GENETIC STUDIES OF SEVERAL CHARACTERS IN COLIAS EURYTHEME (LEPIDOPTERA, PIERIDAE)

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Received August 26, 1957

REMINGTON (1954) recently reviewed the previous studies on genetics of *Colias*. The sex-limited "alba" gene, which controls female dimorphism, has been studied in *C. eurytheme* Boisduval, *C. philodice* Latreille, *C. christina* Edwards, *C. myrmidone* Esper, and in *C. erate poliographus* Motschlsky. Besides this well studied gene, inheritance of the following genes has been investigated: "blue-green" and "olive-green" larval color in *C. philodice* (GEROULD 1926); blue-green larval, pupal and adult eye color, recessive white male and female wing color, and egg sterility and food plant sterility. (HOVANITZ 1944); discal spot color on the hind wings in *C. philodice*, *C. eurytheme*, and *C. erate poliographus* (KOMAI and AE 1953; REMINGTON 1955; DEAN 1956; AE 1957); and inheritance of orange and yellow ground coloration in the hybrids between *C. eurytheme* and *C. philodice* (GEROULD 1943; HOVANITZ 1944; REMINGTON 1954). AE (1956) also studied the genetics of hybrids between *C. eurytheme* and *C. interior* Scudder.

As FORD (1945) has pointed out, wild butterflies carry many recessive genes in heterozygous condition, and sib matings in the laboratory produce some recognizable recessive homozygotes.

Certain new hereditary characters in *C. eurytheme* were found by means of sib matings, and their genetics has been investigated. Two of these characters were transmitted into the hybrids between *C. eurytheme* and *C. philodice*. The collection of *Colias* at the American Museum of Natural History, New York, and most of the specimens of GEROULD's genetic studies (1923, *et al.*) at the Peabody Museum of Natural History at Yale University also have been examined. The results are presented and discussed here.

MATERIALS AND METHODS

C. eurytheme orange female, P-62, which was collected at Yale University by the writer, carried the several hereditary characters described below. The progeny from one other *C. eurytheme* and one *C. philodice* in New Haven, and from two *C. eurytheme* in Jackson, Mississippi, were also used in this study.

The rearings were carried on in a rearing room of the Josiah Willard Gibbs Laboratory, at Yale University. The room was kept at 80°F, and fluorescent light illumination was used 14 hours per day. Humidity was kept low and approximately constant in the room.

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Eggs were obtained from females in glass lamp chimneys on potted food plants, placed under the sun or near an incandescent lamp. The larvae were reared in insect cages about 10 inches cubed, of which all four sides were netting and the top was glass. Vetch (*Vicia villosa*) was used as the sole larval food. Matings were obtained in cages near the laboratory window, under either direct sunlight or under strong fluorescent light in the rearing room. A mixture of one part of clover honey and two parts of water was used as food for the adults. The butterflies were stored at 60°F in a dark room when they were not being used for mating or egg laying.

During the rearing, a large number of larvae or pupae often died of diseases. Their symptoms coincide precisely with the description by STEINHAUS (1949) of polyhedroses by virus infection in *Colias*. Therefore, these losses are referred to simply as virus diseases in this paper, although microscopic tests were not performed.

An individual designation was given to each wild female which became the mother of a brood, and to all laboratory reared males and females. The individual designation of a female was also used as the designation of its brood. For instance, the wild *C. eurytheme* female, P-62, produced Brood P-62, and one of the females of this brood, P-62-9, mated with one of its sibs, P-62-2, and produced Brood P-62-9.

RESULTS

"Black-veined" character

On 20 December 1956, one *C. eurytheme* mutant male emerged from a stock from female P-62 which had been continuously inbred by sib matings. Its wing veins on the upper side were almost completely black as in the Monarch butterfly (*Danaus plexippus* L.). The ancestors of this butterfly were carefully examined and further matings and rearings were carried on in order to clarify the genetics of this new mutant (Figure 1).

Figure 2 shows the analysis of ancestors and sibs of the above "black-veined" mutant, P-62-9-7-2, which is one of the four "black-veined" mutants of Brood P-62-9-7. The expression of blackness of veins varied extensively. All veins were black on the upper sides of both wings in the most prominent ones. In some individuals veins of the upper side of the hind wings only were clearly black. Only traces of blackness were found in veins of the central portions of the hind wings in other individuals. In many females in this strain and others, there is a tendency for black lines to extend along veins from the border to the base of the wing. However, no correlation of this female condition with male "black-veined" was recognized.

