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# The Genetics of Some Polymorphic Forms of the Butterflies *Heliconius melpomene* Linnaeus and *H. erato* Linnaeus. I. Major Genes.[1,2]

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(Plate I; Text-figure 1)

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the Station is intended principally for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I." by William Beebe, Zoologica, 1952, Vol. 37, No. 13, pp. 157-184.]

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## I. INTRODUCTION

THE neotropical butterflies *Heliconius melpomene* and *H. erato* (Lepidoptera, Nymphalidae, Heliconiinae) show complex parallel polymorphism in their wing markings (Oberthür, 1902; Eltringham, 1916; Joicey & Kaye, 1916). In connection with research into the biology of the heliconiine butterflies of Trinidad[4], the genetics of several of the polymorphic forms of *H. melpomene* has been investigated. Crosses were made between several forms bred from eggs obtained in the wild in Surinam (Dutch Guiana) and between the Surinam stock and insects from Trinidad, West Indies, where the species is monomorphic. The results show that most of the differences between the major polymorphic forms studied are produced by genes in the same linkage group; analysis of previously described broods of *H. erato* (Beebe, 1955)

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[4]Related in different degrees to the present contribution are the following: Beebe, 1955; Crane, 1955, 1957; Fleming, 1960; Beebe, Crane & Fleming, 1960; Alexander, 1961.1, 1961.2.

shows that the mode of inheritance of analogous patterns in this species is very similar to that in *melpomene*.

This paper deals with differences produced by major genes; a second paper will deal with quantitative variation (Turner, in press).

The preliminary work involved in assembling the material was long and exacting. The difficulties included irregular seasonal scarcities, the intermittent prevalence of disease, the apparent impossibility of hand-pairing the imagos, the usually rapid deterioration of the stock with inbreeding and, finally, the fact that the larvae, being not only non-gregarious but incompatible, had to be reared singly. We would like, therefore, to express particular thanks to the people responsible, through parts of two years at the Trinidad Field Station, for the collection of the stock and the rearing of the resultant broods. Included are Mr. Henry Fleming, who collected the breeding stock on field trips to Surinam and who designed the breeding cages, and the following laboratory assistants: Mesdames Susan Allan, Kathleen Campbell, Frances W. Gibson and Jane S. Kinne, and Misses Constance Carter and Diana Jeffrey.

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Finally, we express our indebtedness to the late Dr. William Beebe for his continued and constructive interest in the general study of the biology of the heliconiines and for his helpful suggestions in the course of the present section of the work.

## II. The Genetics of *H. melpomene*

### A. Materials and Methods

1. *Methods of Collection.* Most of the material for the present contribution was collected in Surinam in December, 1958, and during the same month in 1959. Rearing and breeding were carried out at the Trinidad Field Station. Crosses between Surinam material and *melpomene* from Trinidad were produced by mating stock living near the field station with broods resulting from the eggs and larvae collected in Surinam.

In both years the Surinam collections were made during pronounced dry seasons in the vicinity of Moengo, a bauxite mining community in the northeastern part of the country. Some were taken near the wharf and others close to the Moengo end of a 30-mile road joining Moengo and Albina. The latter town borders the Maroyne (=Maroni) River which separates Surinam from the region of French Guiana from which came the collection described by Joicey & Kaye (1916). The Moengo area has become so well known to us over a period of years that individual food plants, located in previous seasons in an area of some 20 square miles, could be revisited daily.

Eggs and larvae of *H. melpomene* were exceedingly scarce during the two collecting periods under consideration, each of which lasted one week. The total catch numbered 13 in 1958 and 17 in 1959; each year the specimens were found in only two small areas; 12 of those taken in 1958 came in fact from a single vine. During each year only two or three imagos were seen flying in the entire area of search. It therefore seems likely that the resultant broods came from not more than several females each season.

2. *Methods of Breeding.* A summary of the material is given in Table I. As indicated, the broods resulting from the eggs collected in December, 1958, were exceedingly subject to disease and/or genetic weakness. In the following year therefore it was thought wise to breed pairs in which the males and females in the generation came from localities separated by approximately 10 miles. Abnormalities, particularly crumpled wings and the inability to emerge fully from the pupal case, as well as a number of forms of disease and/or abnormalities in the larvae, frequently appear in the second inbred generation.

Even when inbreeding is avoided, however, as in our nongenetical studies, it is not possible ever to plan definitely to rear particular species of heliconiines, including *H. melpomene*, in any given season, because of the frequent occurrence

Table I.

| Year | Eggs Laid | Eggs Hatched | % Hatched | Larvae Successfully Reared | % Larvae Successfully Reared of Eggs Laid | % Larvae Successfully Reared of Eggs Hatched |
|---|---|---|---|---|---|---|
| 1959 | 248 | 227 | 91.6% | 25 | 10.1% | 11% |
| 1960 | 2,605 | 1,832 | 70.4% | 777 | 29.8% | 44.7% |



of disease. Sometimes a year of abundance is followed by two poor seasons as in the material under consideration; sometimes good and poor years alternate; rarely do two good years follow each other. Evidence is accumulating that poor seasons occur simultaneously in Surinam and Trinidad, and that both the annual amount of rain and the condition of the food plants are involved.

