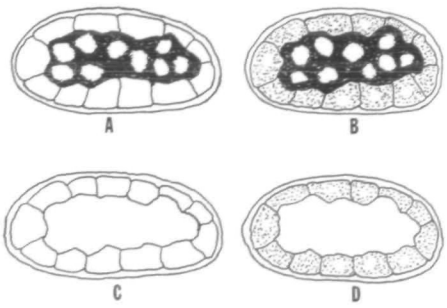

Figure 2. Transverse sections of feather branches with proposed melanin pigment distributions to account for the observed affects of the genes for both the blue and dun colors: (A) pearl and royal purple, (B) blue, (C) dun, and (D) blue-dun.

guinea fowl is similar to that of the budgerigar, I would suggest that the mode of action of the guinea fowl *i* and *d* genes may be the same as the budgerigar genes mentioned by Simon (1971) in the previous paragraph.

If this suggested mechanism is in fact true, then the difference between the blues and the darker colored pearl and royal purple guinea fowl is the presence of tiny melanin particles in the cells of the horny keratin outer layer of the feather branches of the blue-colored birds, whereas in the darker phenotypes these melanin particles are absent. Both color types however have densely packed melanin particles in the center of the feather. The action of the dun gene would then be to remove the densely packed melanin particles from the center of the feather branches. In the case of the blue phenotype, the color would now change to a pale blue color. These proposed gene actions are illustrated in Figure 2.

If the dun color is in fact the result of the lack of the densely packed melanin particles, then this would also imply that the dun-type pigment is normally always in the feather and that it is only able to be seen when the darker pigment is not produced. Proof of this proposed mechanism for the gene action of both the blue and dun genes (*i* and *d*) could easily be verified with some electron micrographs of these features. However, I am now retired, and so this proof must be left for someone else to obtain. Some other mechanism may be responsible for the results seen in this study, but I feel that the proposal suggested here best fits the results seen in this study and previously reported feather pigment work done by others and reported in the literature.

Not only has this study determined the inherited basis of the dun feather color, but it has led to suggested mechanisms for the gene action of both the blue (*i*) and dun (*d*) genes.

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The Journal of Heredity 1996:87(2)

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Received July 11, 1994
Accepted July 7, 1995

Corresponding Editor: James Bitgood

The Genetics of the Mimetic Coloration in the Butterfly *Heliconius cydno weymeri*

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The genetic bases of the wing color pattern in the neotropical butterfly *Heliconius cydno weymeri* were investigated. Evidence from F_1 broods of wild-caught females indicates that the studied subspecies is composed of two mimetic forms, *weymeri* and *gustavi*, which differ mainly by single allele substitution of major phenotypic effect. Three additional Mendelian genes are hypothesized to possess alleles that contribute to Müllerian mimicry with two alternative model species (mimicked by the two mentioned polymorphic forms), and a fifth one is hypothesized to possess alleles that are mimetically irrelevant. Segregation occurred at all five putative loci. Most of the broods show ratios consistent with simple Mendelian segregation. Broods inconsistent with simple Mendelian inheritance can be explained by (1) a possible epistatic interaction between some of the hypothesized loci and/or the modifier effect of two addtional genes; and (2) a possible effect of sex on the expression of one of the hypothesized loci. There is evidence that the genetic system has evolved epistatic interactions in order to facilitate mimetic resemblance. There is no evidence of linkage between mimetically relevant loci except for one pair of these. This is the first report on the genetic bases of the wing color pattern variation of the species *Heliconius cydno.*

Evolutionary biologists have debated for a long time whether adaptations result from the accumulation of many allele substitutions of small effect ("micromutationism") or from the accumulation of gene substitutions of large effect (which I could call "macromutationism"; Charlesworth et al. 1982; Goldschmidt 1940; Orr and Coyne 1992). In order to account for the evolution of adaptations, it is fundamental to elucidate the genetic bases of concrete examples of these biological attributes and infer the nature, number, and magnitude of the gene substitutions that conform them.

A good example of an adaptation is Müllerian mimicry which is the phenotypic close resemblance between two, or more, distasteful relatively distantly related species. The wing pattern of *Heliconius* butterflies represents an excellent case of Müllerian mimetic coloration shaped mainly by natural selection (Benson 1972; Brown et al. 1974; Mallet 1986, 1989; Mallet and Barton 1989a,b; Mallet et al. 1990). These insects can be cultured and used for studying the genetic bases of a major adaptation (Müllerian mimicry), through hybridization experiments and genetic analysis, providing valuable information that may contribute to resolve the debate between micro- and macromutationists mentioned above. In this article I present results on the genetic bases of the Müllerian mimetic wing color pattern variation in the butterfly subspecies *Heliconius cydno weymeri* (for a list of studies involving *Heliconius* genetics, see Mallet 1993). Furthermore, this is one of the most detailed studies on the genetics of a *Heliconius* species, involved in Müllerian mimicry simultaneously with another member of *Heliconius* and one of the subfamily Ithomiinae (see below), in which the polymorphism does not seem to be maintained through the interaction of natural hybridization between differentiated subspecies, and selection on mimetic patterns, as in most of races of *Heliconius* studied by Turner and

Figure 1. Left: *Heliconius erato chestertonii* (top) and *Elzunia humboldt regalis* (bottom). Right: *Heliconius cydno weymeri* form *gustavi* (top) and *Heliconius cydno weymeri* form *weymeri* (bottom).

Mallet (Mallet 1986, 1989; Sheppard et al. 1985; Turner 1971a,b).

