THE EFFECT OF CERTAIN ENVIRONMENTAL FACTORS AND CHEMICALS ON THE MARKINGS OF *PIERIS RAPAE* (PIERIDAE)

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INTRODUCTION

It is well known (Klots, 1951; Comstock and Comstock, 1943) that the dark markings of *Pieris rapae* (Linnaeus) are somewhat reduced, or even entirely absent (form *immaculata*), in the spring brood, which emerges from overwintering pupae. Therefore, the markings are capable of being diminished by particular factors that are involved in the spring brood, and the present work is essentially an attempt to reduce the markings by means of light and temperature variations and, especially, incorporation of certain chemicals in the larval diet.

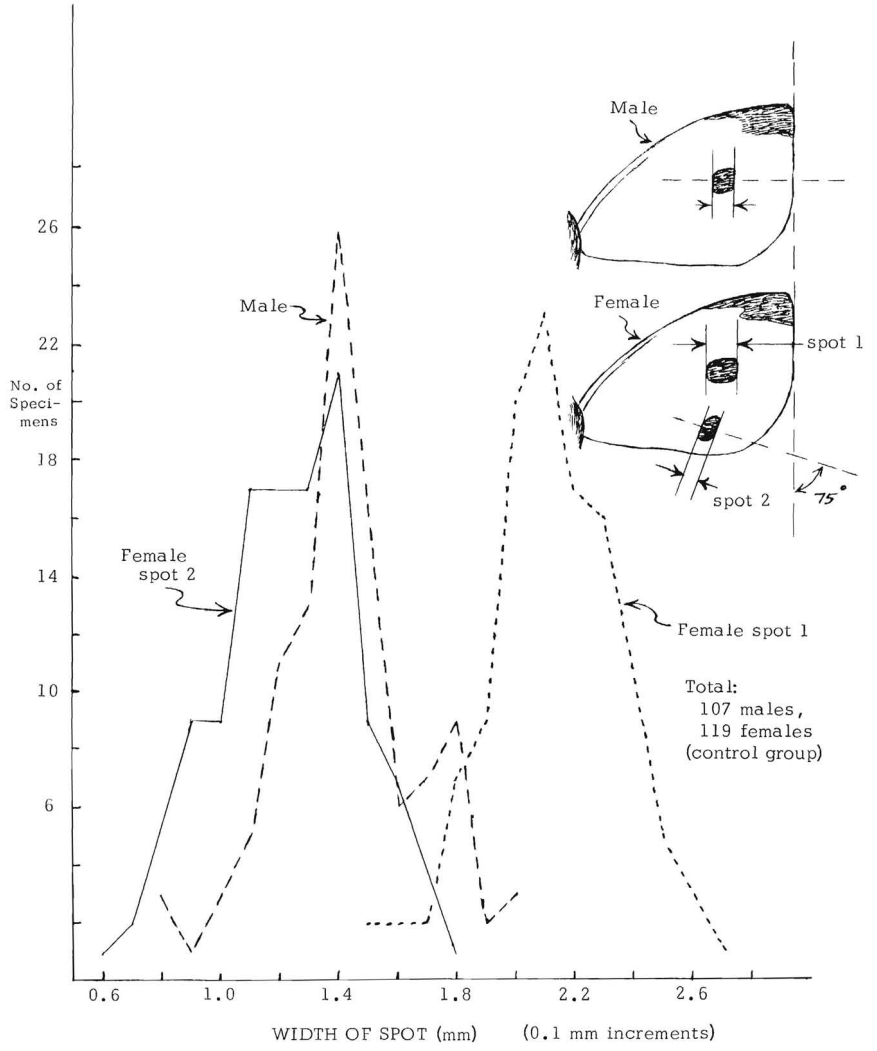
REARING PROCEDURE

In the course of the experiments, five consecutive broods were reared indoors beginning with eggs laid by females captured at Morristown, New Jersey (July 2, 1964) and Doylestown, Pennsylvania (July 4, 1964). Inbreeding was not very close, because a number of pairs from each brood were mated and the eggs of mixed parentage reared together to give the next brood. There was no evidence of declining vigor in the development rates; the durations of stages for the second and fifth broods (control groups) were not greatly different, as seen below.

Brood	Date Eggs Laid	Relative Humidity (%)	Temp. (°F.)	No. of Days			
				Eggs Laid	Hatched	Pupation	Eclosion
2	July 31, 1964	51–66	68–86	0	3	21–27	29–31
5	Nov. 15, 1964	36–53	67–75	0–2	3–7	21–24	30–34

Incidentally, typical S-shaped curves were obtained by plotting length of the largest larva vs. time; in the case of the first brood, the largest larva increased from one mm (calculated to be about 2×10^{-5} grams) at hatching to a maximum of 25 mm (0.25 grams) in the course of 14 days, and the same increase in length took place in the case of the third brood in 15 days. The length of the smallest larva in the third brood increased from two mm on the seventh day after hatching to only 10 mm on the 19th day, showing the wide range in growth rates among individuals.

