry (see Chapter 1). However, direct evidence for aposematic selection comes from only a handful of studies, including field simulations with brightly coloured distasteful artificial prey (Greenwood et. al. 1989) and selection experiments utilizing a single tropical aposematic species (*H. erato* where the co-mimetic species *H. melpomene* was rare, Benson 1972; Mallet and Barton 1989a; Mallet et. al. 1990). Experimental evidence for the benefit of signal sharing *between* Müllerian mimic species has not been unequivocally demonstrated in the field.

In this chapter I utilize a field experiment to test whether Müllerian mimicry exists. I exploit the distribution of *H. eleuchia*, *H. sapho*, and polymorphic *H. cydno* in Western Ecuador (Chapter 1) to ask the question, does selection favour different colour-pattern morphs of the unpalatable species *H. cydno*, when present with one or the other of their putative Müllerian comodel species (*H. eleuchia* or *H. sapho*)? In addition to testing the tenets of Müllerian mimicry, demonstration of divergent selection generated by two or more comodel species on different morphs of single species will verify that divergent selection can

favour polymorphism in warningly coloured species normally subject to strong selection favouring a single common morph.

In the presence of yellow *H. eleuchia*, populations of *H. cydno* are monomorphic for the matching colour (yellow) but not pattern (Chapter 4). This led to the prediction that selection is stronger on *H. cydno* colour than pattern. Using a reciprocal transfer experiment, I estimate the strength of natural selection against rare colour-patterned *H. cydno* when they are present with one of two comodel species *H. eleuchia* or *H. sapho*. I examine but find no evidence that differential emigration of rare colour-pattern *H. cydno* morphs explains the observed effect. Additionally, I explore the effect of the relative density of *H. cydno* morphs on their fitness. I argue that the presence of strong divergent natural selection to match two different *Heliconius* comodels is consistent with the current distribution of colour polymorphism in Western Ecuadorian *H. cydno*.

This work addresses two processes: positive frequency-dependent selection, which favours a single morph within a population because it is more common; and Müllerian mimicry, which is the outcome of positive frequency-dependent selection between multiple species of distasteful organisms such that they converge on a common colour pattern. Earlier field experiments have demonstrated that positive frequency-dependent selection favours the common morph within one aposematic species, *H. erato* (Benson 1972; Mallet and Barton 1989a). However, the present field experiment provides evidence that divergent frequency-dependent selection favours different morphs of a single species, *H. cydno*, that are Müllerian mimics of two different comodels. As far as I am aware, this is the first experimental test of the selective value of signal sharing *between* Müllerian mimic species--the *benefit* of Müllerian mimicry.

Materials and methods

Study Sites

I captured yellow and white *H. cydno* butterflies from two source sites and released them at four sites in Western Ecuador (Table 3.1). At three release sites (Agua Caliente, Manta Real, and Maquipucuna) only yellow *H. cydno* occurs naturally with the yellow comodel *H. eleuchia.* At a fourth site, Tinalandia, all four morphs of *H. cydno* occur at low density with both comodels *H. eleuchia* and *H. sapho* (Chapter 4). At Tinalandia the relative frequency and density of the two comodel species varies seasonally and annually, possibly due to variation in host plant growth (Chapter 4). During the release at Tinalandia, *H. sapho* and white *H. cydno* outnumbered yellow butterflies (either *H. eleuchia* or yellow *H. cydno*) by 12:1.

Source sites for experimentally released butterflies were chosen from sites where *H. cydno* is polymorphic and occurs at high density. Two sites were utilized, El Copal (sources for three releases) and El Padrino (source for one release; Table 3.1).

Experimental Methods

Up to 30 pairs of experimental (locally rare colour at the targeted release site) and control (locally common colour at the same release site) butterflies were captured from a source site. Captive butterflies were temporarily held in numbered glassine envelopes in collection boxes. Butterflies were fed saturated sucrose solution every four hours during daylight. After four to six hours butterflies were individually numbered on their ventral hind-wing margins using a black permanent marker (Sharpie ultra fine point) and then released into cages. To minimize desiccation, butterfly cages were misted with filtered water. Caged butterflies were also fed a saturated sucrose solution immediately before dusk, in the morning, and every four hours during the day.

Butterflies were transported to release sites as soon as sufficient numbers were collected (median 3 days, range 1 to 6 days). Butterflies were packed in fresh envelopes in airtight plastic containers containing moist chemical-free tissue. These containers were stored in a 12V DC auto cooler maintained at 18°C. For each replicate, butterflies were transferred between source and release sites by vehicle (four to six hours of driving time) over one night. Prior to release I noted each individual's sex, colour, pattern, wear, wing tears and unique number of each butterfly (Benson 1972). Butterflies were then photographed both dorsally and ventrally. Both experimental and control butterflies were sterilized to prevent the introduction of novel genes into the release site. I used a single drop of cyanoacrylate glue ("super glue") to glue male claspers together at their posterior junction. This prohibited effective grasping of the female as well as preventing eversion of the aedeagus for sperm transfer. Terminal abdominal segments of females were glued partially shut preventing complete eversion of the ovipositor. Females treated by this method are unable to lay undamaged eggs. Tests in the laboratory indicate that these methods are 100% effective at preventing male mating and 100% effective at preventing successful oviposition (D. D. Kapan and M. Medina, unpublished data). Sterilized butterflies survived for over 30 days in the lab and up to 33 days in the field (D. D. Kapan and M. Medina, unpublished data). Finally, butterflies were held for one night prior to release in hanging cages and provided with ample moisture by misting and kitchen scrub pads moistened with saturated sucrose solution.

Paired experimental and control butterflies were matched for the number of days held and for approximate wing wear. Pairs were released along forest access trails and creeks at areas likely to be adopted by transferred butterflies such as light gaps with passion vine host plants, cucurbit vine adult feeding flowers, or known roost areas. After release, individual *Heliconius* butterflies

usually disperse a short distance and then establish small home ranges in which they roost, feed, mate, and lay eggs (Turner 1971; Mallet 1986).

Previous mimicry experiments have indicated that the total number of butterflies released in an area can affect the outcome; releasing too many experimental butterflies in a given area results in decreased selection when compared to controls owing to predator learning (Brower, Cook and Croze 1967; Benson 1972). For a preliminary assessment of this possibility, I released butterflies at low density (163 to 193m between release pairs) in three sites and at high density (average ~ 42 meters between release pairs) in a fourth site (Maquipucuna).

Butterflies were released either in an afternoon and the following morning (site 1) or throughout a single day (sites 2 to 4; see Table 3.1 for release dates). Each experimental replicate consisted of nine to 16 pairs released at a single study site. In a few cases, when odd numbers of butterflies were available, matched triplets were released together. Total numbers released depended on study site size (measured as distance of trail through suitable habitat; see Table 3.1 for total number of butterflies). Both sexes were utilized in the experiment due to the scarcity of butterflies at source sites. Released butterflies that were unable to fly moderately strongly (sustained 10 second flight or strong initial flight if less than 10 seconds) were recaptured immediately and eliminated from the experiment. To estimate dispersal distances, each individual release point was flagged and mapped onto study site maps (scale 1:1000). Trails and linear distances between release points and resighting localities were mapped using a 50m tape, 75m optical range-finder, ½ degree sighting compass, and clinometer. Maximum dispersal distances plus 1 were *ln*-transformed and compared by a standard two-sample t-test with a two-tailed alternative hypothesis.

All release points were revisited the day after release. On a given day all release points were searched on a rotating basis except at Tinalandia where every release

point was visited approximately every 1.5 days. Only one experiment was carried out at a time. Butterflies were resighted by myself and one to three trained assistants (per replicate) with 10-power binoculars or a 20-power spotting telescope. Upon resighting, the identification number, phenotype and location of each butterfly was noted. Each experiment was followed for a minimum of 13 days or until marked butterflies were no longer detected.

Statistical Analysis

Likelihood model

Classic mark-recapture methodology was used to estimate resighting and survival differences between experimental (rare colour) and control (common colour) butterflies. A model incorporating survival differences must take into account that many individual *Heliconius* butterflies are never resighted. Others are found at the release point or nearby and are resighted frequently (Mallet and Barton 1989a). A simple equation for the decline of the number of butterflies (N) as a function of time (t) is:

$$N_t = N_{t=0} P_E e^{-\lambda t}$$

where P_E represents the probability of establishment to $t = 1$, $e^{-\lambda t}$ is the probability of survival to t days, λ is the death rate and $N_{t=0}$ is the number of butterflies released (Mallet and Barton 1989a). Early loss (mortality and emigration) may be higher than subsequent mortality due to naïve predators, a butterfly's lack of familiarity with the release site and emigration. An additional resighting parameter (α) is necessary to estimate the survival parameters. This parameter determines the probability (θ_i) of encountering an experimental or control butterfly on day i as a function of study effort (E_i), $\theta_i = \alpha E_i$ (E_i is estimated as the total number of native butterflies of the three study species encountered on day i, Mallet and Barton 1989a).

I used maximum-likelihood to estimate these three parameters (α, P_E and λ) for each experimental group (for details and full likelihood equations see Mallet and Barton 1989a: 431). Likelihood assesses the probability of the data given hypothesized parameter combinations in the above model (Edwards 1992). I fitted the model to the data for each treatment and sex separately for each site (16 possible resight parameters and 32 possible survival parameters). Data from both sexes were combined at Agua Caliente (experimentals) and Tinalandia (experimentals and controls) because no females in these treatment classes established ($P_E = 0$, λ undefined). This model has the maximum number of parameters and is called the "full" model.

Empirical 2-unit support ellipses (hereafter support limits) for P_E and λ are obtained by encircling the set of all values of P_E and λ whose *ln*-likelihoods are within two units of the maximum. Support limits are analogous to 95% confidence limits for any one parameter (Edwards 1992). Life expectancy is then given by initial survival (P_E) multiplied by the estimated life-span after establishment ($1/\lambda$, Mallet and Barton 1989a) Support limits for life expectancy are found by calculating life-expectancies for pairs of P_E and λ found along the two-dimensional support limits for P_E and λ, then locating the minimum and maximum values. Support limits were located on a 49 by 49 grid of log-likelihood values using the contour function in S-PLUS (Statistical Sciences 1995).

Goodness-of-Fit

The Goodness-of-Fit (GOF) of the full model is assessed by comparing observed numbers of butterflies on a given calendar day (R_i) with those predicted by the full model ($\hat{R}_i = \hat{N}_i\theta_i$) using a χ^2 test with the degrees of freedom equal to i days of observation minus the number of parameters estimated from the data (Burnham 1987). Visual inspection of the residuals and

GOF tests indicates that the full model fits the data and thus represents a reasonable starting point for analysis (χ^2_{66}= 59.133, P=0.713; Lebreton et. al. 1992).

Testing effects of experimental treatment and other factors

To test for a difference between experimental and control butterflies in P_E and λ (a "treatment effect"), whether P_E and λ differ between *sites* and *sexes*, and whether there were interactions among these factors (e.g., did the experimental effects differ by site), I used a method similar to stepwise regression (Lebreton et. al. 1992). The full model is Logit (α, P_E, λ) = TREATMENT * SITE * SEX (where Logit = log[x/(1-x)] for x equal to α, P_E, λ, Lebreton et. al. 1992). This gives a total of 48 possible unique parameters to estimate (ignoring the three un-estimable λ's due to zero establishment, see above). Recall that the model with the maximum number of estimable parameters is called the full model. This model has too many parameters in that not all main effects and interactions are significant. Starting with the full model I reduce the number of parameters by dropping any non-significant interactions and main effects. Finally, with this reduced model I tested for a treatment effect if it was retained during the model reduction. This model reduction can be carried out in two nearly equivalent ways: through formal tests such as the analysis of deviance (ANODEV) and the likelihood-ratio test (LRT, McCullagh and Nelder 1989; Lebreton et. al. 1992) or through Akaike Information Criterion (Akaike 1973). The Akaike information criteria (AIC) (Akaike 1973; Lebreton et. al. 1992) is calculated as minus twice the *ln*-likelihood of a model plus two times the number of parameters in that model (Lebreton et. al. 1992). Models with the lowest AIC are generally superior, explaining the most data variation with the fewest parameters. Comparisons between models which are not nested are possible only with the AIC. The model reduction approach is valid, indeed better than standard

buildup of models with stepwise regression, because buildup of models (from simple to complex), can lead to non-optimal model selection and bias (Lebreton et. al. 1992).

Because the saturated model fit the data well (as indicated by GOF tests) survival differences between colour and pattern morphs (and site and sex) were assessed with likelihood ratio tests (LRT) instead of ANODEV (Lebreton et. al. 1992; Skalski, Hoffman, and Smith 1993). I used the AIC to compare non-nested capture models (Lebreton et. al. 1992; see results). I reduced the full model by comparing its *ln*-likelihood to a model that postulates effects are similar (additive) across sites or treatments using an LRT, where δL is the differences in the *ln*-likelihoods of these two models and $2\delta L$ (G) is approximately distributed as chi-square (Lebreton et. al. 1992). If the treatment effect did not drop out, this implies it is significant. The AIC and LRT model reduction method selected the same model. Once the appropriate reduced model was identified (including site and treatment, see results), I tested for a difference in a survival parameter (e.g., P_E) between experimental-colour and control-colour butterflies, by comparing the reduced model with different maximum-likelihood estimates of P_E for experimental-colour and control-colour butterflies with a model that includes only a single P_E for both groups of butterflies.

Researchers commonly assume all butterflies are equally likely to be recaptured immediately after initial release (Mallet and Barton 1989a). In this experiment, experimental and control groups did not differ in handling, site of release, or other factors except for those of interest (e.g., phenotype). However, butterflies with the rarer experimental colour may be more noticeable to researchers than control butterflies that match the most common local butterflies. I tested for this possibility by comparing resight probabilities for treatment and control butterflies known to be alive and on the study site. Following the suggestions of

Lebreton et. al. (1992), I conducted the aforementioned model reduction on α first because live experimentals may be more detectable than live controls. I set α's to the same value within each site, sex, or treatment unless the AIC comparisons indicated significant differences between site, sex, or treatment classes, in which case α's were set to their MLE values (see results).

Programming details

All likelihoods were evaluated using a Visual Basic function (Microsoft Corporation 1994b) written by the author. To locate global maxima, *ln*-likelihoods were passed to SOLVER, a function maximizing routine running under Microsoft Excel (Microsoft Corporation 1994a). All runs using different initial parameter values converged on the same maximum likelihood estimate. Visual inspections of profiled parameters indicated that all likelihood functions were smooth and continuous with a single global maximum.

Overall, these statistical methods follow closely those of Mallet and Barton (1989a), with suggested improvements from other authors (Burnham et. al. 1987; Lebreton et. al. 1992; Skalski et. al. 1993).

Results

Proportion surviving

Figure 3.1 indicates the observed proportion of individuals resighted after initial release in each treatment ($N_i/N_{i=0}$) for each study site. In all cases the experimentals (dashed line) are below the controls (solid line) although the differences at Maquipucuna are smaller. This supports the hypothesis that each colour morph of *H. cydno* is a Müllerian mimic of its respective comodel.

Resighting probability

Estimates of the resighting coefficient α differed between experimental (α =.0103) and control (α =.00798) butterflies (G_1 = 4.59, P = 0.032, AIC =

309.16, 2 parameters): experimental butterflies are easier to locate. The sampling interpretation for this difference in resighting probability is simple; the colour of experimental butterflies differs from the more common locally coloured butterflies (natives and controls). For example, with the average effort of 40 butterflies seen daily this translates into a 10% greater probability of resighting an experimental than a control butterfly (recall $\theta_i = \alpha E_i$ thus a 22.5% difference in α translates into an approximately ~10% absolute difference in θ_i, the daily probability of resighting). This difference was in the same direction at Maquipucuna, Agua Caliente, and Tinalandia (the probability of resighting an experimental butterfly at these sites was approximately 0.09, 0.07 and 0.17 higher than controls assuming a daily effort of 40 butterflies seen). At one site (Manta Real) α (controls) was 0.00214 higher than experimentals (assuming 40 butterflies seen, a 10% difference in the other direction). Additional inspection of Manta Real data revealed this difference was due to frequent resightings of surviving Manta Real female butterflies whose release points were frequently visited.

To avoid potential biases from assigning improper capture probabilities to females versus males at Manta Real and experimental versus control butterflies at the three study sites, an intermediate model for resighting ("α intermediate") was chosen. This model included treatment differences in α (control $\alpha = 0.00743$, experimental $\alpha = 0.0106$) estimated across all replicates but Manta Real ($G_1 = 5.63$, $P = 0.0176$) and α estimated separately for males ($\alpha = 0.00687$) and for females ($\alpha = 0.0132$) at Manta Real, the only site where sex differences were noted ($G_1 = 4.08$, $P = 0.0435$). The AIC for the α intermediate model was lowest (AIC = 306.00, four parameters). To avoid bias, these significant effects in resighting probability were utilized to calculate the daily probability of capture (θ_i) for the analysis of survival parameters below.

Survival

Disappearance rates support the hypothesis that selection strongly favours control individuals whose warning colour matches the most common comodel. These differences were in the predicted direction: control butterflies had a higher P_E and a lower λ than experimentals (Table 3.2). Over all sites, maximum likelihood estimates of life expectancy (P_E/λ) were 14 days for controls and five days for experimentals (a 64% lower life expectancy of experimentals, Table 3.2). The life-expectancies estimated from P_E/λ are 3.5 to 6 times lower than those reported by Mallet and Barton (1989a). This is most likely due to increased handling (including sterilization) on released butterflies in my experiment (which is why I included control and experimental butterflies [handled equally] to look for relative survival differences). The support limits in P_E and λ overlap slightly at all sites indicating a slight overlap in estimated life expectancy (Figure 3.2). The AIC analysis indicated the best combination of P_E and λ included additive effects of site and treatment with 12 parameters (site + treatment model). All estimable interactions were not statistically significant. There also was no main effect of sex ($G_4 = 6.60$, $P = 0.16$), thus sex is not discussed further. An overall test for differences in P_E and λ between experimental and control butterflies using this model was statistically significant ($G_4 = 10.72$, $P = 0.03$).

It could also reflect the finer detail in my data where λ is estimated on a daily basis while Mallet and Barton revisited their study sites weekly. Finally, *H. cydno* is larger and more wide-ranging than *H. erato* (used in Mallet and Barton's work) and thus may be lost to the experiment quicker.

Effects of Density

The average distance between release pairs at site 2 (Maquipucuna) was 42 meters. This differed four-fold from sites 1, 3 and 4 where this distance was, on average, 173 meters (range 163 to 193 meters; see methods). These distances

reflect real differences in release density (measured as relative or absolute encounter rates). The released experimental and control butterflies averaged 3% (95% confidence limits on mean [2.6%, 3.4%]) of the total butterflies encountered per day at low density sites. At Maquipucuna, the released experimental and control butterflies averaged 52% (95% confidence limits on mean [47.7%, 55.5%]) of the total butterflies encountered on a daily basis. Absolute encounter rates of released experimental and control butterflies also reflect this difference. At low density sites an average of 2.1 (± 0.52 SE) released butterflies were resighted daily whereas at Maquipucuna an average of 18.2 (± 1.32 SE) released butterflies were resighted daily. A simple model accounting for this difference in the number of butterflies released per unit distance and the resultant density differences calculates treatment effects across sites 1, 3 and 4 (low density sites) and within site 2 (high density) separately (see Figure 3.3).

At high density there were no overall differences (in both P_E and λ) between experimental and control butterflies ($G_4 = 1.72$, $P = 0.79$). The life expectancy for experimentals and controls were both approximately equal (16 and 17 days, respectively, Table 3.2).

As predicted, at the low density sites the experimental butterflies had lower P_E and higher λ than control butterflies ($G_2 = 8.92$, $P = 0.0115$). This test is valid because experimental treatments did not vary among low density sites (e.g., there was no Site * Treatment interaction) and similarly, there were no significant differences in P_E and λ among the three low-density sites (e.g., there was no main effect of site $G_6 = 2.87$, $P = 0.82$). At low density sites, the maximum likelihood estimate of life expectancy was 2 days for experimentals (support limits 0.81 to 4.5 days) and was 12 days for controls (support limits 4.4 to 52.6 days, an 84% lower experimental life expectancy). These limits overlap slightly (0.1 day) if we allow P_E and λ to take on any pair of values determined by the

ellipses in Figure 3.2, because more than one combination of P_E / λ can lead to the same estimated life expectancy.

Experimentals had a much higher death rate (λ) than controls at the low density sites (Table 3.2). At the high density site (Maquipucuna), there were no differences between experimentals and controls in λ. The three low density sites and the high density site had a similar treatment effect in P_E (experimentals lower than controls, Table 3.2). This trend suggests that the effect of increasing density is to obliterate differences between control and experimental butterflies in λ but not P_E. Figure 3.3 shows that the strength of selection quickly drops off as release density increases.

Dispersal

There were no detectable dispersal differences between experimental and control butterflies. Table 3.3 summarizes movement data for all resighted butterflies. Plots of the distance that individuals moved from site of release as a function of time indicate no increase after the first one or two days (data not shown). Some released butterflies actually returned after being resighted away from their release point. The mean of the maximum distance each resighted butterfly moved did not differ between experimental and control butterflies ($t_{41} = 0.42$, $P = 0.68$ [on *ln*-transformed data]). Resighted experimentals maximum distance averaged 105 (range 0 to 552) meters versus 118 (range 0 to 506) meters for controls. Overall, white butterflies had a slightly lower (but non-significant) mean dispersal distance than yellow butterflies (80 vs. 146 meters, respectively). This trend was in the same direction in all sites. Thus, dispersal cannot account for greater disappearance of experimental butterflies because all differences were not significant, and white butterflies (low dispersal) acted as experimentals in three out of four cases. Similarly, at the fourth site (Tinalandia), the higher mean

maximum distance of yellow experimentals was due entirely to one butterfly which moved 552 meters.

Discussion

In this study I found marked overall differences in disappearance rates (P_E and λ) between experimental and control butterflies. When the effects of between-site variation due to density are taken into account, differences between experimentals and controls in λ were even greater. The effect of treatment is also apparent when viewing the raw data (Figure 3.1). What accounts for this effect? I consider two possibilities.

Dispersal or selection

Differential loss of experimental versus control butterflies may be due to differential dispersal of experimentals from release sites, or to death resulting from predation or a different factor correlated with experimental treatment. I assessed the importance of differential dispersal by comparing whether resighted experimental (colour) butterflies were found further from their release localities than controls. All comparisons indicated no significant difference in dispersal between experimental and control butterflies. These data do not rule out differential long-distance (> 600 meter) dispersal occurring in the first 24 hours after release. However, this is unlikely because vigorous searches of likely resighting areas up to 1km away from the periphery of the study area revealed only one butterfly (a control) approximately 200 meters from its release site. Even within each study area remarkably few resighted butterflies flew long-distances. Dispersal is not likely to account for the differences in establishment of the experimental and control butterflies. To do so, dispersal would have to be correlated with a butterfly's treatment (colour) and be relatively large in magnitude. Data on butterfly movement among resighted butterflies show no indication of this trend. Thus, butterfly disappearance is not likely to have been

due to dispersal. Although I have no direct evidence, the most likely factor that remains is predation.