The eggs and larvae collected in Surinam were brought to Trinidad by air and reared in the laboratory by the same general method previously described (Crane, 1955, pp. 168-170; Beebe, Crane & Fleming, 1960, pp. 113-115). Briefly, this consists of rearing each caterpillar in an individual dish, because of the cannibalistic habits of the early instars and the frequent aggressive behavior of larger larvae. Low stender dishes, 60 × 28 mm. in size and with ground glass lids, are ideal containers during the first three to four instars. When adequate space and glassware become a problem the early instars are also reared in 3 × 1-inch vials, with the uncorked mouths pushed into a tray of damp sand. Humidity, a most important factor in the rearing of heliconiines, is difficult to control when the vials are used. In the stender dishes and in the 4 × 4 × 2.5-inch glass refrigerator dishes used for the last one or two instars, humidity is furnished by small sections of cotton dental wads, which are saturated and squeezed partly dry. Larvae also drink from drops of water left on the sprinkled leaves.

The normal foodplant of the species both in Surinam and in Trinidad is *Passiflora laurifolia* L. Eggs have been collected also in the field and reared in the laboratory on three other species of the same genus, *P. auriculata* HBK, *tuberosa* Jacquin and *lonchophora* L.

Before the prepupal wandering phase, larvae for genetical study are furnished with a piece of firm wire netting laid beneath the glass cover of the dish. Healthy larvae climb on the netting and usually spin the pad toward the middle of the screen, pupation normally taking place the following morning. Pupae are kept undisturbed for 24 hours, after which each screen with its label is transferred to the top of a jar or tumbler above a well-soaked cotton wad.

The approximate usual durations of the developmental stages are as follows: Egg, 4 days; larval instars, 15; pupal, 9 to 10. Further details are given in Beebe, Crane & Fleming, *loc. cit.*

The night before emergence, if the imago is certainly not to be bred, the screen with the pupa is transferred to the top of a cylinder made of soft wire netting and set in a dish of damp sand. After emergence the butterfly is allowed to dry the newly expanded wings for several hours before being placed, wings folded, in a transparent plastic envelope, and chloroformed. About 24 hours later, when *rigor mortis* has passed, the butterfly is removed, the wings opened out flat with forceps, and the insect returned to the envelope. In the absence of a drying cabinet the envelopes may be stored in a cardboard carton and, without uncovering, exposed daily to hot sun or the top of a warm oven. Abundant and constant paradichlorobenzine is a tropical necessity, not only to ward off pests but to prevent mold. The prompt spreading of the wings and storage of the insect in fully transparent envelopes eliminates both pinning and traditional spreading techniques; considerable time is thus saved, while shipping, sorting and study are all facilitated.

Butterflies intended for breeding are treated as follows. Males are released, as soon as the wings dry, into isolation cages of wire mesh measuring at least six feet on a side. When more than one male is kept temporarily in the same cage, the specimens are marked with waterproof, quick-drying lacquer. The males are allowed to mature to the requisite age for mating before being placed in a cage with a female, since courtship is then more likely to take place, particularly between individuals of low vitality. No male mates before the second morning after emergence, at the imaginal age of about 48 hours; 72 hours is the normal age in *melpomene* while 96 hours is occasional. Males are placed singly in breeding cages that measure at least 9 × 9 × 8 feet, preferably on the night before breeding is expected to take place, so that any shock of handling is minimized and time allowed for recovery. The insect should never be picked up between bare fingers or chased with a net; a strip of wax paper will insulate wings from the fingers or a small net may be used after the insect has come to rest.

Females to be bred are placed either in small isolation cages or directly in the breeding cage. Mating can occur in this species at any imaginal age from about 1½ hours to about 12 days. Females are most attractive on the first and second days. Males may be bred at least four times with healthy offspring resulting. They are not known to mate on successive days, although mating is often repeated when one day has elapsed between the pairings. Successful breeding has resulted in males more than two months old.

It has not been found possible to pair any heliconiine by hand[5], and they have not been

[5] *Dione juno*, which rarely mates or oviposits in captivity, can be hand mated and will readily lay when

mated successfully in cages much smaller than the size specified above. Females will however lay eggs in normal numbers in cages measuring about six feet in each dimension. Single individuals of either sex may be kept when necessary for two or, rarely, three days in cages about three feet on a side; however, they must be fed, beginning on the second morning, with flowers placed on a shelf near the cage top in the brightest corner. If the butterflies are kept longer in such cramped quarters, their vitality proves to be impaired when eventually they are moved to the breeding cages.

Details of the maintenance of butterfly insectaries will be found in Crane & Fleming, 1953, and Crane, 1955, 1957. The principal factors ensuring success, regardless of cage size, are: abundant natural flower food; abundant humidity, maintained through sprinkling when necessary; foliage, or at least growing grass, inside the cage; and ample sun and shade.