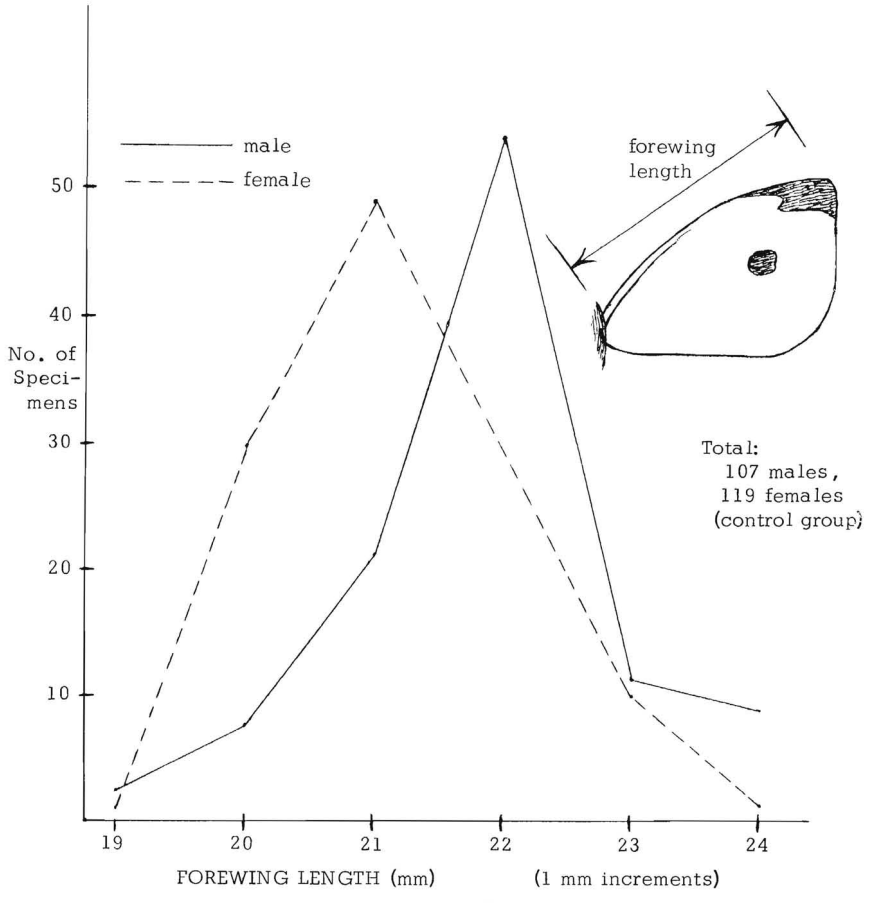
The larvae were fed cabbage leaves from refrigerated heads and were reared in cardboard boxes (4.5 inches high and 9 inches square with a gauze window 5.5 inches square in the lid) in diffuse light.



EXPLANATION OF GRAPH 1

Frequency curves for wing spot widths in *Pieris rapae* (L.).

Mating was accomplished by exposing about 10 to 75 adults (roughly equal numbers of the sexes) in a screen cage (16 inch cube) to direct sunlight for one or two days. Then cabbage leaves were hung up on the sunny side of the cage for two or three successive days to obtain a yield of several hundred fertile eggs. In certain cases, a single pair was mated by exposing to sunlight in a jar, and the offspring were reared separately,



EXPLANATION OF GRAPH 2

Frequency curves for wing length in *Pieris rapae* (L.).

but since variation in the markings for offspring of a single pair was as great as the general variation, groups of mixed parentage were used for most purposes.

Chemicals were fed by sprinkling a fine powder from salt shakers, liberally onto the cabbage leaves. The chemicals were obtained from Matheson, Coleman, and Bell Co., with the exception of hydroquinone (from B. and A. Division, Allied Chem. Corp.) and 4-chlororesorcinol (from Koppers Co.; recrystallized to give a capillary melting point of 108.5–110° C.). Chemical feeding was initiated when the larvae had reached a length of 7–23 mm (3–8 mm in the case of tyrosine and phenylalanine).

TABLE 1
EFFECT OF PUPAL COLOR AND OF REFRIGERATION OF PUPAE ON ADULT WING SPOTS

MALES

Pupal Color	Refrigerated	Wing Length (mm)	No. Specimens	Spot (mm)			
				Range	Average	AD	SD
green	no	23	5	1.0–1.6	1.3	0.14	0.19
brown	no	22	9	0.9–1.9	1.3	0.24	0.29
green	yes	22	8	1.3–1.7	1.5	0.14	0.15
brown	yes	22	6	0.9–1.6	1.4	0.33	0.35

FEMALES

Pupal Color	Refrigerated	Wing Length (mm)	No. Specimens	Spot 1 (mm)			
				Range	Average	AD	SD
green	no	22	7	2.1–2.5	2.3	0.13	0.15
brown	no	22	3	1.8–2.9	2.2	0.43	0.51
green	yes	21	4	1.9–2.5	2.1	0.25	0.26
brown	yes	21	5	1.8–2.5	2.1	0.20	0.24

Pupal Color	Refrigerated	Wing Length (mm)	No. Specimens	Spot 2 (mm)			
				Range	Average	AD	SD
green	no	22	7	1.2–1.6	1.3	0.07	0.13
brown	no	22	3	0.9–1.9	1.3	0.40	0.44
green	yes	21	4	0.9–1.6	1.2	0.22	0.26
brown	yes	21	5	0.8–1.4	1.2	0.18	0.22

MEASUREMENT OF MARKINGS

In order to provide a quantitative parameter, the width of the forewing spots (one in the male, two in the female) was measured, and, since the spots are variable even among the offspring of a single pair, it was necessary to express results statistically by means of a range, an average, an average deviation (AD), and a standard deviation (SD). As will be seen (Tables 1–4), no emphatic reduction in the average was encountered for any test group. Intensity of the markings, as opposed to width, was not measured but was found to be diminished markedly, especially for the apical area, in certain cases (Plates 1–3).

