VARIATIONS IN THE MARKINGS OF *PIERIS RAPAE* (PIERIDAE) INDUCED DURING THE PUPAL STAGE

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Introduction

The present work represents the conclusion of a study which has involved observations on the effects of feeding larvae certain chemicals (Kolyer, 1966) and on the effects of environmental factors (Kolyer, 1969) on the wing markings of the European cabbage butterfly *Pieris rapae* (Linnaeus). Specific objects were to note variations in markings as a result of (1) reduced-temperature storage of pupae of various ages, as an extension of previous work (Kolyer, 1969), (2) brief, barely sublethal heating of pupae, and (3) exposure of pupae to short-wave ultraviolet (UV) radiation. Incidental observations, e.g. on larval disease and the yellow form of *P. rapae*, also are included.

Experimental Procedures

Rearing. Final instar larvae were reared on an artificial diet at 79 ± 2°F and 45 ± 5% relative humidity under continuous cool white fluorescent light. Larval development was completed on cabbage leaves from refrigerated heads (see Kolyer, 1966); rearing room conditions were: 70–81°F, approx. 20–33% rel. hum., diffuse sunlight (March 6–April 17, 1969, Convent, New Jersey).

Refrigeration of pupae. As in previous work (Kolyer, 1969), pupae were packed carefully along with facial tissues in fiber mailing cans, which were covered with polyethylene bags to exclude moisture and stored in a refrigerator at 32–36°F for 154–156 days (approx. 5 months).

Heat treatment of pupae. Two test tubes, one inside the other, were immersed in hot water in a Dewar (vacuum-jacketed) vessel and allowed to equilibrate so that the temperature of the air within the inner tube approximated the temperature of the bath. Then pupae were added to the inner tube and allowed to remain for the specified time periods.

UV irradiation of pupae. Pupae were exposed, righthand wing case up, at a distance of 2 inches from an 8-watt glow discharge (mercury) lamp bulb giving light principally at 254 millimicrons. The intensity is given as 17 microwatts/cm.^{2} at 1 meter, which is "sufficient for effective air, surface, and liquid disinfection" (Anonymous, 1965). Temperature rise of a mercury thermometer bulb 2 inches from the lamp was considered negligible (0.5° C after 40 min. exposure).

Results and Discussion

Variability of markings. The great variation in markings among individuals of *P. rapae* was mentioned in the earlier work. Wing length of this species varies from year to year (Petersen, 1947), and such variation also may be true of the proportion of "immaculata" (spotless form) males in the spring brood. Of 21 males taken at Flemington, New Jersey, on May 1, 1965, 11 were "immaculata" by the arbitrary criterion of less than 10 black scales in the forewing spot (Kolyer, 1966). In contrast, only 4 of 28 males taken on April 21–27, 1969, at Morristown, New Jersey (29 miles northeast of Flemington) were "immaculata" (No. 1, Plate I). Refrigeration of nondiapause pupae at 34–39°F for 5 months gave 5 "immaculata" of 21 males (Kolyer, 1969), thus surpassing natural conditions in producing this form in at least one instance.

Controls—deformed head, yellow form. The control pupae were allowed to remain under rearing room conditions (cited above), and all adults eclosed in 9–12 days. Adult forewings are shown in No. 3, Plate I, and Nos. 5 and 6, Plate II.

The first control group (No. 3, Plate I) included an otherwise normal female with undeveloped head (no proboscis, palpi, or antennae), shown in No. 4, Plate I. A similar result was noted with a male adult fed the dye *safranin bluish* as a larva (Kolyer, 1965).

During completion of rearing of the second control group (Nos. 5 and 6, Plate II), a yellow-green larva, markedly different in shade from the other (grass-green) larvae, was isolated and reared to pupation. In 9 days a yellow-form male eclosed (the complete specimen in No. 5, Plate II). Since this was the only yellow form among 149 adults which eventually eclosed from the batch of larvae in question, the probability of having picked the yellow-form larva by chance alone was only 1.3%. A recessive yellow form of the larva of *Pieris napi* (L.) has been described (Gladman, 1962), but adults in this case are normal in appearance. Incidentally, the complete specimen (male) in No. 3, Plate I, also is a yellow form.