Density, predator learning, and the form of selection

What accounts for the large first day loss of experimental butterflies indicated by their lower P_E relative to control butterflies at all sites (Table 3.2)? If these differences are not due to dispersal then selection against experimentals acts soon after release. Greater early loss (large differences in P_E) due to differential predation by naive bird predators could account for this difference if birds began to learn rare colour patterns subsequent to initial sampling (Brower, Cook and Croze 1967; Benson 1972; Mallet and Barton 1989a).

Predator learning could also account for the difference in life expectancy estimates between site two (Maquipucuna) and the three low density sites (see Table 3.2). Few individual bird predators may be responsible for selection against rare colour butterflies at each study site (Mallet and Barton 1989a; D. D. Kapan, pers. obs). Predators at site two (Maquipucuna) may have quickly learned to avoid the more tightly packed experimental butterflies producing a difference between experimental and controls in P_E but not in λ (see Figure 3.2). Birds at the low density sites would have encountered fewer released butterflies than those at high density sites. As a result, they may have taken longer to learn to avoid experimental butterflies, causing the higher experimental death rate (λ) at these sites. This suggests that the selection does not scale linearly with increasing density, but instead may be proportional to the inverse of density ($1/N$), and thus may be hyperbolic as Müller originally hypothesized (Müller 1879; Mallet pers. comm., see Figure 3.3). Future experiments need to further explore the effects of the density of a given novel warning-colour signal on the strength of aposematic selection.

Strength of selection

The differential loss of experimental versus control butterflies at low-density sites indicates natural selection against rare warning-colour patterns. If I assume that a reduction in experimental life expectancy is equivalent to selection, then the selection coefficient s = 1 - [experimental life expectancy /control life expectancy] (Mallet and Barton 1989a). For colour, accounting for both differences in P_E and λ gives estimates of s = .84 at low-density sites (range s =.77 to > .9), whereas at the high density site, experimental and control butterflies had essentially identical life expectancies (s = .06). Over all the sites combined, s = .64. If selection is equated with only the death rate parameter (λ), then estimates of s against experimentals are slightly lower at all sites (s = .40) and at low-density sites (s = .73, compare P_E/λ versus $1/\lambda$ in Table 3.2).

The benefit of Müllerian mimicry

In the present study I tested the benefit of Müllerian mimicry: does one bad tasting species provide an umbrella of protection for another? This differs from previous mimicry experiments with unpalatable warningly coloured insects (Benson 1972; Mallet and Barton 1989a) where the most common comodel (of a mimetic pair) was either made non-aposematic by painting over the warning-colour pattern or moved into a different mimicry environment set by its own species. These experiments measured the strength of aposematic selection acting within a single species *H. erato*, one of the prerequisites for Müllerian mimicry, rather than the benefit of Müllerian mimicry that *H. cydno* derives from resembling one of two comodel species.

Benson (1972) manipulated the wings of *H. erato* to reduce their resemblance to control sham-manipulated and native *H. erato* and likely rarer native *H. melpomene* comodels. In a later sample of nearly 5000 captured *Heliconius* from a nearby study site, *H. erato* outnumbered *H. melpomene* by three to one (Gilbert 1991).

Benson found that experimental (manipulated) butterflies survived 22% poorer than controls in the first year (Benson 1972; values from Mallet and Barton 1989a). Assuming *H. melpomene* was rarer than *H. erato* at Benson's study site then the increased survival he observed in sham manipulated controls was derived from resembling conspecific native *H. erato* rather than its comodel *H. melpomene*. In either case, Benson concludes that his experiments "demonstrate that selection was operating to promote monomorphism in the experimental populations and that the process was consistent with the Müllerian mimicry hypothesis."

In a similar study, Mallet and Barton (1989a) moved *H. erato* either parallel to (control) or across (experimental) a hybrid zone between two parapatric colour-pattern races of *H. erato*. Consistent with Benson's study and my study, they found that strong selection acted against races with novel warning-colour patterns compared to controls (s =.52 [range .25 to .71] Mallet and Barton 1989a). Mallet and Barton (1989a) were the first to demonstrate that aposematic selection could stabilize narrow warning-colour hybrid zones between races of the same species. However, Mallet and Barton's study sites were also dominated by *H. erato*, approximately 75% of all native butterflies captured at the four study sites were *H. erato* (Mallet et al. 1990). The one study site (Tarapoto) where *H. melpomene* was dominant (57%, Mallet et. al. 1990) showed no difference in life expectancy for experimental versus control *H. erato* (Mallet and Barton 1989a). Thus, their experiments provide a demonstration of the value of looking like a conspecific (aposematic selection, see Chapter 4) but do not unequivocally demonstrate the value of mimicking a putative Müllerian comodel (true Müllerian mimicry, see Chapter 4). In my study, I measured the effect of the Müllerian comodels on the fitness of different colour-morphs of *H. cydno*. At low density sites control comodels (the locally common species) outnumbered native *H. cydno*, accounting for 79.1% (95 % confidence limits of mean [77.4%,

80.7%]) of average daily encounters. In absolute terms control comodels were abundant at low density sites where 29.3 (± 3.42 SE) butterflies were seen daily (see Chapter 4). Therefore control comodels dominated at low-density sites where selection was observed. I conclude that selection measured in my study was largely due to the effect of the Müllerian comodels on the fitness of different colour-morphs of *H. cydno*. This provides the first experimental evidence of which I am aware that aposematic selection operates *between* species, favouring Müllerian mimicry (i.e., morphs matching local comodels survived longer than those that did not).

Selection pressures found in my study were similar to those estimated in different studies of *Heliconius* mimicry. My study measured selection against rare colour-pattern morphs. In nature, the experimental design is equivalent to a rare long-distance dispersal event or an extreme comodel population fluctuation (Mallet and Barton 1989a; Chapter 4). The magnitude of estimates of selection are large although not outside the range of aposematic selection operating within *Heliconius* species, measured either directly in the field on trans-located individuals (range of s =.25 to .71, Mallet and Barton 1989a), or inferred from measures of the width of hybrid zones between races of *Heliconius* species thought to be stabilized by a combination of selection on warning colouration and gene flow (range of $s > .4$ to nearly 1, Mallet 1989; Jiggins et al. 1997).

Mallet and Barton (1989a) also review estimates of selection in mimicry experiments. They found that detectable selection on warning colouration ranged from $s = .22$ to $s = .7$ in several studies that used phenotypic manipulations or release experiments to test the intra-specific value of warning colouration. In almost all of these experiments higher numbers of butterflies or moths were released on a per-site basis than in my study. Large numbers of

releases may improve the statistical ability to detect selection, whereas reducing the strength of selection due to predator learning (as discussed above).

My study focused on colour (white or yellow), a one-locus two allele trait, with yellow being recessive to white (Nijhout, Wray and Gilbert 1990; Chapter 2). The pattern differences discussed in this paper are probably also due to two major alleles at a single locus (Chapter 2). Many controls butterflies matched comodels in both colour and pattern, whereas many experimentals differed from comodels in both colour and pattern. If the selection measured in this experiment is due to colour and pattern differences acting in an additive manner, this represents very strong selection on a per-locus basis (s = .32 per locus). However, separate estimates of the survival contributions of pattern were not made, because most control and experimental butterflies differed in both colour and pattern (e.g., white-band and yellow-triangle butterflies dominated the experiment).

Relevance of natural selection to polymorphism

Strong selection against rare colour-pattern morphs identified in this study indicates that the presence of either comodel species (*H. eleuchia* or *H. sapho*) favours the corresponding mimetic morph of *H. cydno* (yellow-triangle or white-band) where they co-occur. Does this imply selection for polymorphism in Western Ecuador? Strong selection favouring the appropriate colour-pattern combination determined by local comodels should tend to eliminate variation unless *H. cydno* either occurs with both comodels and the latter are common, or occurs with only one co-model at a site but is linked to other populations by gene flow.

Weak selection at high density is also consistent with the existence of relatively stable high-density polymorphic populations in the absence of comodels, such as that at the El Copal source site in the present study. The latter possibility is

supported by the lack of selection when butterflies were released at high-density. Also, high-density *H. cydno* only sites are within the historic range of *H. eleuchia* and *H. sapho*. Many populations of these two comodels (and *H. cydno*) have been destroyed due to relatively recent forest destruction (Dodson and Gentry 1991).

Three forms of divergent selection may be important in maintaining *H. cydno* polymorphism: disruptive selection within sites at one time, temporally variable divergent selection within sites, and spatially variable divergent selection between sites. The present study measured the existence of the latter two. In addition, selection may often be lower than recorded in low density sites in this study because of the increased density of *H. cydno* relative to either comodel species (see Chapter 4).

Conclusions

In this chapter I demonstrate that divergent natural selection favours morphs of *H. cydno* which match locally dominant comodel species. This provides unique evidence for the benefit of signal sharing between the *H. cydno* morphs and their comodels—in other words—the benefit of Müllerian mimicry.

Figure Legend

Figure 3.1. The observed proportion of *H. cydno* treatment classes resighted after the initial release. Controls (——) and experimentals (-----) are graphed against experimental day for Manta Real, Maquipucuna, Agua Caliente and Tinalandia.

Figure 3.2. *Ln*-likelihood profiles for probability of establishment (P_E) and exponential death rates (λ) of released butterflies at each study site, at low density sites (1, 3 and 4 summed), and at all sites combined. Butterflies with lower life-expectancies have lower estimates of P_E (down) and higher estimates of λ (to the right). Study sites are Manta Real (1), Maquipucuna (2), Agua Caliente (3), and Tinalandia (4). The maximum likelihood estimates for P_E and λ are indicated by ● (controls) and ■ (experimentals). The 2-unit support limits (approximate 95% confidence intervals for any one parameter) are indicated by ellipses (controls [——] and experimentals [-----]).

Figure 3.3. Selection as a function of density of release. Selection, s = 1 - [experimental life expectancy /control life expectancy], for each study site (sites numbered as in Figure 3.2 and Table 3.1). The density of release was measured as the number of butterflies released per 100m of trail. The estimate of control life expectancy at site 4 (Tinalandia) was undefined ($\lambda = 0$). In this graph λ at site 4 was set to .008 making the control life expectancy equal to 43 days, an average life span for native butterflies (unpublished data). Without this change the control life expectancy would be infinity ($s = 1.0$), thus this change is conservative. The solid line [——] estimates selection proportional to the inverse of density (N, in this case $s = 1/N^{1.8}$), whereas the dotted line [.....] assumes a linear decrease in selection ($s = -.22N + 1.09$).

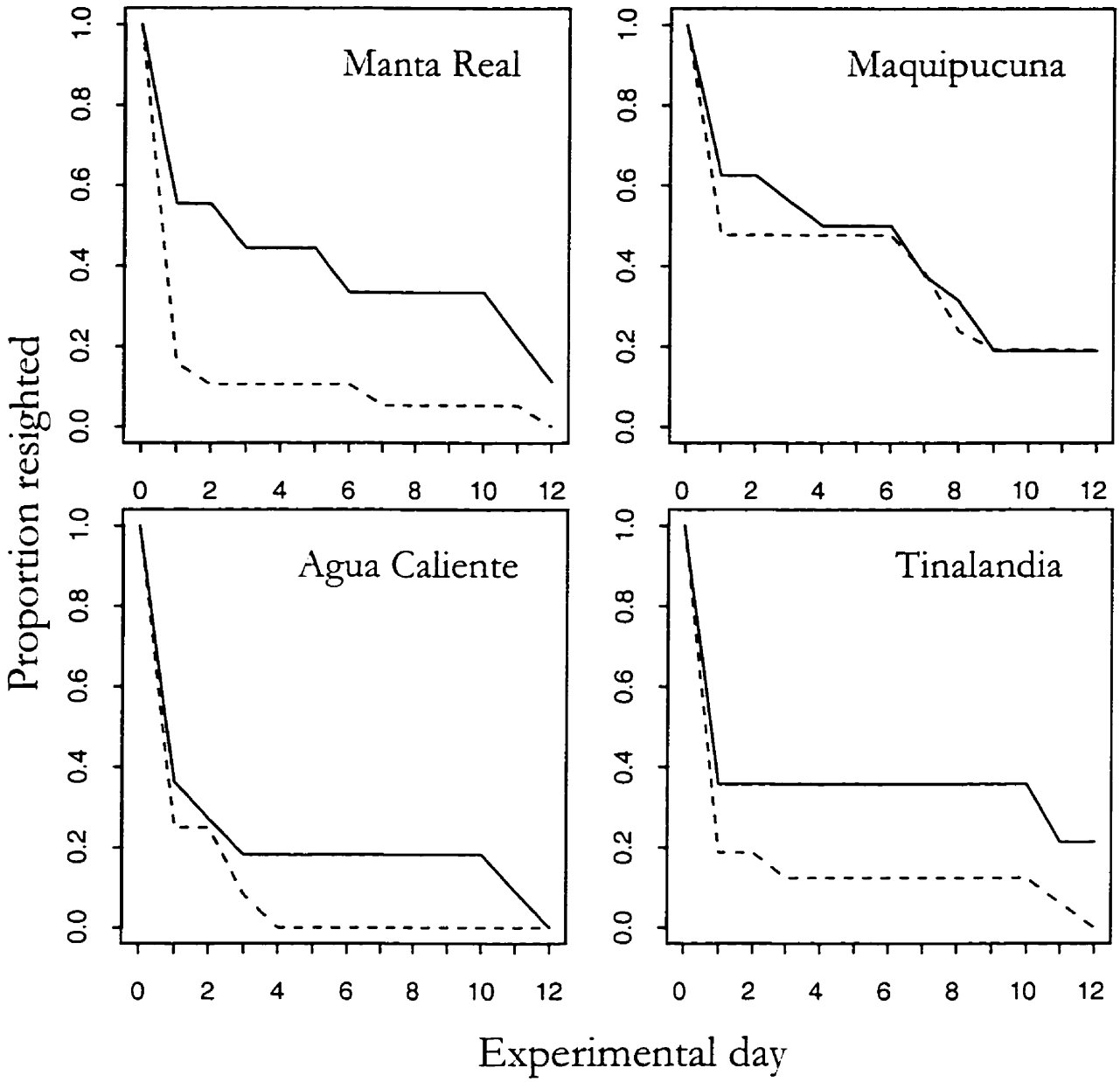

Figure 3.1. The observed proportion of *H. cydno* treatment classes resighted after the initial release (day 0). For sample sizes see Table 3.1.

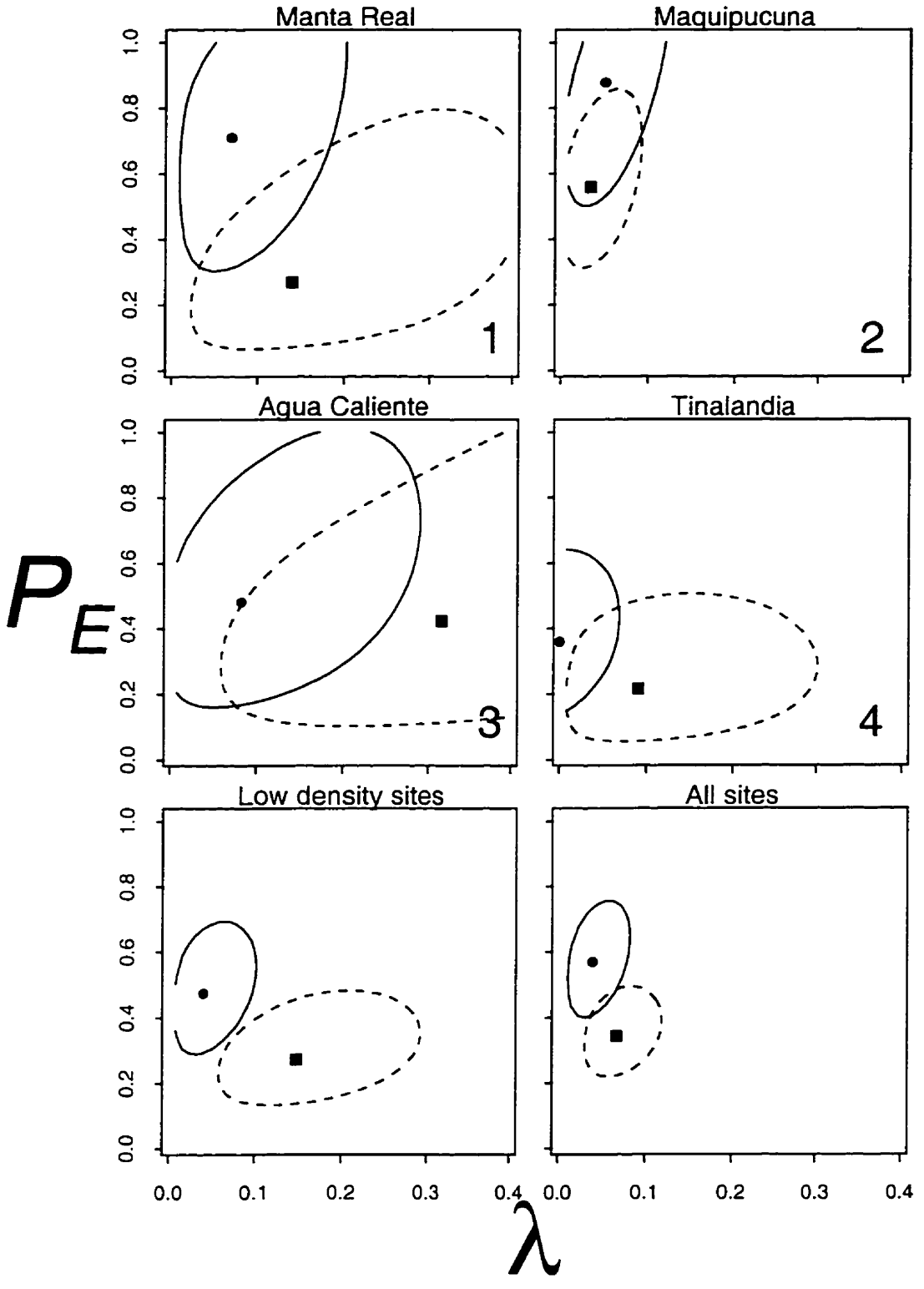

Figure 3.2. *Ln*-likelihood profiles for probability of establishment (P_E) and exponential death rates (λ) of released butterflies at each study site, at low density sites (1, 3 and 4 summed), and at all sites combined.

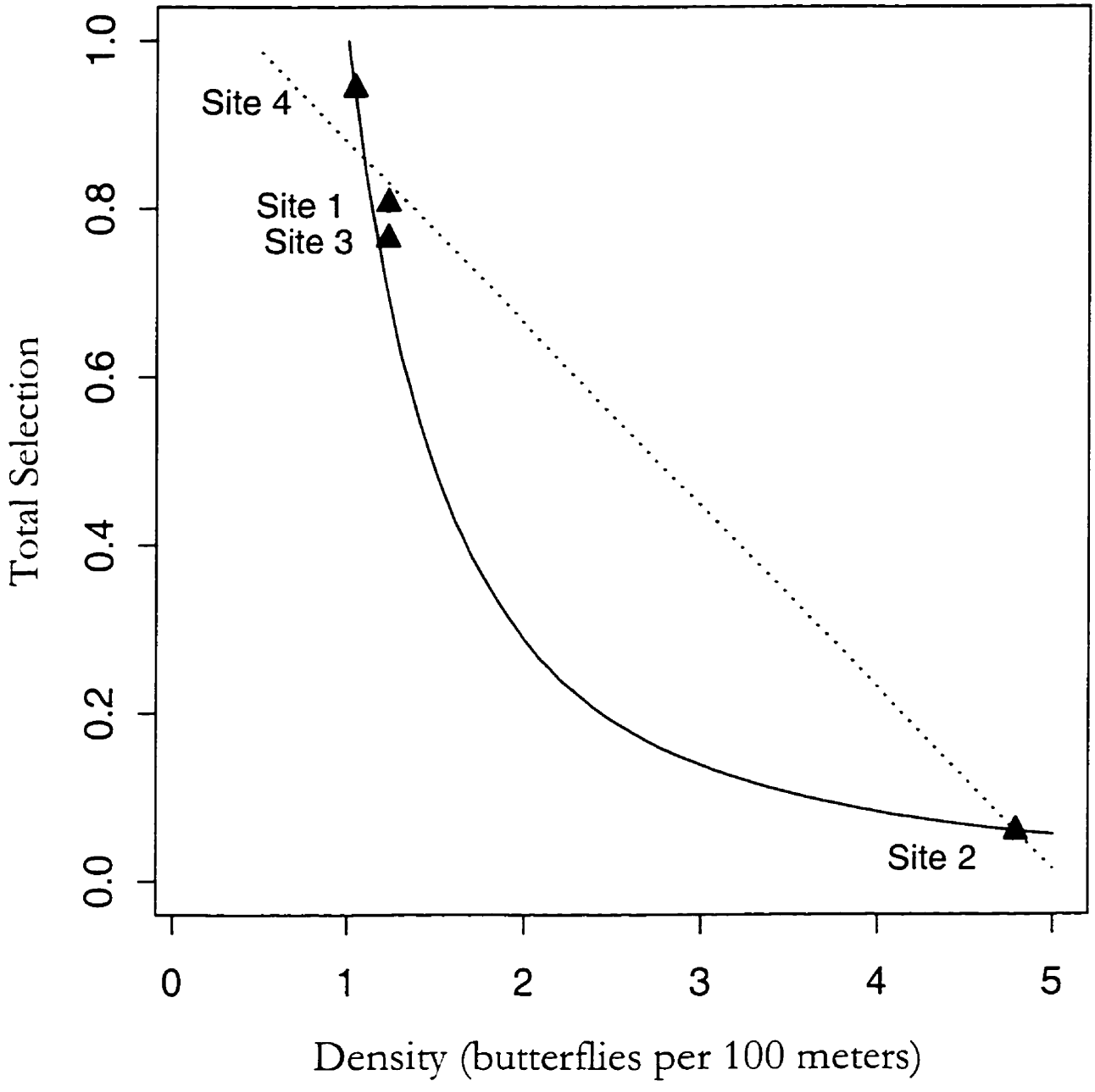

Figure 3.3. Selection as a function of density of release. Selection, s = 1 - [experimental life expectancy /control life expectancy], for each study site (sites numbered as in Table 3.1).

Table 3.1 Details of experimental replicates. Treatments: C = controls, E = experimentals.