No female has ever been found to lay eggs on any plant not belonging to the genus *Passiflora*. The maximum number of eggs laid by a single *H. melpomene* was 196 over a period of fifty-five days. One to four eggs are usually laid per day, rarely more, beginning on the eighth to fifteenth day after emergence. The major egg-laying period is finished after about two weeks; later in the female's life eggless days may become frequent. Eggs are collected every afternoon to avoid predation by ants.

It is doubtless unnecessary to emphasize the importance for care and detail in labelling at every step of the study, from the gathering of eggs, whether wild or cage-laid, to the final labels on the envelopes holding the imagos. Self-adhesive labels are used on the rearing dishes while metal garden markers work well on breeding and egg-laying cages out-of-doors.

3. *Methods of Study*. The wing pattern of *melpomene* is made up of a number of elements which vary independently and which occur in most if not all possible combinations, each one of which has a separate taxonomic name (Seitz, 1913; Joicey & Kaye, 1916). Rather than become involved in nomenclatural difficulties, we have adopted the procedure recommended by Camp and Gilmour (Gilmour, 1958) in another context, of keeping the genetic and taxonomic names completely separate; this system also has the advantage that the same notation may be used for *H. erato*. The various pattern elements we studied are therefore designated by English names, with appropriate symbolic letters, as follows:—

Ray (R): the presence of four to six red radiate marks on the discal part of the upperside of the hindwing (Pl. I, Fig. 1);

nonray (r): the absence of such red rays (Pl. I, Figs. 2-5);

Dennis (D): the presence of extensive red areas at the base of the forewings on the upper and under sides, and on the base of the hindwings on the upperside (Pl. I, Figs. 1-3);

nondennis (d): the absence of such red areas (Pl. I, Figs. 4-5);

Wide-band (B): the presence of a broad red band extending from the costal edge of the forewing towards the inner angle (Pl. I, Figs. 1, 2 & 4);

narrow-band (b): the presence of a narrow red band extending from the costal edge of the forewing toward the inner angle (Pl. I, Figs. 3 & 5).

In addition there was much quantitative variation in the width and shape of the forewing bands and in the amount of yellow pigment in the discal area of the forewing, which will be discussed in the second paper (Turner, 1962).

In the course of the present study, Crane has been responsible for the Trinidad aspects, including methodology, pair selection and supervision of rearing and recording. The work of analysis, on the other hand, has been accomplished altogether by Turner at the University of Liverpool.

## B. Results

Data for all the broods reared will be found in Tables II and III. In the light of our findings we have deduced the genotypes of most of the parent butterflies, although in some instances, marked with an asterisk, it has been possible only to indicate the most likely genotype, assuming that crossing-over has not occurred. Note that as the Trinidad population is monomorphic all Trinidad butterflies must be homozygous at the major loci.

1. *Inheritance of the Ray Pattern*. The only brood in which this pattern occurred (Table II, 1) shows that it is probably inherited as a single factor, one of the parents of the brood being a recessive homozygote, the other a heterozygote, but it is not possible to tell from the data

confined in a black silk-organza sleeve. *Heliconius numata* and *H. erato* have been hand mated and the latter has laid in a sleeve. However, both species are refractory in these respects and the techniques are not recommended unless the normal methods have failed.



Table II. Breeding Data for *H. melpomene* (First Series). (1959)

| Brood No. | ♂ Parent | | ♀ Parent | | Brood | | | |
|---|---|---|---|---|---|---|---|---|
| | Brood or Origin | Phenotype | Origin | Phenotype | BDR | Bdr | bdr | Total |
| 1 | Surinam | Bdr | Surinam | BDR | ♂ 0 | 1 | 0 | 1 |
| | | | | | ♀ 3 | 4 | 0 | 7 |
| | | | | | 3 | 5 | 0 | 8 |
| 2 | Surinam | Bdr | Surinam | bdr | ♂ 0 | 2 | 6 | 8 |
| | | | | | ♀ 0 | 3 | 2 | 5 |
| | | | | | 0 | 5 | 8 | 13 |
| 3 | Surinam | Bdr | Surinam | Bdr | ♂ 0 | 2 | 0 | 2 |
| 4 | 3 | Bdr | Trinidad | Bdr | ♂ 0 | 1 | 0 | 1 |
| 5 | Surinam | Bdr | Trinidad | Bdr | ♂ 0 | 1 | 0 | 1 |

whether the gene is dominant or recessive; the use of the capital R for the Ray pattern is therefore provisional. For reasons explained under Section 4 below, it is apparent that the locus is not sex-linked.