Larval disease. A minority of larvae evidenced black spots (appearing under magnification as black-rimmed pits) on the integument. Two of these larvae were sent to Mr. G. M. Thomas of the University of California (see Acknowledgments) for a disease diagnosis. The larvae were found to suffer a bacteriosis caused by a strain of the rather common insect pathogen *Serratia marcescens* (Bizio). This strain was nonchromogenic in culture, and association of the disease with the black spots is speculative. Such dark spots on larvae usually are symptomatic of microsporidian

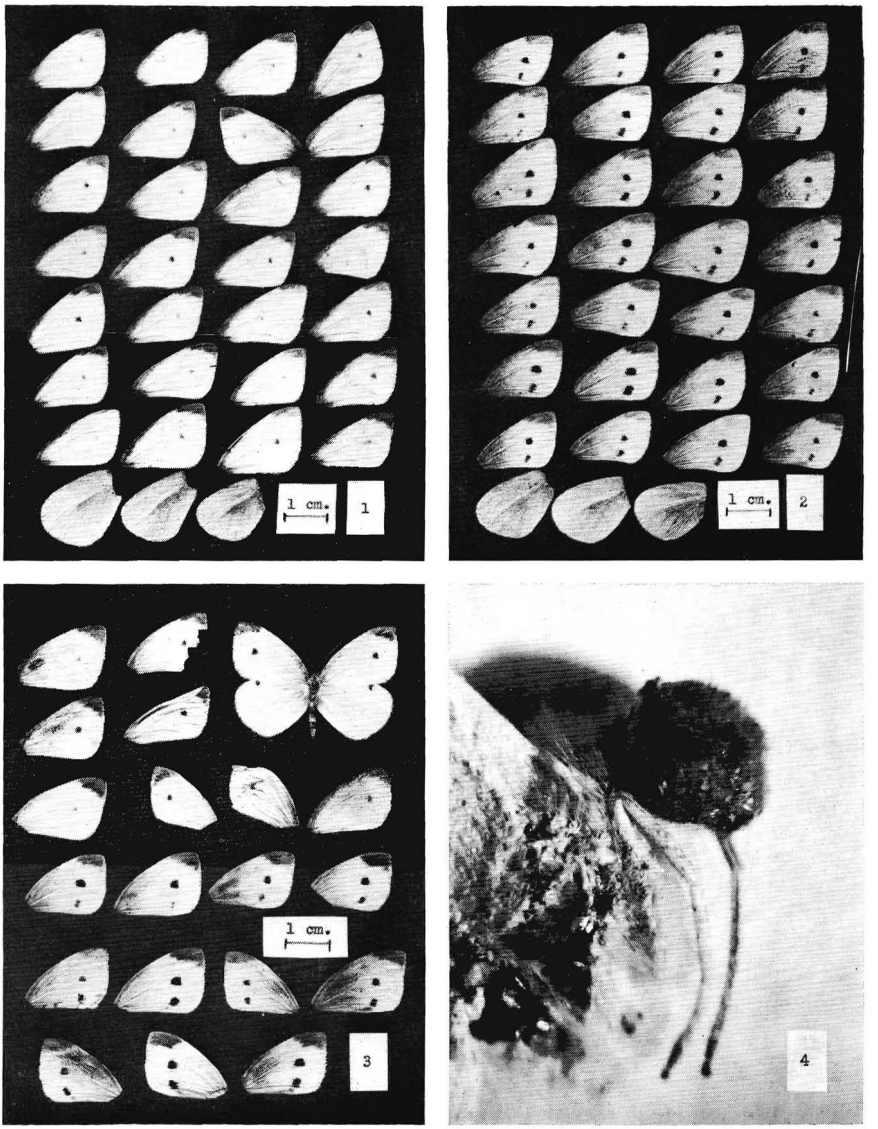

PLATE I

Specimens of *Pieris rapae* (L.). 1, 2, Forewings (and underside of hind wings in bottom rows) of series taken at Morristown, New Jersey, on April 21–27, 1969; 1, ♂ ♂; 2, ♀ ♀; 3, control group (pupae developed at 70–81° F) for Nos. 7–9, Plate II; 4, ♀ from preceding control group with undeveloped head, photographed through 16× microscope.

infections, but no protozoans were found, nor was there evidence of virus, fungi, or nematodes.

Some of the spotted larvae died, or yielded deformed pupae which died, but others gave normal pupae and adults. One adult was sent to Mr. Thomas. Thorough examination of the tissues and blood revealed no microbial etiology; a complete analysis showed no bacteria, protozoans, virus, fungi, or nematodes.

Refrigeration of pupae. In the previous work (Kolyer, 1969), nondiapause pupae were refrigerated (34–39°F) at an age of 12–24 hours from pupation (final molt); after 5 months these pupae were allowed to develop at room temperature and yielded adults with significantly reduced (spring brood) markings.

