

HYBRIDS BETWEEN *COLIAS EURYTHEME* AND *C. INTERIOR* (PIERIDÆ)

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Hybrids in the genus *Colias* present problems in the fields of genetics, ecology, and evolution. Studies of the hybrids between *C. eurytheme* and *C. philodice* have been published by GEROULD (1923, 1943, 1946), HOVANITZ (1943, 1944a, 1944b, 1948, 1949a, 1949b) and REMINGTON (1954), and the mechanism of it was clarified partly. This paper is a preliminary report on a study of hybrids between *C. eurytheme* and *C. interior*. *C. interior* is a more northern species than *C. eurytheme* which feeds upon the Sour Top Blueberry (*Vaccinium canadense*) and other *Vaccinium*. Recently the possible existence of hybrids between *C. interior* and *C. philodice* in the field was suggested (Hovanitz 1949b), and later a female, combining the characteristics of *interior* and *philodice*, was taken near Cheboygan, Michigan, June 29, 1951, with a large number of typical specimens of *interior*; Dr. A. B. KLOTS suggested that it must be a hybrid (Voss 1954).

MATERIAL AND METHODS

The process of obtaining intra- and interspecific matings of *Colias* which the writer used is the following. Healthy males, usually 6 to 12, are placed in the usual insect cage, of which its upper side is also of netting, under the sun, at 8:00-9:00 A. M.. New females usually emerge from late morning to afternoon. As soon as a female emerges, she is placed into the cage of males under the sun. A copulation usually takes place within 30 minutes, if it occurs, although it may occur much later occasionally.

About 500 pupæ of *C. eurytheme* and *C. philodice* were raised in the green house of the University of Notre Dame, in several insect cages, 30 cm. cubed. White Clover was used as a food plant. These pupæ were brought to the Biological Station, University of Michigan, Cheboygan, Michigan, on June 28, 1954. Many females emerged between June 29 and July 9.

Matings were tried in the above mentioned way, at Mud Lake and at Elliot's Creek, where *C. interior* is usually found, and outdoors at the Biological Station, using females of *C. eurytheme* which emerged at the laboratory and wild *C. interior* males which were collected at Mud Lake or Elliot's Creek. Females emerged within 24 hours, and pupæ which were just about to emerge were brought to Mud Lake or Elliot's Creek. *C. interior* males were placed in the cage of females as soon as they were collected. Usually 6—12 males and 6—12 females were in the cage at a time. Both *interior* males and *eurytheme* females were exposed at least for 2 hours under the sun. *C. interior* males were active only under the bright sun in the cages, as in the field. About 70 males of *interior*, in all, were used for matings on two afternoons at Elliot's Creek, two afternoons at Mud Lake, and four mornings and two afternoons outdoors at the Station. Only at Elliot's Creek, where two bright afternoons were spent in the effort, did the *interior* males readily try to copulate with

eurytheme females. On the second afternoon at the Creek (July 9), one *interior* male, caught several minutes before, copulated from 2:08 to 3:15 P. M. with an orange *eurytheme* female which had emerged within 30 minutes. Mating outdoors at the Station was tried, with mating between *eurytheme* females and males as a control. Although a total of 13 matings were obtained between *eurytheme* females and males during the period without any difficulty, no mating between *eurytheme* females and *interior* males was obtained during the same period.

The females which were used for the matings were of the species *C. eurytheme*, either the white or orange forms. Some of them might have had a series of *C. philodice* genes, because they were the progeny of the wild *eurytheme* females which were caught in a place where *eurytheme* and *philodice* were both abundant. But the majority of these were phenotypically *eurytheme*. It was impossible to use *philodice* for mating, because almost all of the *philodice* adults emerged before any *interior* was found in the field.

RESULTS

The orange female of *C. eurytheme* (W. 15. 62) which was mated to *C. interior* laid eggs on the leaves of White Clover at Notre Dame. Thirty-two eggs were laid on July 12 (group A), 25 on July 13 (group B), 26 on July 14—20 (group C). Less than 10 eggs (between 6 and 9) were excluded from the above count because they failed to turn red and were presumably infertile. One of the causes that eggs failed to turn red was observed to be sucking by small insects or arachnids. Towards the end of egg laying, there is also a possibility that the mother butterfly did not receive enough sperm from the *C. interior* male. It is difficult to determine the egg fertility under this condition, but even if it is presumed that all eggs which did not turn red were non-fertile, the percentage of non-fertile eggs is about 10%, because the total number laid was about 90.