2. *Inheritance of the Dennis Pattern*. A large number of broods show that this pattern is produced by a single dominant gene which is not sex-linked. For example, in Table III, broods 3, 6 and 27, all of which are D × D, give a satisfactory approximation to the ratio 3 D:1 d. The dominance of the *D* gene is confirmed by brood 23, and broods 14, 22, 24, 26 and 32 among others conform to the 1:1 backcross ratio, so confirming that a single gene is involved. Broods 22, 24 and 26 also show that the gene is autosomal, for if it were sex-linked all males would be nondennis and all females Dennis. The anomalous individuals in brood 7 are assumed to be the result of an error.

3. *Inheritance of the Width of the Bands*. Similarly the difference between a Wide-band and a narrow-band is produced by a single autosomal locus, the factor for Wide-band being dominant. In Table III, among others, broods 4, 9, 10 and 22 are seen to be $F_2$ generations, corresponding to the 3:1 ratio, broods 13, 23 and 29 are backcross generations and brood 13 demonstrates the absence of sex-linkage. Again the anomalous individual (brood 28) is assumed to result from an error.

4. *Linkage of Ray, Dennis and Band Factors*. The three loci controlling the patterns Ray and Dennis and the width of the band are all in the same linkage group. Thus in Table II, brood 1 is apparently a backcross for the Ray and Dennis factors and departs significantly from independent assortment ($P < .01$ by Fisher's exact test); there are no crossovers. The maximum crossover value, estimated on the assumption that if one more butterfly had emerged it would have been a crossover, is 11%, with a standard error of ± 11%. It is therefore highly unlikely that the COV is more than 30% and it may be much lower. We stated earlier that the Ray locus was not sex-linked; the reason for this conclusion is now obvious.

The data themselves do not exclude the possibility that the Ray locus is on a separate chromosome from the Dennis locus, but that the *R* gene can only express itself in the presence of the *D* gene; however we think this unlikely, as there is a variety of *H. melpomene* in which the Ray pattern occurs independently of the Dennis pattern (var. *contiguus*, see Eltringham, 1916, plate XII, 26). As this variety apparently occurs only in Ecuador (Joicey & Kaye, 1916: p. 420), it is possible that the COV between *D* and *R* is very low.

Similarly the *D* and *B* loci are linked, as is shown by broods 22 and 24 (*P* for independent assortment less than .0003). If crossovers had occurred they could have been detected by inspection of the phenotypes in broods 4, 15, 17, 22, 24, 27 and 30 (Table III); in these broods no butterflies have appeared in the crossover class (bd) and as at least one, and in some broods probably both parents in each cross are repulsion heterozygotes, it is not possible to obtain a reliable estimate of the crossover value. A total of 11 butterflies from broods 2, 14, 17 and 32 (Table III) has been crossed in such a way that their genotypes can be determined from their progeny; of these, 3 are carrying crossover chromosomes, two of them *bd* and one *BD* (the original chromosomes in the crosses

TABLE III. BREEDING DATA FOR *H. melpomene* (SECOND SERIES). (1960).
All insects in this table are nonray (r)

| Brood No. | ♂ Parent | | | ♀ Parent | | | Brood | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Brood or Origin | Phenotype | Genotype | Brood or Origin | Phenotype | Genotype | | BD | Bd | bD | bd | Total |
| 1 | "S" Surinam | Bd | ? | "B" Surinam | ? | ? | ♀ | 0 | 4 | 0 | 0 | 4 |
| 2 | "D" Surinam | BD | bD/Bd | 1 | Bd | Bd/?d | ♂ | 0 | 3 | 0 | 0 | 3 |
| | | | | | | | ♀ | 2 | 3 | 0 | 0 | 5 |
| | | | | | | | | 2 | 6 | 0 | 0 | 8 |
| 3 | 32 | BD | bD/Bd* or BD/Bd | 2 | BD | BD/Bd or bD/Bd* | ♂ | 23 | 8 | 0 | 0 | 31 |
| | | | | | | | ♀ | 26 | 10 | 0 | 0 | 36 |
| | | | | | | | | 49 | 18 | 0 | 0 | 67 |
| 4 | 17 | BD | BbDd | 3 | BD | BbDd | ♂ | 0 | 1 | 0 | 0 | 1 |
| | | | | | | | ♀ | 5 | 1 | 2 | 0 | 8 |
| | | | | | | | | 5 | 2 | 2 | 0 | 9 |
| 5 | 3 | BD | ? | 3 | BD | ? | ♂ | 11 | 0 | 0 | 0 | 11 |
| | | | | | | | ♀ | 5 | 0 | 0 | 0 | 5 |
| | | | | | | | | 16 | 0 | 0 | 0 | 16 |
| 6 | 3 | BD | ?D/Bd* | 3 | BD | ?D/Bd* | ♂ | 10 | 1 | 0 | 0 | 11 |
| | | | | | | | ♀ | 9 | 3 | 0 | 0 | 12 |
| | | | | | | | | 19 | 4 | 0 | 0 | 23 |
| 7 | 3 | Bd | Bd/Bd* | 3 | Bd | Bd/Bd* | ♂ | 0 | 4 | 0 | 0 | 4 |
| | | | | | | | ♀ | 1 | 2 | 0 | 0 | 3 |
| | | | | | | | | 2 | 7 | 0 | 0 | 9 |
| 8 | "M" Surinam | Bd | Bd/bd | "E" Surinam | Bd | Bd/Bd | ♂ | 0 | 25 | 0 | 0 | 25 |
| | | | | | | | ♀ | 0 | 17 | 0 | 0 | 17 |
| | | | | | | | | 0 | 42 | 0 | 0 | 42 |
| 9 | 8 | Bd | Bd/bd | 8 | Bd | Bd/bd | ♂ | 0 | 2 | 0 | 1 | 3 |
| | | | | | | | ♀ | 0 | 1 | 0 | 0 | 1 |
| | | | | | | | | 0 | 3 | 0 | 1 | 4 |
| 10 | "M" Surinam | Bd | Bd/bd | "H" Surinam | Bd | Bd/bd | ♂ | 0 | 10 | 0 | 1 | 11 |
| | | | | | | | ♀ | 0 | 10 | 0 | 2 | 12 |
| | | | | | | | | 0 | 20 | 0 | 3 | 23 |



