EFFECTS OF PHOTOPERIOD ON *COLIAS EURYTHEME*

by SHIGERU ALBERT AE

INTRODUCTION

Colias eurytheme Boisduval is an extremely variable butterfly, and many names have been given for its seasonal forms. Furthermore, extensive natural hybridization of this species with *C. philodice* Latreille makes its variation even more complicated. HOVANITZ (1945) has reported that differences of temperature, humidity, irradiation, and food plants produce variations of the size, and of pattern of this butterfly.

The present paper reports the results of controlled experiments to test the effects on larval color, adult size, and wing color of *C. eurytheme* produced by the difference between 14 and 10 hours fluorescent light illumination per day during larval and pupal development. These experiments were suggested by the observation that the fall and spring form ("ariadne") of *C. eurytheme* is obtained in mid-winter under hot greenhouse conditions, indicating that temperature is not the principal factor producing this form. Photoperiod then appeared most likely (Gerould, 1943; Remington, unpubl.).

MATERIALS AND METHODS

Two *C. eurytheme* orange females were sent in late January 1957 from Jackson, Mississippi, by Mr. BRYANT MATHER. Mississippi stock was preferred to Connecticut material, since *C. philodice* is very rare in the South, and color characters of *C. eurytheme* would be relatively pure (see Discussion). Broods P-77 and P-78 were established from them. Three females of Brood P-78 (P-78-4, P-78-8, and P-78-12) were crossed with males of Brood P-77. The eggs obtained from them were used in these experiments.

All rearings described in this paper were carried out at the Josiah Willard Gibbs Research Laboratory at Yale University. The windowless rearing room was kept at 80°F. and 14 hours fluorescent light illumination per day automatically regulated by a time-clock. For the contrasting conditions of 10 hours light per day, a light-proof compartment was set up in this room with fluorescent lights in it regulated at 10 hours of light per day. The conditions other than the photoperiod were kept exactly the same. The light intensity was controlled so that the insect cages under both 14 and 10 hours of light got the light of the same intensity per unit of time. Humidity was kept low and approximately constant in the room.

Cubic cages ten inches on a side were used for the rearing. The top of each cage was glass and the four sides were of netting. Potted seedlings of vetch (*Vicia villosa*) were used as the sole larval food.

RESULTS

A. *Black patches on spiracular line of 5th instar larvæ.*

There is one prominent difference between larvæ reared in 14 and 10 hours conditions. At the 5th instar, black patches along the spiracular line are more or less prominent in all larvæ (more than 60) reared in 10 hours of light. No black patches were found in any larvæ (more than 40) reared

TABLE I. FOREWING LENGTH AND BLACK-BORDER INDEX

Brood Number	Rearing Condition	Food Plant Condition	Sex	Number Examined	Forewing Length	Black-border Index
P-78- 4	14 M	14 M	♂	7	25.47±1.04	17.26±2.15
			♀	8	26.84±1.03	19.82±2.03
P-78- 4	10 M	10 M	♂	3	23.00±0.89	11.10±2.82
			♀	0		
P-78- 4	14 L	14 L	♂	8	25.36±1.26	16.24±1.64
			♀	4	25.73±1.47	19.44±0.30
P-78- 4	10 L	10 L	♂	6	22.07±1.33	9.20±2.79
			♀	8	23.50±1.94	18.59±2.74
P-78- 8	14 M	14 M	♂	7	24.20±0.51	12.88±1.72
			♀	2	26.85±0.92	18.39±0.30
P-78- 8	10 M	10 M	♂	7	23.70±1.67	9.05±2.01
			♀	8	24.64±1.18	19.54±1.51
P-78- 8	14 L	14 L	♂	2	24.60±0.85	13.81±0.67
			♀	1	24.40	18.85
P-78- 8	10 L	10 L	♂	11	23.25±1.01	8.21±1.34
			♀	3	24.80±1.13	17.73±2.11
P-78- 8	14 L	10 L	♂	1	25.80	11.24
			♀	3	25.53±0.63	22.20±1.79
P-78- 8	10 L	14 L	♂	3	22.60±1.25	8.12±0.64
			♀	1	22.50	20.00
P-78-12	10 L	10 L	♂	6	24.97±1.15	11.75±2.60
			♀	6	27.48±1.28	19.99±1.62
P-78-12	14 H	10 L	♂	6	25.62±1.51	12.57±2.71
			♀	7	28.41±1.35	19.54±1.37
P-78-12	10 L	Greenhouse (April)	♂	6	24.33±1.05	10.21±3.13
			♀	13	27.17±1.61	19.24±1.30