Release Site Altitude (m) Latitude & Longitude	Source Site Latitude & Longitude	Experimental dates	Treatments	Numbers of butterflies		Mean known lifespan of resighted butterflies (days)
				Total released	Total resighted	
Manta Real (400 - 500m)	El Copal (885m)	15/16-July-93	C (yellow)	9	5	8.80
2° 34.73' S. 79° 20.92' W.	**0° 52.81' S. 79° 04.96' W.**	28-Jul-93	E (white)	18	3	6.67
Maquipucuna (1150 -1300m)	El Padrino (775m)	03-Aug-94	C (yellow)	16	10	9.80
0° 7.42' N. 78° 37.77' W.	**0° 0.73' S. 78° 59.21' W.**	03-Sep-94[1]	E (white)	21	10	11.90
Agua Caliente (200 350m)	El Copal	23-Nov-94	C (yellow)	11	4	6.00
2° 37.07' S. 79° 28.99' W.		05-Dec-94	E (white)	12	3	2.33
Tinalandia (600-800m)	El Copal	24-Jun-95	C (white)	14	5	10.60
0° 17.88' S. 79° 3.26' W.		07-Jul-95	E (yellow)	16	3	8.00
		Overall	C	50	24	9.13
			E	67	19	8.95
			Total	117	43	

[1] Maquipucuna was last visited 2/3-September-94 and only one experimental butterfly was detected. To maintain relatively equal contributions of each site to the overall likelihood estimates the data was analyzed up to 23 August 1994 (the last regular visit at this site).

Table 3.2 Maximum likelihood estimates of survival rates.

Site	Group[1]	P_E	λ	Life expectancy (P_E/λ days)	($1/\lambda$ days)
Manta Real	C(yellow)	0.710	0.0703	10	14
	E(white)	0.268	0.1403	2	7
Maquipucuna	C(yellow)	0.877	0.0505	17	20
	E(white)	0.559	0.0343	16	29
Agua Caliente	C(yellow)	0.481	0.0833	6	12
	E(white)	0.423	0.3169	1	3
Tinalandia	C(white)	0.360	0.0000	---[2]	---[2]
	E(yellow)	0.216	0.0907	2	11
Low density	Control	0.475	0.0404	12	25
(sites 1,3 & 4 combined)	Experimental	0.273	0.1483	2	7
All sites combined	Control	0.570	0.0403	14	25
	Experimental	0.344	0.0674	5	15

[1] Controls (C) versus Experimentals (E).

[2] Life expectancy (P_e/λ) undefined.

Table 3.3 Dispersal data for resighted butterflies.

	Experimentals	Controls
Resighted only at release point	5	9
Resighted away from release point [1]	14	15
mean distance travelled (m) [2]	105	118
mean distance travelled (m) of butterflies resighted away from their release point [3]	142	188

[1] butterflies seen over 35 meters (maximum detectable distance from original release point).

[2] calculated as the maximum distance each individual moved from release point.

[3] excludes those that didn't disperse (zero maximum distance).

Polymorphic Müllerian Mimicry: the case of *Heliconius cydno*

Abstract

In Western Ecuador, *H. cydno* is polymorphic for wing-colour and pattern and co-occurs with two comodel species, *H. eleuchia* and *H. sapho*. In this chapter I demonstrate that high frequencies of yellow morphs of *H. cydno* are associated with high density of yellow *H. eleuchia*, whereas high frequencies of white *H. cydno* are associated with high densities of white *H. sapho*. I also show temporal changes in phenotype frequency at several study sites. At one site, Tinalandia, changes in *H. cydno* colour-frequency are correlated with changes in comodel frequency. Changes in allele frequencies at colour and pattern loci, estimated from the phenotype data, suggest selection favours combinations of colour and pattern that match one local comodel at only one site (Bilsa). At three sites having both comodels present, linkage-disequilibrium between colour and pattern alleles is in the direction predicted by divergent selection due to Müllerian mimicry (10 out of 12 values positive, but only one is statistically significant). At one site without comodels their are three negative and four positive linkage disequilibrium values. Geographic comparisons of the relative hind-wing band-width of different *H. cydno* morphs suggest disruptive selection when both comodels are present and directional selection when only one comodel (*H. eleuchia*) is present. These results are consistent with the hypothesis that divergent selection generated by two comodels helps maintain the polymorphism in *H. cydno* at some sites. Surprisingly, several high-density *H. cydno* populations are polymorphic in the absence of comodels. I suggest this pattern occurs because high density itself decreases the strength of frequency-

dependent selection (i.e., morphs of the lowest frequency occur in sufficient numbers to educate predators). High density may weaken selection but polymorphism in *H. cydno* is not expected to persist over the long term in the absence of comodels. The long-term maintenance of variation at high density *H. cydno* only sites requires either weak selection and gene flow from polymorphic sites with comodels or another undiscovered factor that helps balance the polymorphism.

Introduction

In this chapter I utilize field data to test the hypothesis that co-occurrence with more than one warningly coloured species (known as comodels) helps maintain polymorphism in *Heliconius cydno.* I call this the polymorphic Müllerian mimicry hypothesis. In butterflies, Müllerian mimic species generally are monomorphic at any one site and share their warning colour with one or more species also present at the site (see Chapter 1; Ford 1971). These Müllerian mimics generally display one of up to five or six modal colour-pattern phenotypes (know as mimicry rings, Papageornis 1975; Turner and Mallet 1996). In contrast to the majority of Müllerian mimics, some species show apparent warning-colour polymorphism. Examples of polymorphic Müllerian mimics can be found in African acraeid and danaid butterflies (Owen et al. 1994), temperate North American bumblebees (Plowright and Owen 1980) and ladybird beetles (Coccinellidae, Brakefield 1985).

Perhaps the most striking examples of polymorphic Müllerian mimics come from several species within the New World genus *Heliconius.* One example is *H. numata*, a polymorphic *Heliconius* species found throughout the Amazon basin. This member of the 'tiger-pattern' mimicry ring is hypothesized to be a Müllerian comodel of many different distasteful Ithomiine species that vary greatly in abundance over time and space (Brown and Benson 1974). Temporal

and spatial variation in comodel abundance has been used to explain the maintenance of polymorphism in *Heliconius numata* (Brown and Benson 1974). A similarly patterned species, *H. ethilla*, occurs in Trinidad where two major morphs appear to mimic different Ithomiine butterflies (Sheppard 1963; Turner 1968a,b; Ehrlich and Gilbert 1973; and L. E. Gilbert unpublished data). A related species, *Laparus doris* (formerly *Heliconius doris*), has three morphs distributed throughout the Neotropics. Two *L. doris* morphs appear to be co-mimetic of species belonging to two different mimicry complexes (Turner 1965; Turner 1968b). In Colombia, *H. cydno* polymorphism is transient. Two polymorphic forms of *H. cydno* (*weymeri* and *gustavi*) coexist in the Cauca Valley and are hypothesized to be mimetic of two separate comodels, one comodel that was historically abundant in less disturbed forest in the last century and one comodel that has become abundant and replaced the other in areas of recent habitat destruction (Linares 1997).

The examples above suggest that divergent selection to match more than one comodel species may account for polymorphic Müllerian mimicry in *Heliconius* species. This hypothesis makes several general predictions: One, a species may be polymorphic when multiple comodels are present (with rare monomorphisms taking longer to reach fixation due to the presence of multiple comodel species, Brown and Benson 1974; Turner 1977) and should be monomorphic when only one comodel is present (e.g., *H. erato* and *H. melpomene*, Sheppard et. al. 1985). Two, similarly, spatial variation in mimetic morph frequencies should be correlated with changes in the abundance of corresponding comodels across sites (Brown and Benson 1974; Turner 1977; Owen et. al. 1994). Three, temporal variation in relative abundance of comodels at any one site will alternately favour one morph then the other (Brown and Benson 1974; Papageornis 1975, Plowright and Owen 1980, Owen et. al. 1994). Four, in a species that is polymorphic at more than one warning-colour pattern locus, allele

frequency changes due to selection generated by comodel abundance variation should generate correlated changes in allele frequency at warning colour and pattern loci. This should favour a single colour and pattern when one comodel predominates (correlated selection), or individuals with colour-pattern elements that match one or the other comodel when more than one comodel is abundant (linkage disequilibrium). Intermediate "mismatch" morphs should be eliminated. Five, for more continuous traits these predictions also hold, long-term association with two comodels should promote a genetic correlation (revealed by a phenotypic correlation) between warning colour and pattern elements that confer resemblance to each comodel. Association with only a single comodel should promote directional changes in traits that confer resemblance to that comodel.

In this chapter I test these predictions in a polymorphic Müllerian mimic species *H. cydno*. I summarize *H. cydno*'s polymorphism and the natural history of its comodels. I then test predictions with special reference to the patterns of co-occurrence of *H. cydno* and its two comodels (*H. eleuchia* and *H. sapho*). To test the predictions of the polymorphic Müllerian mimicry hypothesis, I first analyze data on the presence/absence of *H. cydno* polymorphism and the co-occurrence of *H. eleuchia* and *H. sapho* across Western Ecuador. I then use observations of *H. cydno* morph frequency and the density of the two comodel species collected over five years (1992 to 1997) to test the above predictions for spatial variation, temporal variation, correlated selection and disruptive selection. I also use data on relative hind-wing band-width to look for the signature of selection depending upon which comodels co-occur with *H. cydno* in a given population.

To conclude, I discuss the general phenomenon of polymorphic Müllerian mimicry and speculate why polymorphism persists in the absence of micro-sympatric comodels.

Methods and Materials

Field methods

Much of Western Ecuador is deforested, and as of 1988 less than 8% of land surface below 900m was still covered with native primary forest (Dodson and Gentry 1991). During the last decade over half of this remaining habitat may have been lost (C. A. Dodson, pers. comm.). I searched for sites with sufficient forest cover to harbour *H. cydno* and its comodels in Western Ecuador by consulting researchers in Ecuador, Great Britain, and North America (J. Brown, J. Shihan, J. Decuex , R. Justicia, R. Ontaneda, S. Platonoff, G. Onore, C. Dodson, J. Mallet, and A.V.Z. Brower pers. comm.); by consulting 1:50,000 scale topographic maps (Instituto Geographica Militar, Quito, Ecuador); and by visiting nearly all major and many minor roads accessible to four-wheel drive vehicle, from Alto Tambo (0° 51' N, 78° 29' W) in NW Ecuador, to the transition from moist to dry forest at 3° 25' S, 79° 45' W along the Andean foothills in SW Ecuador. In addition I visited the slopes of the Cotocachi-Cayapas biosphere reserve along Rio Santiago via canoe as well as coast range reserves at Tonchingue (along the coast south of Esmeraldas 0° 55' N, 79° 55' W), Bilsa Reserve (in the Montañas de Mache south of Esmeraldas), and Cerro Blanco west of Guayaquil. During these surveys I located 30 sites where at least one of the three study species was found (Appendix 4.1). I thoroughly searched an additional 10 potential sites where none of the three study species were found. Sites with more butterflies were generally visited for longer periods, and some sites were visited repeatedly. Individual site visits lasted from one to 20 consecutive days between 1992 and 1997. Wet season samples (mid-January through mid-May) were not taken.

Heliconius butterflies inhabit forest light gaps where host plants, adult feeding plants, sunny flight corridors, and roosting areas are most likely to be found

(Gilbert 1972; Mallet et. al. 1987; Gilbert 1991). These light gaps often coincide with trails, creeks, roads and rivers along which I traveled to collect the data.

Presence and abundance of the three study butterfly species may be determined in part by the occurrence of their respective host plants (Gilbert 1991). All *Heliconius* butterflies have host plants in the family Passifloraceae, most in the genus *Passiflora* (Brown 1981). I noted *Passiflora* as I proceeded through the forest. Host plants for *H. eleuchia* (*P. macrophyllum*) and *H. sapho* (*P. pittieri*) were scored as present if they were found at a study site over the five years of the study, and absent if they were not detected during a minimum of five days searching in 1992 to 1997 for *P. macrophyllum* and five days searching since 1994 for *P. pittieri*. Detection of the latter species is difficult, and its definite absence can only be ascertained by exhaustive collecting. *H. cydno* is a generalist that will utilize nearly all species of *Passiflora* including *P. macrophyllum* and *P. pittieri*.

Although the absolute density of butterflies has long been considered of fundamental importance in mimicry (Müllerian 1879, Sheppard and Turner 1977), comparative work on polymorphic Müllerian mimicry (Owen et. al. 1994; but see Brown and Benson 1974), and simple mathematical (Huheey 1976) and population genetics models (Gavrilets and Hastings 1997) assume density to be constant and compare only the relative frequency of different morphs or species despite the authors' recognition that density is important. Neotropical birds are likely to encounter butterflies in proportion to their density (Chai 1986). Although some species may differ in catchability (Brown and Vasconcellos-Neto 1976), palatability (Chai 1990), or memorability of their patterns (Mallet and Barton 1989b), this is probably not the case with the three *Heliconius* species in this study (D. D. Kapan, pers. obs.). Unless the overall density of comodels is constant or comodel density is perfectly negatively correlated, the relative comodel frequency will not accurately reflect changes in the selective

environment (Turner 1977; Turner, Kearny, and Exton 1984). *H. eleuchia* and *H. sapho* vary over a high range in density probably due to variation in the abundance and phenology of their host plants (D. D. Kapan, pers. obs.).

Thus, for between site comparisons I adopt density of each comodel as a measure of the selective value of a given Müllerian mimetic warning signal rather than relative comodel frequency because density is neither constant nor perfectly negatively correlated between comodels. Morph frequency of *H. cydno* is the appropriate response variable. To estimate species density and morph frequency, I continuously counted all three study butterfly species that were encountered (Brown 1972). Four morphs of *H. cydno* (white-band, white-triangle, yellow-triangle, yellow-band; see Figure 1.1) were identifiable on the wing with the naked eye or with 10-power Bausch and Lomb binoculars. Some butterfly phenotypes were identified to colour only (especially during June through mid October 1992). To double check the accuracy of visual phenotype identification, I captured a sample of butterflies with multi-section tropics nets (BioQuip Corporation) using standard techniques (Benson 1972; Gilbert 1972; Ehrlich and Gilbert 1973; Gilbert 1991) and recorded the phenotype details. To ensure that these captured butterflies were not double counted, I individually marked them on the ventral hind-wing with numbers (0-999) using a Sharpie Ultra fine-point indelible marker (Sanford Corporation). I then measured the relative hind-wing band-width of these butterflies using a dial caliper as described in Chapter 2. Marked butterflies were then released for future resighting or recapture in other phases of the study (see Chapter 3).

H. cydno morph frequency was estimated from all individual encounters (captures and sightings) and from unique first-time captures only. I used the former estimates because it is much easier to visually "capture" than physically net *H. cydno* and its comodels. The two estimates were generally consistent except when

the sample size of the capture-only data was small. Density was estimated as the number of butterflies of a given species encountered per unit time (converted to eight hour days) at a given study site. This method of estimating density, although approximate, is sufficient to distinguish between sparse and dense populations.

Statistical methods

The frequency of different *H. cydno* morphs and the relative frequency of comodel species was estimated from the total number of encounters (all captures and sightings) of each morph (or species) at a given study site for a span of contiguous dates (up to 20 consecutive days). Separate estimates were generated for each additional trip to those sites that were visited multiple times. For all comparisons at a single site, visits in which the median sample dates were less than one generation apart (27 days from egg to egg, assuming it takes three days to mate and lay) were pooled. These visit samples (November and December 1994, June and July 1995 at Tinalandia, and July and August 1993 and June and July 1995 at El Copal) are likely to be autocorrelated and were only inspected graphically rather than included in statistical analyses. Data for each unique site visit are referred to as the "Sites" data set. This data set was utilized to cross-tabulate *H. cydno* and comodel co-occurrence as well as to calculate allele frequencies and gametic correlations for some study sites.

Geographic and time series data often show some degree of autocorrelation (Sokal and Oden 1978; Sokal, Jacquez and Wooton 1989; Diggle 1990). Comodel populations and *H. cydno* morph frequencies may be similar between two study sites due to shared environmental factors and gene flow. Comodel distribution and density is dependent on the presence and abundance of their host plants. *H. cydno* phenotype frequencies alone may show autocorrelation due to gene flow, a common selective environment imposed by comodels or both.

Spatial and temporal autocorrelation were therefore investigated before proceeding with further analysis.

Spatial autocorrelation was assessed by calculating the spatial autocorrelation coefficient Moran's *I* (Sokal and Oden 1978) for comodel density and *H. cydno* phenotype frequencies. Moran's *I* is calculated for a group of sites whose inter-site distances fall into a particular distance class. The value of *I* as a function of distance class for 1992, when the highest number of study sites (20) was visited during one field season, is shown in Figure 4.2. All distance classes for *H. eleuchia* and *H. sapho* density had non-significant values of *I* (Figure 4.2a, b; relative comodel frequency also showed no spatial autocorrelation, data not shown). The only detectable between-site autocorrelation was in the proportion of yellow *H. cydno* at distances less than 33 kilometers ($I = +0.54$, $Z = 2.26$, $P = 0.024$, Figure 4.2c). This autocorrelation in *H. cydno* proportion quickly breaks down with increasing distance (33 to 66 kilometers $I = 0.12$, $Z = 0.96$, $P = 0.34$; Figure 4.2c). Sites greater than 33 kilometers apart have relatively independent colour-morph frequency, although there is a tendency for values of *I* to be negative from 66 through 300 kilometers and the variance is so high that none of these values is significantly different from zero (Figure 4.2). These patterns were identical when Moran's *I* was calculated with all data (summed over time). The only detectable spatial autocorrelation was in *H. cydno* colour phenotype frequency between those sites closer than 30 kilometers (data not shown).

Temporal autocorrelation is also present in the data. One source is repeated measurement of the same butterflies if sampling periods fall within a butterfly's lifetime (mean minimum life-span of a related butterfly *H. ethilla* was found to be approximately 50 days, Ehrlich and Gilbert 1973; this agrees with observed life-spans of *H. cydno*, D. D. Kapan unpublished data). For overall regression analyses (see below) I avoided this source of temporal autocorrelation by

summing encounter counts taken within the early dry season (May to August) and late dry season (September through early January). Both time periods represent sufficient time for up to five generations of *H. cydno* to breed thus allowing for the possibility of substantial morph frequency change. As a result, each seasonal period (early/late) contributed a single observation to regression analyses.

Correlations remaining between these temporal blocks were not corrected further. If such temporal correlations are due to fixation of a particular allele, this will inflate the degrees of freedom for hypothesis tests. Care was taken to note these circumstances, although model fitting was carried out with the full data set (see results). Data from sites less than 33 km apart and within a given season (early dry season May to August and late dry season September to December/January) were lumped into a single study area/time observation to remove spatial autocorrelation and provide greater temporal replication. This is called the "Area" data set to distinguish it from the finer scale "Site" level measurements (see below). Appendix 4.2 summarizes the 29 site/time combinations in the Area data set.

To test the polymorphic Müllerian mimicry hypothesis I regressed the phenotype frequency of *H. cydno* against comodel density. I utilized samples from the Area data set that met either of the following two criteria: at least 10 *H. cydno* co-occurred with both *H. eleuchia* and *H. sapho* (n = 3 areas) or at least 10 *H. cydno* co-occurred with *H. eleuchia* (n = 2 areas). This reduced the Area data to 18 site/time combinations. Sites without comodels were not included in the regression analyses. I performed two separate multiple logistic regressions (binomial response variable), one for colour and one for pattern, to assess how well comodel density predicted *H. cydno* colour and pattern morph frequency.

Allele frequencies and Linkage disequilibrium

I estimated allele frequencies assuming two loci (colour and pattern) each with two alleles (Chapter 2) from the counts of the four main *H. cydno* morphs using the Hardy-Weinberg model. I did this for each unique visit (Site data set) to four sites where polymorphic *H. cydno* populations either locally co-occurred with two comodels (Tinalandia, Bilsa and El Padrino) or with no comodels (El Copal). I estimated the allele frequencies p_W, q_w for W/w alleles at the colour locus and p_B, q_b for the B/b alleles at the pattern locus respectively (see Chapter 2) by fitting the proportions of the four observable phenotypes (white-band, white-triangle, yellow-band and yellow-triangle) to the Hardy-Weinberg model using maximum likelihood (Hill 1974; Weir 1990; Edwards 1992). With only four phenotype classes, the maximum likelihood estimator for allele frequency is equal to the square-root of the frequency of a given recessive colour or pattern phenotype in the population. For instance, q_w the frequency of the recessive *w* (or "yellow") allele is equal to the square-root of the frequency of yellow butterflies in the population. Due to complete dominance, these allele frequency estimates may be quite biased (owing to an undetectable excess or deficit of heterozygotes) however this problem is somewhat alleviated by relatively high frequency of recessive colour (yellow) and pattern (triangle) traits in the populations. Allele frequency change over time at a single site can be due to selection, drift, non-random mating, and sampling error. I look to see whether these changes are in the direction predicted by Müllerian mimicry: towards *W* and *B* (white-band) to match white *H. sapho*, or towards *w* and *b* (yellow-triangle) to match yellow *H. eleuchia*. Mismatches would not support the Müllerian mimicry hypothesis: towards *W* and *b* (white-triangle), or towards *w* and *B* (yellow-band).

From these allele frequencies the maximum likelihood estimator of linkage disequilibrium (*D*) can be calculated. Linkage disequilibrium is the difference of the observed frequencies for "*wb*" gametes (equal to the square root of the

frequency of yellow-triangle butterflies [*wwbb* genotypes] in the sample; Chapter 2) and the expected value from the estimated allele frequencies ($q_w q_b$). The maximum possible disequilibrium value (D_{max}), given the allele frequencies, is the minimum of the product of expected gamete frequencies $p_W p_B$ or $q_w q_b$ when $D > 0$ and minimum of $p_W q_b$ or $p_w q_B$ when $D < 0$ (Lewontin 1964; Hedrick 1987; Hartl and Clark 1989). Positive values of D indicate that *w* and *b* alleles (and *W* and *B* alleles) are positively associated in gametes and negative numbers indicate that these are negatively associated (Weir 1990). I expect disruptive selection to generate positive linkage disequilibrium between "*w*" and "*b*" and "*W*" and "*B*" alleles (e.g., yellow-triangle and white-band phenotypes are favoured). Since values of D depend upon the allele frequency at each locus (Hedrick 1987; Lewontin 1988), I report two standardizations of linkage disequilibrium, the gametic correlation coefficient $R = D/\sqrt{(p_W q_w p_B q_b)}$ and related scaled linkage disequilibrium coefficient $D' = D/D_{max}$ (Lewontin 1964; Mallet et. al. 1990). Neither R or D' are independent of allele-frequency because the denominator in both terms changes as a function of allele frequency (Lewontin 1988; Mallet et. al. 1990) so comparison between times and sites (with different allele-frequencies and different D) must be made with caution. I assess the significance of D (any deviations from that expected under the Hardy-Weinberg model) by estimating G (twice the difference of the negative *ln*-likelihoods of the two parameter model [q_w, q_b] and the saturated model [q_w, q_b, D]) on one degree of freedom (Edwards 1992; Mallet et. al. 1990). G is approximately distributed as chi-square and is preferable to a chi-square test conducted on phenotype frequencies when sample sizes in any one category are low (Zar 1984). Owing to complete dominance for both colour and pattern traits, this test is not very powerful (although the large fraction of homozygous recessive individuals [yellow and triangle] somewhat ameliorate this problem). Despite these difficulties, I proceed with the analysis to determine if disequilibrium, even if underestimated, is in the direction predicted by mimicry.