TABLE III. BREEDING DATA FOR *H. melpomene* (SECOND SERIES). (1960). (Continued)
All insects in this table are nonray (r)

| Brood No. | ♂ Parent | | | ♀ Parent | | | Brood | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Brood or Origin | Phenotype | Genotype | Brood or Origin | Phenotype | Genotype | | BD | Bd | bD | bd | Total |
| 11 | 10 | bd | bd/bd | 10 | Bd | Bd/?d | ♂ | 0 | 2 | 0 | 0 | 2 |
| 12 | "Number 2" Trinidad | Bd | Bd/Bd | 10 | bd | bd/bd | ♂ | 0 | 14 | 0 | 0 | 14 |
| | | | | | | | ♀ | 0 | 18 | 0 | 0 | 18 |
| | | | | | | | | 0 | 32 | 0 | 0 | 32 |
| 13 | 9 | bd | bd/bd | 12 | Bd | Bd/bd | ♂ | 0 | 1 | 0 | 3 | 4 |
| | | | | | | | ♀ | 0 | 2 | 0 | 1 | 3 |
| | | | | | | | | 0 | 3 | 0 | 4 | 7 |
| 14 | "D" Surinam | BD | bD/Bd | "P" Surinam | Bd | Bd/Bd | ♂ | 11 | 20 | 0 | 0 | 31 |
| | | | | | | | ♀ | 14 | 13 | 0 | 0 | 27 |
| | | | | | | | | 25 | 33 | 0 | 0 | 58 |
| 15 | 14 | BD | bD/Bd* | 14 | BD | bD/Bd* | ♂ | 0 | 1 | 0 | 0 | 1 |
| 16 | "Number 1" Trinidad | Bd | Bd/Bd | 14 | BD | bD/Bd* | ♀ | 1 | 0 | 0 | 0 | 1 |
| 17 | 14 | BD | bD/Bd | 14 | BD | bD/Bd | ♂ | 1 | 0 | 0 | 0 | 1 |
| | | | | | | | ♀ | 3 | 1 | 5 | 0 | 9 |
| | | | | | | | | 4 | 1 | 5 | 0 | 10 |
| 18 | 14 | Bd | Bd/Bd* | 14 | BD | bD/Bd* | ♀ | 1 | 0 | 0 | 0 | 1 |
| 19 | 14 | Bd | Bd/Bd* | 14 | Bd | Bd/Bd* | ♂ | 0 | 7 | 0 | 0 | 7 |
| | | | | | | | ♀ | 0 | 1 | 0 | 0 | 1 |
| | | | | | | | | 0 | 8 | 0 | 0 | 8 |
| 20 | 14 | Bd | Bd/Bd* | 14 | Bd | Bd/Bd* | ♀ | 0 | 1 | 0 | 0 | 1 |
| 21 | 32 | Bd | Bd/Bd* | 14 | Bd | Bd/Bd* | ♂ | 0 | 36 | 0 | 0 | 36 |
| | | | | | | | ♀ | 0 | 35 | 0 | 0 | 35 |
| | | | | | | | | 0 | 71 | 0 | 0 | 71 |
| 22 | 8 | Bd | Bd/bd | 14 | BD | bD/Bd | ♂ | 8 | 10 | 5 | 0 | 23 |
| | | | | | | | ♀ | 8 | 17 | 6 | 0 | 31 |
| | | | | | | | | 16 | 27 | 11 | 0 | 54 |
| 23 | 8 | Bd | Bd/bd | 17 | bD | bD/bD | ♂ | 2 | 0 | 2 | 0 | 4 |
| | | | | | | | ♀ | 5 | 0 | 2 | 0 | 7 |
| | | | | | | | | 7 | 0 | 4 | 0 | 11 |