First, I present evidence indicating that *H. c. weymeri* is composed of two major interfertile mimetic polymorphic forms: *weymeri* and *gustavi*. Although this is the first report that documents experimentally that these two phenotypes are polymorphic forms of a single interbreeding population, it was suggested before that they are Müllerian comimics of two different toxic species: *weymeri* of the ithomiine butterfly *Elzunia humboldt regalis* and *gustavi* of *Heliconius erato chestertonii* (Eltringham 1916; Fassl 1912) (Figure 1). *H. c. weymeri* and its putative comimics are sympatric in several localities in the southern part of the Cauca valley in the Andes of Colombia (Brown 1979; Linares 1989; Torres and Takahashi 1983). Second, I describe five putative loci that control color pattern elements, four of which possess alternative alleles that seem to contribute to mimicry with the two mentioned toxic comimics of *H. c. weymeri*, and one that I assume to be mimetically irrelevant. Finally, I conclude that my results do not support exclusively the micromutationists' view nor that of the macromutationists, but a mixture of the two.

Materials and Methods

Between 1984 and 1994 I performed genetic experiments in four insectary cages in La Vega (Colombia), a village 50 km northwest of Santafé de Bogotá, and in greenhouse insectaries atop Patterson Laboratory, University of Texas (Austin). Mated wild-caught females from eight localities surrounding Cali (Dagua, La Cumbre, Río Aguacatal, Villa Carmelo, Pance, Popayán, Río Quilichao and Corinto) were kept in isolation laying eggs. Fresh males and virgin females to be used as parents of broods came from experimental populations established from mated females caught in three places: Río Aguacatal, Río Quilichao, and Dagua. Desired broods were obtained by isolating the two potential parents in an insectary cage. To collect broods these mated female parents (of hybrid broods) were singly isolated in an insectary cage so that, the data presented here came from 11 families whose parents and offspring were of known phenotype. The putative genotypes of the family members was inferred through visual examination with a stereoscope and analysis of the segregation patterns obtained in particular broods. Chi-square was utilized to test goodness of fit hypotheses. Heterogeneity between the distribution of genotypes by sexes for a single locus or a pair of loci was tested with a Monte Carlo simulation 20,000 times (Lewontin and Felsenstein 1965). Unless stated otherwise, the reader can assume that the sexes are homogeneous. In *Heliconius* (Suomalainen et al. 1973; Turner and Sheppard 1975), as in many other Lepidoptera (Robinson 1971) chiasmata and crossing-over occur only in the homogametic sex, the male. Thus, I have assumed that the occurrence of recombination between two loci in a doubly heterozygous *H. cydno* female is strong evidence of no linkage between them. Throughout the analysis, I assumed the simplest genetic model, while conceding the possibility that larger broods might reveal more complex gene–phenotype relationships. Also, I used the subindex number 1 to denote those alleles

that control the presence of melanic elements of the wing color pattern.

Passiflora edulis, P. biflora, and *P. caerulea* (Passifloraceae) were used for egg laying and mass rearing of broods. Eggs were collected daily in Austin and every three days in La Vega. Larvae were reared in groups of about ten individuals in separate containers with abundant food. *Psiguria* spp. (Cucurbitaceae) and *Lantana* spp. (Verbenaceae) flowers were provided as nectar and pollen sources for adults. Further details of insectary maintenance are given by Turner (1974).

Results and Discussion

Evidence of Polymorphism

Usually *Heliconius* species are monotypic in a given locality. However, in the southern part of the Cauca valley, the butterfly *Heliconius cydno weymeri* possesses two major forms *gustavi* and *weymeri* which co-occur in several localities as polymorphic populations. These forms differ in that *H. cydno weymeri* f. *gustavi* has a black forewing, whereas *H. cydno weymeri* f. *weymeri* has two white bands in the medial area of the forewing (Figure 1). Although pure-breeding stocks of both forms can be established in the laboratory, in the field they often segregate within sibships: of 46 females that I caught in the wild, 22 produced both *weymeri* and *gustavi* among their F_1 offspring. Such evidence shows that these two forms are indeed conspecific and form single interbreeding populations.

Genetics and Mimetic Relevance of the Studied Wing Pattern Elements

Character gustavi/weymeri. It corresponds to the two major forms of *H. c. weymeri* just described: *weymeri* and *gustavi.* The weymeri phenotype shows two bands of white scales in the medial area of the forewing (Figure 2, top row, right); in the gustavi phenotype this same area is replaced by melanic scales (Figure 2, top row, left).

Based on the results presented (Table 1), a major locus with two alleles is hypothesized to control the morphological difference between the mentioned two phenotypes: L^GL^G genotype controls gustavi and L^CL^C controls weymeri. The possible heterozygote L^GL^C appears to be distinguishable from the homozygote L^GL^G in that the first, nearly always (but see below), shows expression of a bit of white scales in the medial area of the forewing, between the cubital veins 1 and 2 (Figure 2, mid column, the single white dot in the anterior wing). These conclusions are supported by the following results: in two crosses (broods 7 and 11) between a putative homozygote L^CL^C and a heterozygote there is no significant deviation from the expected 1:1 ratio ($P > .17$ in both cases, df = 1). In addition, a cross between two putative heterozygotes (brood 9) does not deviate significantly from the expected 1:2:1 ratio ($P = .98$, df = 2) and seven crosses (numbers 1, 2, 3, 4, 5, 8, and 10), between possible homozygous parents, produce the expected phenotype (and thus genotype).