Phenotypic correlation of colour pattern with hind-wing band-width

Relative hind-wing band-width (RHWBW) was measured as the length of the white or yellow trailing edge (BW) along the M3 wing vein expressed as a proportion of the total length of the M3 wing vein (BW to M3; see Chapter 2 and Figure 1.1). This size corrected measurement is highly correlated with the residuals of a regression of BW on M3 within sites but is better than residuals for between site comparisons because its calculation does not depend on the reference population. I transformed this measurement before statistical tests by taking the arcsine of the square root of RHWBW (Zar 1984). To assess the hypothesis that white-band butterflies have a narrower RHWBW than yellow-triangle butterflies in the presence of two comodels, I use a one-tailed t-test on the Bilsa RHWBW data. I also use the Bilsa data to perform an analysis of variance to determine if RHWBW variation is correlated most to colour, pattern, or both. I use one-tailed t-tests on site means or variances of RHWBW to assess the effects of different comodel configurations, which vary by site, on RHWBW.

Results

Background: Natural history of Ecuadorian Heliconius cydno *and its comodels* H. eleuchia *and* H. sapho

Across lowland Western Ecuador south of 1° N latitude these two colour (white and yellow) and two pattern (triangle and band) forms result in four different colour-pattern phenotypes (Figure 1.1). Two of these colour-pattern morphs of *H. cydno* (yellow-triangle and white-band) appear to match two different sympatric monomorphic warningly coloured *Heliconius* comodels, *H. eleuchia* and *H. sapho*, respectively (Figure 1.1; Figure 4.1). One *H. cydno* population in NW Ecuador (Maquipucuna) and all populations south of 1° S along the Andean foothills in Southern Ecuador are sympatric with only one comodel, yellow *H. eleuchia*, and are monomorphic for yellow (Fig. 4.1; Appendix 4.1). Finally, in

NW Ecuador several sites harbour dense polymorphic populations of *H. cydno* in the local absence of either comodel (Figure 4.1; Table 4.1).

Host-plant and comodel distribution

H. eleuchia and *H. sapho* belong to the *H. sapho* subgenus, which specializes on woody *Passiflora* vines in the subgenus *Astrophea* (Brown 1981; Longino 1986). In Western Ecuador *H. eleuchia* specializes on *Passiflora macrophyllum* (Spruce ex Mast.; D. D. Kapan, unpublished oviposition, rearing and field eclosion records). *H. sapho* also specializes on a single host plant, *Passiflora pittieri* (Mast.), in Western Ecuador (D. D. Kapan, unpublished field eclosion and rearing records).

There appears to be a correspondence between these two comodel species' distributions and that of their host plants, although sampling of the latter is difficult (Appendix 4.1; D. D. Kapan, unpublished data). *P. macrophyllum* is a riparian treelet that occurs in relatively undisturbed moist forest from sea level to greater than 1300 meters elevation in Western Ecuador (D. D. Kapan, unpublished data). *P. macrophyllum* grows most commonly along small shaded streams in the forest understory (D. D. Kapan, pers. obs.). *P. pittieri* is a canopy vine with a known distribution in Western Ecuador ranging from 250 to 800 meters elevation (D. D. Kapan, unpublished data). Both localities where *P. pittieri* grows (Bilsa and Tinalandia) have virgin forest. Based on *H. sapho* distribution in Western Ecuador, *P. pittieri* only occurs within several forest reserves and other patches of undisturbed forest such as mountain tops and cliff-sides. Field surveys indicate the distribution of *P. pittieri* is nested within the distribution of *P. macrophyllum*. Wherever the more restricted *P. pittieri* is found, so is *P. macrophyllum* (see Appendix 4.1). The nested distribution of these two host plants is likely why the distribution of *H. eleuchia* and *H. sapho*, which depend upon them, is nested. Table 4.1 illustrates this relationship; wherever *H. sapho* occurs (three sites, two of which are known to have *P. pittieri*), we also find

H. eleuchia. However, *H. eleuchia* occurs in the absence of *H. sapho* (four sites all of which are known to have *P. macrophyllum* and not *P. pittieri*).

Polymorphism in H. cydno *and comodel co-occurrence*

H. cydno polymorphism in colour, but not pattern, is correlated with the occurrence of *H. eleuchia* and *H. sapho* (Table 4.1). All three sites that harboured *H. eleuchia* and *H. sapho* also harboured both colour morphs of *H. cydno.* All four *H. cydno* populations co-occurring with only *H. eleuchia* were monomorphic for yellow, the comodel's colour ($P = 0.029$, Fisher exact test). However, *H. cydno* was polymorphic in wing pattern whether two comodels were present or just one (Table 4.1), but, the proportion of band butterflies was always very low (Appendix 4.1). *H. sapho* did not occur at any site without *H. eleuchia*, and correspondingly, no *H. cydno* population is monomorphic for white.

Surprisingly, *H. cydno* was polymorphic for colour at five study sites lacking either comodel species and it was polymorphic for pattern at least two of these (Table 4.1). Polymorphism in the absence of both comodels is not predicted by the theory of warning colouration (see discussion). Polymorphism where comodels are absent may result from gene flow from nearby sites where comodels were historically abundant. The current distribution of *H. sapho* is restricted compared to its historic distribution (Figures 4.1b and 4.1c) lending some credence to this hypothesis (see discussion).

H. cydno *morph frequency versus comodel density*

The frequency of yellow *H. cydno* and of triangle *H. cydno* should be positively correlated with *H. eleuchia* density and negatively correlated with *H. sapho* density. This expectation is clear because the densities of *H. eleuchia* and *H. sapho* are partially inversely related (Figure 4.3; based on 18 samples with at least one comodel and over 10 *H. cydno* present per sample period). Sites with the highest densities of *H. eleuchia* lack *H. sapho.* Sites with the highest density of *H. sapho*

have intermediate to low densities of *H. eleuchia*. My test of these predictions uses the Area data set (Appendix 4.2).

As predicted, frequency of yellow *H. cydno* was positively correlated with *H. eleuchia* density and negatively correlated with *H. sapho* density (Figure 4.4; based on 18 site/time combinations with comodels and sufficient data).[1] The yellow morph reaches fixation at the highest *H. eleuchia* densities, where *H. sapho* is absent (Figure 4.3). The strength of these correlations was tested with multiple logistic regression of *H. cydno* colour-morph frequency against the density of both comodels (Table 4.2). Overall this regression model explains almost half of the variance ($R^2 = 0.413$). This test is not completely independent of the test of polymorphism versus comodel presence and absence (Table 4.1) because where *H. cydno* occurs with *H. eleuchia*, we see fixation of yellow. When only sites with two comodels are utilized, the correlation becomes weaker, remaining in the right direction for *H. eleuchia* but becoming essentially flat for *H. sapho*. Thus, it appears comodel density is a predictor of colour frequency when all sites are considered together, and *H. eleuchia* density remains influential when sites with two comodels are considered separately. At these latter sites the presence of *H. sapho* predicts polymorphism (as in Table 4.1.) but does not predict morph frequency.

In contrast to colour, the proportion of triangle *H. cydno* butterflies does not correlate with the density of either comodel (Figure 4.5). Trends were in the right direction but were extremely weak and not statistically significant (see Table 4.2 and Figure 4.5). The proportion of triangle butterflies varies much less than the colour morph frequencies between sites in Western Ecuador ($P_{triangle}$ range [0.60, 1] vs. P_{yellow} range [0.25, 1]).

Temporal variation in morph frequencies within sites

Time variation in *H. cydno* morph frequency may be correlated with comodel density variation (Figure 4.6). However, temporal replication within sites is limited; only Bilsa and Tinalandia have sufficient change in the frequency of comodels to allow a test.

At Tinalandia the densities of comodels are nearly perfectly negatively correlated with each other when either are greater than approximately three per day (r = -0.833, t_5 = -3.373, P = 0.0198). Thus, I adopt comodel frequency as the predictor variable at Tinalandia because any change in the proportion of *H. eleuchia* relative to all comodels reflects differences in relative abundance of the two comodels and comodel frequency allows a better visualization (one graph vs. two) at Tinalandia. Comodels at Bilsa are also negatively correlated but with only four samples, this is far from certain. Within Bilsa, no patterns are revealed with either density or frequency correlations.

At Tinalandia, time variation in proportions of yellow *H. cydno* morphs follows temporal changes in comodel frequency (Figure 4.6a). The data are five large samples from late dry season 1994 through mid dry season 1995 at Tinalandia. A lag in the proportion of yellow *H. cydno* is indicated by the counterclockwise rotation. Bilsa also has large changes in the relative frequency of comodels over the period August 1994 through January 1997, but there is no correlated change in the frequency of *H. cydno* colour morphs (see Figure 4.6b).

Allele frequency change and gametic correlations

H. cydno may have higher dispersal and a more stable population size than either comodel species. If this is true, *H. cydno* may integrate comodel population

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[1] Note apparent outlier in lower panel of Figure 4.4 (at P_{yellow} ~ 0.8 and density of *H. sapho* > 30) corresponds to an intermediate density of *H. eleuchia* (~ 18 per group day) and is thus

fluctuations over a wider area or longer time scale than that measured at a local study site like Bilsa. Allele frequency changes in colour and pattern give us a another way to test whether difficult to measure large-scale changes in comodel abundance affect *H. cydno*'s polymorphism. Correlated selection on *H. cydno* generated by comodel replacement over time should result in simultaneous increases or decreases in allele frequency at p_W and p_B. That is, correlated selection should lead to increases in white-band phenotypes with decreases in yellow-triangle phenotypes or vice-versa. This trend, indicated by linear regression of phenotype frequency versus time, is seen at Bilsa and El Copal but not Tinalandia (see Figure 4.7). Data are insufficient to determine whether this is the case at El Padrino (Figure 4.8b). At the allele level, correlated selection at the colour (*W*, *w*) and pattern (*B*, *b*) loci should favour *WB* if *H. cydno* is tracking a general increase in *H. sapho*, and favour *wb* if it is tracking a general increase in *H. eleuchia*. Figure 4.9 shows plots of estimated allele frequency change over four study sites. There was no tendency for co-variation in allele frequency change from one time to the next (arrows bounce back and forth). However, the main direction of change at Bilsa (towards *WB*) is consistent with mimicry with *H. sapho*, whereas the main direction of change (towards *wb*) at El Copal is not interpretable in terms of mimicry because there are no local comodels at that site (Figure 4.9).

Sympatry with two comodels should generate disruptive selection favouring those attributes of *H. cydno* that result in a match to the comodels (e.g., white-band and yellow-triangle if both comodels are present) over hypothesized "mismatch" morphs (white-triangle and yellow-band). This may be detected as positive linkage disequilibrium (*D*) and gametic correlation (*R*) between the *w* (for yellow) and *b* (for triangle) alleles, and between the *W* (for white) and *B* (for

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influenced by both high density of *H. sapho* and moderately high density of *H. eleuchia*. This is accounted for in the multiple logistic regression model.

band) alleles, where *H. cydno* is polymorphic. Positive gametic correlations are expected at sites with two comodels, and *D* should not differ from zero at sites with no comodels. Table 4.3 shows allele frequency and gametic correlation estimates from four sites where *H. cydno* is polymorphic. Two comodels are present at Tinalandia, Bilsa, and El Padrino, whereas El Copal has no sympatric comodels. Sites with both comodels (Tinalandia, El Padrino and Bilsa) tend to have positive *D* values (10 of 12 observations), whereas El Copal has four positive and three negative values. Although in the predicted direction, contrasts between sites with comodels and the one site without comodels do not differ in the sign of *D* and *R* values (Table 4.3). Average *R* values are 0.104 for sites with comodels and 0.003 for El Copal without comodels. However, only two *D* values are statistically significant, both positive (Bilsa in July 1995 and at El Copal in July 1995; see Table 4.3). In conclusion, disequilibrium measures, although in the right direction, do not support the prediction that the presence of two comodels generates two adaptive peaks (favouring a gametic correlation between colour and pattern) in *H. cydno* populations. If there is disruptive selection, favouring white-band and yellow-triangle at sites with two comodels, it must be weak.

Phenotypic correlation of colour pattern with hind-wing band-width

Heliconius eleuchia has a wide hind-wing band (mean RHWBW = 0.77 ± 0.001 SE), and *H. sapho* has a narrow hind-wing band (band mean RHWBW = 0.39 ± .0001 SE). Disruptive selection generated by the presence of these two comodels should be reflected in the relative hind-wing band-width of different *H. cydno* morphs. I test two predictions using the Bilsa data. First, the relative hind-wing band-width of yellow-triangle *H. cydno* should be greater than RHWBW for white-band *H. cydno*. At Bilsa the mean RHWBW of yellow-triangle is 0.57 ± 0.0126 SE, and for white-band *H. cydno*, it is 0.51 ± 0.0231 SE (t_{27} = -2.16, P = 0.0199). This indicates that, as predicted, yellow-triangle *H.*

cydno have wider hind-wing bands than white-band *H. cydno*. These differences (~ .06) in mean RHWBW between extreme morphs of *H. cydno* are much smaller than the difference between *H. eleuchia* and *H. sapho* in mean RHWBW (0.38).

Is *H. cydno* relative hind-wing band-width at sites with two comodels related to pattern or colour differences? An analysis of variance of Bilsa data reveals that RHWBW is mostly related to pattern and not colour. The means (± SE) for each pattern (triangle = 0.568 ± 0.043 and band = 0.519 ± 0.071) are different, whereas colour differences (yellow = 0.560 ± 0.038 and white = 0.557 ± 0.044) are negligible. Pattern alone explains the variance (interactions and main effect of colour were not significant). A linear model relating three levels of pattern (band, intermediate and triangle) to hind-wing band-width was significant ($F_{1,86}$ = 5.73, P = 0.0188).

Second, is the variance in *H. cydno* RHWBW at sites with two comodels greater than comodel-free sites? Bilsa, with comodels, has a higher variance (σ^2 = 0.107) than El Copal, without comodels, (σ^2 = 0.020, Figure 4.10). The variance in RHWBW at Tinalandia (the other site with two comodels) is also higher (σ^2 = 0.087). A two sample t-test comparing the variance of Bilsa (above) and Tinalandia (0.0969) with those of El Copal (above) and La Hesperia (0.0250), where comodels are absent, is significant (t_2 = 6.43, P = 0.0117; see Figure 4.10).

Third, mean *H. cydno* RHWBW is also higher in sites with *H. eleuchia* only (0.66) than at sites with both comodels (0.56; t_2 = 5.46 , P = 0.016; Figure 4.10). This difference is due to an increase in mean RHWBW of yellow morphs at all yellow sites, not simply a loss of white-band and white-triangle butterflies.

In conclusion, *H. cydno* RHWBW variation is consistent with Müllerian mimicry with two comodels at sites with both *H. eleuchia* and *H. sapho* and with only one comodel where it co-occurs only with *H. eleuchia*.

Discussion

Results from Chapter 3 indicate that divergent selection generated by Müllerian mimicry with two different comodels favours different colour-morphs of *H. cydno*. This raises the possibility that positive frequency-dependent selection to match two different comodels may help maintain colour-pattern diversity within polymorphic *H. cydno*. This is the polymorphic Müllerian mimicry hypothesis. I find support for several predictions of this hypothesis. *H. cydno* was polymorphic for colour and pattern in the presence of two comodel species. When it occurred with only a single abundant comodel (*H. eleuchia*), *H. cydno* was monomorphic for the predicted colour (yellow) but not for pattern. I found an association between colour frequencies and comodel density: the proportion of yellow *H. cydno* was positively correlated with *H. eleuchia* density and negatively correlated with *H. sapho* density (weaker). However, no similar relationship between comodel density and wing-pattern was found. The colour results show that co-occurrence with a single comodel eliminates variation as predicted by classical mimicry theory. When *H. cydno* co-occurs with two comodel species, it is always polymorphic across a range of comodel densities (Figure 4.4 and Table 4.1). Therefore divergent selection may operate on colour within areas where two comodels occur consistent with selection pressures measured in Chapter 3. Although direct site to site correlations in relative frequency of morphs and comodels are weak, two comodels appear to provide an umbrella of protection for both colour morphs of *H. cydno* across a broad area of Western Ecuador. In support of this pattern, at one site, Tinalandia, where *H. cydno* is generally rare with respect to the comodel species, colour-morph frequency tracked comodel frequency over a period of nearly a year (see Figure 4.6).

Estimates of allele-frequency change and linkage disequilibrium were less conclusive. Allele frequency change was in the direction predicted if tracking a global increase in *H. sapho* at Bilsa where *H. sapho* has become more abundant

since 1994 but allele frequency change from period to period was not directly correlated with *H. sapho* change at this site. Also, allele frequency change at El Copal was in the direction favouring yellow and triangle phenotypes despite the absence of local comodels. Strong linkage disequilibrium between alleles at colour and pattern loci does not exist.

Relative hind-wing band-width variation is consistent with the polymorphic Müllerian mimicry hypothesis. Results supported the four predictions for relative hind-wing band-width: One, within sites with two comodels yellow-triangle morphs have greater relative hind-wing band-widths than white-band butterflies; Two, the variance in RHWBW at sites with two comodels is greater (possibly due to disruptive selection) than sites with a single comodel (*H. eleuchia*); Three, *H. cydno* butterflies at yellow-only sites have greater RHWBW than *H. cydno* butterflies from sites with both comodels; and four, this difference may be due to directional selection on RHWBW itself because yellow-triangle butterflies at yellow-only sites have greater relative hind-wing band-widths than yellow-triangle butterflies from sites with both comodels.

Overall, it appears that Müllerian mimicry between different *H. cydno* colours and their respective comodels is relatively important, whereas *H. cydno* pattern morphs appears to be more mimetically neutral. However, the correlation between colour pattern and RHWBW reveals that there may be some selection on pattern details. This indicates pattern may be a more important to mimetic resemblance with comodels than revealed by the morph frequency correlations alone. These results support the action of Müllerian mimicry primarily on colour and weakly on hind-wing band-width expression relative to pattern in *H. cydno*.

Anomalies

Data on morph frequency correlations and phenotypic correlations between pattern and RHWBW and evidence from Chapter 3 support the potential for

divergent selection to help maintain variation in areas where two comodel species co-occur with *H. cydno*. However, variable allele frequency change and a lack of strong linkage disequilibrium at sites with two comodels, and the presence of polymorphic populations in the absence of comodels, indicate that divergent selection does not fully explain the maintenance of polymorphism in Western Ecuador's *H. cydno*. Differences in mean RHWBW between the putative co-mimetic morphs of *H. cydno* at sites with two comodels are six times smaller than the average differences in RHWBW between comodels, which suggests that disruptive selection, if present, is not wholly effective at moving morphs of *H. cydno* to two separate adaptive peaks (by generating a bimodal distribution of *H. cydno* hind-wing band-width). Additionally, inferring that disruptive, directional, or stabilizing selection are the causes is difficult due to a lack of sufficient site-level replication of different hypothesized selective environments generated by patterns of co-occurrence with the different comodel species (n = 2 in all cases). However, an even greater anomaly is the presence of polymorphism in the absence of comodels. I discuss this next.

H. cydno *polymorphism in the absence of comodels*

Polymorphism is unexpected in the local absence of comodels because positive frequency-dependent selection should eliminate the rarest morphs. Transplant experiments confirm the presence of strong positive frequency-dependent selection against rare colour-pattern morphs of *H. cydno* (Chapter 3). Nevertheless, *H. cydno* was polymorphic for colour in five study sites found to have no comodels (Table 4.1). Three of these sites were visited only once each for less than three days, so it is possible that comodels were temporarily rare during the visit. However, two sites, La Hesperia (3 visits, 51 encounters P_{yellow} = 0.57, $P_{triangle}$ = 0.89) and El Copal (7 visits, 825 encounters P_{yellow} = 0.34 , $P_{triangle}$ = 0.81) were sampled intensively, sufficient to conclude that comodels were indeed absent.

Two explanations of the anomaly of comodel-free polymorphism come to mind. First, positive frequency-dependent selection may be weak when *H. cydno* density is high and both colour morphs are common. This hypothesis was suggested by Brown and Benson (1974) and is consistent with two separate lines of evidence here. The two main comodel-free polymorphic *H. cydno* sites have consistently high densities, and the evidence from the divergent selection experiment in Chapter 3 suggests weak or no selection at high-release density.

When site data are summed across all visits, the monomorphic *H. cydno* and *H. eleuchia* sites have a lower *H. cydno* density than the comodel-free *H. cydno* sites. The mean number of encounters per day is approximately 1, 6 and 10 for all yellow sites (Agua Caliente, Maquipucuna and Manta Real, respectively), where *H. cydno* and *H. eleuchia* co-occur, versus approximately 17 to 42 per day at two comodel-free sites (La Hesperia and El Copal, respectively). The density of *H. cydno* at these comodel-free sites is comparable to the total density of these three species of *Heliconius* butterflies at Bilsa or Tinalandia (approximately 28 and 40 per day, of which *H. cydno* made up approximately 53% and 22% at each site respectively). This suggests that where either *H. cydno* or the two comodel species are abundant, weak selection, due to this high density, slows the approach to monomorphism.

Data from other polymorphic Müllerian mimic systems are consistent with density data in my study. Brown and Benson (1974) note that some unpalatable South American ithomiid species are also very abundant and polymorphic. They cite as good examples of this phenomenon morphs of *Mechanitis lysimnia polymnia* and *M. L. mazaeus* and *Hypothyris euclea.* Polymorphism in these species does not appear to be the result of secondary hybridization. Brown and Benson explicitly state the hypothesis that high butterfly density ameliorates positive frequency-dependent selection against rare (in terms of frequency) warning-colour morphs:

> This phenomenon may indicate that very common unpalatable species free themselves from effective stabilization by predator selection for uniformity in colour pattern. Thus the approximately constant number of individuals take by predators before they learn that the patterns are associated with unpalatability would be small compared with the total number of insects in the local populations, and genetic recombinants and other sources of variability could not be eliminated (Brown and Benson 1974: 221).

Brown and Benson (1974) note that *H. numata* occurring in undisturbed forest is relatively rare, whereas in secondary forest clearings, *H. numata* proliferates on abundant food plants leading to dense concentrations. Oddly enough, they do not explicitly state that high *H. numata* abundance is an important factor aiding maintenance of *H. numata* polymorphism. However, they note that the unusually high density may contribute to polymorphism of Müllerian mimic *Acraea* species in Africa. Paradoxically, Owen et. al. (1994) do mention locally abundant concentrations of the *Acraea* species in their study but do not comment on the significance of density for the maintenance of polymorphism in these polymorphic Müllerian mimics (see below).

The second line of evidence that high density decreases the strength of frequency-dependent selection comes from the divergent selection experiment presented in Chapter 3. By releasing a high density of experimental and control butterflies at Maquipucuna (an all-yellow site) I found only an initial loss of experimental butterflies (Chapter 3). Initial loss (probability of establishment, P_E) was in the predicted direction (experimentals lower than controls), whereas subsequent exponential death rate (λ) did not differ between experimental and control butterflies. This resulted in no detectable selection when compared with three low-density release replicates of the experiment (Figure 3.3). In addition, the densities of experimental and control butterflies released at Maquipucuna

(estimated by the encounter rate method during the first eight days of the experiment) averaged approximately 18.2 (±1.3 SE) per group day. This density was within the range of approximately 17 to 42 butterflies per group day, found at high-density *H. cydno*-only populations La Hesperia and El Copal, where selection is presumably weak.