Brood P-62-9-7 was a very productive brood, and a total of six sib matings and three other kinds of matings were obtained. Some males were mated two or three times, and two females were mated twice. Although some of the matings were completely sterile, a large number of progeny, which included many "black-veined" mutants, was obtained. Table 1 shows the direct progeny from

FIGURE 1.—Three “black-veined” mutants of *Colias eurytheme*. Normal male is shown in lower right hand corner.

TABLE 1

F_1 from females of Brood P-62-9-7 (B)

Brood	Male parent	Male parent phenotype	Offspring				Total
			Females	Males			
				Bv	Tr	No	
B-3	B-4	No	7	0	0	3	10
B-5	B-12	No	3	1	0	3	7
B-9	B-1	Tr	11	3	1	4	19
B-10	P-76-8	No	10	0	0	12	22
B-13	B-4	No	0	3	0	1	4
B-18	B-15	No	4	0	1	8	13
B-21	B-9-5	Bv	1	0	3	1	5

the above matings. Sterile matings and other matings which failed to produce adult progeny were excluded from the table. Brood P-62-9-7 was designated as Brood B in this table and hereafter.

Figure 3 shows a genealogy of a part of the “black-veined” strain which produced an all “black-veined” brood. Figure 4 shows a genealogy of another part of the “black-veined” strain which included one *C. philodice* male, P-76-8, and

FIGURE 2.—Genealogy of a “black-veined” strain (AE I). No-normal, Tr-trace black-veined, Bv-Black-veined.

an unrelated *C. eurytheme* female, P-78-4-102, which was from Mississippi stock.

The writer found two wild *C. eurytheme* males which show “black-veined” in trace expression in the collection of the American Museum of Natural History. One was collected by LICK BROOK at Ithaca, N.Y., on 19 August 1928. Another was collected by H. J. SANFORD at Manahawkin, Ocean County, N.J., on 15 October 1948.

The writer also examined most of the specimens reared by J. H. GEROULD in his genetic studies of *C. eurytheme* and *C. philodice* (1923), now deposited in the Peabody Museum of Natural History at Yale University. Two strains of *C. philodice* proved to include “black-veined” males. Figures 5 and 6 show their genealogies. No difference was found in expression of “black-veined” in *C. eurytheme*, *C. philodice* and hybrids between them.

These studies clearly indicate that “black-veined” is a hereditary character, which is recognized only in males. Phenotypes vary in intensity of expression of the “black-veined” character. The most probable interpretation for the trait is that it is a single recessive. Table 2 shows results obtained from matings in which the genotypes of parents were fairly certain, as derived from their ancestors or progeny. The mating between a strongly “black-veined” male,

FIGURE 3.—Genealogy of a "black-veined" strain (AE II).

TABLE 2

*F*₁ of *Colias* crosses of "black-veined" strain

Brood	Male parent	Male offspring	
		Normal	"black-veined"
a. $Vv \times Vv$			
P-62	wild	17	6
1909 2w	1a ⁶	13	3
1910 a	wild	12	2
1910 e	a ⁷	51	4
b. $Vv \times vv$			
P-62-35	P-62-15	12	6
P-62-9-7 (B)	P-62-9-8	5	5
B-9	B-1	4	4
B-21	B-9-5	1	3
B-10-17-4	B-3-4-19	1	6
c. $VV \times vv$			
P-78-4-102	B-3-4-26-8	23	0
d. $vv \times vv$			
B-9-10	B-9-5	0	11

FIGURE 4.—Genealogy of a "black-veined" strain (AE III). B = P-62-9-7.

B-3-4-26-8, and an unrelated (to "black-veined" strain) female, P-78-4-102, produced 23 normal males (Figure 4, Table 2). This suggests complete dominance of the wild type allele of the "black-veined" locus. It is difficult to interpret the ratio of 51 normal : four "black-veined" (Figure 6 & Table 2) in GEROULD's brood 1910e. Possible explanations include a reduced penetrance of genes for "black-veined" in homozygous individuals, or a low viability of the homozygotes. The observed 13 : 3 and 12 : 2 ratios for theoretical 3 : 1, and 12 : 6 for presumed 1 : 1 ratio fit the above interpretation. Furthermore, female B-21 and B-10-17-4 could have been recessive homozygotes, judging from their ancestors, on the assumption that one normal male in each brood was a

FIGURE 5.—Genealogy of a "black-veined" strain (Gerould I).