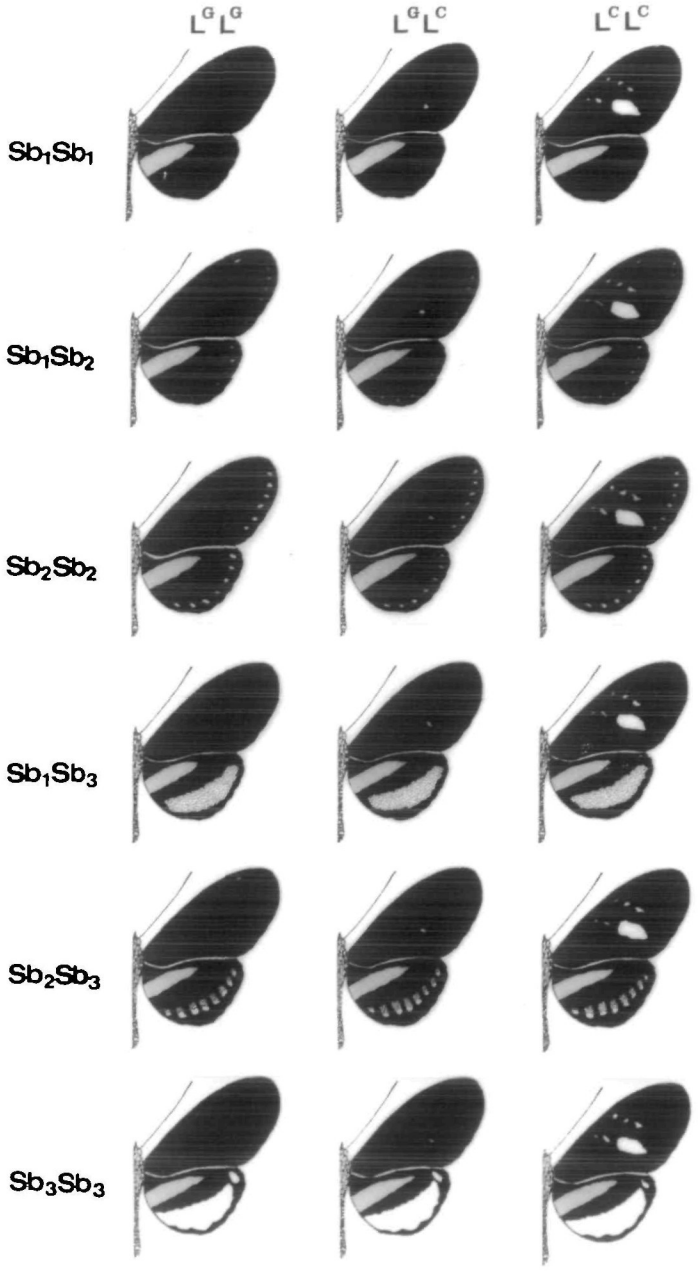

Figure 2. Illustration of the interaction between the L^G/L^C and $Sb_1/Sb_2/Sb_3$ genes. The single white dot in the forewing, mid column, is nearly always typical of L^G/L^C heterozygotes. The heterozygotes Sb_1Sb_3 and Sb_2Sb_3, fourth and fifth rows, respectively, always have a light black "shadow" in the submarginal region of the hindwing illustrated by a multitude of tiny black dots.

As I mentioned, allele L^G is not completely dominant over L^C: the heterozygote nearly always shows a bit of white scales in the medial region of the forewing in either or both surfaces (Figure 2). This conclusion is supported by the fact that four putative heterozygous parents (broods 7, 9, and 11) and all 100 possible heterozygous offspring (Table 1) fulfill the rule. However, allele L^G may rarely show complete dominance over allele L^C: the female parent of brood 6 should be L^GL^G, given its phenotype, but should be L^GL^C, given that the phenotypic ratio of its F_1 brood

Table 1. F_1 totals (males/females) in 11 families of four mimetically relevant wing pattern elements

Code of genotype	Genotype F_1 offspring	1 Cross: male × female 33 × 26	2 37 × 38	3 38 × 48	4 37 × 48	5 47 × 48	6 28 × 12	7 45 × 21	8 3 × 1	9 10 × 13	10 40 × 51	11 10 × 41
1	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_1$								3/9	0/1		
2	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_1Yl_2$									0/1		
3	$L^GL^CSb_1Sb_1Wo_1Wo_2Yl_1Yl_1$								9/0			
4	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_1$									1/0		
5	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_2$									0/6		
6	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_1$									1/0		
7	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_2$									1/0		
8	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_1Yl_1$						0/4			0/1		3/2
9	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_1Yl_2$									0/3		0/2
10	$L^GL^CSb_1Sb_1Wo_1Wo_2Yl_1Yl_1$						7/1			4/1		4/0
11	$L^GL^CSb_1Sb_1Wo_1Wo_2Yl_1Yl_2$						2/0			1/0		
12	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_1$						0/3					
13	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_2$									0/4		
14	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_1$						8/0			5/0		
15	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_2$									2/0		
16	$L^GL^CSb_1Sb_3Wo_1Wo_1Yl_1Yl_1$											0/4
17	$L^GL^CSb_1Sb_3Wo_1Wo_1Yl_1Yl_2$											0/1
18	$L^GL^CSb_1Sb_3Wo_1Wo_2Yl_1Yl_1$											2/1
19	$L^GL^CSb_1Sb_3Wo_1Wo_2Yl_1Yl_2$											3/0
20	$L^GL^CSb_2Sb_2Wo_1Wo_1Yl_1Yl_1$							0/8				
21	$L^GL^CSb_2Sb_2Wo_1Wo_1Yl_1Yl_2$							0/1				
22	$L^GL^CSb_2Sb_2Wo_1Wo_2Yl_1Yl_1$							10/5				
23	$L^GL^CSb_2Sb_2Wo_1Wo_2Yl_1Yl_2$							3/5				
24	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_1Yl_1$									1/0		
25	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_1Yl_2$	2/3					0/6					
26	$L^GL^CSb_1Sb_1Wo_1Wo_1Yl_2Yl_2$	7/2										
27	$L^GL^CSb_1Sb_1Wo_1Wo_2Yl_1Yl_1$						4/0					1/1
28	$L^GL^CSb_1Sb_1Wo_1Wo_2Yl_1Yl_2$						4/0			2/2		4/4
29	$L^GL^CSb_1Sb_1Wo_2Wo_2Yl_1Yl_1$											1/2
30	$L^GL^CSb_1Sb_1Wo_2Wo_2Yl_1Yl_2$		4/2									0/1
31	$L^GL^CSb_1Sb_1Wo_2Wo_2Yl_2Yl_2$		3/0									
32	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_1$						0/1					
33	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_1Yl_2$	3/2					0/3				2/2	
34	$L^GL^CSb_1Sb_2Wo_1Wo_1Yl_2Yl_2$	2/4										
35	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_1$						0/1			1/0		
36	$L^GL^CSb_1Sb_2Wo_1Wo_2Yl_1Yl_2$						4/3			3/2	2/4	
37	$L^GL^CSb_1Sb_2Wo_2Wo_2Yl_1Yl_2$		9/10		4/5						1/2	
38	$L^GL^CSb_1Sb_2Wo_2Wo_2Yl_2Yl_2$		3/4	2/2	1/2							
39	$L^GL^CSb_1Sb_3Wo_1Wo_1Yl_1Yl_2$										1/2	
40	$L^GL^CSb_1Sb_3Wo_1Wo_2Yl_1Yl_1$											2/2
41	$L^GL^CSb_1Sb_3Wo_1Wo_2Yl_1Yl_2$										6/2	2/3
42	$L^GL^CSb_1Sb_3Wo_2Wo_2Yl_1Yl_1$											1/2
43	$L^GL^CSb_1Sb_3Wo_2Wo_2Yl_1Yl_2$										2/1	6/0
44	$L^GL^CSb_2Sb_2Wo_1Wo_1Yl_1Yl_2$							0/3				
45	$L^GL^CSb_2Sb_2Wo_1Wo_2Yl_1Yl_1$							2/2				
46	$L^GL^CSb_2Sb_2Wo_1Wo_2Yl_1Yl_2$							17/11				
47	$L^GL^CSb_2Sb_2Wo_2Wo_2Yl_1Yl_2$		1/1		4/4	10/16						
48	$L^GL^CSb_2Sb_2Wo_2Wo_2Yl_2Yl_2$		3/3	3/5	5/5	17/8						
49	$L^GL^CSb_2Sb_3Wo_1Wo_1Yl_1Yl_2$										0/2	
50	$L^GL^CSb_2Sb_3Wo_1Wo_2Yl_1Yl_2$										2/3	
51	$L^GL^CSb_2Sb_3Wo_1Wo_2Yl_2Yl_2$											
52	$L^GL^CSb_2Sb_3Wo_2Wo_2Yl_1Yl_2$										1/4	
53	$L^GL^CSb_3Sb_3Wo_1Wo_1Yl_1Yl_2$										2/2	
54	$L^GL^CSb_3Sb_3Wo_1Wo_2Yl_1Yl_2$										2/5	
55	$L^GL^CSb_3Sb_3Wo_2Wo_2Yl_1Yl_2$										2/1	