Results from other experiments designed to test the selective advantage of aposematic colouration to Müllerian mimic species are also consistent with the hypothesis that high density of warningly coloured insects decreases positive frequency-dependent selection. Benson (1972) blackened the red forewing patch on distasteful warningly coloured *H. erato* in Costa Rica, eliminating their resemblance to other *H. erato* or any other local warningly coloured butterfly. During the first replicate of this experiment in 1968, he observed a statistically significant reduction in rank minimum longevity of manipulated *H. erato* butterflies when compared to sham manipulated controls with unaltered appearance. When he replicated this experiment a second time, longevity of experimentals was not significantly different from the controls. Benson speculated that resident bird predators may have remembered experimentals from the previous year. If this explanation is true, his second replicate was equivalent to elevating the total density of experimental butterflies experienced by local bird predators, and thus could account for the lack of difference he noted between experimental and control survival.

Mallet and Barton (1989) reported a reciprocal transplant experiment to measure the selective pressure against two races of *H. erato* moved across their shared warning-colour hybrid zone. Their experiment revealed stronger differences in the initial loss of butterflies (P_E) measured from release to the first visit to their study sites than the subsequent loss of butterflies measured from the first visit until the end of the experiment. I also found this pattern in the high-density

replicate of the selection experiment (Chapter 3). Their data are consistent with rapid learning of transferred butterflies' novel colour-patterns by local bird predators that subsequently ignore them. This evidence and experiments on predator learning of the rufous-tailed jacamar, a specialized Neotropical insectivore thought to be important in the evolution of mimicry (Chai 1986, 1988, 1990, 1996), support the idea that bird predators can rapidly learn new colour patterns which would explain reduced selection at high density.

Gene-flow and clines

The second hypothesis to account for anomalous polymorphism in the absence of comodels is long-distance gene flow. Despite density's potential to reduce the strength of selection, polymorphism in high density comodel free populations is not stable. Positive frequency-dependent selection should still slowly lead to fixation of the most common morph. Continued maintenance of polymorphism under these conditions implies some variation-restoring factor at work. One obvious candidate is gene flow. No direct evidence for gene flow in *H. cydno*-only populations exists. However, the spatial autocorrelation analysis indicates that colour frequencies are correlated in nearby populations (Figure 4.1). This autocorrelation is likely due to local gene flow or a combination of spatially autocorrelated selection and gene-flow.

Gene-flow has been estimated for *H. melpomene*, a closely related species with similar body size (Mallet et. al. 1990). Mallet and his colleagues found that the standard deviation in *H. melpomene* parent-offspring distances (σ, a measure of moderate gene flow) was 3.7 km, calculated from measures of cline-width between parapatric races of *H. melpomene* in Peru (Mallet et. al. 1990). Zones of polymorphism (clines) are proportional to σ/s. Therefore the broad areas of polymorphism across Western Ecuador suggest a combination of gene flow and weak selection acting at sites that are comodel-free yet polymorphic, or where

either of the two comodels are common. Otherwise, some combination of increased gene flow from source sites and intermediate selection is necessary to explain the presence of polymorphic *H. cydno*-only sites.

Dominance and random mating

Dominance coupled with random mating may help maintain local *H. cydno* morph frequency variation in the absence of comodels. *H. cydno* colour and pattern are under simple genetic control (Chapter 2). Both colour and pattern loci show variation consistent with complete dominance (Chapter 2). Under these conditions, frequency-dependent selection favouring greater than 50% dominant phenotypes does not eliminate recessive alleles because these are completely masked in the heterozygote state. It becomes increasingly difficult to remove recessives as dominant allele frequency rises. This may help explain polymorphism for colour at comodel-free sites, which generally have greater than 50% white (dominant allele = *W*) butterflies. This scenario does not work for band alleles (*B*), which are never common enough in Western Ecuador to account for 50% of butterflies. Without immigration from sites where they are favoured, band patterns would presumably go extinct due to frequency-dependent selection.

Specialization versus generalization

The presence of *H. cydno* at high density without comodels, and at sites with comodels (Bilsa), the lack of strong linkage-disequilibrium between colour and pattern, the lack of strong separation in relative hind-wing band-width and the imperfect matching of *H. cydno* forewing patterns to their comodels (Figure 1.1) suggest that some constraint has prevented *H. cydno* from evolving near perfect resemblance to either comodel as found in other areas of its range outside Ecuador. One possibility is a selective constraint. *H. cydno* colour morphs (yellow and white) relatively close match the colour of *H. eleuchia* and *H. sapho*,

whereas fore- and hind-wing patterns do not. One explanation is that selection on pattern may be weak. White-triangle may be a more general purpose morph that gains protection from resemblance to both comodels (white *H. sapho* and triangle *H. eleuchia*) as well as from its overall higher density. This is consistent with the rank order of abundance of morphs from Western Ecuador; where all three species broadly co-occur, white-triangle is most abundant, whereas yellow-triangle, white-band, and yellow-band are decreasingly less abundant. Changes in morph frequency are usually seen in trade-offs between yellow-triangle and white-band (potential *H. eleuchia* and *H. sapho* specialists, Figures 4.7b, c), whereas white-triangle appears to remain relatively constant. Finally, white-triangle RHWBW spans the range of both yellow-triangle and white-band (although it has a higher mean) at sites with two comodels suggesting it is under the mimetic influence of both *H. eleuchia* (wide RHWBW) and *H. sapho* (narrow RHWBW). Thus the presence of two comodels may provide protection for a general Müllerian mimic phenotype (white-triangle) as well as two specialist combinations (yellow-triangle and white-band). This hypothesis, that white-triangle is a generalist phenotype, could be falsified with learning experiments using captive wild birds and fitness measures on wild butterflies of each morph at sites with both comodels.

It is likely that some combination of reduced selection and variation restoration due to gene flow, dominance and possibly mimetic generalization explains the persistence of polymorphism at anomalous comodel-free *H. cydno* sites.

Conclusions

My study provides evidence supporting the polymorphic Müllerian mimicry hypothesis. There is evidence of a correlation between the presence of two comodels and the maintenance of polymorphic populations of *H. cydno* revealed by 1) cross-tabulation of comodel presence/absence and *H. cydno* polymorphism

and 2) to a limited extent by the geographic correlation between *H. cydno* polymorphism and the comodel density. At one site (Tinalandia) *H. cydno* colour frequency tracked relative abundance of comodels over one year. Measures of the phenotypic correlation between relative hind-wing band-width and colour-pattern within and between sites were consistent with predictions from mimicry theory. However, other measures were not consistent with strong Müllerian mimicry operating at all times and places. Allele frequency changes at both the colour and pattern loci were in the direction predicted by Müllerian mimicry at only one site. Similarly, measures of gametic correlations do not suggest that strong disruptive selection on colour-pattern combinations is generated by the simultaneous presence of two comodel species. Without comodels, positive frequency-dependent selection should result in monomorphism. But several populations of *H. cydno* are polymorphic in the local absence of comodels. These sites have a high *H. cydno* density that may reduce the strength of frequency-dependent selection against rare morphs sufficiently that gene flow from nearby populations can help maintain polymorphism. One possibility is that *H. cydno* is selectively constrained by the presence of two comodels and its own relatively high density. If one phenotype (a generalist white-triangle) gains protection from both comodels then other more specialist phenotypes (yellow-triangle and white-band) may not be able to evolve perfect resemblance (by linkage of colour-pattern genes) to their respective comodels, *H. eleuchia* and *H. sapho*. Thus, despite some evidence that divergent selection to match two locally co-occurring comodels exists (see above and Chapter 3), it is difficult to conclude that divergent selection is the main factor currently maintaining *H. cydno* polymorphism across all of Western Ecuador. Globally, phenotype frequencies of *H. cydno* in NW Ecuador are very likely the result of historic divergent selection to match two comodels that probably operated consistently until major habitat destruction disrupted many populations. Weak selection at high-density sites populated by high proportions of dominant phenotypes, may

forestall the loss of variation over evolutionarily short time periods of several decades.

Figure Legend

Figure 4.1 The current distribution of *H. cydno* morphs (yellow and white), *H. eleuchia* and *H. sapho* based on present study, as well as the historical distribution of *H. eleuchia* and *H. sapho* based on museum survey by Brown (1979). Each slice on the pie chart represents the presence of a corresponding morph of *H. cydno* (see inset key). Numbered localities plotted by actual latitude and longitude (dots) for present study. Size of pie is proportional to population size for present study (see Appendix 4.1 for locality information). The pie size for historical data is not proportional to population size and represents simple presence/absence for historical data lumping specimen localities by 0'30' latitude/longitude grids..

Figure 4.2 Moran's *I* versus distance category for density of yellow comodel *H. eleuchia* (A.) white *H. sapho* (B.) and phenotype frequency of yellow *H. cydno* (out of the total *H. cydno* population) versus distance (C.). Moran's *I* was calculated for distance categories with boundaries at > 0, 33, 66, 100, 200, 300, and greater than 400 kilometers.

Figure 4.3 Correlation of *H. eleuchia* density versus *H. sapho* density. Density measured as encounters per group day. 18 site/time combinations in area data set shown.

Figure 4.4 The response of *H. cydno* colour morph frequency to variation in the abundance of two comodel species, *H. eleuchia* and *H. sapho*. The top panel shows the values for proportion of yellow butterflies versus the density of *H. eleuchia* per group day and the bottom panel versus density of *H. sapho* per group day. Vertical lines indicate ± 1 profile standard-error for each estimated frequency.

Figure 4.5 The response of *H. cydno* pattern morph frequency to variation in the abundance of two comodel species, *H. eleuchia* and *H. sapho*. The top panel shows the values for proportion of triangle butterflies versus the density of *H. eleuchia* per group day and the bottom panel versus density of *H. sapho* per group day. Vertical lines indicate ± 1 profile standard-error for each estimated frequency.

Figure 4.6 Variation in the proportion of yellow *H. cydno* morphs as a function of proportion of *H. eleuchia* (out of the total comodels) at Tinalandia during 1994/1995 and Bilsa during 1994 to 1996/97. Median sample dates are at least one month apart for each of the five sample periods. Bars are +/- 1 profile standard error for the binomial distribution (Edwards 1992).

Figure 4.7 Time variation in the proportion of different *H. cydno* morphs (white-triangle △, yellow triangle ▲, white-band □, yellow-band ■) for three study sites Tinalandia (A.), Bilsa (B.), and El Copal (C.) from 1992 through 1997 (note: panels are not all displayed on the same time scale). Lines connect observations for Tinalandia. For Bilsa and El Copal lines are linear regression fits of each proportion versus time (for illustrative purposes).

Figure 4.8 Time variation in the proportion of different *H. cydno* morphs (white-triangle △, yellow triangle ▲, white-band □, yellow-band ■) for three study sites Maquipucuna (A.), El Padrino (B.), and Manta Real (C.) from 1992 through 1997 (note: panels are not all displayed on the same time scale). For panel B. lines are a linear regression of date versus each proportion.

Figure 4.9 Allele frequency estimates for four study sites found in Table 4.3: Tinalandia (A.), Bilsa (B.), El Padrino (C.) and El Copal (D.). Arrows proceed from first to last median sample date at each site (found in Table 4.3).

Figure 4.10 Relative hind-wing band-width for *H. cydno* (bars) and *H. eleuchia* (.....) and *H. sapho* (- - -) from sites with two comodels, Tinalandia and Bilsa (A.), sites with no comodels, El Copal and La Hesperia (B.), and sites with one comodel *H. eleuchia*, Yanu Yacu and Manta Real (C.). Bars represent frequency histogram of *H. cydno* RHWBW and lines represent density function of *H. sapho* or *H. eleuchia* RHWBW respectively. Asterisks (*) indicate means of the *H. cydno* distributions.

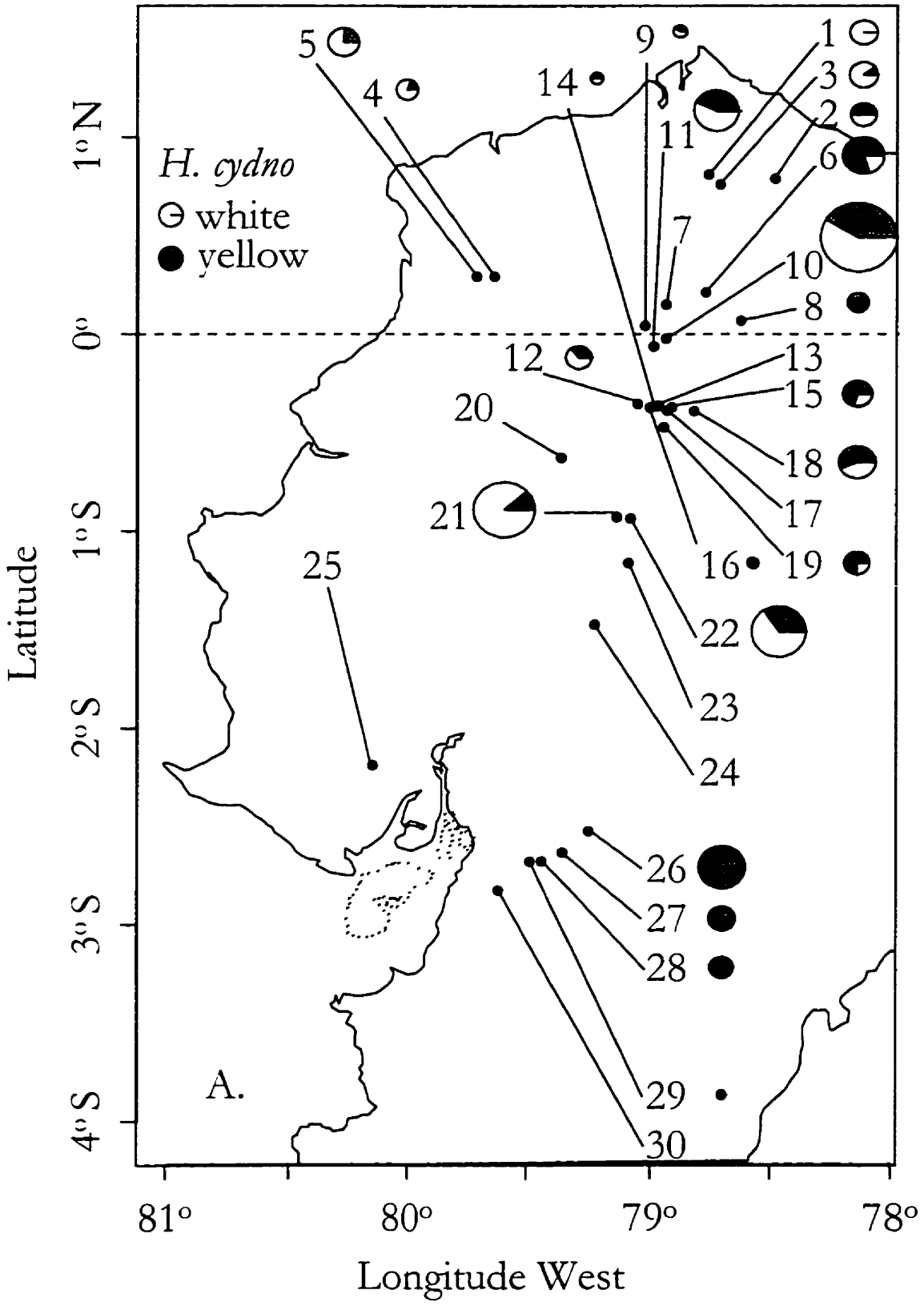


Figure 4.1 The current distribution of (A.) *H. cydno* morphs (yellow and white) and (B.) *H. eleuchia* and *H. sapho* based on present study and (C.) the historical distribution of *H. eleuchia* and *H. sapho* based on museum survey by Brown 1979. (Continued next page).

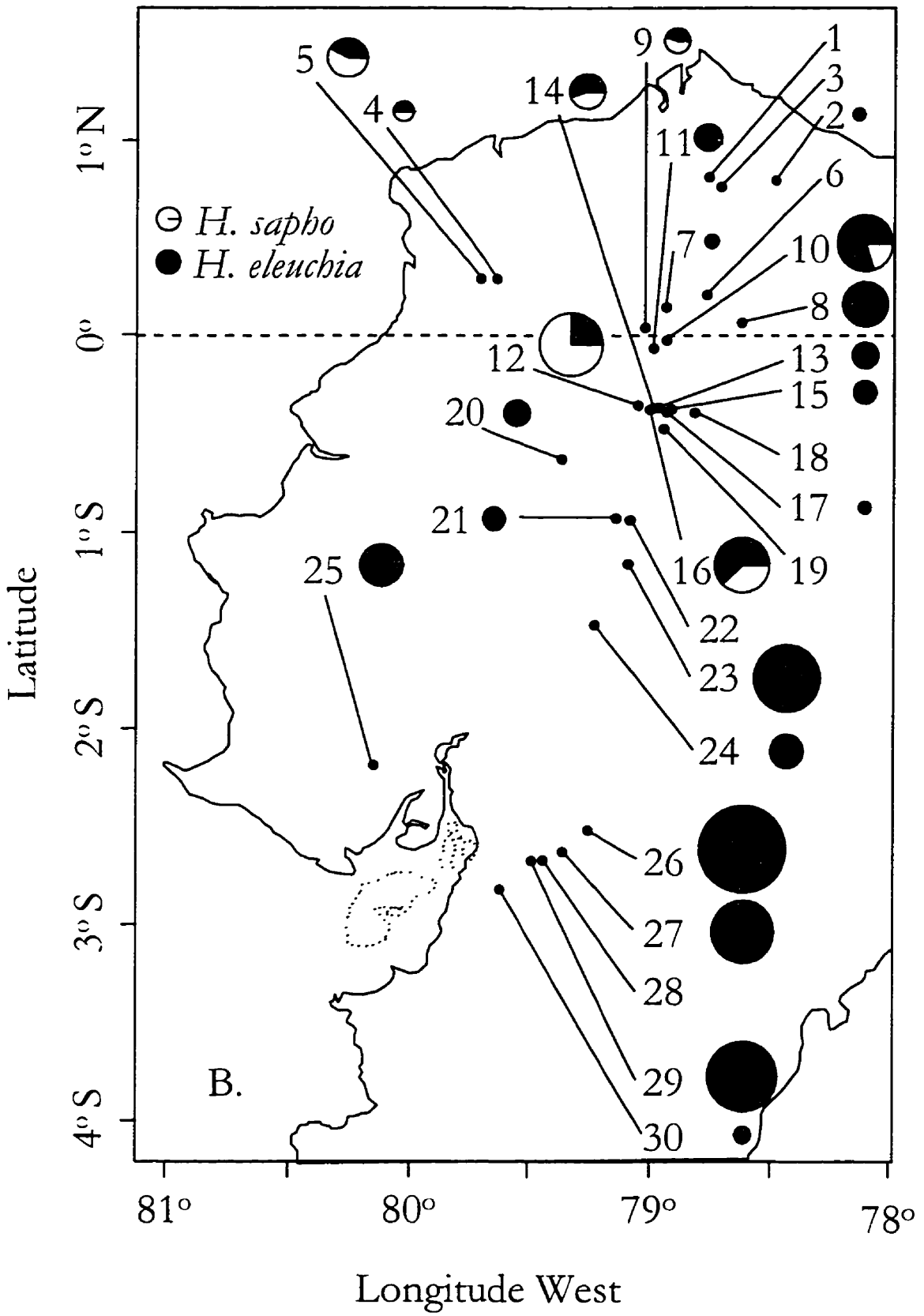
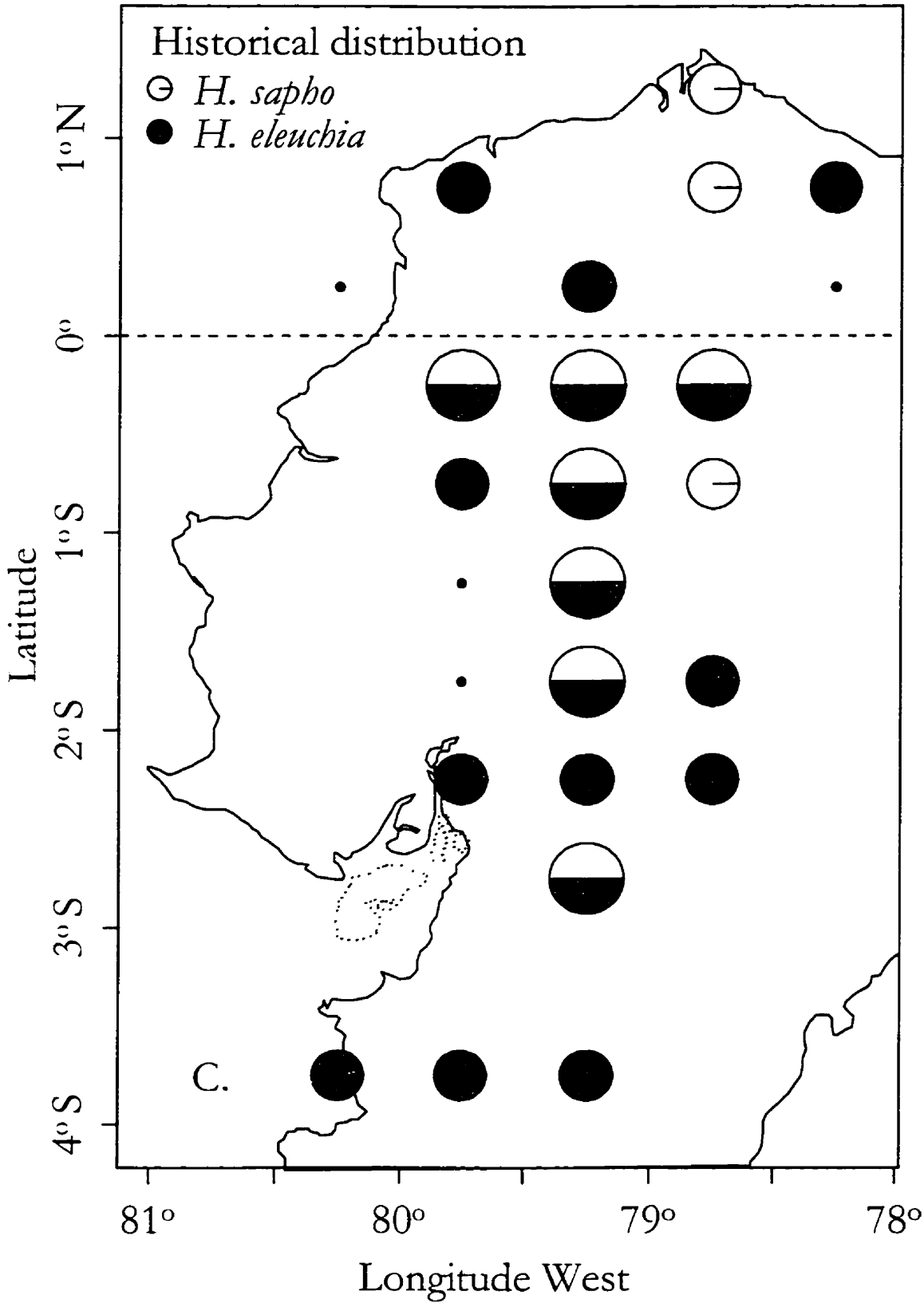


Figure 4.1 The current distribution of (A.) *H. cydno* morphs (yellow and white) and (B.) *H. eleuchia* and *H. sapho* based on present study and (C.) the historical distribution of *H. eleuchia* and *H. sapho* based on museum survey by Brown 1979. (Continued next page).