14 M—14 hours continuous light illumination per 24-hour day, light intensity about 6,300 foot candles.
10 L—10 hours continuous light illumination per 24-hour day, light intensity about 3,400 foot candles.
H—light intensity about 7,100 foot candles.

in 14 hours of light. However, the black patches disappeared at the end of the instar in about half of the larvæ (more than 12) in Brood P-78-12 reared in 10 hours of light.

B. *Adult size.*

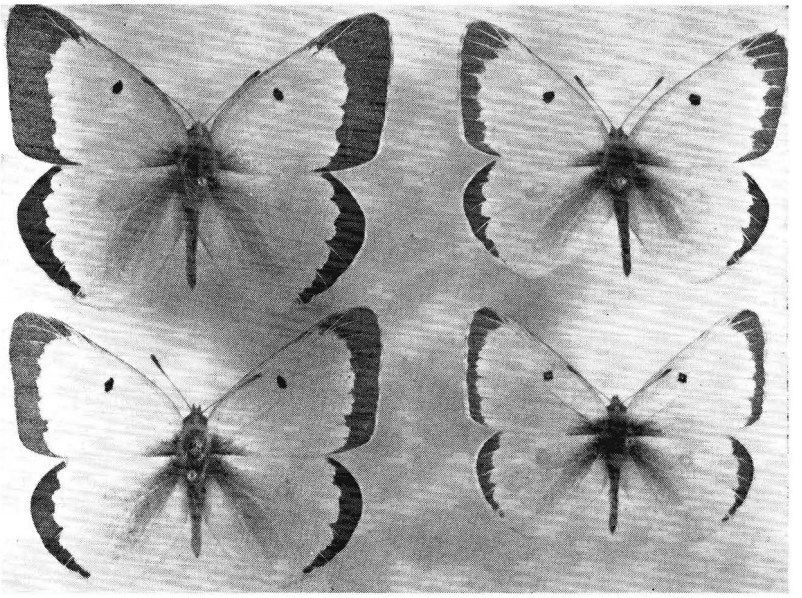
The forewing lengths were measured for a comparison of sizes of adults. Adult butterflies reared in the 14 hour photoperiod seem larger than adults reared in the 10 hour photoperiod in both sexes in Broods P-78-4 and P-78-8 (Table 1). A larger sample is necessary for a more definite distinction.

C. *Melanin color on wings.*

1. *Black border.*

For a comparison of the width of the black border, a *black border index* was calculated: this is 100 times the ratio of the black border width to the

forewing length. For uniformity, the black border width was measured on the second vein from the tornus of the forewing. The results on Table 1 show clearly a definite difference in the males: the border of the butterflies reared in the 10 hour photoperiod is much narrower than the border of the butterflies reared in 14 hours of daily light (Fig. 1). The data show no difference in females, although more data might reveal a slight difference.



Effects of photoperiod on black margins of *Colias eurytheme* males. Left, photoperiod 14 hours (6300 foot candles); right, photoperiod 10 hours (6300 foot candles). Above, from Brood P-78-4; below, from Brood P-78-8.

2. *Melanin suffusion of the under side of the hindwing.*

Melanin suffusion of this area is definitely more intense in the butterflies reared in the 10 hour photoperiod than in the 14 hour conditions in both sexes in Broods P-78-4 and P-78-8. This difference is clear-cut, and no intermediate was obtained. The number of butterflies examined is the same as in Table 1.