The spots were measured as shown in Graph 1 by means of a 6-power comparator with 0.2 mm scale divisions (Edmund Scientific Co., Barrington, N.J., No. 30,169 in Catalog 645). Estimation was necessary because the edges of the spots are not very sharply defined, but good agreement was obtained among controls (Tables 1–3).

TABLE 2
VARIABILITY IN SIZE OF WING SPOTS

MALES

Group	Wing Length (mm)	No. Specimens	Spot (mm) Range	Average	AD	SD
Control	22	107	0.8–2.0	1.4	0.18	0.25
Offspring of single pair	22	6	0.9–1.7	1.4	0.15	0.24
Wild sample[1]	23	25	1.0–1.9	1.5	0.16	0.22
Wild (Spring Brood[2])	22	21	0.6–1.3[3]	1.0[3]	0.16[3]	0.20[3]

FEMALES

Group	Wing Length (mm)	No. Specimens	Spot 1 (mm) Range	Average	AD	SD
Control	21	119	1.5–2.7	2.1	0.18	0.24
Offspring of single pair	21	11	1.5–2.5	2.0	0.24	0.29
Wild sample	22	4	1.5–2.3	2.0	0.25	0.30
Wild (Spring Brood)	22	15	1.4–2.2	1.8	0.17	0.21

Group	Wing Length (mm)	No. Specimens	Spot 2 (mm) Range	Average	AD	SD
Control	21	119	0.6–1.8	1.2	0.20	0.24
Offspring of single pair	21	11	0.4–1.5	1.1	0.20	0.27
Wild sample	22	4	1.2–1.8	1.5	0.20	0.22
Wild (Spring Brood[2])	22	15	0.7–1.8	1.2	0.30	0.33

[1] Collected at Doylestown, Pennsylvania, October 4, 1964.
[2] Collected at Flemington, New Jersey on May 1, 1965.
[3] These values are for the 10 specimens with spots; the others (11) had no spots (0–10 black scales in area where spot should be).
NOTE: The spring brood females tended to have dusky basal regions on the forewing and weak or absent apical markings.

Forewing lengths were measured as shown in Fig. 2 and averaged for each set of wings. Then the spot measurement for each wing was normalized to the mean wing length by multiplying by the quotient of the mean length divided by the particular length. Average deviation (AD) and standard deviation (SD) calculations indicated that approximately normal curves were obtained (Graph 1).

PUPAL COLOR

Pupal color was studied to some extent, the main interest being in checking for correlation with adult markings.

In many cases, pupae were definitely either brown or pale green, but

TABLE 3
EFFECT OF LIGHT AND TEMPERATURE ON SIZE OF WING SPOTS
(Wing lengths normalized to 22 mm for males, 21 mm for females)

MALES

Group	No. Specimens	Spot (mm)			
		Range	Average	AD	SD
Control (1); 18 hrs. light/day	107	0.8–2.0	1.4	0.18	0.25
Control (2); as above	16	1.1–2.0	1.5	0.25	0.30
Reared in darkness (1)	11	0.7–2.1	1.2	0.30	0.38
Reared in darkness (2)	18	1.3–2.2	1.6	0.13	0.19
Darkness and cold[1]	7	1.2–1.7	1.4	0.17	0.19