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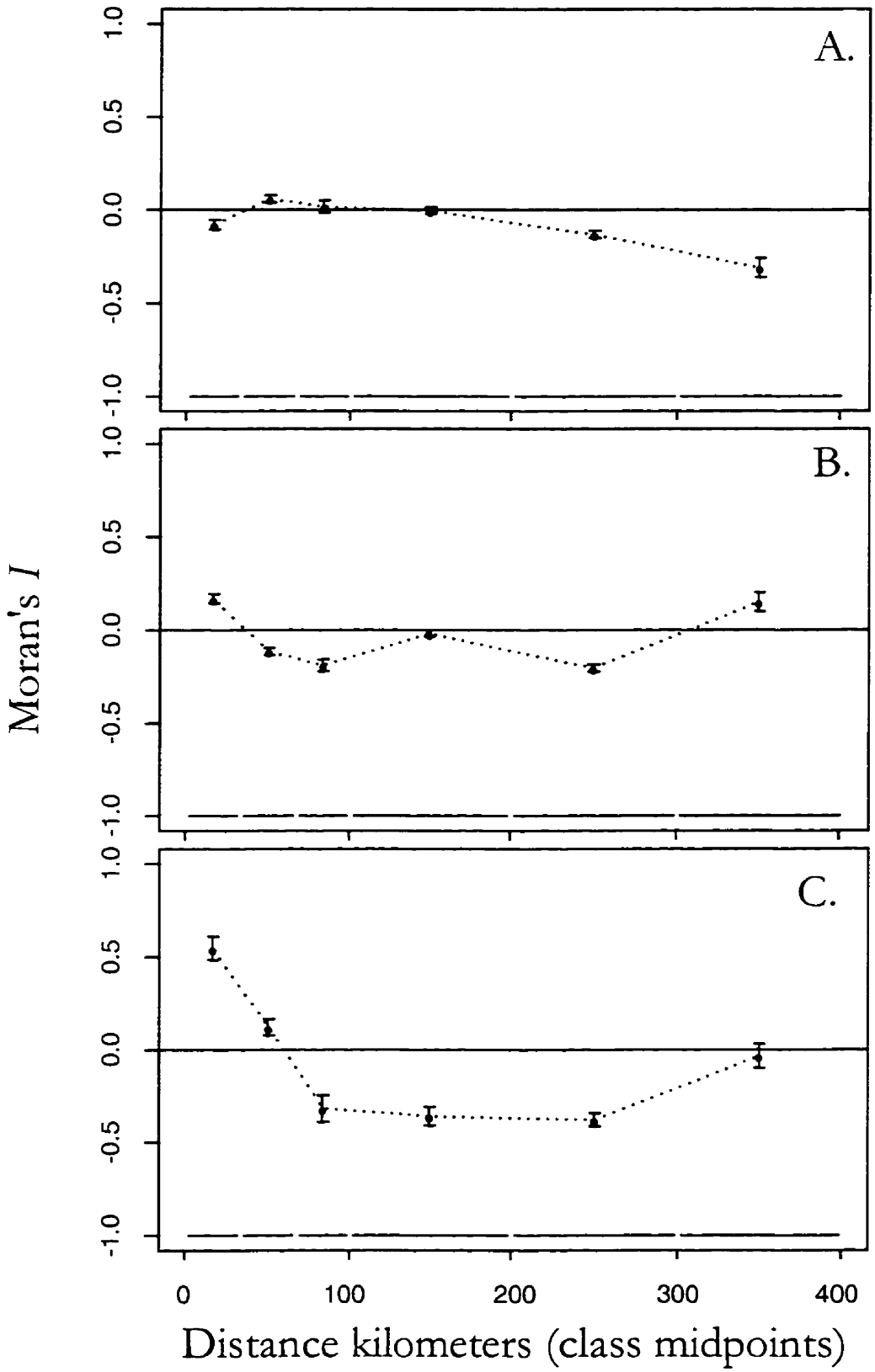

Figure 4.2 Moran's *I* versus distance category for density of yellow comodel *H. eleuchia* (A.) white *H. sapho* (B.) and phenotype frequency of yellow *H. cydno* (of the total *H. cydno* population) versus distance (C.).

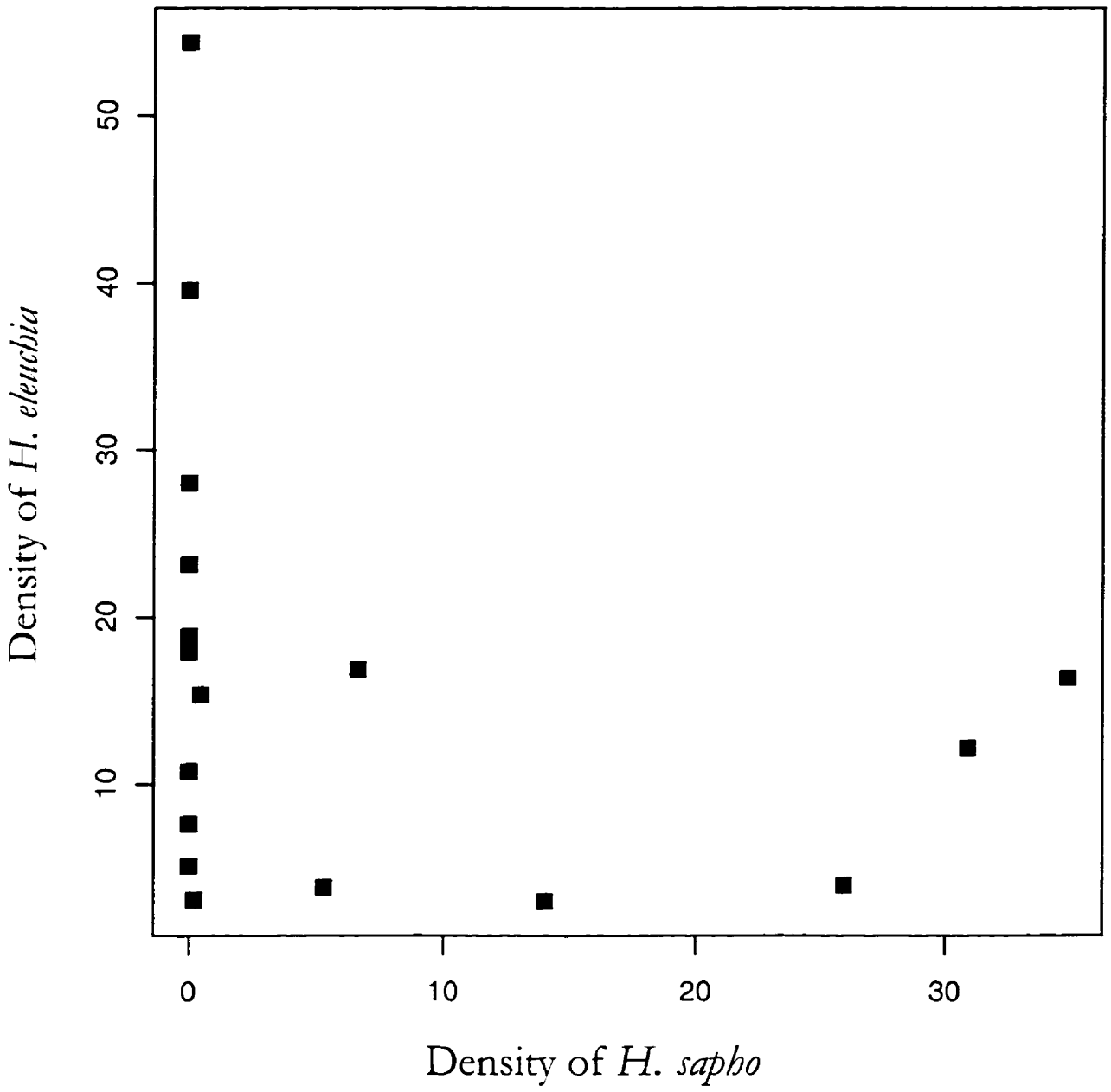

Figure 4.3 Correlation of *H. eleuchia* density versus *H. sapho* density. Density measured as encounters per group day.

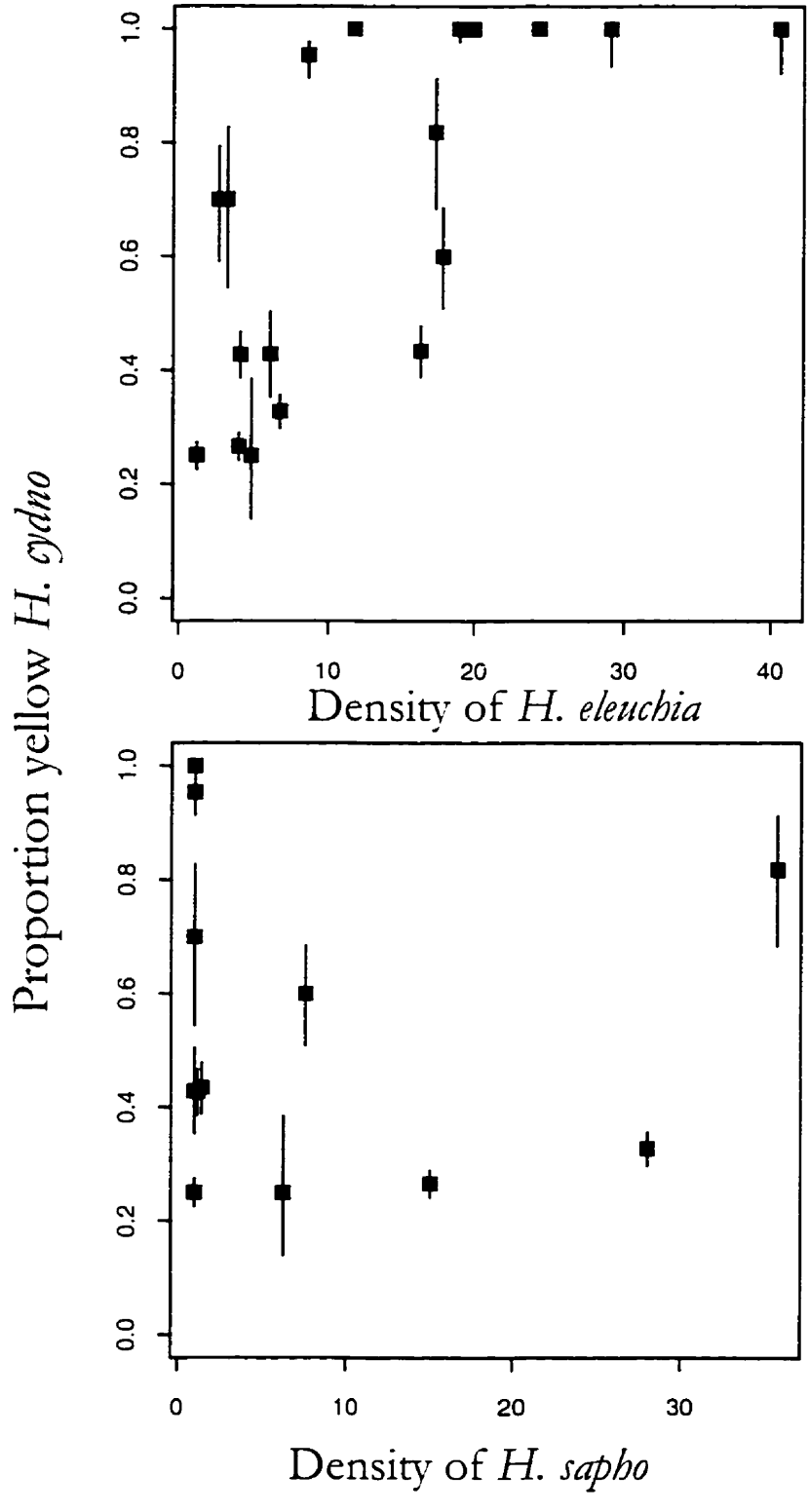

Figure 4.4 The response of *H. cydno* colour morph frequency to variation in the abundance of two comodel species, *H. eleuchia* and *H. sapho*.

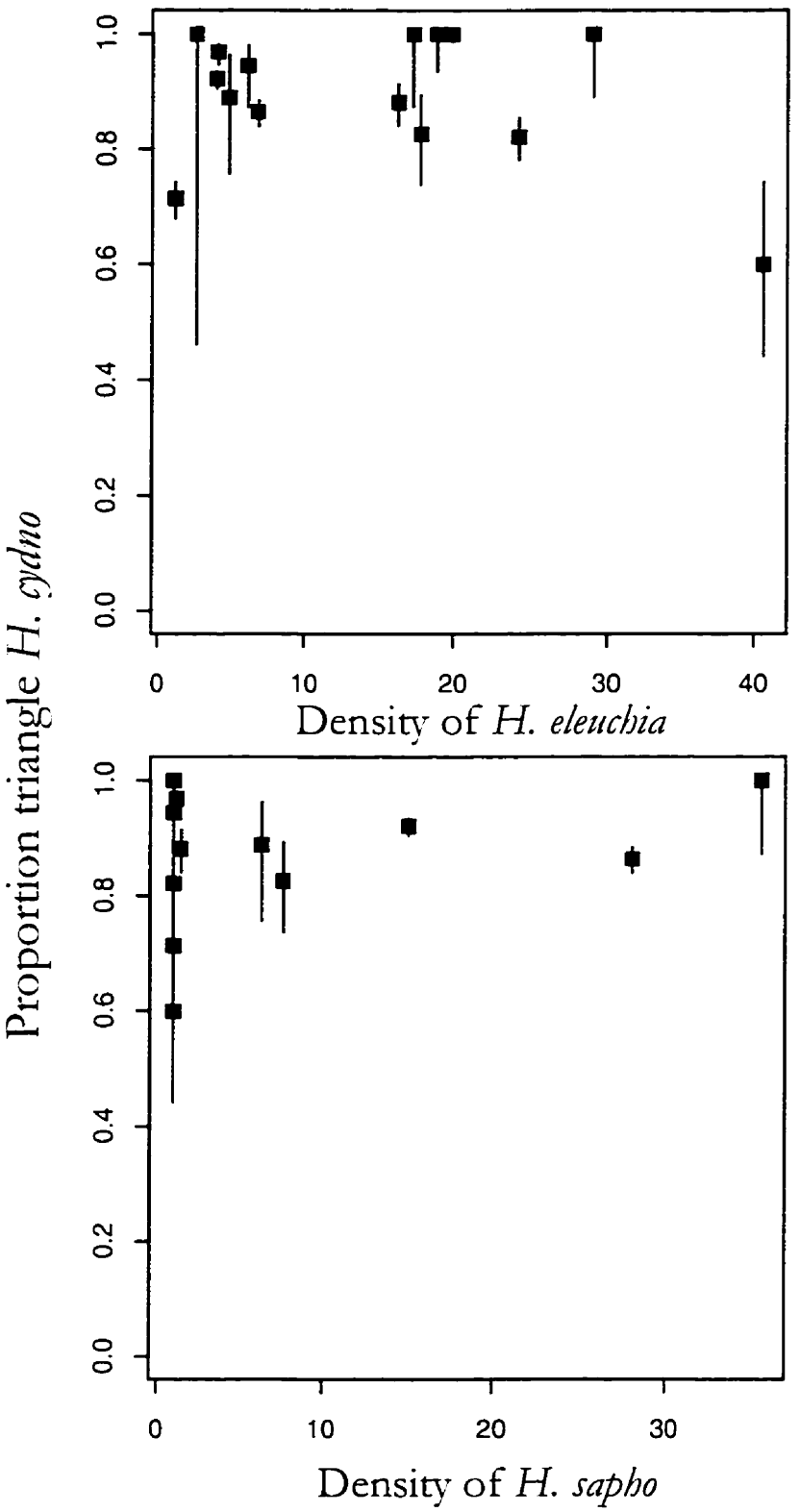

Figure 4.5 The response of *H. cydno* pattern morph frequency to variation in the abundance of two comodel species, *H. eleuchia* and *H. sapho*.

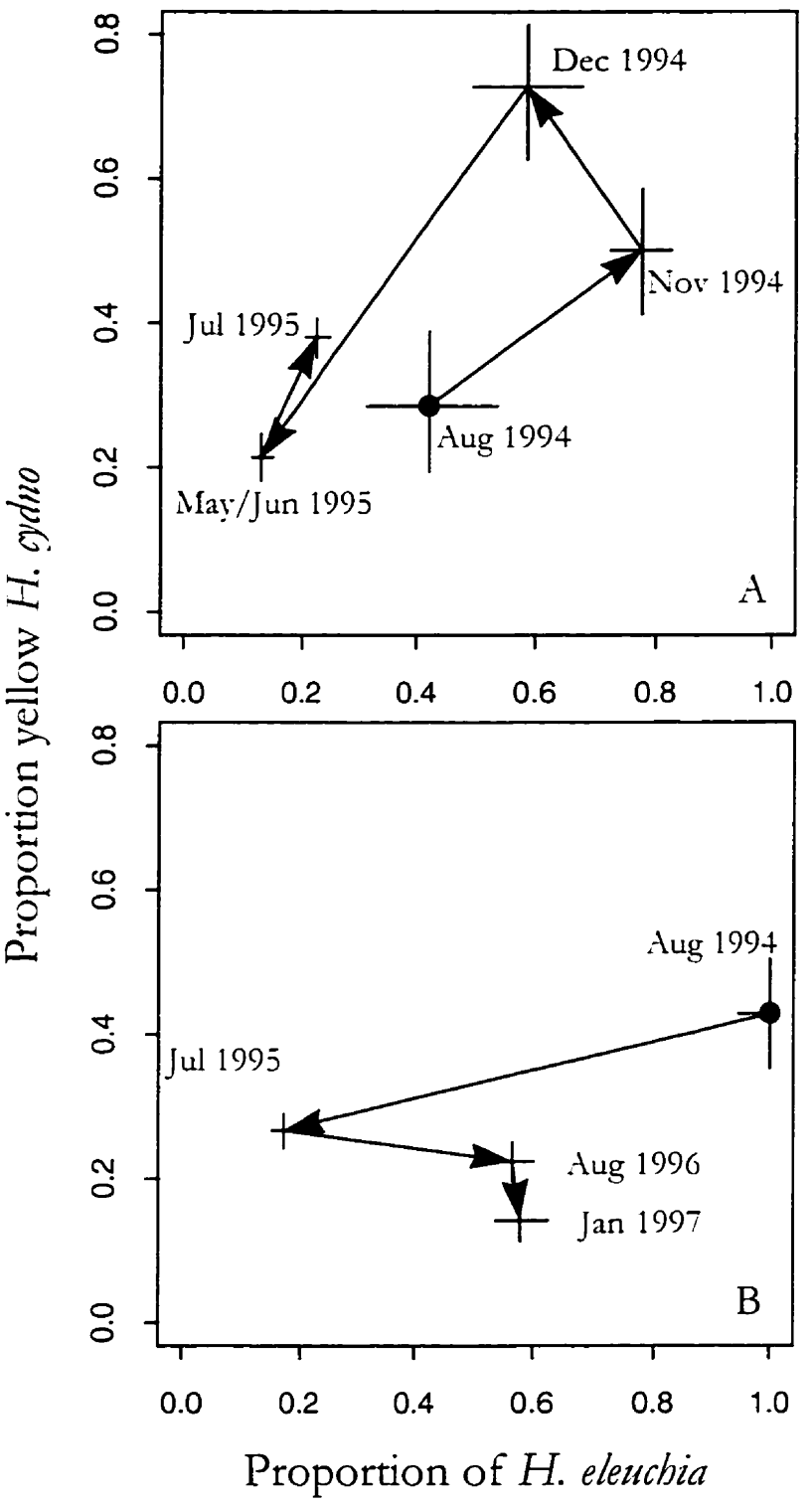

Figure 4.6 Variation in the proportion of yellow *H. cydno* morphs as a function of proportion of *H. eleuchia* (out of the total comodels) at Tinalandia during 1994/1995 and Bilsa during 1994 - 1996/97.

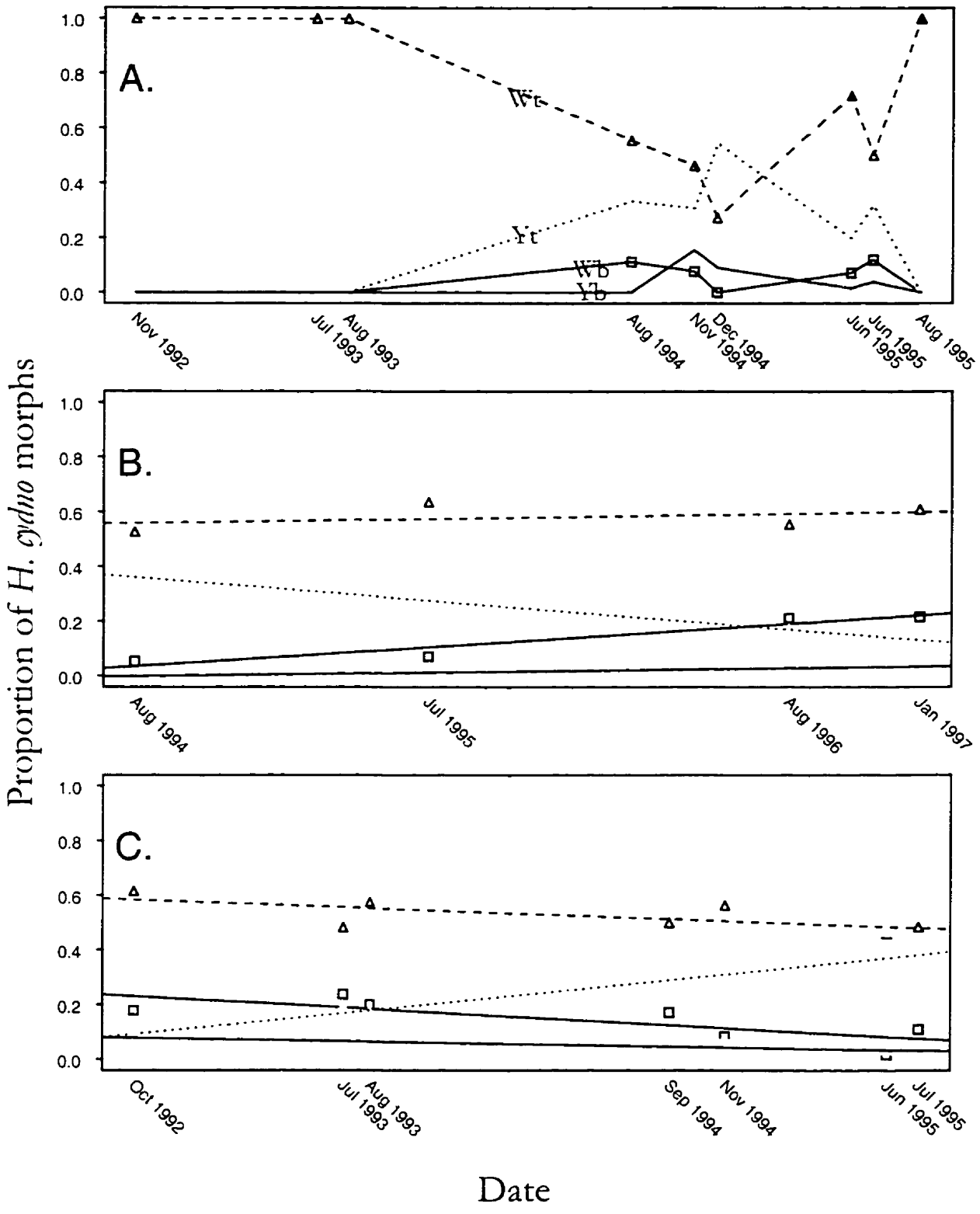

Figure 4.7 Time variation in the proportion of different *H. cydno* morphs (white-triangle △, yellow triangle ▲, white-band □, yellow-band ■) for three study sites Tinalandia (A.), Bilsa (B.), and El Copal (C.) from 1992 through 1997 (note: panels are not all displayed on the same time scale). Lines connect observations for Tinalandia.