Groups A and B were bred in the greenhouse, and group C was bred in a special growth room which was kept at 75° F., 75% relative humidity, and 10 hours fluorescence light illumination per day. The eggs from intraspecific matings of *C. eurytheme* were raised at the same time in both places as controls.

None of the fertile eggs died before hatching in groups A and B, and only a few died, just before hatching, in group C. No deaths of larvæ were observed in the first and second instar stages in any of the three groups.

From group A, 26 hybrid males emerged in 6 days (August 8—13). The growth rates of these males equalled the growth rate of 8 control *C. eurytheme* males which emerged from August 10 to August 18. But the growth rate of the other six larvæ of group A was extremely slow. Although one of them continued to grow and emerged as a female on August 26 (control—11 *C. eurytheme* females from August 9 to 17), the others stopped growing at the 3rd or 4th instar.

Sixteen pupæ were obtained from group B with the ordinary growth rate. Ten males emerged from these, while six were kept in the refrigerator. (These six pupæ died before winter in the refrigerator.)

Including groups A and B, 15 larvæ of the 3rd and 4th instars were counted on August 24. None were feeding. Nine of these larvæ were refrigerated from August 24 to August 31, and removed to the greenhouse again. They did not begin to feed and died one by one, as did the other six which were not refrigerated.

The larvæ of group C, in the growth room, grew at the same rate as the controls until the 3rd instar, but they stopped feeding at the 3rd instar (a few in 4th instar) and remained largely motionless for about 10 days at the beginning of August. They were removed to the greenhouse on August 17. They were kept in the greenhouse only during daytime, when the temperature was 80° to 100° F., and were removed to the growth room at night to prevent chilling. After this was repeated for several days, they began to feed again one by one from August 21 to 23. After the evening of August 24, they were kept in the growth room continuously. Because of this non-feeding stage, the first larva of the hybrids entered the 5th instar 22 days later than the first one of the *C. eurytheme* controls. By August 24 the number of larvæ had decreased to 12 from the initial number of 26 eggs. The first butterfly emerged on September 5 and the last one on October 2. The total number was 10, and all were males. The other two seemed to grow very slowly but they died around the middle of October, without reaching 5th instar.

From the control brood which was raised in the growth room, 13 males emerged from August 11 to 19, and 8 females emerged from August 14 to 19.

There was only one prominent difference observed in the shape or coloration of egg, larva, and pupa of the hybrids in comparison with the control (*C. eurytheme*). This difference is in the larva. At the 4th instar, the hybrids showed a crimson line along the spiracular fold, but not so bright, while *C. eurytheme* had a white line. At the 5th instar, the hybrid larvæ showed a very clear crimson line, but *C. eurytheme* showed an orange line. This might be an expression of intermediacy of larval coloration, since *C. interior* larvæ have a bright crimson line.

The size of adult male hybrids raised in the greenhouse is the same as for *eurytheme* which emerged in the greenhouse during the same period. The pterine coloration was intermediate between *eurytheme* and *interior*, namely slightly orange. The orange pigment is distributed over most of the upper side of both wings. The individual variation of intensity of orange coloration is very slight among the 26 males. The color grade [9 (=most red)—0 (=yellowest) of HOVANITZ] is 5, and rather closer to 4 than to 6. Submarginal dark spots (lacking in *interior* but present in *eurytheme*), and the dark spot on the costa of the hind wing (usually lacking in *interior* but present in *eurytheme*) exists in the hybrid, and the discocellular spot on the hind wing in the hybrid is double (single in *interior* and usually double in *eurytheme*). These characters are uniform among the 26 hybrid males, and all of these spots are comparatively weak in comparison with similar spots of *eurytheme*. The author did not examine the color grade of control *eurytheme* in the greenhouse, since no reduction from typical *eurytheme* was found by simple checking. It will be possible to examine them in detail, because the samples are being saved.

Unfortunately the one hybrid female failed to extend her wings. Melanin coloration and the shape of the tip of the abdomen expressed clearly the female character. Though it is very difficult to decide the orange color grade, it seems to show intermediate coloration. All of the larvæ of F_1 hybrids which showed the ordinary growth rate emerged as males. These made up more than half of the F_1 population. Only one of the larvæ with the slower growth rate emerged, and this was a female. Hence it may be that all or most of the slow-growing larvæ were females.