TABLE III. BREEDING DATA FOR *H. melpomene* (SECOND SERIES). (1960). (Continued)
All insects in this table are nonray (r)

| Brood No. | ♂ Parent | | | Brood | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Brood or Origin | Phenotype | Genotype | | BD | Bd | bD | bd | Total |
| 24 | 9 | Bd | Bd/bd | ♂♂ | 11 | 23 | 15 | 0 | 49 |
| | | | | ♀♀ | 12 | 22 | 11 | 0 | 45 |
| | | | | | 23 | 45 | 26 | 0 | 94 |
| 25 | 19 | Bd | Bd/Bd | ♂♂ | 1 | 4 | 0 | 0 | 5 |
| | | | | ♀♀ | 1 | 0 | 0 | 0 | 1 |
| | | | | | 2 | 4 | 0 | 0 | 6 |
| 26 | 9 | bd | bd/bd | ♂♂ | 0 | 0 | 14 | 8 | 22 |
| | | | | ♀♀ | 0 | 0 | 13 | 9 | 22 |
| | | | | | 0 | 0 | 27 | 17 | 44 |
| 27 | 17 | BD | BbDd | ♂♂ | 4 | 4 | 1 | 0 | 9 |
| | | | | ♀♀ | 0 | 2 | 1 | 0 | 3 |
| | | | | | 5 | 6 | 2 | 0 | 13 |
| 28 | 23 | bD | bD/bd | ♂♂ | 0 | 1 | 2 | 1 | 4 |
| | | | | ♀♀ | 0 | 0 | 5 | 1 | 6 |
| | | | | | 0 | 1 | 7 | 2 | 10 |
| 29 | 12 | Bd | Bd/bd | ♂♂ | 2 | 3 | 0 | 3 | 8 |
| | | | | ♀♀ | 4 | 7 | 2 | 5 | 18 |
| | | | | | 7 | 10 | 2 | 9 | 28 |
| 30 | 3 | BD | BbDd | ♂♂ | 6 | 10 | 2 | 0 | 18 |
| | | | | ♀♀ | 10 | 2 | 1 | 0 | 13 |
| | | | | | 16 | 12 | 3 | 0 | 31 |
| 31 | 32 | Bd | Bd/Bd* | ♂♂ | 0 | 32 | 0 | 0 | 32 |
| | | | | ♀♀ | 0 | 23 | 0 | 0 | 23 |
| | | | | | 0 | 55 | 0 | 0 | 55 |
| 32 | "D" Surinam | BD | bD/Bd | ♂♂ | 3 | 3 | 0 | 0 | 6 |
| | | | | ♀♀ | 1 | 1 | 0 | 0 | 2 |
| | | | | | 4 | 4 | 0 | 0 | 8 |

*Indicates that the genotype is not known for certain. The genotype given is the most probable, assuming that crossing over has not occurred.
Words and letters within quotation marks are the designations of wild-caught butterflies.
Discrepancies in the total numbers of offspring have been produced by insects which could not be scored for sex.



being *bD* and *Bd*). Again the amount of information is so small that no reliable estimate of the crossover value can be obtained.

From Tables II and III it will be seen that the following chromosomes, genotypes and phenotypes have been observed in our broods (not considering the *R* locus):

Chromosomes: *BD, bD, Bd* and *bd;*

Genotypes: *BD/Bd, Bd/Bd, bD/bD, Bd/bd, bD/bd, bD/Bd* and *bd/bd;*

Phenotypes: BD, bD, Bd and bd.

That is, all possible phenotypes and chromosomes occurred, and all but three of the possible combinations of those chromosomes. Taking the Ray pattern into account, the following phenotypes were found:

BDR, BDr, bDr, Bdr, bdr.

Other phenotypes have been found in the wild (Joicey & Kaye, 1916).

## III. THE GENETICS OF *H. erato*

### A. MATERIALS AND METHODS

Data presented by Beebe (1955) of broods reared from wild individuals of *H. erato* give information about the inheritance of various elements of the pattern in this species. The following symbols have been used in tabulating his results:—

B: a wide forewing band, as in *melpomene;*

$B^b$: a complicated, broken forewing band, different from anything in the *melpomene* material;

D: red pigmentation of the base of the forewing, similar to that of Dennis in *melpomene,* but lacking the basal red on the hindwing;

d: absence of this red pigmentation;

R: Ray patterns, similar to those in *melpomene,* but extending into the basal area of the hindwing;

r: the absence of such red rays;

(y): indicates that the feature with which the symbol is bracketed is yellow instead of red; thus if B is a red forewing band, (By) is a yellow one;

w: presence of prominent white spots in the forewing band.

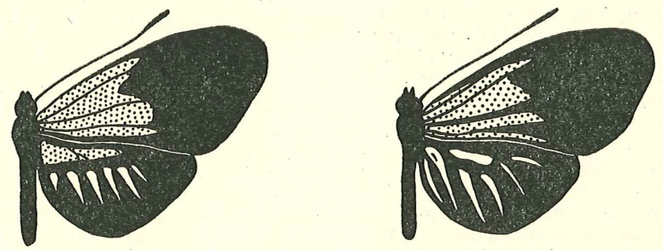
The difference between the Dennis and Ray patterns in the two species is worthy of special note and is illustrated in Text-fig. 1; for illustrations of the other patterns see the plates in Beebe's paper.