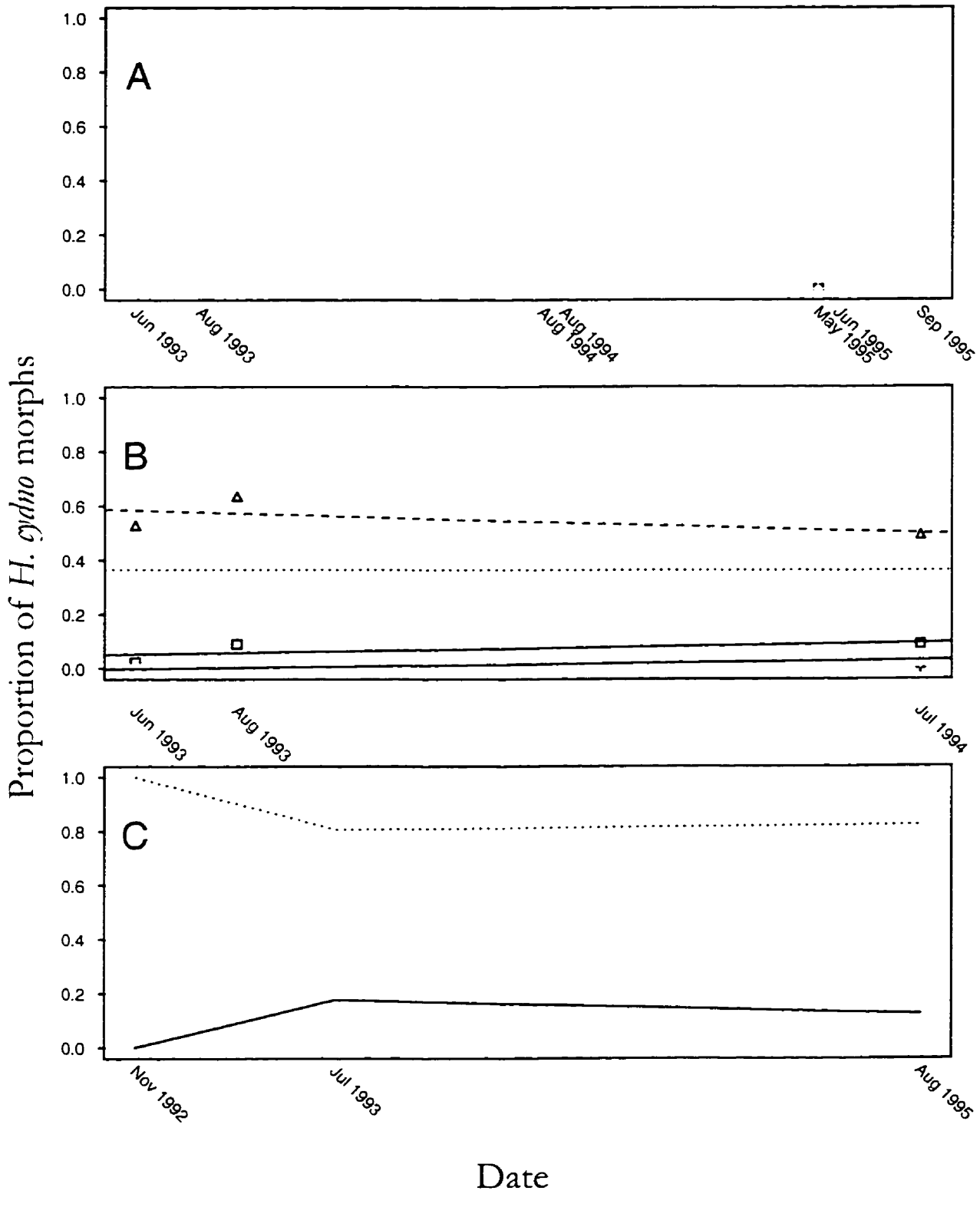

Figure 4.8 Time variation in the proportion of different *H. cydno* morphs (white-triangle △, yellow triangle ▲, white-band □, yellow-band ■) for three study sites Maquipucuna (A.), El Padrino (B.), and Manta Real (C.) from 1992 through 1997.

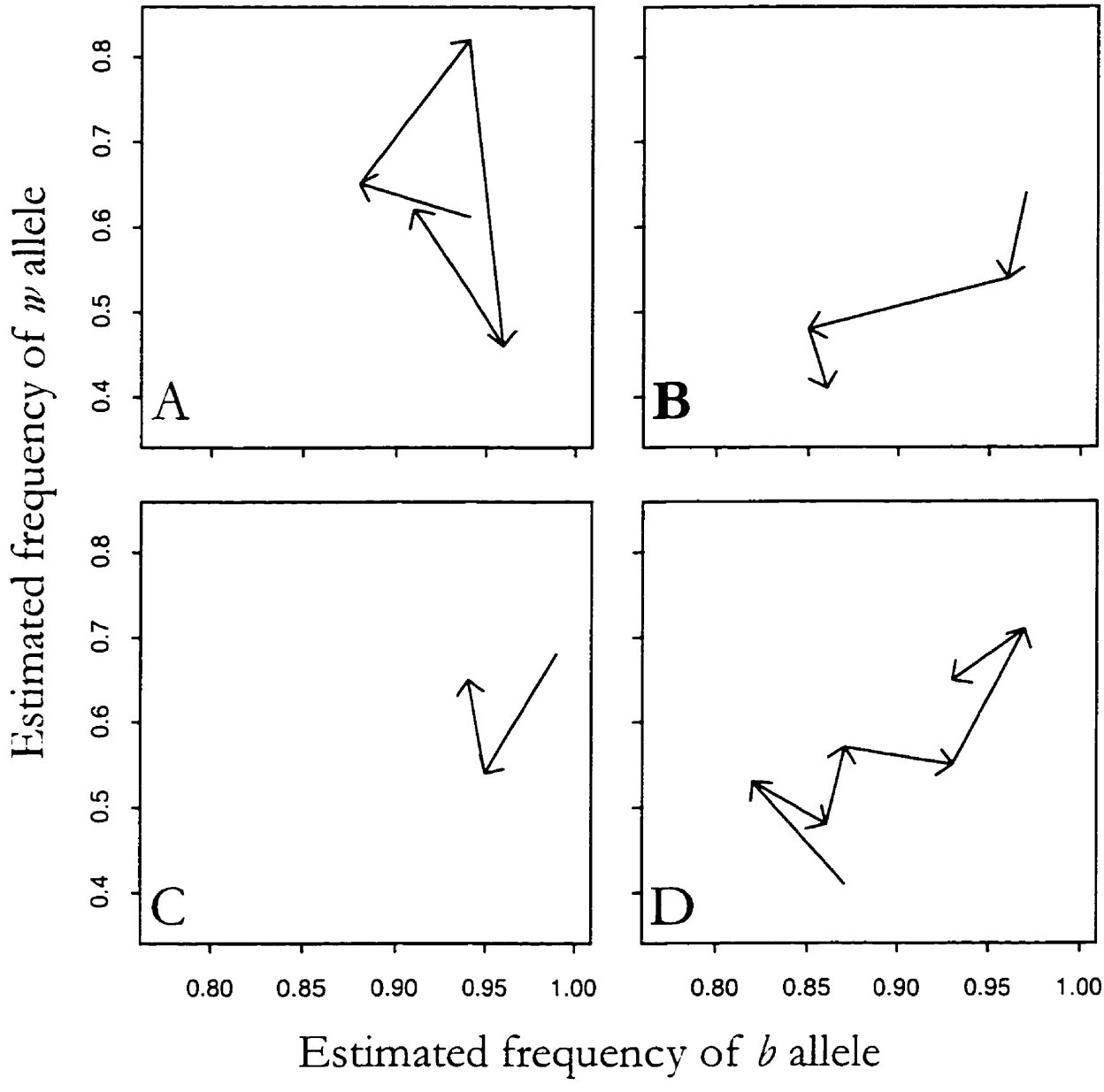

Figure 4.9 Allele frequency estimates for four study sites found in Table 4.3: Tinalandia (A.), Bilsa (B.), El Padrino (C.) and El Copal (D.).

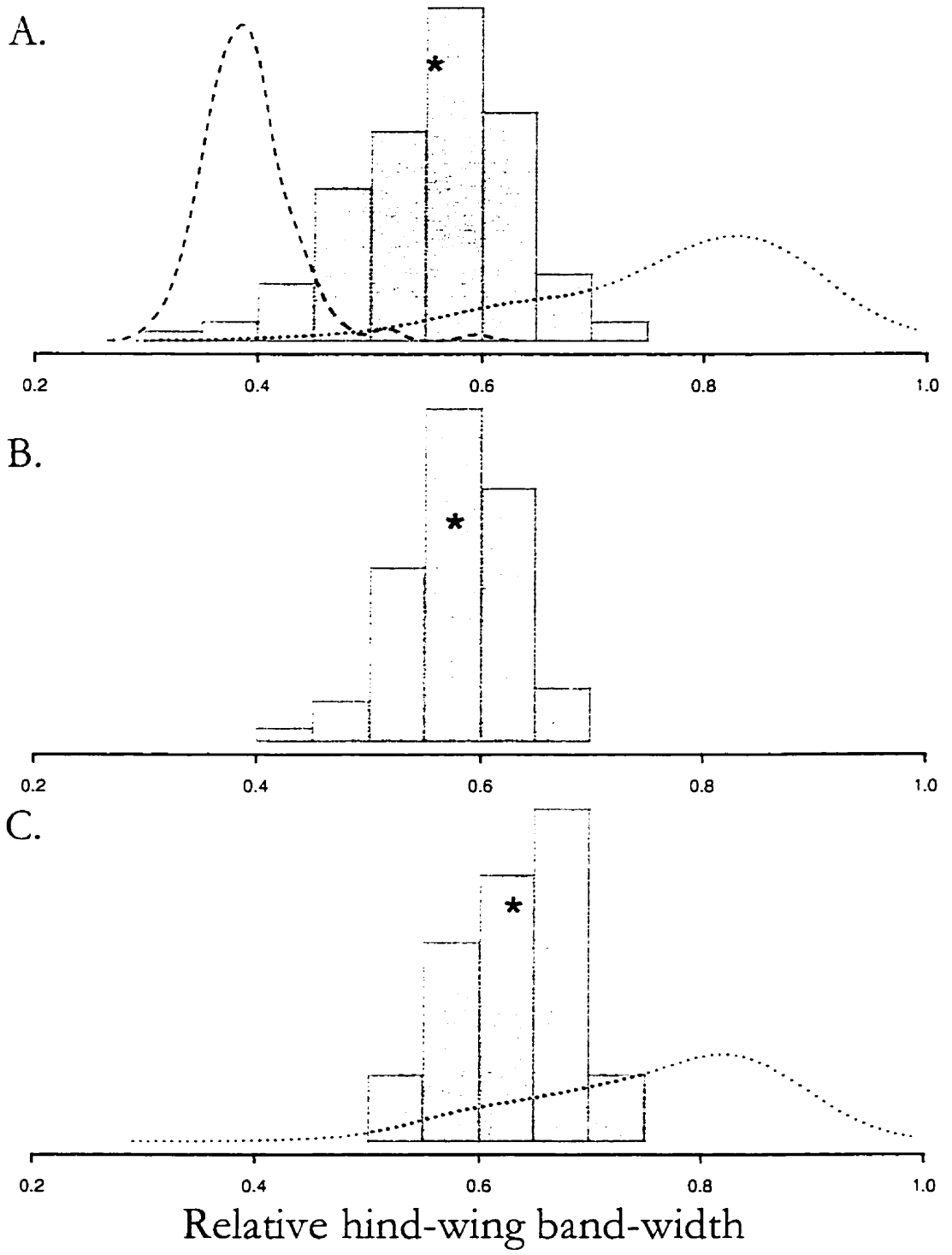

Figure 4.10 Relative hind-wing band-width for *H. cydno* (bars) and *H. eleuchia* (.....) and *H. sapho* (- - - -) from sites with two comodels, Tinalandia and Bilsa (A.), sites with no comodels, El Copal and La Hesperia (B.), and sites with one comodel *H. eleuchia*, Yanu Yacu and Manta Real (C.).

Table 4.1 Co-occurrence of *H. cydno* colour and pattern polymorphism with the presence/absence of *H. eleuchia* and *H. sapho* from 12 sites in Western Ecuador.[1]

	H. cydno state			
Comodels present	polymorphic for color	monomorphic yellow	polymorphic for pattern	monomorphic triangle
H. eleuchia only	0	4	3	0
H. eleuchia and *H. sapho*	3	0	3	0
neither	5	0	2	1
total sites	8	4	8	1

[1] Only sites with in which at least 10 *H. cydno* were encountered are included. Sites where comodels are rare (at least 1 but fewer than 10 total encounters) are not included.

Table 4.2 Multiple logistic regression of morph frequency as a function of density of comodels.

Color	terms	degrees of freedom	residual df	F-value[1]	P
proportion yellow morph	$\log_e$(*H. eleuchia* + 1)	1	15	15.14	0.001
	$\log_e$(*H. sapho* + 1)	1	15	5.95	0.028
Pattern					
proportion triangle morph	$\log_e$(*H. eleuchia* + 1)	1	12	0.02	0.90
morph	$\log_e$(*H. sapho* + 1)	1	12	0.09	0.76

[1] F-value calculated by dropping term from overall model ($\log_e$(*H. eleuchia* +1) + $\log_e$(*H. sapho* +1)). These values were very similar to those generated from sequential addition of model terms since the effects were relatively balanced.

Table 4.3 Linkage disequilibrium (D) estimates from *H. cydno* phenotype counts at four polymorphic sites (Tinalandia, Bilsa, El Padrino, and El Copal).

Site	Date[1]	estimated frequency of *w* allele[2] (q_w)	estimated frequency of *b* allele[2] (q_b)	Disequilibrium[3] $D = (P_{YT} - q_w q_b)^{1/2}$	Maximum disequilibrium[4] D_{max}	$D' = D / D_{max}$[5]	$R = D / (p_w q_w p_B q_b)^{1/2}$	P
Tinalandia	Aug-94	0.58	0.94	0.0330	0.0330	1.0000	0.2879	0.35
	Nov-94	0.68	0.88	-0.0395	0.0395	-1.0000	-0.2575	0.42
	Dec-94	0.84	0.95	-0.0085	0.0085	-1.0000	-0.1034	0.54
	May/June 95	0.46	0.96	0.0043	0.0198	0.2148	0.0421	0.77
	Jul-95	0.60	0.92	0.0179	0.0505	0.3531	0.1319	0.14
Bilsa	Aug-94	0.62	0.97	0.0176	0.0176	1.0000	0.2192	0.31
	Jul-95	0.52	0.96	0.0173	0.0203	0.8499	0.1787	0.0021
	Aug-96	0.48	0.86	0.0100	0.0682	0.1462	0.0570	0.51
	Jan-97	0.39	0.87	0.0277	0.0501	0.5526	0.1699	0.14
El Padrino	Jun-93	0.67	0.99	0.0079	0.0079	1.0000	0.1555	0.12
	Aug-93	0.52	0.95	0.0243	0.0243	1.0000	0.2310	0.41
	Jul-94	0.64	0.94	0.0159	0.0400	0.3966	0.1365	0.32
El Copal	Oct-92	0.43	0.86	-0.0440	0.0808	-0.5441	-0.2548	0.29
	Jul-93	0.52	0.83	0.0109	0.0909	0.1200	0.0577	0.58
	Aug-93	0.47	0.87	0.0075	0.0635	0.1186	0.0443	0.83
	Sep-94	0.55	0.88	0.0209	0.0665	0.3145	0.1294	0.38
	Nov-94	0.56	0.92	-0.0127	0.0342	-0.3706	-0.0953	0.53
	Jun-95	0.72	0.97	0.0061	0.0079	0.7673	0.0817	0.44
	Jul-95	0.63	0.93	0.0268	0.0429	0.6244	0.2188	0.012

[1] Median date of visit.

[2] The allele frequency q_w (for *w* or "yellow" alleles) and q_b (for *b* or "triangle" alleles) was estimated using hypothesized genetic basis for color and pattern (Chapter 2) to fit expected phenotype frequencies to the data (Weir 1990).

[3] Disequilibrium (D) was estimated as the difference between the observed gametic frequencies estimated from the double recessive phenotype and the expected gametic frequencies estimated from the allele frequencies predicted to fit the HW model (Weir 1990).

[4] D_{max} is the minimum of the product of expected gamete frequencies $p_w p_B$ or $q_w q_b$ when $D > 0$ and minimum of $p_w q_b$ or $p_w q_B$ when $D < 0$.

[5] The observed disequilibrium expressed as a fraction of the maximum possible value given allele frequencies $D' = D / D_{max}$ (Hartl and Clark 1989).

Appendix 4.1 Distribution of morphs of *H. cydno*[1] and comodels (*H. eleuchia* and *H. sapho*) and their respective host plants (*P. macrophyllum* and *P. pittieri*) in Western Ecuador listed from NW to SE.

Site information									Total butterflies encountered								Comodel Hostplants encountered[2]	
						Altitude	total days		*H. cydno*[1]						*H. eleuchia*	*H. sapho*		
#	Study Site	Latitude			Longitude	(m)	visited		Wt	Wb	Yt	Yb	W'	Y	yellow	white	*P. macrophyllum*	*P. pittieri*
1	Estero Potico	0° 52' N			78° 46' W	70	1		1	0	0	0	0	0	0	0	?	?
2	Alto Tambo	0° 51' N			78° 29' W	1030	2		2	0	3	0	2	1	1	0	?	?
3	El Salto, Rio Santiago	0° 49' N			78° 43' W	125	3		12	0	2	0	5	0	0	0	yes	?
4	Road to Bilsa	0° 21' N			79° 38' W	400	2		2	1	1	0	0	0	1	1	yes	?
5	Bilsa	0° 21' N			79° 43' W	600	42		407	95	145	14	56	17	266	374	yes	yes
6	Los Cedros Reserve	0° 16' N			78° 47' W	1200	2		0	0	0	0	5	19	0	0	?	?
7	Jim and Merideth's House	0° 12' N			78° 56' W	500	1		0	0	0	0	0	0	1	0	yes	?
8	Maquipucuna	0° 7' N			78° 38' W	1220	69		0	0	76	2	0	183	667	0	yes	no
9	Endesa	0° 6' N			79° 2' W	680	3		0	0	0	0	3	2	4	5	?	?
10	Rio Achiote	0° 2' N			78° 56' W	880	1		7	0	2	1	3	4	4	1	?	?
11	El Padrino	0° 0' S			78° 59' W	810	12		85	9	66	2	48	40	47	1	yes	?
12	Tinalandia	0° 18' S			79° 3' W	670	41		149	26	78	10	16	16	258	767	yes	yes
13	Pipeline E. of Alluriquin	0° 19' S			78° 58' W	800	1		0	0	0	0	0	0	1	0	?	?
14	N. above Alluriquin	0° 19' S			78° 59' W	880	1		0	0	0	0	1	1	5	4	?	?
15	Road to Dos Rios	0° 19' S			78° 55' W	1050	5		0	0	1	0	6	13	7	0	no	?

[1] *H. cydno* morphs: white-triangle, white-band, yellow-triangle, yellow-band or white (no pattern noted), yellow (no pattern noted) see Figure 1.

[2] Visit was insufficient to determine the presence or absence of this hostplant. For *P. macrophyllum* a minimum of 4 days at a study site since 1992 was necessary to determine absence. For *P. pittieri* a minimum of 4 days searching since 1994 was necessary to classify the plant as "absent" (see methods).

[3] *P. pittieri* was not found in comprehensive surveys conducted by Calaway Dodson to construct a Flora for this site (Dodson and Gentry 1978).

(continued on next page)

Appendix 4.1 (continued) Distribution of morphs of *H. cydno*[1] and comodels (*H. eleuchia* and *H. sapho*) and their respective host plants (*P. macrophyllum* and *P. pittieri*) in Western Ecuador listed from NW to SE.

	Site information						Total butterflies encountered								Comodel Hostplants encountered[2]	
				Altitude	total days	*H. cydno*[1]						*H. eleuchia*	*H. sapho*	*P. macrophyllum*	*P. pittieri*
	Study Site	Latitude	Longitude	(m)	visited	Wt	Wb	Yt	Yb	W	Y	yellow	white		
16	Parides Hill	0° 19' S	79° 0' W	699	7	0	0	4	0	0	5	69	41	yes	?
17	S. above La Union del Toachi	0° 20' S	78° 56' W	1000	1	0	0	0	0	0	0	1	0	?	?
18	La Hesperia	0° 20' S	78° 49' W	1130	4	17	3	25	2	2	2	0	0	no	?
19	Rio Esmeraldas	0° 25' S	78° 57' W	1240	2	0	0	1	0	3	8	0	0	?	?
20	Rio Palenque	0° 34' S	79° 22' W	176	3	0	0	0	0	0	0	13	0	yes	no[3]
21	0530 Creek	0° 52' S	79° 8' W	530	3	15	2	3	0	5	0	2	0	yes	?
22	El Copal	0° 53' S	79° 5' W	886	19	314	85	180	30	147	69	0	0	no	no
23	Road to El Corazon	1° 6' S	79° 5' W	1055	1	0	0	0	0	0	0	1	0	?	?
24	Chazo Juan	1° 25' S	79° 14' W	1100	1	0	0	0	0	0	0	3	0	yes	?
25	Cerro Blanco	2° 8' S	80° 9' W	100	3	0	0	0	0	0	0	33	0	yes	?
26	Yanu Yacu	2° 28' S	79° 15' W	500	2	0	0	9	0	0	5	26	0	yes	?
27	Manta Real	2° 35' S	79° 21' W	400	19	0	0	130	25	0	21	519	0	yes	no
28	New road above Agua Caliente	2° 37' S	79° 26' W	980	1	0	0	1	0	0	0	0	0	yes	?
29	Agua Caliente	2° 37' S	79° 29' W	280	10	0	0	6	4	0	0	333	0	yes	no
30	San Miguel Agua Caliente	2° 46' S	79° 37' W	330	1	0	0	0	0	0	0	1	0	yes	?