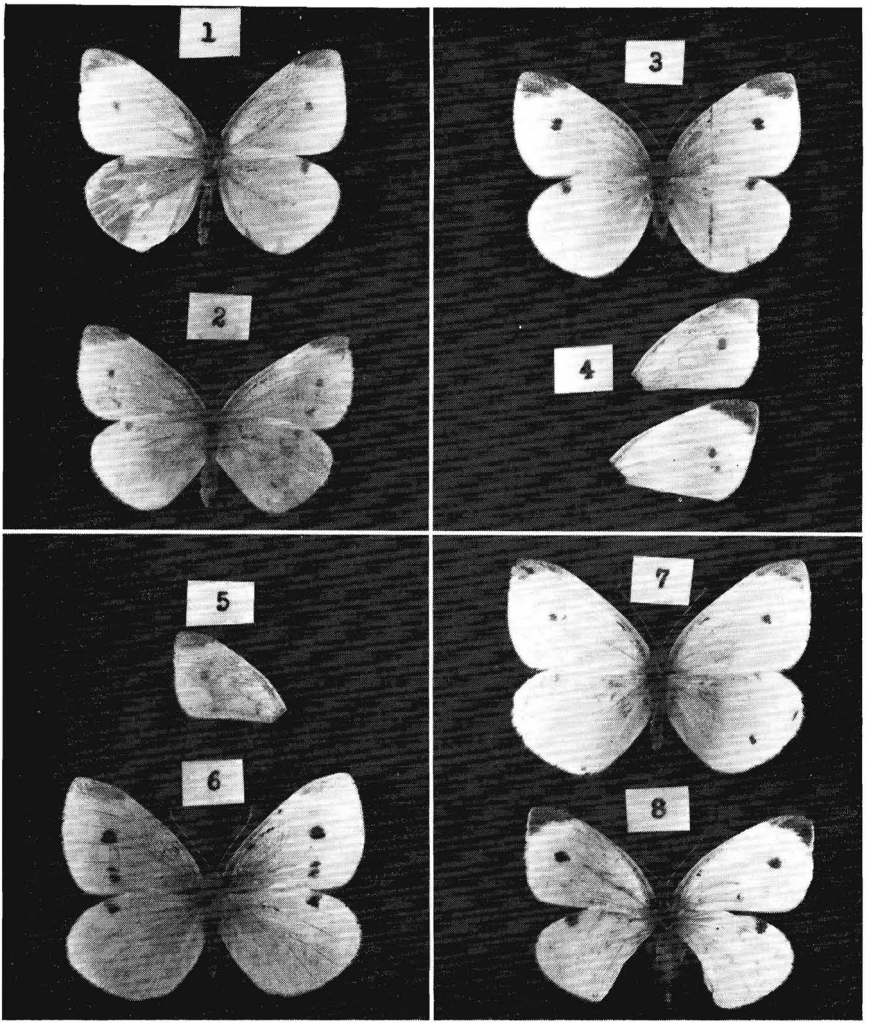
FEMALES

Group	No. Specimens	Spot 1 (mm)			
		Range	Average	AD	SD
Control (1)	119	1.5–2.7	2.1	0.18	0.24
Control (2)	18	1.4–2.6	2.0	0.25	0.33
Reared in darkness (1)	6	1.7–2.1	1.8	0.15	0.21
Reared in darkness (2)	16	2.0–2.9	2.5	0.23	0.26
Darkness and cold[1]	10	1.9–2.7	2.3	0.18	0.22
Group	No. Specimens	Spot 2 (mm)			
		Range	Average	AD	SD
Control (1)	119	0.6–1.8	1.2	0.20	0.24
Control (2)	18	0.6–2.2	1.3	0.32	0.40
Reared in darkness (1)	6	0.7–1.4	1.1	0.20	0.25
Reared in darkness (2)	16	1.0–1.9	1.5	0.19	0.24
Darkness and cold[1]	10	0.9–1.7	1.4	0.19	0.23

[1] The larvae, after reaching 3–8 mm, were reared at 37–68° F. and 38–78% rel. humidity, in darkness. Pupae were kept at 33–68° F. and 44–80% rel. humidity, in darkness, until eclosion.

there were all variations between, so that the colors often had to be judged subjectively, making the following numbers only approximate. Also, there was a shift toward green (see below) as the pupae matured. Ultimately, of course, darkening before eclosion obscured the original colors.

Sex ratio.—A pair that had eclosed from green pupae was mated, and the resulting larvae gave 16% green among the male pupae (total of 25) (36% after 3 days) and 33% green among the female pupae (total of 15) (60% after 3 days). This shows that neither sex necessarily has an essential monopoly on the green color. Incidentally, larvae are easily sorted by sex by means of the subcutaneous dorsal markings (presumably testes)



EXPLANATION OF PLATE I

Pieris rapae (L.) specimens with unusual markings. 1, ♂, larva fed 4-chlororesorcinol, light markings; 2, ♀, larva fed *p*-aminobenzoic acid, unusually light markings; 3, ♀, larva fed *p*-aminobenzoic acid, spot 2 absent; 4, ♂, larva fed cysteine·HCl, no black scales in spot of upperside shown; 5, ♂, larva fed ascorbic acid, no black scales in spot; 6–8, reared at reduced temperatures (see "dark and cold" group, Table 3), 6, ♀, light apical markings, 7, ♂, light markings, 8, ♂, pupal case on abdomen.

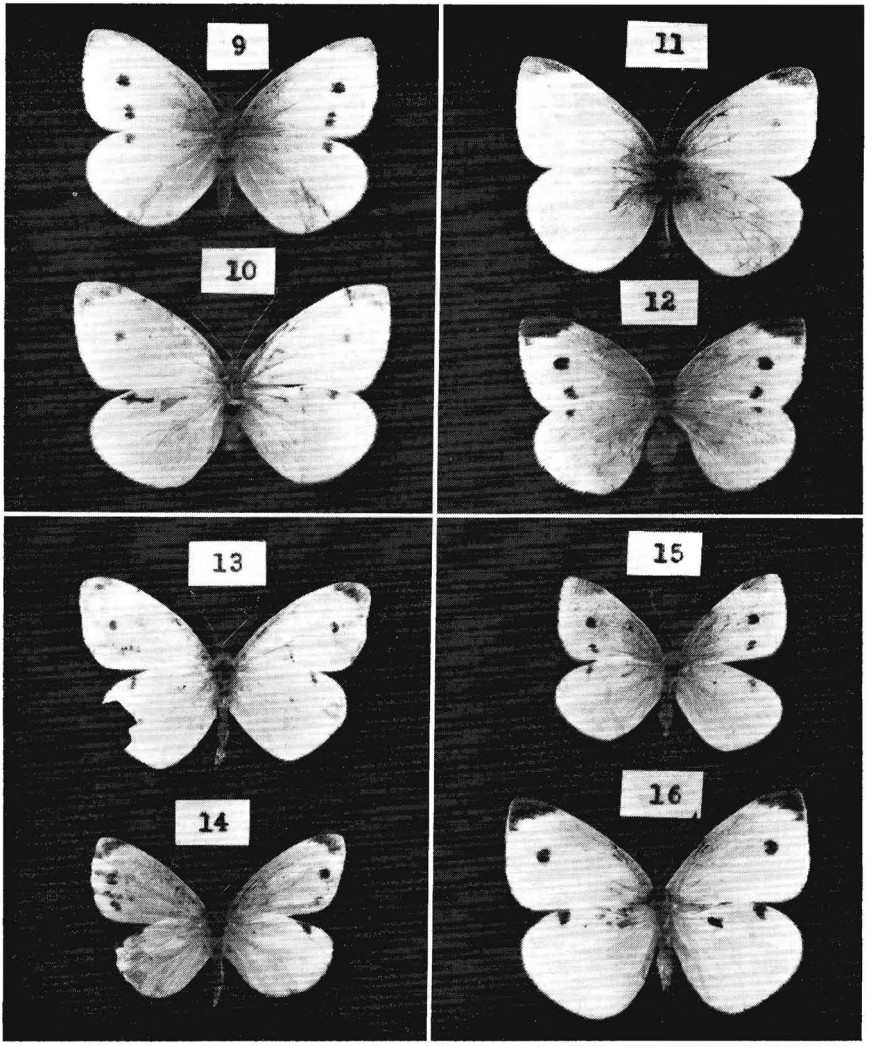
in the male. In one case, 27 male larvae were sorted from 17 females, the sexes being verified when the adults eclosed.