3. *Forewing discal spot.*

Some of the butterflies reared in the 10 hour photoperiod lost some of the melanin pigment on this spot and orange color appeared on the spot. The same tendency was also found in a few butterflies reared in the 14 hour conditions.

4. *Melanin suffusion of the upper side of the hindwing.*

Melanin suffusion on this area was usually more intense in females reared in 10 hour conditions than in 14 hour conditions. However, this difference was not so clear-cut as in melanin suffusion of the under side of the hindwing. No difference was found in males.

5. *Melanin suffusion of the under side of the forewing.*

There was a tendency toward more intense melanin suffusion in this area in males reared in 14 hour conditions than in 10 hour conditions. No difference was found in females.

6. *Others.*

No differences were found in melanin suffusion on the basal region of the upper side of the forewing, melanin suffusion on the apex of the upper side of the forewing, submarginal dark spots, and the dark spots on the costa of the hindwing.

TABLE 2. ORANGE COLOR GRADES

Brood	Rearing Condition	Food Plant Condition	Sex	Color Grades 9	8	7	6	5	4	3	2	1	0	Total
P-78- 4	14 M	14 M	♂	1	1	4	0	1	0	0	0	0	0	7
			♀	0	3	3	1	0	1	0	0	0	0	8
P-78- 4	10 M	10 M	♂	0	0	2	1	0	0	0	0	0	0	3
			♀	0	0	0	0	0	0	0	0	0	0	0
P-78- 4	14 L	14 L	♂	0	1	4	2	1	0	0	0	0	0	8
			♀	0	0	3	1	0	0	0	0	0	0	4
P-78- 4	10 L	10 L	♂	0	0	0	5	1	0	0	0	0	0	6
			♀	0	2	3	2	1	0	0	0	0	0	8
P-78- 8	14 M	14 M	♂	0	0	0	7	0	0	0	0	0	0	7
			♀	0	0	2	0	0	0	0	0	0	0	2
P-78- 8	10 M	10 M	♂	0	0	7	0	0	0	0	0	0	0	7
			♀	0	1	4	2	1	0	0	0	0	0	8
P-78- 8	14 L	14 L	♂	0	0	2	0	0	0	0	0	0	0	2
			♀	0	0	0	1	0	0	0	0	0	0	1
P-78- 8	10 L	10 L	♂	0	0	8	3	0	0	0	0	0	0	11
			♀	0	1	2	0	0	0	0	0	0	0	3
P-78- 8	14 L	10 L	♂	0	0	1	0	0	0	0	0	0	0	1
			♀	0	1	1	1	0	0	0	0	0	0	3
P-78- 8	10 L	14 L	♂	0	0	3	0	0	0	0	0	0	0	3
			♀	0	0	1	0	0	0	0	0	0	0	1
P-78-12	10 L	10 L	♂	0	0	6	0	0	0	0	0	0	0	6
			♀	0	1	1	0	0	0	0	0	0	0	2
P-78-12	14 H	10 L	♂	0	0	5	1	0	0	0	0	0	0	6
			♀	0	1	4	0	0	0	0	0	0	0	5
P-78-12	10 L	Greenhouse (April)	♂	0	0	4	1	0	1	0	0	0	0	6
			♀	1	1	2	0	1	0	1	0	0	0	6

D. *Pterines on the wings.*

1. *Orange color grades.*

Orange color grades (9 = most red, 0 = yellow; HOVANITZ correspondence) are shown in Table 2. Although the data are not conclusive because of the small number examined, there seems to be no difference in orange color grades produced by a difference of photoperiod. The appearance of color grades as low as 3 to 6 from *C. eurytheme* stock is notable, but hybridization with *C. philodice* as a cause seems unlikely (see Discussion).