FIGURE 6.—Genealogy of a "black-veined" strain (Gerould II).

recessive homozygote, which had its normal expression decreased by a low penetrance. Therefore, 1 : 3 and 1 : 6 ratios for 1 : 1 do not necessarily indicate an excess of recessive homozygotes, but rather a modification from 0 : 1 ratio.

Glueless female

Four females, out of nine in Brood B which laid eggs, failed wholly or partially to glue their eggs on the leaves of food plants. This tendency was transmitted to their progeny extensively. Figure 7 shows its genealogy. *G* indicates glue, namely individuals which glued their eggs on the leaves, and *g* indicates glueless (wholly or partially). Females which were not examined were excluded from

FIGURE 7.—Genealogy of a "glueless" strain. G-glue, g-glueless.

this genealogy. Some wild females which were kept at 60°F in a dark room for about one month or more also failed to glue eggs during the experiments. However, glueless females of this genealogy had been kept in the 60°F room for only a few days or less before they failed to glue eggs. Therefore, its hereditary basis is fairly clear. Although the data are not sufficient to justify a discussion of genes conditioning this character, the most probable interpretation is a six-limited, single dominant gene, because this character seems to carry through males and crossing between glueless female B-10 and unrelated *C. philodice* male produced a glueless female and two glue females.

Male sterility

Thirteen matings were obtained from seven males of Brood B. Table 3 shows the results of egg fertility from these matings. Two males mated three times and the other two males mated twice. Five matings produced only infertile eggs (did not turn red), and one mating produced only one fertile egg out of 44 laid. The above six matings were from males B-2 and B-14. Two females, B-5 and B-13, mated with B-2, later mated again with males respectively B-12 and B-4 and produced fertile eggs. One female, B-18, mated with B-14, later mated again with B-15 and produced fertile eggs. Figure 8 illustrates the situation. It is clear that the sterilities of these six matings are the result of sterility of males B-2 and B-14. Since the above seven males were all sibs and the progeny of the two continuous sib matings, these sterilities could have been the results of one or two pairs of recessive genes.

TABLE 3
Egg fertility of mates of Brood P-62-9-7 (B) males

Parents		Eggs laid	Fertile eggs	Egg fertility percent
Males	Females			
B-1	B-9	184	142	77.2±3.09
B-2	B-5	21	0	0.0
B-2	B-13	11	0	0.0
B-2	P-70-6-9	31	0	0.0
B-4	B-3	124	99	79.8±3.61
B-4	B-13	135	54	40.0±4.22
B-12	B-5	46	36	78.3±6.08
B-14	B-18	21	0	0.0
B-14	B-16	44	1	2.3±2.26
B-14	B-9-12	17	0	0.0
B-15	B-18	78	70	89.7±3.44
B-19	B-22	56	13	23.3±5.65
B-19	B-9-14	50	18	36.0±6.79

From the rearings and matings of the progeny of the above matings, large numbers of completely sterile matings were obtained, mainly by continuous sib matings (Table 4). Although it was not determined whether females or males were responsible for these sterilities, many of the above sterile matings could be the results of the same gene(s) which caused the sterilities of B-2 and B-14.

Reductions of fertility and hatchability

On the other hand, the general reduction in fertility progressed with continuation of sib matings (Table 5). The egg fertility fluctuated extensively among the different matings within the same generation, but steadily declined with generation after generation. This could be interpreted as homozygosity of many recessive genes which as polygenes reduce egg fertility.

FIGURE 8.—Genealogy of sterile males, B-2 and B-14.

TABLE 4

Ratio of number of crosses of egg fertility less than five percent to total number of crosses in continuous sib matings from female P-62

Generation	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇
Ratio	0/1	0/2	2/4	4/10	2/7	7/10	1/1

The reduction of egg hatchability seems to follow the same line as the above. Although the hatchability was not recorded in detail, no reduction was observed in the first and second generations of sib matings, about 75 percent hatch was observed in third, and a fluctuation between 75 to 50 percent hatch in the fourth, and between 75 to 25 percent hatch in fifth and sixth were observed. This could also be polygenic.