Inheritance.—Larvae obtained from a pair which had eclosed from green pupae produced 21% green pupae (of total of 39), while brown-pupa parents gave larvae which yielded 28% green pupae (of total of 108). A single pair of brown-pupa parents gave larvae which produced 35% green pupae (of total of 31). Therefore, under the conditions of this particular test, pupal color of the parents had no control over pupal color of the offspring. However, it has been reported (Harrison, 1928) that the green pupal color, inhibited by orange light, in *Pieris napi* (L.) and *Pieris brassicae* is inherited.

Photoperiod.—Larvae which were reared and pupated in darkness gave only brown pupae (of total of 93). With 18 hours of light/day, offspring of a single pair (from brown pupae) gave 24% green pupae (of total of 17), while the combined offspring of several brown-pupa pairs produced 30% green (of total of 330). With 10 hours light/day the offspring of a single pair of brown-pupa parents produced 29% green pupae (of total of 17). Thus it seems that reduction of the photophase from 18 to 10 hours had no pronounced effect but that complete absence of light gave only brown pupae. That darkness causes brown pupae in *Pieris rapae* has been reported (Okamoto, 1960). This reference also states that the pupal color is determined by photostimuli in the fifth instar larva and that the wavelength and quantity of light reflected from the pupal site is of great importance. However, in the present work there was no definite trend towards green pupae in those regions (such as the gauze window) of the rearing boxes that received the most light.

Chemical effects.—Larvae fed phenylalanine gave 7% green pupae (of total of 29), and those fed tyrosine gave 24% green (of total of 17), while the control group gave 22% green (of total of 135). Increased melanin formation caused by phenylalanine (see discussion below) would explain the low proportion of green pupae, though this is a doubtful rationalization.

In another series of experiments, the following melanogenesis inhibitors gave the percent green pupae in parentheses: 4-chlororesorcinol (61%, of total of 23), *p*-aminobenzoic acid (26%, of total of 31), cysteine hydrochloride (14%, of total of 8), and ascorbic acid (8%, of total of 24). The control gave 38% (of total of 24). There is no obvious correlation in these data; the very high proportion of green pupae obtained with 4-chlororesorcinol might be rationalized as inhibition of pigmentation, but there would seem to be an opposite effect with the other melanogenesis inhibitors. It is to be understood that the biochemical reaction sequences which may be initiated by these chemicals are unknown.



EXPLANATION OF PLATE II

Pieris rapae (L.) specimens with unusual markings. 9–10, larvae reared at reduced temperatures (see "dark and cold" group, Table 3), 9, ♀, light apical markings, 10, ♂, light markings; 11–12, larvae exposed to ultraviolet light (mostly 366 millimicrons) for total of 18 hours, 11, ♂, spot very light, almost absent, 12, ♀, pupal case on abdomen; 13–15, larvae fed tyrosine, 13, ♂, apical black scales partly missing, 14, ♀, spot 1 nearer margin, 15, ♀, apical markings very light; 16, ♂, larva fed phenylalanine, markings normal.

Effect on adult markings.—Pupae which were definitely either brown or green were selected and allowed to develop and eclose under ambient conditions (72–81° F., 36–55% relative humidity) or were placed (within three days following pupation) in refrigeration (0–2° C., 100% relative humidity) for one week and then allowed to develop and eclose under ambient conditions (69–81° F., 35–55% relative humidity). The time from pupation of the first to eclosion of the last was 12 days for unrefrigerated pupae and 19 days for refrigerated pupae. The results are given in Graph 1. No significant variation in size of the forewing spots was found with respect to pupal color (or as a result of refrigeration under the specified conditions).

ADULT WING MARKINGS

Variability of spots.—Graph 2 shows the variability in the size of the spots for a large control group of mixed parentage, the offspring of a single pair, and a series of wild specimens collected at Doylestown, Pennsylvania, on October 4, 1964. Frequency curves for the control group of 226 specimens are shown in Graph 1. It is seen that normal frequency curves are approximately defined by plotting the number of specimens having a particular size spot vs. spot size in 0.1 mm increments. For the control group, normal curves also are produced by plotting number of individuals with a particular length vs. wing length in 1 mm increments as shown in Graph 2. For wing length, AD was 0.70 for males or females, and SD was 1.1 for males and 0.96 for females. This means that at least 99% of wing lengths will fall within 15% (for males) or 14% (for females) of the mean, while, for the same control group, the variance is 54% for the male spot, 34% for female spot 1, and 60% for female spot 2. Thus, the spots are much more variable than the wing lengths. It will be noted that the SD in the tables lies between 0.15 and 0.38 for the male spot, 0.13 and 0.51 for female spot 1, and 0.13 and 0.44 for female spot 2.