does not deviate significantly from a 1:1 ratio (P = .88, df = 1).

With regard to mimicry, the L^G/L^C locus is probably the most important. Because allele L^G is responsible for the presence of a broad area of melanic scales in the forewing, it controls most of the resemblance with respect to *H. e. chestertonii,* which also has a black (iridescent black-bluish) forewing (Figure 1). The alternative allele L^C controls the presence of two white bands in the medial area of the forewing, a pattern that is also present in *E. h. regalis* (Figure 1).

Character submarginatus/no submarginatus/white submarginal band. Submarginatus consists of seven to eight white dots in the submarginal region of the fore- and hindwing (dorsal and ventral surfaces; Figure 2, third row). No submarginatus is the absence of the above mentioned white dots and their replacement by melanic scales (Figure 2, first row). White submarginal band is a broad white band only in the hindwing (dorsal and ventral surfaces), and the absence of white submarginal dots in the forewing and their replacement by melanic scales (Figure 2, sixth row).

Based on the results presented (Table 1), a major locus with three alleles is hypothesized to control the morphological difference between the mentioned three phenotypes: Sb_1Sb_1 genotype controls no submarginatus, Sb_2Sb_2 controls submarginatus, and Sb_3Sb_3 controls white submarginal band. There is no complete dominance, but putative allele Sb_1 tends to be dominant over Sb_2 and the two of them tend to be dominant over Sb_3. However, all heterozygotes involving the Sb_3 allele show a submarginal "shadow" as though the expression of the color of melanic

scales in this region of the hindwing was not as intense as in the homozygotes Sb_1Sb_1 (Figure 2, represented by a multitude of tiny black dots in the heterozygotes Sb_1Sb_3 and Sb_2Sb_3). These conclusions are supported by six crosses (broods 1, 3, 4, 6, 9, and 11) between a putative homozygote (either Sb_1Sb_1 or Sb_2Sb_2) and a heterozygote (either Sb_1Sb_2 or Sb_1Sb_3) which do not show a significant deviation from the expected 1:1 ratio ($P > .16$ in all six cases, df = 1). In addition, a cross between two possible (Sb_1Sb_2) heterozygotes (brood 2) does not deviate significantly from the expected 1:2:1 ratio ($P = .38$, df = 2). Three broods (numbers 5, 7, and 8) are crosses between putative homozygotes for the same genotype (either Sb_1Sb_1 or Sb_2Sb_2), and all three produce the expected phenotype (and thus genotype). Finally, in brood 10 the expected four phenotypes are obtained in a 1:1:1:1 ratio ($P = .97$, df = 3).

With regard to mimicry, the $Sb_1/Sb_2/Sb_3$ locus is the second (after L^G/L^C) in importance. Allele Sb_1, responsible for the presence of melanic scales in the submarginal region of the hind- and forewing, contributes to mimicry with *H. e. chestertonii* which also has black (iridescent black-bluish) color in this region of its wings (Figure 1). Allele Sb_2 controls the presence of a series of seven to eight white dots in the submarginal region of the hind- and forewing and a very similar pattern appears to be found in *E. h. regalis* (Figure 1). Allele Sb_3 does not contribute to mimicry with any of the mentioned comimics and is typical of a different subspecies, *H. c. cydnides* (not shown in this publication) that occurs in the northern part of the Cauca valley. This is the only genetic element described here that is not typical of *H. c. weymeri* but is present in the broods because Dagua (see above) is located within a hybrid zone involving *H. c. weymeri* and *H. c. cydnides* (Linares 1989). In the latter subspecies allele Sb_3 contributes substantially to mimicry with respect to its comimic *H. eleuchia eleuchia* (not shown here).