2. *Tip of the forewings.*

Reduction of orange color at the tip of the forewings among the butterflies reared in 10 hours of photoperiod was prominent. For uniformity, the orange color was measured in the third cell from the apex of the forewing along the outer margin. Table 3 shows the results. Five arbitrary reduction grades were used. For grade A the third cell is entirely yellow. For grade B the third cell has orange pigment only on the veins surrounding the cell. For

TABLE 3. REDUCTION OF ORANGE COLOR AT TIP OF FOREWING

Brood	Rearing Condition	Food Plant Condition	Sex	Reduction Grades A	B	C	D	E	Total
P-78- 4	14 M	14 M	♂	0	0	0	1	6	7
			♀	0	2	2	3	1	8
P-78- 4	10 M	10 M	♂	0	0	2	1	0	3
			♀	0	0	0	0	0	0
P-78- 4	14 L	14 L	♂	0	0	0	1	7	8
			♀	0	1	1	2	0	4
P-78- 4	10 L	10 L	♂	0	1	1	4	0	6
			♀	0	0	4	4	0	8
P-78- 8	14 M	14 M	♂	0	0	0	3	4	7
			♀	0	0	2	0	0	2
P-78- 8	10 M	10 M	♂	0	0	2	4	1	7
			♀	0	1	4	3	0	8
P-78- 8	14 L	14 L	♂	0	0	0	0	2	2
			♀	0	0	0	1	0	1
P-78- 8	10 L	10 L	♂	0	1	5	5	0	11
			♀	0	0	0	2	0	2
P-78- 8	14 L	10 L	♂	0	0	0	0	1	1
			♀	0	2	1	0	0	3
P-78- 8	10 L	14 L	♂	0	1	1	1	0	3
			♀	0	0	1	0	0	1
P-78-12	10 L	10 L	♂	0	0	5	1	0	6
			♀	0	0	0	2	0	2
P-78-12	14 H	10 L	♂	0	0	0	2	4	6
			♀	0	2	1	2	0	5
P-78-12	10 L	Greenhouse (April)	♂	0	2	2	2	0	6
			♀	0	1	1	4	0	6

grade C less than half is orange. For grade D more than half is orange. For grade E the cell is completely covered by orange pigment. The difference is definite between males reared in 14 and 10 hour conditions, although it is not clear in females.

E. *Indirect effects of photoperiod through food plants.*

Several striking alterations of the phenotype of *C. eurytheme* by different photoperiods having been found, it became essential to determine whether the changes are produced by direct action of the photoperiod on the insects or by chemical changes in the plants induced by the photoperiod and passed on to the insects.

To examine this effect, food plants were interchanged for some larvæ of Brood P-78-8. On alternate days, larvæ which were being reared in the 14 hour photoperiod were transferred to food plants raised in 10 hour conditions, and vice versa. Larvæ of Brood P-78-12 were divided into the three groups in the beginning of larval life. The first group was reared at 10 hours (intensity about 3,400 foot candles) of light illumination on plants grown at 10 hours (intensity about 3,400 foot candles) of light. The second group was reared at 14 hours (intensity about 7,100 foot candles) of light on plants grown at 10 hours (intensity about 3,400 foot candles) of light. The third group was reared at 10 hours (intensity about 3,400 foot candles) of light illumination on plants raised in a greenhouse in April. Vetch raised in a greenhouse in April had darker green color than vetch raised in a rearing room and resembled vetch in a field in summer.

The results are shown in Tables 1, 2, and 3. The melanin suffusion of the under side of the hindwing and of the upper side of the hindwing in females and all other photoperiod-sensitive characters discussed above (sections A-D) indicate no indirect effects of photoperiod. Butterflies reared in the 14 hour photoperiod show the same characters in spite of differences in the photoperiod of the food plants, as far as the writer's experiments go, and the same results appear for butterflies reared in the 10 hour conditions.

F. *Developmental rates.*

Developmental rates were approximately the same throughout the experiment, regardless of conditions and broods. This characteristic is apparently dependent primarily on temperature.