### B. RESULTS

Beebe's results are summarized in Table IV and permit the following conclusions:—

1. The female parent of Brood B, being from Trinidad where the species is monomorphic, must have been homozygous for all major wing-pattern loci. This means that in this brood the factors for the D pattern, R pattern and $B^b$ pattern (Broken-band) were all dominant and the male parent was homozygous. Unless complex factor interaction is involved, the gene for yellow coloring of the forewing band is recessive to the gene for red coloring.

2. It is therefore likely that brood D is a backcross for the *D* and *R* factors, the unknown male parent having been a heterozygote ($P > .5$). *D* and *R* could be recessive, as the fit to an $F_2$ ratio is also good ($P > .2$), but in view of the result for brood B this seems unlikely. The brood gives a strong suggestion of linkage between the *D* and *R* loci. Assuming that the brood is a double backcross, *P* for independent assortment is less than .001, and the maximum value for the crossover rate is 8% ± 7%. The data do not exclude the possibilities that (a) Dennis and Ray are pleiotropes of one gene, or (b) *D* and *R* are on separate chromosomes but that one can only express itself in the presence of the other; though both these alternatives seem unlikely because of the existence of specimens



TEXT-FIG. 1. Difference between Ray and Dennis elements in *H. melpomene* (left) and *H. erato* (right). Ray element white, Dennis element stippled.

TABLE IV. BREEDING DATA FOR *H. erato*. (BEEBE'S DATA).

| Brood Letter | ♂ Parent Phenotype and Origin | ♀ Parent Phenotype or Genotype and Origin | Brood | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $B^b$ DR | $B^b$ DRw | ($B^b$y) Dr | BDR | Bdr | Total |
| A | ? Surinam | $B^bDr$ Surinam | ♀ 0 | 0 | 1 | 0 | 0 | 1 |
| B | ($B^b$y)DR Surinam | *Bdr/Bdr* Trinidad | 13 | 0 | 0 | 0 | 0 | 13 |
| C | ? Surinam | Bdr Surinam | ♂ 1 | 0 | 0 | 0 | 0 | 1 |
| | | | ♀ 0 | 1 | 0 | 0 | 2 | 3 |
| | | | 1 | 1 | 0 | 0 | 2 | 4 |
| D | ? Surinam | Bdr Surinam | ♂ 0 | 0 | 0 | 3 | 5 | 8 |
| | | | ♀ 0 | 0 | 0 | 2 | 3 | 5 |
| | | | 0 | 0 | 0 | 5 | 8 | 13 |
| E | ? Surinam | Bdr Surinam | ♂ 0 | 0 | 0 | 0 | 2 | 2 |
| | | | ♀ 0 | 0 | 0 | 0 | 2 | 2 |
| | | | 0 | 0 | 0 | 0 | 4 | 4 |

Insects designated "?" were the wild mates of the females and were never seen.

in which Dennis occurs without Ray (vars. *dryope* and *cybelina;* see Seitz, 1913, Pl. 78) and in which Ray occurs without Dennis (var. *vesta*; see Seitz, 1913, p. 393).

3. Brood C therefore also provides evidence of linkage between the *D* and *R* loci, and between these two loci and the factor, apparently $B^b$, which is affecting band-width in this brood; it is not possible to prove or estimate the linkage (*P* for independent assortment greater than .1). It is unlikely that the $B^b$ and D patterns are pleiotropes of the same gene, or appear linked because of factor interaction, as they can occur independently (vars. *callicopis* and *erythraea;* Seitz, 1913, Pl. 78).

4. Nothing can be said about the inheritance of white coloration.

5. There is no evidence for or against sex-linkage.

6. One reservation must be made: the apparent linkage of the various loci could have been produced even if the loci were not linked, if the females had mated twice in the wild; double matings have been suggested by Ford (1936) to explain anomalous broods reared from wild *Papilio* females.

## IV. DISCUSSION

The polymorphisms of *Heliconius erato* and *H. melpomene* are of exceptional interest from a number of points of view. The species are undoubtedly aposematic (Crane and refs., 1955, preliminary observations; and L. P. Brower, J. V. Z. Brower & C. T. Collins, in prep., experimental study). Almost certainly the species are also Müllerian mimics and are involved in mimetic relationships of amazing complexity with numerous other Lepidoptera (Eltringham, 1916). Again, the hue red, which in the most usual form of both species is present as a forewing band, has high value in releasing courtship behavior; in fact an all-red model of about the size of a normal butterfly acts as a supernormal stimulus (Crane, 1955, on *H. erato* and in prep. on *H. melpomene.*) Finally, evidence is emerging (Beebe, Crane & Fleming, 1960; Alexander, 1960.1; and Crane *et al.* unpubl.) that *H. melpomene* and *H. erato* are closely related.