Character white oval/no white oval. White oval appears in the medial area of the forewing between the cubital vein 2 and the second anal vein (dorsal and ventral surfaces; Figure 3, third column). No white oval is the absence of white oval and its replacement by melanic scales (Figure 3, first column).

Based on the results presented (Table 1) a locus with two alleles is hypothesized to control the morphological difference between the mentioned two phenotypes: Wo_1Wo_1 genotype controls no white oval and Wo_2Wo_2 controls white oval. The putative heterozygote nearly always shows an intermediate phenotype (a speckling of white scales where the white oval appears) but the sex of the individual possibly affects the expression of this locus (see below). These conclusions are supported by the following results: five crosses (broods 1, 2, 3, 4, and 5) between putative homozygotes of like genotype produce the expected phenotype (and thus genotype). Two crosses (broods 10 and 11) between putative heterozygotes do not deviate from the expected 1:2:1 ratio ($P > .84$ in both cases, df = 2).

Four broods (number 6, 7, 8, and 9) are inconsistent with the hypothesis of a single locus with two alleles controlling the absence and presence of the white oval. The distribution of the putative genotypes by sexes is heterogeneous in all of them. They are the result of a cross between a putative Wo_1Wo_2 male and a Wo_1Wo_1 female and the F_1 offspring of all of them show a significant excess of males of the parental male genotype (Wo_1Wo_2) and a deficiency of males of the parental female genotype (Wo_1Wo_1). This suggests a possible effect of sex in the expression of the alleles of the Wo_1/Wo_2 locus. Assuming that the genotypes of the parents are correct, due to this hypothesized sex effect, many of the F_1 Wo_1Wo_1 males may show a speckling of white scales where the white oval is expressed and may be misclassified as heterozygotes. Another explanation for some of these broods is a possible epistatic interaction between the Wo_1/Wo_2 locus and a sex linked modifier gene. However, future experiments will have to explore if other hypotheses like differential viability of the males and females of different genotypes, or other gene-gene and/or gene-environment interactions, can account for broods 6, 7, 8, and 9.

With regard to mimicry the Wo_1/Wo_2 locus seems to make a minor, but important, contribution. Allele Wo_1 improves the phenotypic resemblance with respect to *H. e. chestertonii,* which has a totally black (black-bluish) forewing (Figures 1 and 3). Because allele Wo_2 is responsible for the presence of a white oval, it improves phenotypic resemblance with respect to *E. h. regalis,* which has a relatively large white spot in the medial area of the forewing (Figures 1 and 3).

Character yellow line/no yellow line. Yellow line is the presence of a line of yellow scales along the vein that defines the dis-

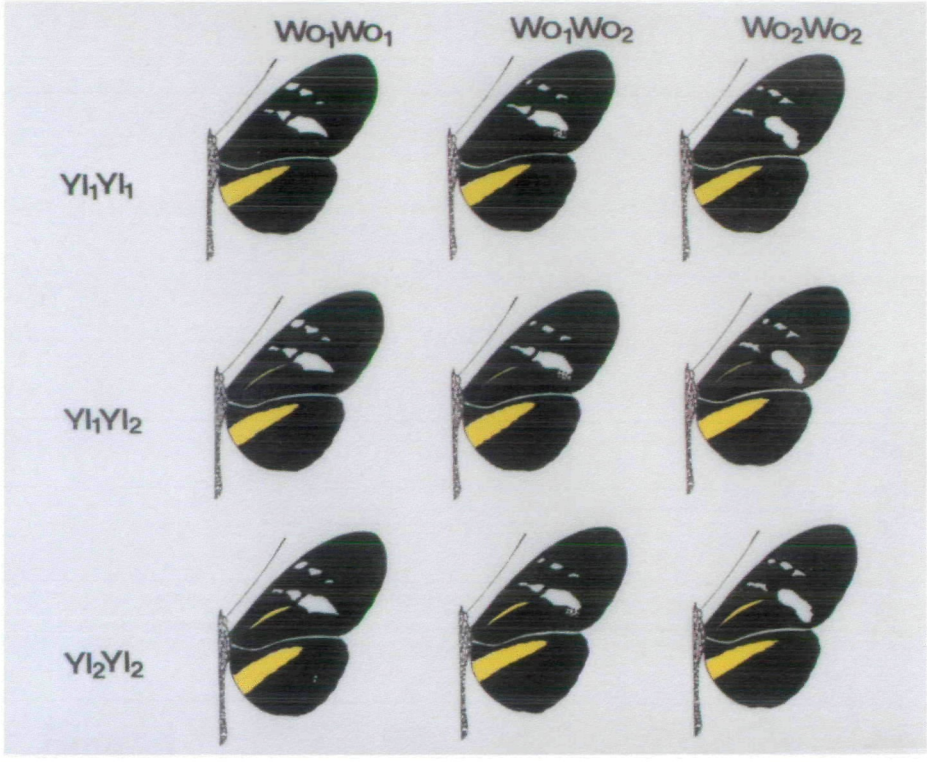

Figure 3. Illustration of the interaction between the Wo_1/Wo_2 and Yl_1/Yl_2 genes. The heterozygote Yl_1/Yl_2 typically shows a smaller yellow line than the Yl_2/Yl_2 homozygote, mid row. The heterozygote Wo_1/Wo_2 typically shows an intermediate expression of the white oval, illustrated by a few tiny black dots, mid column.

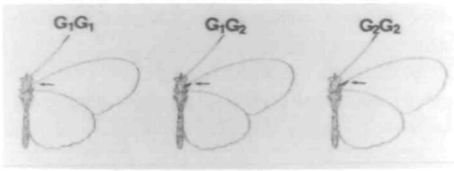

Figure 4. Incomplete dominance of the red dot in the forewing costal vein (basal underside) controlled by the G_1/G_2 locus. The presence of the tiny red dot is illustrated by a tiny black dot.

cal cell of the forewing (Figure 3, third row). No yellow line is the absence of the above mentioned yellow scales and their replacement by melanic scales (Figure 3, first row).