DISCUSSION

Although rearings were started with at least 20 first instar larvæ in each group with different conditions, many larvæ and pupæ were killed by virus diseases, and the number of adults obtained was irregular and was very small in some groups. However, it is clear that differences in photoperiod directly affect larvæ and (or) pupæ and produce very different phenotypes among genetically similar butterflies. The differences between broods under identical conditions are almost negligible in comparison with differences produced in different photoperiods, in the writer's experiments.

According to a letter from Mr. BRYANT MATHER to Dr. JOHN H. GEROULD in 1956, a sample of 231 *C. eurytheme* and *C. philodice* from the state of Mississippi from where the material for the present experiments came, consisted of 118 orange and 2 yellow males and 64 'alba", 45 orange, and 2 yellow females. Therefore, there is a slight possibility that low orange color grades could be the results of genes from *C. philodice*. There is, however, no correlation between low orange color grades and a reduction of orange color at the tip of the forewing.

The results of these experiments do not coincide with HOVANITZ's findings (1945) for some characters. His data indicate no change in black border and an increase in melanin suffusion of the under side of the hindwing with increase of irradiation period. However, the conditions which were used by HOVANITZ were: a) continuous light; b) 10 hours light per day; and c) absolute darkness. If the effect of light is simply accumulative, continuous light may act as an extreme case of long photoperiod. However, the writer's data indicate that the important factor is the photoperiod itself; *i.e.,* differences in light intensity (7,100; 6,300; and 3,400 foot candles) produced no notable effects (Tables 1, 2, & 3). It is also known that continuous lighting does not act as an extreme case of a long photoperiod in the diapause of saturniid moths (Tanaka, 1952). Therefore, the data of HOVANITZ and the writer are perhaps not comparable.

SUMMARY

Three broods of *Colias eurytheme,* resulting from outcrossing between F_1 individuals from 2 Mississippi females, were reared at 14 and 10 hours of fluorescent light per day in an 80°F. constant temperature room. The intensities of light were varied, and food plants (vetch) of different photoperiodic histories were also used. The results are as follows.

1. While 5th instar larvæ in the 10 hour photoperiod had black patches on the spiracular line, the same stage at 14 hours lacked them.
2. The forewing length (*i.e.,* presumably size) seems to be larger in the 14 hour conditions than in the 10 hour conditions.
3. The black border of the upper side of the wings was wider in the 14 hour conditions than in the 10 hour photoperiod.
4. Melanin suffusion of the underside of the hindwing was more intense in the 10 hour conditions than in the 14 hour conditions.
5. Melanin suffusion of the upper side of the hindwing in females seems to be more intense in the 10 hour conditions than at 14 hours.
6. No notable photoperiodic difference was found in orange color grades (intensity).
7. A reduction of orange color at the tip of the forewing in males was more prominent in the 10 hour conditions than in the 14 hour conditions.
8. Indirect effects of photoperiod through food plants seems to be negligible in comparison with its direct effects on larvæ and pupæ.
9. No difference was found in developmental rates.

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SYNONYMY OF TWO AFRICAN EUTELIINÆ (NOCTUIDÆ)

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1. *"Pacidara venustissima."*

P. venustissima has been the unequivocal designation for the largest known euteliine in the world, ever since WALKER first proposed it in 1865. It was, therefore, something of a shock to discover that both the genus and species names are antedated by sixteen years, the earlier names prominently proposed, and accompanied by excellent colored figures, in no less a journal than the *Proceedings of the Zoological Society of London*. The type, further, is stated to have been placed in the British Museum collection. Since neither WALKER nor HAMPSON make any mention of it, it is likely that the specimen met with misadventure sometime before they arrived on the scene.

For discovery of this synonymy I owe a posthumous debt to the late W. J. HOLLAND, who had penned both name and reference on the label affixed to one of his specimens. The name, without reference, in someone else's hand, appears on another specimen, received from ROLAND TRIMEN, and I suspect that this was the source of HOLLAND's information.

The rectified synonymy of the genus and species is as follows:

Genus *Caligatus* Wing

Caligatus Wing 1850, *Proc. Zool. Soc. London* 1849: 104 (type: *Caligatus angasii* Wing 1850).