In those features studied so far, both polymorphisms are apparently controlled by loci in the same linkage group and are therefore polymorphisms of the type described by Sheppard (1953). At least two of the elements resulting in more extensive red markings than usual are dominant in both species, while that responsible for much reduced forewing bands is recessive.

We have emerging, therefore, in the study of these two species a number of related factors to be considered in our further examination of the biology and evolution of these and other heliconiines. First, the probable value of red as a warning hue associated with aposematism. Second, the definite value of the same hue in courtship behavior, additional amounts of red having, up to a certain size, extra stimulating value in experimental situations. Third, the dominance, as shown in the present contribution, of certain elements responsible for red in excess of the usual forewing band. Fourth, the apparent occurrence of polymorphic forms exhibiting more than a single red forewing area of moderate size in few geographic localities, compared with the wide distribution of the species. Fifth, our field observations (unpublished and incomplete) in Surinam, indicating that individuals with the larger areas of red markings are uncommon to rare in the field. Sixth, mimicry. Seventh, multilocus polymorphism with linked genes.



## V. SUMMARY

1. The butterflies *Heliconius melpomene* and *H. erato* are highly polymorphic; this paper, the first of two, describes differences produced by major genes.

2. In *H. melpomene* three major loci, all linked on the same autosome, have been discovered. They are:

*B;* affecting the width of the band on the forewing;

*D*: producing a red suffusion on the base of fore- and hindwing; and

*R*: producing red rays on the hindwing.

One of the crossover values appears to be low (less than 30%); the other has not been determined, although crossovers have occurred.

3. In *H. erato* the evidence is less certain but it seems that there are three loci, all linked, which produce patterns analogous to those produced by the three linked loci in *melpomene*. There are slight but important differences in the effects of *D* and *R* in the two species, and the *B* locus gene which narrows the band is dominant in *erato* but recessive in *melpomene*.

4. In *erato* there appears to be a single recessive factor which changes the color of the forewing band from red to yellow.

5. The interest of the polymorphisms in connection with courtship behavior, aposematism and linkage are noted.

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## EXPLANATION OF THE PLATE

### PLATE I

Specimens of *Heliconius melpomene* showing the major pattern elements discussed in this paper. All the butterflies are red and black, except that the diffuse C-shaped or triangular mark in the forewing cell of Figs. 3 and 5, and two small areas near the costal edge of the forewing of Fig. 3, are yellow; these yellow marks will be discussed in the second paper (Turner, 1962).

FIG. 1. Wide-band, Dennis, Ray (BDR).

FIG. 2. Wide-band, Dennis, nonray (BDr).

FIG. 3. Narrow-band, Dennis, nonray (bDr).

FIG. 4. Wide-band, nondennis, nonray (Bdr).

FIG. 5. Narrow-band, nondennis, nonray (bdr).



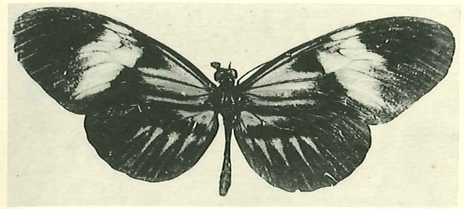

FIG. 1

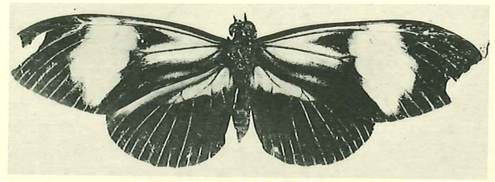

FIG. 2

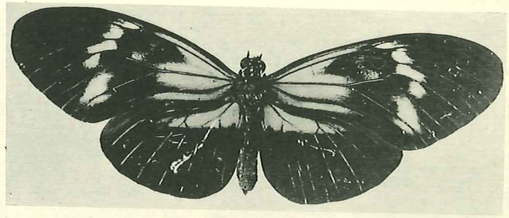

FIG. 3

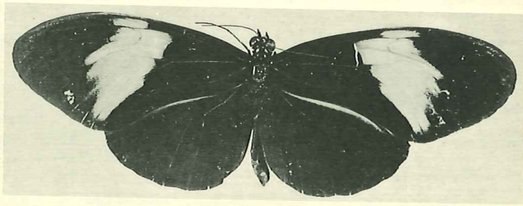

FIG. 4

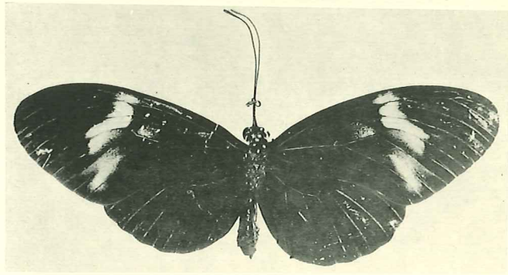

FIG. 5

THE GENETICS OF SOME POLYMORPHIC FORMS OF THE BUTTERFLIES HELICONIUS MELPOMENE LINNAEUS AND H. ERATO LINNAEUS