Based on the results presented (Table 1) a locus with two alleles is hypothesized to control the morphological difference between the mentioned two phenotypes: Yl_1Yl_1 genotype controls no yellow line and Yl_2Yl_2 controls yellow line. The possible heterozygote appears to have nearly always an intermediate yellow line, indicating incomplete dominance between the two alleles of this locus. These conclusions are based on the following experiments: seven crosses (broods 1, 2, 4, 5, 6, 7, and 11) between a putative homozygote (either Yl_1Yl_1 or Yl_2Yl_2) and a heterozygote do not deviate significantly from the expected 1:1 ratio ($P > .09$ in all seven cases, df = 1). Two broods (numbers 3 and 8) are crosses between putative homozygotes of like genotype (either Yl_1Yl_1 or Yl_2Yl_2), and in both cases the expected phenotype is obtained (and thus genotype). Brood 10 is a cross between the two possible different homozygotes and, as expected, all offspring are intermediate. Finally, although brood 9 does not deviate significantly from a 1:1 ratio, the sexes cannot be pooled since they are heterogeneous. This could be due in part to epistasis between the L^G/L^c and Yl_1/Yl_2 loci (see below).

With regard to mimicry the Yl_1/Yl_2 locus, together with Wo_1/Wo_2, makes a minor, but important, contribution. Allele Yl_1 improves the mimicry with respect to *H. e. chestertonii,* which has a totally black (black-bluish) forewing (Figures 1 and 3). Because allele Yl_2 controls the presence of the yellow line, it improves mimicry with respect to *E. h. regalis,* which also has a similar trait (Figures 1 and 3).

Character red dot/no red dot. Red dot is a small red-brown spot in the basal area of the forewing costal vein, only on the ventral surface (Figure 4, right drawing). No red dot is the absence of the red dot and its replacement by melanic scales (Figure 4, left drawing).

Table 2. F_1 totals (males/females) in 11 families of the character red dot/no red dot

Brood number	Putative genotype of the parents		Putative genotype of the F_1 offspring			Test ratio	χ^2 probability
	Male	Female	G_1G_1	G_1G_2	G_2G_2		
1	G_1G_2	G_2G_2	0	9/6	5/5	1:1	0.31
2	G_1G_2	G_1G_2	8/6	15/14	0	—	—
3	G_1G_1	G_1G_2	2/2	3/5	0	1:1	0.24
4	G_1G_2	G_1G_2	2/7	9/7	3/2	1:2:1	0.54
5	G_1G_1	G_1G_2	14/13	13/11	0	1:1	0.67
6	G_1G_2	G_1G_2	11/4	11/11	7/7	1:2:1	0.60
7	G_1G_2	G_2G_2	0	23/17	9/18	1:1	0.11
8	G_1G_1	G_1G_2	9/5	3/4	0	1:1	0.12
9	G_1G_2	G_2G_2	0	14/12	8/8	1:1	0.12
10	G_2G_2	G_1G_2	0	13/14	10/16	1:1	0.89
11	G_1G_2	G_1G_2	6/9	17/11	6/5	1:2:1	0.71

Based on the results presented (Table 2), a locus with two alleles is hypothesized to control the color difference between the mentioned two phenotypes: G_1G_1 genotype controls no red dot and G_2G_2 controls red dot. The putative heterozygote nearly always shows an intermediate red dot indicating incomplete dominance between these alleles. These conclusions are supported by the following results: seven crosses (broods 1, 3, 5, 7, 8, 9, and 10) between putative homozygotes (either G_1G_1 or G_2G_2) and heterozygotes do not deviate significantly from the expected 1:1 ratio ($P > .11$ in all seven cases, df = 1). Three crosses (broods 4, 6, and 11) between putative heterozygotes do not deviate significantly from the expected 1:2:1 ratio ($P > .54$ in all three cases, df = 2).

One brood, number 2, shows a significant deviation from the expected 1:2:1 segregation ratio. It could be explained by hypothesizing an autosomal modifier locus with two alleles: one that dilutes the expression of G_2 in homo- and heterozygous condition and another that permits full expression of G_2. If both parents were heterozygous at these two loci the expected phenotypic ratio in this cross, assuming independent assortment, would be 3:1 of intermediate red dot to its absence. The F_1 brood does not deviate significantly from a 3:1 ratio ($P = .25$, df = 1). However, additional hypotheses like other gene-gene (including a recessive lethal with pleiotropic effect on the presence of the red dot) and/or gene-environment interactions will have to be ruled out as possible explanations for brood 2.

Because the red dot is not present in either of the comimics and its phenotypic effect seems to be very small (it appears somewhat hidden in a groove formed by the forewing costal vein), I have assumed that it is mimetically irrelevant. The presence of this minor character in populations of *H. c. weymeri* is puzzling to me, but it may be a useful "marker" for future population genetics studies.

Analysis of Linkage and Other Relationships Between Mimetically Relevant Loci

Some broods are informative for all the six possible linkage relationships and there is no evidence indicating that any pair of genes are located in the same chromosome except L^G/L^c and Wo_1/Wo_2 (but see below). This conclusion is supported by the following results: both, broods 6 and 9, with female parents $L^GL^cSb_1Sb_2$, do not deviate significantly from the null hypothesis of no linkage (brood 6, expected 1:1:1:1, $P = .91$, df = 3; brood 9, expected 1:1:2:2:1:1, $P = .45$, df = 5). In brood 9, female parent $L^GL^cYl_1Yl_2$, six segregates are obtained rather than the four that should be observed if the genes were in the same chromosome (sexes are heterogeneous). Brood 7, female parent $L^GL^cYl_1Yl_2$, deviates significantly from the null hypothesis of no linkage (expected 1:1:1:1, $P < .001$, df = 3), but four segregates are obtained, indicating that the genes cannot be in the same chromosome. Brood 10, female parent $Sb_2Sb_3Wo_1Wo_2$, does not deviate significantly from the null hypothesis of no linkage (expected 1:1:1:1:2:2:2:2:1:1:1:1, $P = .99$, df = 1). In brood 11, female parent $Sb_1Sb_3Wo_1Wo_2$, six segregates are obtained rather than the four that should be observed if the genes were in the same chromosomes (sexes are nearly heterogeneous). Both brood 2 and 11, with female parents $Sb_1Sb_2Yl_1Yl_2$ and $Sb_1Sb_3Yl_1Yl_2$, respectively, do not deviate significantly from the null hypothesis of no linkage (brood 2, expected 1:1:2:2:1:1, $P = .062$, df