[1] *H. cydno* morphs: white-triangle, white-band, yellow-triangle, yellow-band or white (no pattern noted), yellow (no pattern noted) see Figure 1.

[2] Visit was insufficient to determine the presence or absence of this hostplant. For *P. macrophyllum* a minimum of 4 days at a study site since 1992 was necessary to determine absence. For *P. pittieri* a minimum of 4 days searching since 1994 was necessary to classify the plant as "absent" (see methods).

[3] *P. pittieri* was not found in comprehensive surveys conducted by Calaway Dodson to construct a Flora for this site (Dodson and Gentry 1978).

Appendix 4.2 Density of *H. cydno*, *H. eleuchia* and *H. sapho* at seven study areas[1] over six time periods[2] from 1992 to 1995 in Western Ecuador.

Study Area	Date Group[2]	total group days visited[3]	Proportion *H. cydno* Yellow	Proportion *H. cydno* Triangle[4]	Density of butterflies encountered *H. sapho*	*H. eleuchia*	*H. cydno*
Bilsa	3	2.7	0.429	0.944	0.0	5.1	15.3
Bilsa	5	15.4	0.266	0.922	14.1	3.0	21.8
El Copal	1	2.4	0.315	0.737	0.0	0.0	30.7
El Copal	2	9.9	0.251	0.714	0.0	0.2	30.5
El Copal	3	0.8	0.308	0.774	0.0	0.0	80.0
El Copal	4	2.7	0.319	0.857	0.0	0.0	51.4
El Copal	5	4.3	0.440	0.900	0.0	0.0	62.3
El Padrino	0	1.9	0.400	NA	2.6	2.1	2.6
El Padrino	2	5.2	0.428	0.969	0.2	3.1	27.9
El Padrino	3	2.3	0.434	0.882	0.4	15.4	53.5
La Hesperia	0	0.9	0.700	NA	0.0	2.2	11.0
La Hesperia	1	1.9	0.700	1.000	0.0	1.6	10.7
La Hesperia	2	0.2	1.000	1.000	0.0	9.1	9.1
La Hesperia	4	0.9	0.696	0.905	0.0	0.0	26.3
La Hesperia	5	2.0	0.464	0.885	0.0	0.0	13.9
Manta Real	1	2.0	1.000	1.000	0.0	28.1	6.1
Manta Real	2	12.0	1.000	0.821	0.0	23.2	10.9
Manta Real	4	7.6	1.000	0.600	0.0	39.6	1.3
Maquipucuna	0	5.8	1.000	NA	0.0	7.6	7.4
Maquipucuna	1	10.7	1.000	NA	0.0	10.8	7.2
Maquipucuna	2	10.6	1.000	1.000	0.0	18.9	9.3
Maquipucuna	3	9.1	1.000	1.000	0.0	17.9	4.1
Maquipucuna	5	6.7	1.000	0.833	0.0	21.5	1.2
Tinalandia	0	5.3	0.750	NA	5.3	2.8	0.8
Tinalandia	1	1.5	0.000	1.000	3.3	30.9	1.3
Tinalandia	2	4.2	0.818	1.000	34.9	16.3	2.6
Tinalandia	3	2.1	0.250	0.889	5.2	3.8	5.7
Tinalandia	4	4.1	0.600	0.826	6.6	16.9	7.3
Tinalandia	5	22.1	0.328	0.864	27.0	5.8	11.2

[1] Study areas are named for central study site and area surrounding it to 33 kilometers away (except La Hesperia see text).

[2] The season/date group (early or late-dry) numbered consecutively from first visit in 1992 (0).

[3] The total effort expended at that study site in group days visited.

[4] The proportion triangle was unestimable for some study sites since only *H. cydno* color was noted.

Conclusion

I compare the results of the present study with those of other examples of Müllerian mimicry between polymorphic members of a single species and monomorphic or polymorphic comodel species. I conclude by summarizing the major results of each chapter with special reference to the problem of polymorphic Müllerian mimics.

Comparisons

Comparisons with other polymorphic Müllerian mimicry systems

Spatial and temporal variation in comodel species has been proposed to explain the maintenance of polymorphism in *H. numata*, that has many morphs in the "tiger-pattern" or sylvaniform mimicry ring coexisting at single sites in the Amazon. Each morph mimics a major colour-pattern variant of a number of tiger-patterned ithomiid butterfly species (Brown and Benson 1974). Ithomiid comodels occur in dense local concentrations in the forest interior. These "swarms" include hundreds of individuals of up to 30 species and are separated by areas of forest that harbour few ithomiid butterflies (Brown and Benson 1974). The composition and predominant warning-colour phenotype in these swarms varies unpredictably with time and place (Brown and Benson 1974). These authors argue that this situation, coupled with the higher temporal and spatial population stability of *H. numata* relative to ithomiid comodels, sets the stage for small-scale variation in selection that could favour increased variability in *H. numata* (Brown and Benson 1974). This may be like the situation in Western Ecuador where the local density of the two comodel species (*H. eleuchia* and *H. sapho*) varies greatly between sites and at some sites over time. These comodel fluctuations may be due to habitat differences generated by host plant

distribution, and temporal variance due to the unpredictable phenology of the comodel's host plants on which *H. eleuchia* and *H. sapho* lay single large clutches of eggs (D. D. Kapan, unpublished data). Like *H. numata*, in Western Ecuador, *H. cydno* is relatively widespread and stable. This sets the stage for polymorphic Müllerian mimicry in *H. cydno*.

Another tiger-patterned species of well-defended *Heliconius* (*H. ethilla* formerly thought to be *H. numata*; see Ehrlich and Gilbert 1973) exists in the northern range of mountains in Trinidad and is polymorphic for brown and yellow markings on the forewing (Brower, Brower and Collins 1963; Sheppard 1963; Turner 1968a, b; Ehrlich and Gilbert 1973; L. E. Gilbert, unpublished data). These forms may belong to two different mimicry groups. The yellow butterflies appear to be mimetic of the ithomiid genus *Tithorea*, whereas brown forms are thought to be mimetic of danaids (*Lycorea* spp.) and brown ithomiids (Turner 1968a, b; L. E. Gilbert, unpublished data). *H. ethilla* polymorphism may be maintained by seasonal fluctuation in predation generated by changes in the different Müllerian comodels, but local change in morph-frequencies do not correlate well with comodel change, although it is very difficult to track Ithomiine populations (L. E. Gilbert, pers. comm.). Over its entire range in Trinidad, *H. ethilla* appears to mimic two separate divergently-patterned comodel groups. This is not unlike patterns of *H. cydno* morph frequency change in Western Ecuador where some sites (e.g., Bilsa) have both comodels and all four morphs of *H. cydno* but no direct local correlation between *H. cydno* morph frequencies and variation in comodel populations.

In other polymorphic *Heliconius* butterflies putative comodels exist, though correlations between the comodels and morph frequencies are weak. Western Ecuadorian *H. cydno* morphs may represent the best documented example of polymorphic Müllerian mimic *Heliconius*.

Quasi-Batesian Mimics: Laparus doris

Another polymorphic Heliconiine is *L. doris*. This relatively uncommon butterfly is distributed throughout Central and South America. Hind-wing colouration in *L. doris* is either red, blue, or rarely a non-mimetic green (DeVries 1987). Polymorphism in this species has been hypothesized to be the result of divergent selection on *L. doris* to match red and blue mimetic complexes that are vertically segregated (Papageorgis 1975; but see Mallet and Gilbert 1995). However, *L. doris* may be a better example of a Batesian mimic, because it is relatively palatable unlike most *Heliconius* (Turner 1968b; Speed 1993; L. Gilbert pers. comm.). Additionally, *L. doris* is unlike *H. cydno* because it remains polymorphic but always at low density (as predicted for Batesian mimics), whereas *H. cydno* occur in solo polymorphic populations at high density (something that would be impossible if it were completely palatable [but see Speed 1993]).

Hybrid zones

H. erato and *H. melpomene* both exhibit limited polymorphisms in narrow hybrid zones between parapatric races (Turner 1971). On a larger scale, very wide hybrid zones between historically separate races of monomorphic Müllerian mimics could be indistinguishable from a sympatrically evolved polymorphism. One possible example of a polymorphism derived through contact between previously monomorphic Müllerian mimics comes from Africa. African *Acraea* butterflies (*A. encedon* and *A. encedana*) are distasteful polymorphic Müllerian mimics of different forms of the well-defended polymorphic *Danaus chrysippus* (Danainae). These butterflies inhabit open savanna habitat. Results of extensive surveys from 1964 through 1991 indicate a relatively close correlation between the rank order abundance of *A. encedana* morphs and their co-mimetic morphs of *Danaus chrysippus* (Owen et. al. 1994). This pattern is not found between *A. encedon* morphs and corresponding *Danaus chrysippus* morphs (Owen et. al. 1994). In this example, these authors believe that the morphs of all three species may

have originated from historically isolated monomorphic populations that have moved into a polymorphic zone of overlap. They suggest that this scenario is possibly the result of relatively recent colonization of modified forest habitats in Uganda and Sierra Leone (over the last several thousand years) from areas of savanna that were once widely separated by forests. However, they acknowledge the possibility that the polymorphisms could have evolved in a sympatric zone of overlap due to an increase in the load that Batesian mimics place on the Müllerian comodels (Owen et. al. 1994). This is not like *H. cydno* unless historically *H. eleuchia* and *H. sapho* were geographically separated in Western Ecuador and became subsequently sympatric due to range expansion of their host plants.

Transient polymorphisms.

Hybrid zones between races that share different comodels in adjacent ranges are similar to transient polymorphisms generated by comodel replacement over time in sympatry. Habitat change causing one comodel species to replace another may cause a transient polymorphism in a third species (Turner 1977; Linares 1996). Linares describes one such transient polymorphism in the Cauca Valley of Colombia where one *H. cydno* morph, *weymeri*, apparently co-mimetic of distasteful ithomiid *Elzunia humboldt regalis*, was replaced with *H. cydno gustavi*, co-mimetic of *H. erato chestertonii*, due to habitat modification driving *Elzunia* extinct while favouring *H. erato*, which is now abundant in the valley. This is also unlike *H. cydno* in Western Ecuador. The host plants of *H. sapho* and *H. eleuchia* co-occur in undisturbed forests in Western Ecuador making it likely that these two comodel species (and their corresponding co-mimetic morphs of *H. cydno*) historically co-occurred for an evolutionarily significant time span (e.g., since the Pleistocene, Brown 1979).

Balancing selection

Balancing selection completely unrelated to Müllerian mimicry could help stabilize polymorphism at high *H. cydno* densities. One possible factor is non-random mating. The rarest morphs could have a mating advantage mediated through multiple mating. If rare females were mated more often than common females, they may gain from stored nutrients (Boggs and Gilbert 1979; Boggs 1981) by increase in fertilization success (Lederhouse and Scriber 1987; Watanabe 1988) or possibly by improved sperm competition (Drummond 1984). Rare females, even if mating randomly, would tend to have increased fecundity as a result of these processes. The first possibility was tested by looking for any mate preferences in the lab amongst butterflies from El Copal. Males approached females of either colour or pattern randomly without respect to their own colour or female frequency in the population (D. D. Kapan, and L. E. Gilbert, unpublished data).

It is possible, however, that extra mating is disadvantageous. *Heliconius* butterfly males are known to transfer male anti-aphrodisiacs to females upon mating (Gilbert 1976), and females spend considerable time rejecting male advances in the field (D. D. Kapan, pers. obs.). If mating is disadvantageous owing to increased predation risk, increased damage to females or simply loss of time (Drummond 1984) then rare females may have an advantage. One potential mechanism is that rare female butterflies have an egg-laying advantage due to a decrease in male harassment. Male harassment has been shown to be frequency-dependent in a polymorphic African swallowtail butterfly *Papilio dardanus* (Cook et. al. 1994). Males pursued female-like males in direct proportion to their relative abundance. The reduced intrasexual harassment was hypothesized to favour female-like males with rare over common morphs (Cook et. al. 1994). Intersexual harassment may work the same way in *Heliconius*, although behavioural data from wild *H. cydno* at high-density El Copal sites did not reveal

any bias in butterfly interactions (D. D. Kapan, unpublished data). The lack of supporting data for non-random mating and its potential benefits to rare-morphs and the relatively good fit of the four phenotype categories to the Hardy-Weinberg distribution suggest that non-random mating is not an important factor in promoting polymorphism in *H. cydno* of Western Ecuador. However, test crosses from offspring of field-mated females are necessary to eliminate non-random mating as a contributing factor to *H. cydno*'s polymorphism in Western Ecuador.

Below I summarize the main findings of my research on polymorphic *H. cydno* and its comodels.

Genetics

In Chapter 2, I investigated the genetic basis for wing colour and pattern polymorphism in *H. cydno*. Building on a large body of *Heliconius* genetics research (Sheppard et al. 1985; Mallet 1989; Nijhout, Wray, and Gilbert 1990), I discovered that a simple genetic model of one-locus and two-alleles for colour and a single locus with two or more alleles (or two loci each with two alleles) accounts for the major variation in forewing pattern. Each of these loci exhibited complete dominance. Additional corroboration of this result was found in Chapter 4, where field-counts of colour-pattern phenotype frequencies fit a simple two-locus, two-allele model in 13 out of 15 samples indicating, at a minimum, that colour and pattern characters are independent. I also found the potential for heritable variation and a possible genetic correlation between relative hind-wing band-width and pattern phenotypes. This too is consistent with results from Chapter 4 (see below).

Dominance makes it difficult to select against heterozygotes in a polymorphic population. This may lead to the protection of recessive yellow alleles at sites with two comodels and high white *H. sapho* numbers (and in greenhouses

selected for white-band)! Hybrids between parapatric races of Müllerian mimics often display codominance, whereas *H. cydno* morphs from Western Ecuador do not. Dominance may make it more difficult to evolve linkage between colour and pattern elements and select for better colour-pattern matches.

Selection

In Chapter 3, I demonstrated the benefit of Müllerian mimicry between *H. cydno* colour morphs and their putative comodels. By utilizing a reciprocal transplant experiment, I was also able to verify that divergent selection is a plausible mechanism to account for the origin and/or the maintenance of polymorphism in *H. cydno.* I also suggest that increasing *H. cydno* density, while keeping the relative morph frequency constant, reduces the strength of positive frequency-dependent selection. This pattern has been noted in past mimicry experiments and comparative studies. Both of these results are further discussed in Chapter 4.

Polymorphic Müllerian mimicry

In Chapter 4, I looked at the relationship between the co-occurrence of *H. cydno* and one or two comodels. Müllerian mimics may experience divergent selection when found in sympatry with more than one comodel (Brown and Benson 1974) or when two comodels exist in different sites bridged by gene flow (Sheppard et. al. 1985). In Chapter 4, I found a correlation between colour morph frequencies of *H. cydno* and the density of *H. eleuchia* and *H. sapho* between sites (relatively strong) and within one site over time (weak). No apparent correlation was found between pattern morph frequencies and either spatial or temporal variation in comodels. Allele frequencies calculated from the phenotype frequencies suggest some correlated selection on colour and pattern elements, although correlated selection is not associated with local comodel change. Thus the evidence is too weak to ascribe allele frequency change to

directional selection due to Müllerian mimicry. Phenotype frequencies fit the Hardy-Weinberg equilibrium indicating no detectable linkage disequilibrium between alleles at the two main colour-pattern loci, and also indicating a lack of strong disruptive selection on colour-pattern within sites. However, phenotypic correlations between *H. cydno* pattern and relative-hind-wing band-width suggest that hind-wing band-width has been influenced in the long-term by disruptive selection to match two sympatric comodels (which have highly divergent hind-wing band size). Hind-wing band-width may have also been affected by directional selection to match the large relative hind-wing band-width of sympatric *H. eleuchia* in the absence of *H. sapho*. Paradoxically, comodel-free sites exist where *H. cydno* polymorphism persisted over the short period of this study. This polymorphism at comodel-free sites should be unstable. High butterfly density may provide a mechanism to decrease the strength of positive frequency-dependent selection. Gene flow from nearby sites may help maintain variation at these enigmatic sites.

Conclusions

Warning colour in Müllerian mimic *Heliconius* species has provided fruitful hypotheses, some of the best patterns, and a few precious direct examples of evolution by Darwin's mechanism of Natural Selection (Müller 1879; Turner 1977; Mallet and Gilbert 1995; Mallet, McMillan, and Jiggins 1997; Mallet 1989). In this study I identified the genetic basis of polymorphism in a single species *H. cydno*. I exploited the unusual spatial distribution of Western Ecuadorian *H. cydno* morphs and their comodels to test the tenets of Müllerian mimicry. Finally, I found supporting evidence for the action of Müllerian mimicry between *H. cydno*'s different morphs and their respective comodel species. However, I also found several anomalies suggesting that Müllerian mimicry does not entirely explain the current distribution of polymorphism in *H. cydno*. I hypothesize that the population density of *H. cydno* and the comodels plays an important role in

decreasing the strength of selection against rare morphs. Western Ecuadorian *H. cydno* appear to be in a unique stage of mimetic evolution where they are influenced by the warning colour-patterns of two different species of *Heliconius* butterflies and the composition of their own polymorphic populations.

Continued study of *H. cydno* and other polymorphic Müllerian mimics will reveal more about the evolution of warning colour and the nature of divergent selection operating on Müllerian mimics when morphs are in a transition generated by temporal changes in comodel species or when morphs persist in broad sympatry with more than one comodel. The theory of warning colour and mimicry allows a researcher to generate predictions and utilize the variation in polymorphic warning-colour systems to test new theories for the maintenance of genetic variation, the operation of frequency-dependent selection, gene flow and drift as well as ecological and behavioral mechanisms related to the polymorphism. Like *Drosophila*, warning colouration in *Heliconius* is an ideal system to actually answer modern characterizations of historically fascinating evolutionary questions.

The ultimate fate of *H. cydno*'s polymorphism and that of the majority of organisms inhabiting the sites visited during this study will, unfortunately, not be determined by adaptive evolution. Elimination of the habitat for the three *Heliconius* species in this study is proceeding rapidly. Hopefully, the diligent conservationists and reserve managers who helped make my study possible will be successful in preventing a total loss of Western Ecuador's incredibly diverse rain forests, thereby preventing evolution by extinction.

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IMAGE EVALUATION
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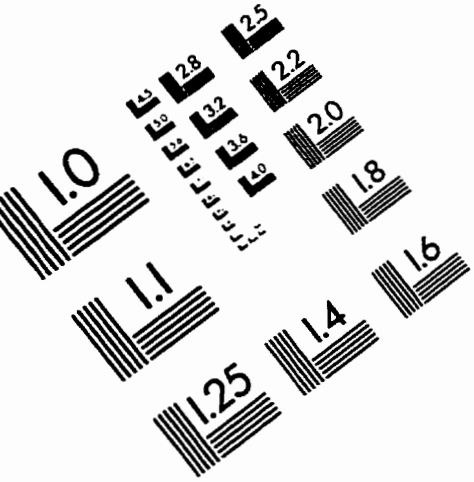

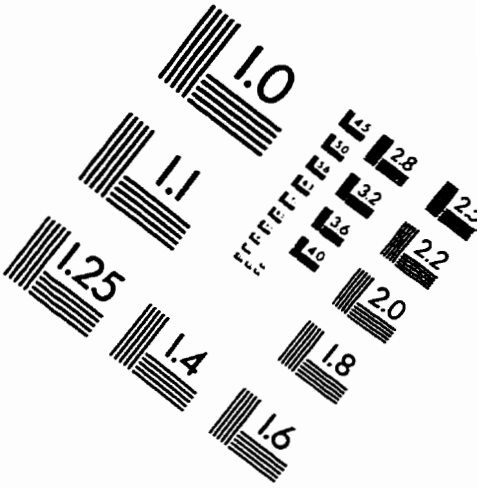

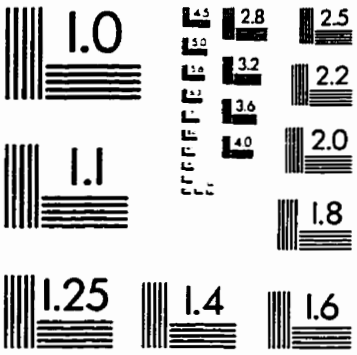

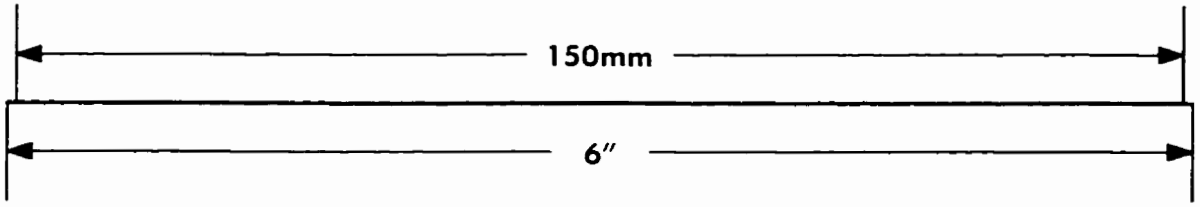

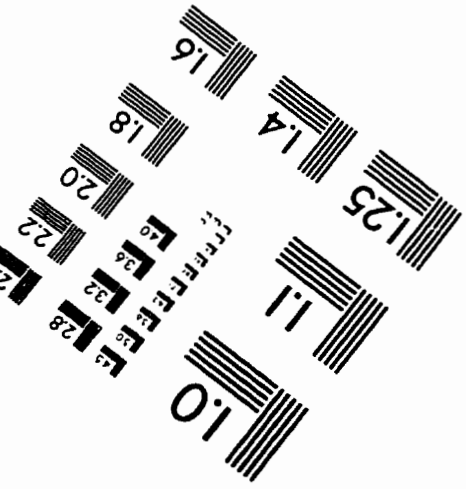

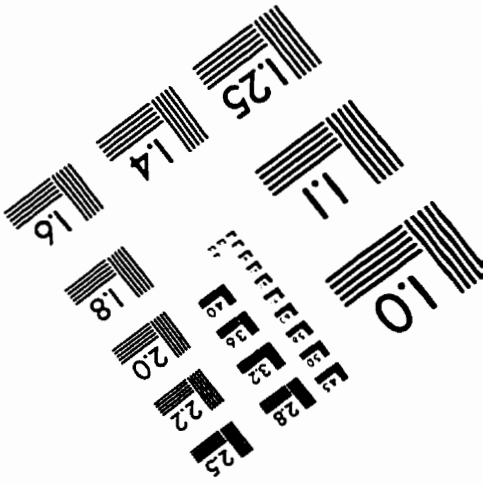

APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989