In the present study, pupae of various ages were refrigerated (32–36°F) for 5 months with the objective of gaining information on the time of determination of the markings. In the meal moth *Ephestia kühniella* (Zeller), and in Lepidoptera in general, the wing pattern is completely determined early in the pupal stage (Magnussen, 1933, and Pohley, 1959). In *P. rapae* pupae the spots have been determined at least by the time of visible deposition of white pigments (pupal age about 135 hours), as shown by the lack of white pigment in the scales within the spot at this time.

Refrigeration results are given in Table 1; two control groups are involved because the larvae had been received from the Department of Agriculture in two separate batches about one month apart. From the 24–34 hour old pupae, eight of twelve normally-expanded males were "immaculata." Refrigerated females tended to lack the apical marking and to have weak spots; in one case both spots and apical marking were almost completely absent (middle wing in 4th row, No. 10, Plate III). Melanization of the underside of the hind wings, as in the spring brood, also occurred (see No. 10, Plate III).

The conclusion is that these nondiapause pupae had to be refrigerated at an age of less than 48 hours to allow suspension of development and eventual eclosion. Also, the lack of reduction of markings on the pupal wings of the 48–56 hour old group (No. 9, Plate II) suggests that the markings have been determined by 48 hours. Concurrent studies indicate that the scales develop at some point between 27 and 87 hours, and it may be that the fate of a scale to be white or black must be decided *before* the scale grows out from the original stem cell. (As described by Lipp (1957), the wing epithelium of the freshly-molted *P. brassicae* pupa is composed exclusively of stem cells which later give the scale and socket arrangement.) Unexpectedly, one male in the 133–143 hour old group managed

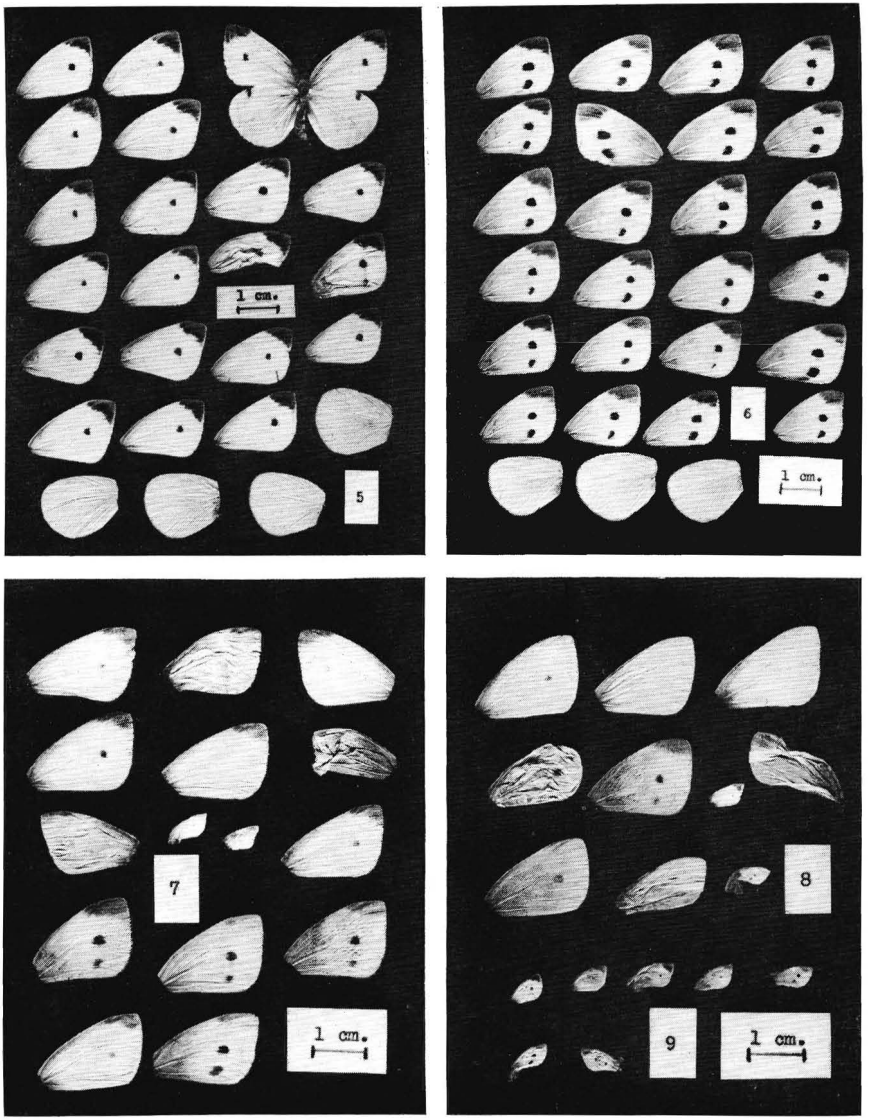

PLATE II

Specimens of *Pieris rapae* (L.). 5, 6, Forewings (and underside of hind wings at bottom) of control group (pupae developed at 70–81° F) for Nos. 10–15, Plate III; 5, ♂ ♂; 6, ♀ ♀; 7–9, forewings of adults from pupae refrigerated at 32–36° F for 5 months, pupae refrigerated at specified time from pupation: 7, 10–18 hours; 8, 24–34 hours; 9, 48–56 hours.