It may be concluded from examining Table 2 that the spots were as variable within a brood as within the general population.

Effect of light and temperature.—Table 3 shows the results for (1) the large control group, raised with 18 hours light/day, (2) another control group, also raised with 18 hours light/day, (3) groups from different broods reared in darkness from the time the larvae reached 4–10 mm in length to eclosion (two males and one female were refrigerated with no noticeable effect on the spots), and (4) a group reared in darkness at reduced temperatures. Note that the lack of effect of refrigeration when initiated after pupation, was mentioned above.

For one brood (1) reared in darkness, the indication is that the spots may have been diminished slightly in size. However, in the other brood

TABLE 4
EFFECT OF CHEMICALS (FED TO LARVAE) ON SIZE OF WING SPOTS

MALES

Chemical	Mortality (%)[1]	No. Specimens	Wing Length (mm)	Spot (mm) Range	Average	AD	SD
DL-β-phenylalanine	88	10	21	1.0–2.0	1.5	0.22	0.28
L-tyrosine	84	6	22	0.8–1.9	1.5	0.33	0.38
4-chlororesorcinol	45	12	22	1.1–1.9	1.6	0.15	0.20
p-aminobenzoic acid	41	13	22	0.8–1.7	1.5	0.19	0.25
L(+) cysteine·HCl	85	3[2]	21	1.2–1.7	1.5	0.25	0.26
L(+) ascorbic acid	0	13[2]	22	1.2–2.0	1.6	0.20	0.24

FEMALES

Chemical	No. Specimens	Wing Length (mm)	Spot 1 (mm) Range	Average	AD	SD
DL-β-phenylalanine	9	21	2.0–2.7	2.4	0.18	0.22
L-tyrosine	10	20	1.5–2.7	1.9	0.29	0.36
4-chlororesorcinol	9	21	1.9–2.6	2.3	0.18	0.22
p-aminobenzoic acid	15	21	1.0–2.6	2.1	0.27	0.39
L(+) cysteine·HCl	3	19	1.9–2.2	2.1	0.10	0.13
L(+) ascorbic acid	9	21	2.0–2.6	2.2	0.11	0.17

Chemical	No. Specimens	Wing Length (mm)	Spot 2 (mm) Range	Average	AD	SD
DL-β-phenylalanine	9	21	1.3–1.9	1.5	0.13	0.18
L-tyrosine	10	20	0.7–1.5	1.1	0.20	0.25
4-chlororesorcinol	9	21	1.0–1.7	1.4	0.10	0.17
p-aminobenzoic acid	15	21	0.8–1.8	1.2	0.13	0.23
L(+) cysteine·HCl	3	19	0.8–1.3	1.1	0.20	0.22
L(+) ascorbic acid	9	21	1.1–1.8	1.4	0.24	0.26

[1] (100) (no. original larvae – no. of adults obtained)/no. original larvae. Hydroquinone and thiourea gave 100% mortality.
[2] There were no black scales in the spot in the case of one specimen.

(2) there was no indication of decrease in size of either the male or female spots, and no change in intensity was evident.

When a group of larvae, starting at 3–8 mm, was reared in darkness at reduced temperatures (down to 33° F.) so that pupation occurred at 32–50 days vs. 24–36 days for the control, and eclosion began at 62 days vs. 33 days for the control, the spots of the adults showed no general decrease in size (Table 3) or intensity. However, certain individuals were lightly marked apically (Figs. 6, 9), as were none of the large control group. One specimen (Fig. 8) retained the abdominal part of the pupal case.