The characteristics of males which were raised in the growth room are as follows. The size of these males was smaller than that of the males which emerged in the greenhouse, but they were equal in size to the *C. eurytheme* controls. The orange color grade was mainly reduced to 3 (8 of grade 3, 1 of grade 4, and 1 of grade 5). The differences in melanin coloration between males raised in the greenhouse and males raised in the growth room were approximately the same as the differences between the typical *C. eurytheme* male and its cold weather form (Klots, 1951). Orange color grades of the control *eurytheme* in the growth room are the following: 7 grade 9, 2 grade 8, 2 grade 7 males and 5 grade 8 females. The reduction of orange color toward the tip of the fore wings of the upper side is somewhat prominent. A part of this brood which was raised in the greenhouse was typical *eurytheme*.

Hybrid males were very active in the cages and copulated readily with orange *C. eurytheme* females. Six backcrosses were obtained within 4 days (August 9 — 12). About 12 females of orange *eurytheme* and about 20 hybrid males were used in the backcross attempts (according to my usual procedure). Three of the above females laid eggs (W.17.22.3, W. 17.22.7, and W.17.23.4), while the other three females died without laying eggs about 10 days after their copulation. W.17.22.3 laid about 140 eggs on the leaves of White Clover, and the fertility of these eggs was about 91%; W.17.22.7 laid about 90 eggs on the leaves of Alfalfa, and the fertility of her eggs was about 98%; and W.17.23.4 laid more than 50 eggs on the leaves of White Clover, but all of them failed to turn red and dried up.

The progeny of W.17.22.3 was divided into 2 groups, designated D and E. Group D was raised in the greenhouse and group E was raised in the growth room from the first instar stage, under the same conditions as the F_1 . White Clover was given to both groups as a foodplant. The progeny of W.17.22.7 was raised in the greenhouse on Alfalfa. The growth rate of the majority of backcross larvæ was the same as that of *C. eurytheme* larvæ which were bred as controls, but a few were very slow like the F_1 hybrids, though no larvæ stopped feeding. In this case the females emerged at the same time as the males, but the butterflies which emerged latest were all females.

DISCUSSION

Since *C. interior* has only one generation a year and has a diapause in the young larval stage, it is difficult to rear, and wild *interior* males were used for the first hybridization trial instead of laboratory bred males. The difficulty

of obtaining matings between *C. interior* and *C. eurytheme* is considered rather natural, since this mating is interspecific.

Although the *interior* male which mated with *eurytheme* has very clear characteristics of *interior*, a doubt remains concerning its identification, since the difference between males of *interior* and *philodice* is rather slight. Repetition of the experiment is required for assurance, but the peculiarity of the life history of the hybrid gives good support for considering it *interior*. The hybrid larvæ entered the non-feeding stage at a high temperature (75 degrees F.). This can be considered as either the inheritance of a character related to the northern range of *interior*, or a peculiar character of this kind of hybrid. Larvæ of *philodice*, *eurytheme*, and of hybrids between *philodice* and *eurytheme* had no diapause in the growth room under the same conditions under which the hybrids between *eurytheme* and *interior* were raised. The long emergence period of the backcross broods might also be a peculiar result of this hybrid crossing, though in this case, the decrease of density of larvæ in the cage may be a cause of it.

C. interior is a *Vaccinium*-feeding species. Therefore, the possible deleterious effects to the hybrid from a White Clover diet must be considered for all results, in spite of the low mortality of the hybrid larvæ in the young stage and the production of fertile hybrid males.

Studies of the characteristics of the backcrossed individuals and their progenies have been made. However, these data are not conclusive and are not presented in this report.

SUMMARY

1. One hybrid mating was obtained between a *Colias eurytheme* orange female raised at Notre Dame, Indiana, and a *C. interior* male collected in Cheboygan County, Michigan.
2. From the eggs from this female, 46 males and 1 female emerged as imagines.
3. Orange coloration intermediate between the deep orange of *C. eurytheme* and the yellow of *C. interior* was observed in the hybrid males.
4. The hybrid larvæ entered a non-feeding stage in the third instar under conditions of 75° F., 75% RH, and 10 hours fluorescent light a day. They began to feed again in the hot greenhouse.
5. Presumed females of the hybrids entered a non-feeding stage in the greenhouse also and failed to survive the non-feeding stage (except one female which emerged).
6. The hybrid males were at least partly fertile, and progeny were obtained by backcrossing to *C. eurytheme* females.

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