= 5; brood 11, expected 1:1:1:1, $P = .88$, df = 3). In brood 9, female parent $Sb_1Sb_2Yl_1Yl_2$, four segregates are obtained as opposed to two that should be observed if the genes were in the same chromosome (sexes are heterogeneous). Broods 1 and 4, male parent $Sb_1Sb_2Yl_1Yl_2$, do not deviate significantly from the null hypothesis of no linkage (in both cases, expected 1:1:1:1, $P > .27$, df = 3). In brood 11, female parent $Wo_1Wo_2Yl_1Yl_2$, six segregates are obtained as opposed to four that should be observed if the genes were located in the same chromosome (sexes are heterogeneous). Brood 11, however, raises the possibility of linkage between L^G/L^c and Wo_1/Wo_2. The male parent is $L^GL^cWo_1Wo_2$ and the four segregates expected if the genes are tightly linked, with conformation L^GWo_1/L^cWo_2, are obtained (six segregates are expected from "loose" or no linkage; sexes are heterogeneous).

Broods 6 and 11 suggest the existence of an epistatic interaction between the L^G/L^c and Yl_1/Yl_2 loci. Without regard to the sex of the parents, they represent a cross of $L^GL^cYl_1Yl_1 \times L^cL^cYl_1Yl_2$ and both deviate significantly from the expected 1:1:1:1 ratio of genotypes $L^GL^cYl_1Yl_1$, $L^GL^cYl_1Yl_2$, $L^cL^cYl_1Yl_1$, and $L^cL^cYl_1Yl_2$, respectively ($P < .05$, df = 3, in both cases). Pooling the two F_1 broods together (2×4 contingency table, $P = .15$, df = 3), the total observed numbers of the four mentioned genotypes are 39:8:18:40, respectively. I basically explain this observation by the existence of an epistatic interaction so that allele L^G precludes the expression of allele Yl_2 (and L^c permits more easily the expression of Yl_2). In fact it is very difficult to observe the yellow line (controlled by Yl_2) in any individual of L^G-phenotype and it generally appears as a tiny specking of yellow scales at the base of the dorsal surface of the forewing.

It is also very likely that there is an epistatic interaction between the L^G/L^c and Wo_1/Wo_2 loci so that allele L^G precludes or diminishes the expression of Wo_2. This hypothesis may be supported for example by brood 11 which segregates genotypes L^G/L^c and L^cL^c in a 1:1 ratio and Wo_1Wo_1, Wo_1Wo_2, and Wo_2Wo_2 in a 1:2:1 ratio (see above). All the Wo_2Wo_2 individuals of this brood turned out to be L^cL^c suggesting that L^G may prevent the expression of Wo_2. Therefore, with respect to the interaction between L^G/L^c and Wo_1/Wo_2, brood 11 can be explained by tight linkage and/or epistasis. However I think that L^G is epistatic over Wo_2 because individuals with L^G-phenotype tend to show reduced expression of the white oval. The existence of the described epistatic interaction between L^G/L^c and both Yl_1/Yl_2 and Wo_1/Wo_2 is adaptive from the point of view of mimicry (see below).

Conclusions

I have described the mimetic phenotypic variation found mostly within the butterfly subspecies *H. c. weymeri*. I have presented strong evidence indicating that the latter is composed of two major polymorphic forms (*weymeri* and *gustavi*) and that a substantial portion of the wing pattern variation is controlled by four putative Mendelian genes that are mimetically relevant and one that does not seem to play any role in mimicry (G_1/G_2). Among those loci that are mimetically relevant there are two that seem to be of major importance and could be classified as macro- allele substitutions: L^G/L^c and $Sb_1/Sb_2/Sb_3$. The other two mimetically relevant genes seem to play a relatively minor role in mimicry and could be classified as micro-allele substitutions with more or less additive effect: Wo_1/Wo_2 and Yl_1/Yl_2.

It is quite possible that the allele L^G is epistatic over alleles Wo_2 and Yl_2, which control the presence of non-melanic elements in the medial area of the forewing (white oval and yellow line, respectively). The epistatic effect just mentioned would be adaptive for mimicry because it would enhance the chances that an individual resembles *H. e. chestertonii* (which also has a black-bluish forewing) even if it is not homozygous for the melanic elements controlled by these two loci. In a similar way, for example, allele Wo_2 would be best expressed in the *weymeri* form (which lacks allele L^G), that is to say in the phenotype where it contributes to mimicry with respect to *E. h. regalis*. Therefore, there is evidence that the genetic system in *H. c. weymeri* may have evolved epistatic interactions that facilitate mimetic resemblance with its comimics. There is also evidence that the sex of an individual affects the expression of the alleles at the Wo_1/Wo_2 locus. There is no evidence of linkage for any of the six possible relationships between mimetically relevant loci except between L^G/L^c and Wo_1/Wo_2.