TABLE 5

Reduction in egg fertility in continuous sib matings from female P-62

Generation	Mother	Father	Eggs laid	Fertile eggs	Egg fertility percent
F ₁	P-62 (wild)	wild	43	43	100.0
F ₂	P-62-9	P-62-2	37	37	100.0
F ₃	P-62-9-7	P-62-9-8	118	112	94.9±2.03
F ₄	B-3	B-4	124	99	79.8±3.61
	B-9	B-1	184	142	77.2±3.09
	B-5	B-12	46	36	78.3±6.08
	B-18	B-15	78	70	89.7±3.44
	B-13	B-4	135	54	40.0±4.22
	B-22	B-19	56	13	23.3±5.65
		Subtotal	623	414	66.5±1.89
F ₅	B-9-9	B-9-1	29	8	27.6±8.30
	B-9-4	B-9-3	16	6	37.5±12.1
	B-9-10	B-9-5	205	94	45.9±3.48
	B-5-5	B-5-2	104	69	66.3±4.64
	B-5-6	B-5-1	107	89	83.2±3.62
		Subtotal	461	266	57.7±2.30
F ₆	B-9-10-18	B-9-10-1	203	51	25.1±3.04
	B-9-10-19	B-9-10-9	15	11	73.3±16.9
	B-5-6-8	B-5-6-2	186	92	49.5±3.66
		Subtotal	404	154	38.1±2.42

DISCUSSION

Three recessive characters, namely, "black-veined", male sterility, and a reduction in egg fertility and hatchability, were found from one wild *C. eurytheme* female, P-62. This may be an indication of extensive heterozygosity in wild *Colias*. The "black-veined" gene seems to be common in nature. However, a homozygote of this gene seems to have a low viability or penetrance in nature, and a higher viability or penetrance in the laboratory. Only two "black-veined" males were found among a very large collection of *C. eurytheme* and *C. philodice* in the American Museum of Natural History, and three "black-veined" strains and a large number of "black-veined" males were obtained from the rearings by GEROULD and the writer. This gene seems interchangeable between *C. eurytheme* and *C. philodice* without altering its phenotype. A possibility that, a slightly "black-veined" individual is a heterozygote, may not be completely denied. However, 23 normal males from a crossing between a strongly "black-veined" male and unrelated female seems to be good evidence that slightly "black-veined" individuals are not heterozygotes, but weakly expressed homozygotes. There is another possibility, that this character is polygenic in control. However, the following evidence may deny this possibility. Female,

B-10, of the "black-veined" strain, was mated to a male of an unrelated *C. philodice* stock, and three females were obtained from a sib mating of their progeny. One of them mated with a strongly "black-veined" male and produced strongly "black-veined" males in the F_1 . There remains a possibility that two pairs of genes are controlling this character. But the frequent appearance of strongly "black-veined" males in different broods may reduce this possibility. One must always consider the possibility that an environmental condition has effected the "black-veined" expression. However, the conditions which the writer used seem never or very seldom to produce a phenocopy of "black-veined" from the wild type allele, because no "black-veined" appeared in a very large number of unrelated individuals (to "black-veined" strain) which were reared in the same room.

The data are not sufficient to discuss their heredity basis for other characters in detail. The most probable interpretations were stated in the previous section. Rearing conditions may reduce egg fertility or hatchability. However, it seems probable that rearing conditions which were used in these experiments were favorable enough that extensive reductions in fertility and hatchability did not take place. Long, continuous sib matings were successful in the rearing room and the butterflies mated very well without help of sunlight. Many of the butterflies laid 100 to 200 eggs in a few days; facilities prevented us from obtaining more eggs. No noticeable reductions of adult sizes or pigmentations took place. When an individual obtained from a continuous sib mating was mated to a nonsib, recoveries of egg fertility and hatchability were sometimes very noticeable.

SUMMARY

1. Four hereditary characters, "black-veined", "glueless", male sterility, and a reduction of egg fertility and hatchability, were obtained from a wild *Colias eurytheme* female, P-62.

2. The "black-veined" character on the wing is a male sex-limited single recessive and has various phenotypes.

3. The above character was transmitted into the hybrids between *C. eurytheme* and *C. philodice*.

4. Two wild-caught "black-veined" *C. eurytheme* males were found from the collection at the American Museum of Natural History.

5. Two "black-veined" strains were found from specimens of *C. philodice* reared during GEROULD's genetic work.

6. The "glueless" character seems to be controlled by one pair of female sex-limited dominant genes.

7. Male sterility seems to be controlled by one or two pairs of recessive genes.

8. A reduction of egg fertility and hatchability seems to be controlled by polygenic systems.

ACKNOWLEDGMENTS

The writer wishes to express his sincere gratitude to DR. CHARLES L. REMINGTON, Department of Zoology, Yale University, for direction of the research and for help in writing this paper. The writer is grateful to MR. MATHER BRYANT of Jackson, Mississippi, providing living *Colias*.

The cooperation of the authorities of the American Museum of Natural History for allowing him to study the *Colias* collections is appreciated.

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