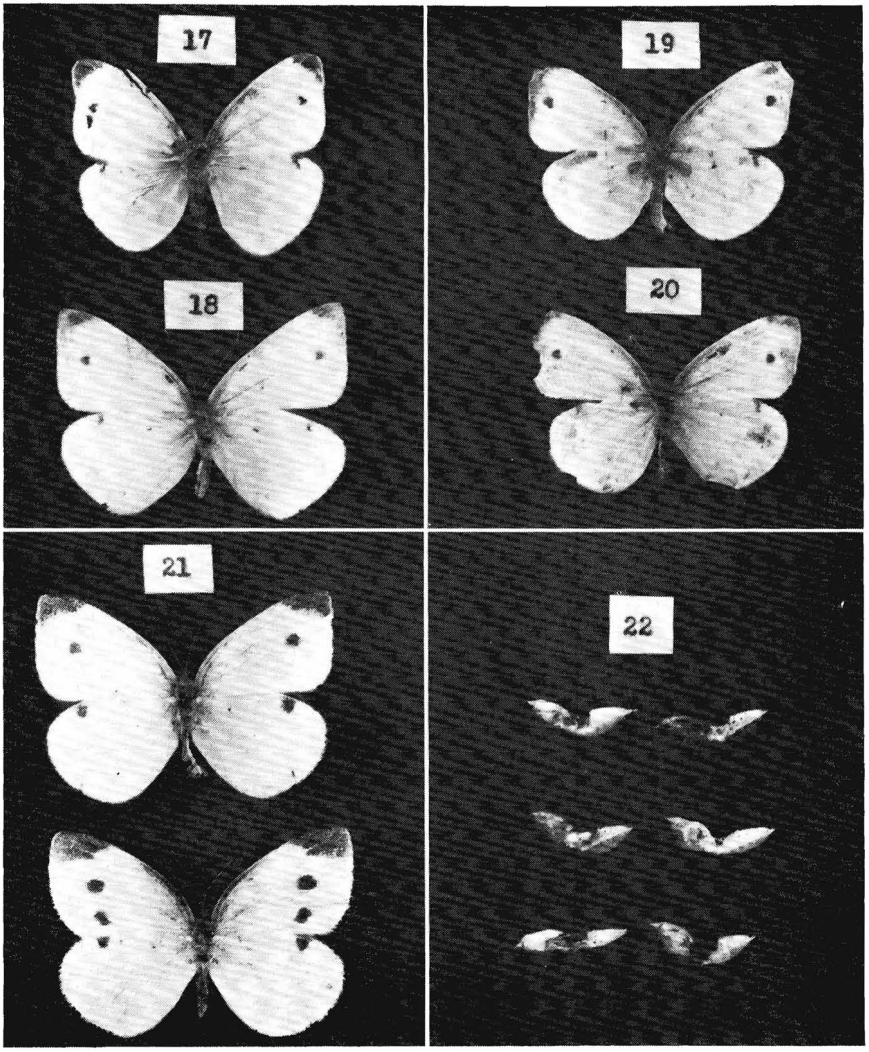
Another group was reared under standard conditions, but the pupae were refrigerated (beginning at 0–4 days after pupation) for 39 days at 32–38° F. (100% relative humidity); the markings were of normal size (averages: male spot 1.4 mm, wing 22 mm; female spot 1, 2.2 mm, spot 2, 1.3 mm, wing 21 mm). Irradiation of larvae with ultraviolet light (mostly 366 millimicrons) for a total of 18 hours, in the case of another group, had no appreciable effect except that one male was lightly marked (Fig. 11) and a female happened to retain the abdominal pupal case (Fig. 12).

It was concluded that those variations of light and temperature which were applied were unsuccessful in consistently reducing the size of the markings from the normal summer-brood range. However, the fact that four of the seventeen specimens reared in darkness at reduced temperature were lightly marked (Figs. 6, 7, 9, 10) suggests that possibly an extension of low-temperature storage (via diapause) following pupation at rather low temperature might produce a consistent effect, as is presumably the natural situation producing the spring brood.

Effect of chemicals.—Curious individual variations were obtained by feeding the larvae certain chemicals active in the process of melanin formation which operates during the pupal stage to produce the dark markings of the adult.

The chemicals were (1) phenylalanine, found in *Pieris brassicae* larvae and food leaves (Stamm and Aguirre, 1955) and starting material for melanogenesis, (2) tyrosine, also a starting material for melanogenesis and found freely in insect blood (Brunet, 1963) and, along with phenylalanine, in silkworm skin (Watanabe, 1956), (3) 4-chlororesorcinol, which causes lack of melanin formation in mosquito larvae (Wallis, 1961) and fish (Kull, Bonorden, and Mayer, 1954), (4) *p*-aminobenzoic acid, an inhibitor of melanogenesis (Lorincz, 1950), (5) cysteine (as the hydrochloride), which is inversely connected with melanin formation in skins of silkworm larvae (Inagami, 1956), (6) ascorbic acid, which inhibits melanogenesis in rabbits (Visetti and Ferrero, 1957) and occurs in cabbage, (7) hydroquinone, an inhibitor of melanogenesis in mice and humans (Denton, Lerner, and Fitzpatrick, 1952), and (8) thiourea, which inhibits melanogenesis in the Planarian eye (Kambara, 1954) and causes abnormal epidermis in the silkworm (Fukuda, 1953). A general discussion of melanogenesis inhibitors and their modes of action is given by Lerner (1953).

In the control group (no chemical fed), the average male spot was 1.5 mm (22 mm wing) and female spots 1 and 2 averaged 2.5 and 1.5 mm (22 mm wing). Therefore, examination of Table 4 shows no general reduction in size of the male spot and little, if any, reduction in size of



EXPLANATION OF PLATE III

Specimens of *Pieris rapae* (L.). 17–20, larvae fed phenylalanine, 17, ♂, spot nearer margin, 18–20, ♂ ♂, apical black scales partly missing; 21, ♂ & ♀, control group, markings normal; 22, pupae from larvae fed phenylalanine, deformed, constricted in middle.

the female spots. Also there was no obvious general decrease in intensity of markings. However, several interesting anomalies resulted (Plates 1–3). Light markings were produced by 4-chlororesorcinol (Fig. 1), *p*-aminobenzoic acid (Fig. 2), tyrosine (Fig. 15), and phenylalanine (Figs.

18–20). The latter compound incidentally produced many deformed (constricted) pupae (Fig. 22), which died. Deformed forewings, bearing the spots near the margin, were produced by phenylalanine (Fig. 17) and tyrosine (Fig. 14). Perhaps the most curious peculiarity was the absence of scales (on upper wing surface) in the male spot of one specimen each from the cysteine hydrochloride and ascorbic acid groups (Figs. 4, 5); there were gray scales on the spot on the underside of the wing, and removal of these would give a transparent "window." Hydroquinone and thiourea were larvicidal and produced no pupae.