TABLE 1. Data on pupae refrigerated for five months.

Pupal Age (hours) When Refrigerated	Control Group No. in Plates	No. Eclosed[1] Fully Expanded Males[2]	Females	Crumpled	No. Died[3]	Notes
10–18	3	6 (1)	4	3	17	Many dead pupae showed light markings.
24–34	3	3 (2)	2	4	21	Ditto.
24–34	5 & 6	9 (6)	15	7	19	Ditto.
48–56	3	0	0	0	29	Some pupal wings showed markings—essentially like summer brood.
78–88	3	0	0	0	29	—
80–86	5 & 6	0	0	0	57	Most had black wing cases.
103–109	5 & 6	0	0	0	51	Some with black wing cases; 5 showed markings —like summer brood.
120–130	3	0	0	0	31	—
133–143[4]	5 & 6	1 (0)	0	0	48	One with black wing cases; 8 showed markings— like summer brood.
Controls	3, 5 & 6	total of 72		2	1	—

[1] Eclosion was completed within 11 days after removal to rearing room conditions 71–83° F, 50–57% rel. hum.).

[2] The number of "immaculata" form is given in parentheses.

[3] Many died after the pupal wing markings had developed. In the case of several pupae (total of 11 for entire column) the adult split the pupal case but failed to emerge.

[4] The wing cases were white (pigment deposited).

to eclose (No. 11, Plate III); the markings seemed not significantly less intense than those of the control males.

Heat treatment of pupae. A fatal high temperature for butterfly pupae has been said to be 39–42° C (Uvarov, 1931), but Kühn (1936) subjected meal moth pupae of various ages to 45° C for 45 minutes to observe effect on the markings, and Schrader (1929) exposed pupae of *Vanessa carye* (Hübner) to 48° C for "a short time" to cause aberrations.

In the present work, pupae of various ages (from 10 to 128 hours) were exposed to air under various conditions within the limits of 36–48° C and 10–30 minutes. Conditions under which all pupae died were (number of pupae, pupal age in hours, temp. in ° C, time in min.): 6, 10–18, 45.3–48.3, 20; 2, 21–27, 43.8–47.0, 20; 4, 23–39, 43.3–46.6, 30. Among conditions which allowed all pupae to live and yield adults were: 4, 13–18, 36.3–39.1, 30; 7, 19–25, 38.9–45.0, 10; 4, 22–28, 41.6–46.4, 20; 2, 24–32, 41.8–45.3, 25. These

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Specimens of *Pieris rapae* (L.). 10, Forewings (and undersides of hind wings for 2 ♂♂ and 2 ♀♀) of ♂♂ (first 3 rows) and ♀♀ from pupae refrigerated (at 24–34 hours from pupation) at 32–36° F for 5 months; 11, forewing of ♂ from pupa