My results are consistent with previous reports on the genetics mainly of *Heliconius erato* and *Heliconius melpomene*, whose races show ubiquitous Müllerian mimicry (Brown and Benson 1974; Mallet 1989; Sheppard et al. 1985; Turner 1968, 1971a,b). Some of the loci described here may be homologous to those of *H. melpomene*: L^G/L^c with N^B/N^N, unbroken versus broken forewing band, G_2/G_1 with D^R/d, radiate versus plain, and Yl_2/Yl_1 with presence and absence of "yellow line or yellow spot" (but I do not think that Yl_1/Yl_2 is homologous to Yb/yb, "yellow hindwing bar") (Sheppard et al. 1985). I do not find any obvious homology in *H. melpomene* with the $Sb_1/Sb_2/Sb_3$ and Wo_1/Wo_2 loci. It is also possible that some of the genes described here may actually represent supergenes. For example, the L^G/L^c and $Sb_1/Sb_2/Sb_3$ loci may be supergenes given that they control mimetic components that cross compartmental boundaries affecting several supposedly independent pattern elements of the wing (Nijhout 1985; Nijhout and Wray 1988; Nijhout et al. 1990; Sibatani 1980).

It has been proposed that the evolution of Müllerian mimetic coloration involves two basic steps: "(i) A mutation in one of the potentially mimetic species produces an approximate resemblance to the other. (ii) As the mutation increases in frequency, the mimicry between its pattern and that of the other species will be improved by selection of the existing polygenic variation in the population, or of few mutants with comparatively small effect" (Sheppard et al. 1985; Turner 1977, 1981, 1987). Although it is unlikely that we will ever know the order in which the various gene substitutions have occurred or make a complete reconstruction of the evolution of mimetic phenotypes, it is reasonable to use the information derived from genetic analysis in order to test theoretical models about the evolution of this biological attribute. The present study has characterized four loci that contribute to mimicry with respect to the comimics of *H. c. weymeri*. Two of these, L^G/L^c and $Sb_1/Sb_2/Sb_3$, would qualify as substitutions of major phenotypic effect or macromutations and two, Wo_1/Wo_2 and Yl_1/Yl_2, as substitutions of comparatively small phenotypic effect or micromutations. These results are consistent with the model for the evolution of Müllerian mimicry mentioned above. The first two alleles could have been responsible for bringing about an approximate resemblance of *H. c. weymeri* with respect to its two model species (the first step of the model), and the last two could have added minor improvement making the resemblance very accurate (part of the second step of the model). There is also evidence that selection on existing polygenic variation, influencing the effect of the major substitutions them-

selves, could have improved the mimicry (also part of the second step). For example, the hypothesized epistatic effect of the L^G/L^c locus over both the Yl_1/Yl_2 and Wo_1/Wo_2 loci could have evolved through the fixation of alleles of very small effect in modifier loci. Therefore, my results do not support exclusively the neo-Darwinian claim that adaptations result from the accumulation of many alleles of small effect (micromutationism) nor the possibility that adaptations result from the accumulation of few alleles of large effect (macromutationism) but a combination of these two views of evolutionary biology.

From the Instituto de Genética, Universidad de los Andes, Calle 18 Carrera 1E, Santafé de Bogotá, Colombia. The research was funded by National Science Foundation grants numbers INT-8610430 and INT-8918819 and by Fondo Colombiano de Investigaciones Científicas y Proyectos Especiales Francisco José de Caldas COLCIENCIAS (Colombia), grants CO-6220-05-00387 and CO-6220-05-008-89. I want to thank the Instituto Vallecaucano de Investigaciones Científicas INCIVA in Cali, particularly F. Arango and L. E. Gilbert, for logistic support. Culture facilities were made possible by NSFDEB 790633 to L. E. Gilbert, University of Texas at Austin. I also thank M. T. Almansa, J. Ayala, A. Barreto, S. Bramblett, K. Brown, E. Constantino, L. C. García, L. P. Moreno, E. W. Schmidt-Mumm, M. Singer, C. D. Thomas, and R. Torres for helping me in various ways. A very special thanks to L. E. Gilbert for teaching me a lot. My institutional affiliations during the period this research was carried out were University of Texas at Austin (graduate student, 1983–1989), Fundación Segunda Expedición Botánica, Colombia (research associate, 1987 and 1990–92), Universidad Javeriana, Colombia (Junior faculty, 1990–1994), and Universidad de los Andes (Director of Genetics Institute, 1994 to the present)

The Journal of Heredity 1996:87(2)

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Received February 24, 1995
Accepted September 6, 1995

Corresponding Editor: Therese Ann Markow

Paternity Exclusion in Koalas Using Hypervariable Microsatellites

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Koala microsatellite loci containing the dinucleotide motif $(CA)_n$ were isolated from a size-fractionated (250–500 bp) koala genomic library and sequenced. Six locus-specific primer pairs were designed and synthesized for DNA amplification using the polymerase chain reaction (PCR). Microsatellite genotyping of 12 individuals generated unique "fingerprints" for each koala. All six microsatellite loci were polymorphic, with a mean of 6.5 ± 0.6 alleles/locus. This level of allelic diversity is capable of generating $>4 \times 10^9$ DNA profiles, making it the most powerful technology for fingerprinting koalas currently available. Observed heterozygosities (H_o) in the eight unrelated individuals surveyed ranged from 0 25 to 0.75, with a mean of 0.54 ± 0.06. Mendelian inheritance of the observed polymorphism was confirmed by family studies. We demonstrate that microsatellite loci are ideal genetic markers for paternity exclusion and pedigree analysis of koalas, which have shown little genetic variation using most other methods.

The determination of parentage of individuals from genetic data has become increasingly popular, because it relates directly to mating behavior, juvenile dispersal, and the management of captive animal populations (Amos et al. 1993; Foltz and Hoogland 1981; McCracken and Bradbury 1977; Meagher 1986). Traditionally, blood group antigens, histocompatibility antigens, and allozymes were employed in these analyses. However, direct examination of DNA sequence polymorphism can distinguish individuals and confirm familial relationships with greater resolving power. This is typified by the detection of DNA polymorphisms using minisatellite "fingerprinting" techniques (Jeffreys et al. 1985), in the positive determination of paternity.

More recently, "fingerprinting" analysis has been performed with simple-sequence repeats, or microsatellites, which are ideal