Thus, the result was a tendency toward depigmentation, perhaps most marked when ingestion was heaviest, with all the chemicals, even though phenylalanine and tyrosine are materials for, rather than inhibitors of, the pigmentation process. This situation is not surprising, however, because strange effects may well result, via obscure biochemistry, from massive overdoses of what normally is ingested in low concentration in the food.

Difference in intensity of markings of the spring and summer broods is said (Pugh, 1934) not to be due to any difference in content of tyrosinase (the enzyme catalyzing melanin formation) in the insect but to some other factor, depending on the temperature at which the pupae are kept. The present work extends this by suggesting that a general change in the markings is not caused by excess phenylalanine or tyrosine (raw materials for melanin) or by inhibitors of tyrosinase.

Therefore, the final conclusion is that the reduced dark pigmentation of the spring brood is not the result of a lowered concentration of phenylalanine or tyrosine in the pupa or in increased concentration of some inhibitor but is due ultimately to the temperature factor, which (with the proper photoperiod) causes the diapause necessary for overwintering.

SUMMARY

1. Pupal color, green or brown, was not correlated with sex nor inherited under the conditions of the test, nor did it affect the markings of the adult. In darkness only brown pupae were produced.

2. Reduced temperature, in conjunction with darkness, caused reduced intensity of markings in about one of every four specimens. Darkness alone (at normal summer temperatures) had no effect on the markings.

3. The feeding of phenylalanine, tryosine, and certain tyrosinase inhibitors to larvae produced sporadic depigmentation effects, including total lack of scales within the male spot, but there was no consistent reduction in intensity of markings.

4. The data suggest that the reduced dark pigmentation of the spring brood of *Pieris rapae* is not the result of lowered concentration of melanin

precursors (since greatly increased concentrations did not increase melanin) or the presence of tyrosinase inhibitors in the pupa but is related to reduced temperature.

Literature Cited

Brunet, P. C. J., 1963. Tyrosine Metabolism in insects. In: Pigment cell; molecular, biological, and clinical aspects. Part II, 1961. Ann. New York Acad. Sci., 100: 1020–1034.

Comstock, J. H., & A. B. Comstock, 1943. *How to Know the Butterflies; a Manual of Those Which Occur in the Eastern United States.* Comstock Publishing Co., Ithaca, New York. [p. 78]

Denton, C. R., A. B. Lerner, & T. B. Fitzpatrick, 1952. Inhibition of melanin formation by chemical agents. Jour. Invest. Dermatol., 18: 119–135.

Fukuda, S., 1953. Effect of thiourea on the silkworm. Zool. Mag., Tokyo., 62: 349–353.

Harrison, J. W. H., 1928. Induced changes in the pigmentation of the pupae of the butterfly *Pieris napi* L., and their inheritance. Proc. Roy. Soc. (London), B 102 (718): 347–353.

Inagami, K., 1956. The formation of the pigments in the silkworm. IX. The relation between the reducing substance content and the melanin formation in some larval markings. Nippon Sanshigaku Zasshi, 25: 128–130.

Kambara, S., 1954. Depigmentation in the eye of Planaria as a result of thiourea treatment. Zool. Mag., Tokyo., 63: 51–54.

Klots, A. B., 1951. *A Field Guide to the Butterflies of North America, East of the Great Plains.* Houghton Mifflin Co., Boston, Mass. [p. 200]

Kull, F. C., R. Bonorden, & R. L. Mayer, 1954. Inhibition of melanin formation in vivo by 4-chlororesorcinol. Proc. Soc. Exptl. Biol. Med., 87: 538–540.

Lerner, A. B., 1953. Metabolim of phenylalanine and tyrosine. Advances in Enzymology, 14: 73–128.

Lorincz, A. L., 1950. The inhibition of melanin formation. Jour. Invest. Dermatol., 15: 425–532.

Okamoto, H., 1960. Studies of the pupal color determination of the common cabbage butterfly, *Pieris rapae crucivora* Boisduval (I). Physiol. and Ecol. (Japan), 9 (2): 84–89.

Pugh, C. E. M., 1934. Tyrosinase in Macrolepidoptera. Biochem. Jour., 28 (4): 1198–1200.

Stamm, M. D., & L. Aguirre, 1955. Aromatic aminoacids and tryptophane in the metamorphosis of *Pieris brassicae* and *Ocnogyna baetica.* Rev. Espanola Fisiol., 11 (1): 69–74.

Visetti, M., & F. Ferrero, 1957. Influence of vitamin C on the pigment of skin grafts. Minerva dermatol., 7: 457–459.

Wallis, R. C., 1961. The effect of phenylthiourea and 4-chlororesorcinol on *Aedes aegypti* larvae. Mosquito News, 21: 187–189.

Watanabe, T., 1956. Dopa and tyrosine in the integument of silkworm larvae. Nippon Sanshigaku Zasshi, 25: 443–444.

REVIVAL OF *Lepidoptera*

This Danish journal, organ of the Lepidopterologisk Forening in Copenhagen, was published from 1946 to 1951. The first issue of Volume 1 of a new series has now appeared, and includes, among other notes, the first part of a series describing and figuring the Danish *Eupithecia.* One or two issues a year are planned. The editor is T. W. Langer. For subscriptions write the Honorary Secretary, Johs. Storm-Olsen, Rødkildevej 14, Copenhagen F., Denmark.—P. F. Bellinger