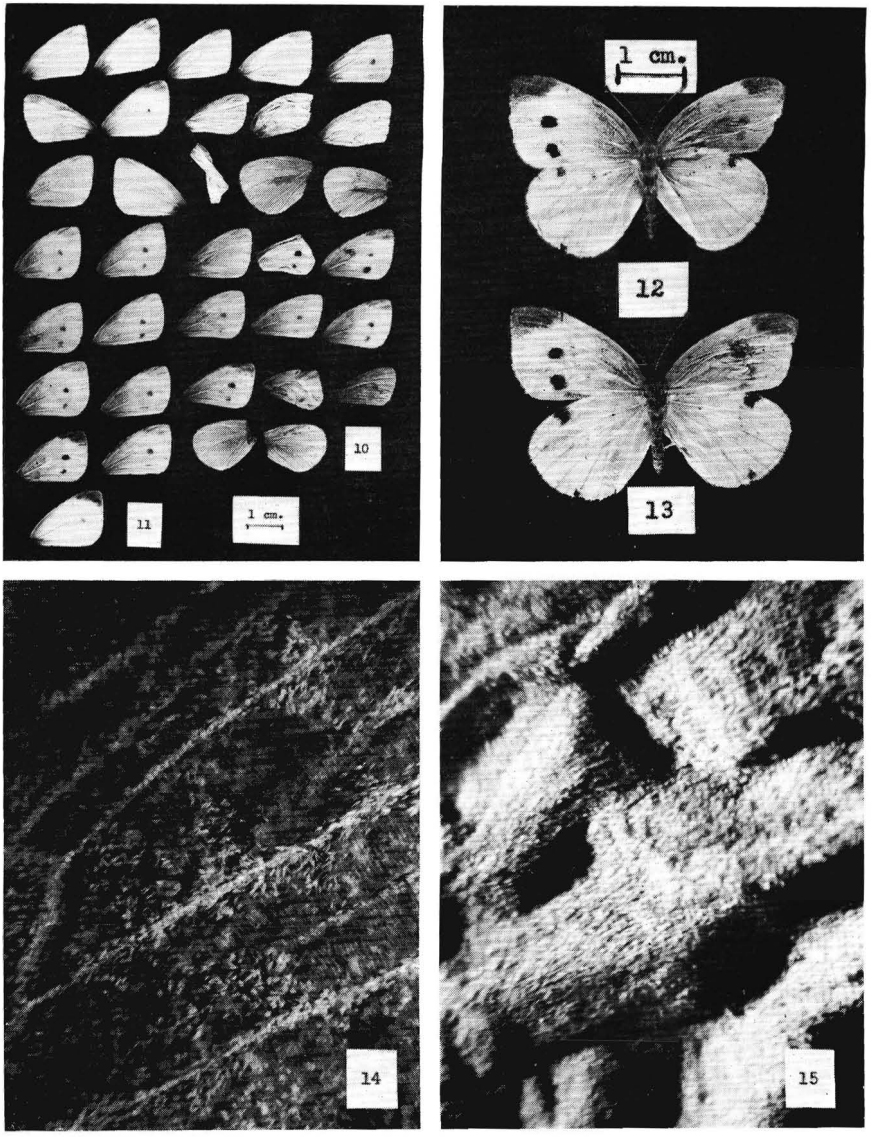

PLATE III

similarly refrigerated at 133–143 hours from pupation; 12, 13, ♀ ♀ from pupae which had right wing cases exposed to UV light (see text); 12, 12 hours exposure; 13, 36 hours exposure; 14, 15, wings of adults from pupae exposed to air at 41.4–47.5° C for 20 min. (see text); 14, largely-scaleless forewing of ♀, photographed through a 16× microscope against a black background; 15, forewing of ♂ with side-lighting to illustrate "bumpy" surface, photographed through 16× microscope.

results suggest that surviving pupae had been exposed to very nearly lethal conditions. However, normal specimens with summer brood markings (like the controls) resulted in almost all instances.

Pronounced structural abnormalities occurred in only one case (conditions: 14, 74–84, 41.4–47.5, 20). Two pupae died, one adult split the pupal case but failed to eclose, 3 pulled partly out of the case, 7 had crumpled wings, and only one (male) expanded normally. One female had particularly wrinkled and distorted wings with a high degree of scalelessness, but the markings (spots) still were visible; No. 14, Plate III, shows the forewing in the region of the spot photographed against a black background. The normally-expanded male had "rough" wings on close examination, due to erection of isolated patches of scales. The other individuals had more or less crumpled wings, in some cases with an unusual "bumpy" appearance though well clothed with scales (as illustrated in No. 15, Plate III).

Dehydration has been suggested to explain changes in meal moth wing pattern caused by heating pupae at about 45° C for 45 minutes (Braun, 1939), but the weight loss for the *P. rapae* pupae heated at 41.4–47.5° C for 20 min. was only 0.3% (14 pupae weighed 2.185 grams before heating, 2.178 grams after heating).

Scale loss as a result of heating pupae has been noted for the meal moth (Kühn and Merkel, 1955). Also, the present results, especially for the female shown in No. 14, Plate III, are reminiscent of the effects, including wrinkling and scalelessness, reported for *Papilio* pupae subjected to beta rays (Kishi, Miwa, and Mori, 1942). In conclusion, heating *P. rapae* pupae failed to change the distribution of melanin but did, in one case, cause considerable structural deformity.

UV irradiation of pupae. Young pupae of the meal moth were irradiated with UV light from a mercury lamp by Köhler (1941) to give disturbances in scale arrangement and transformations in pattern.

Thirty-six *P. rapae* pupae, 50–60 hours old, were placed under the UV lamp as described under "Experimental Procedures" above. Twelve were removed from the light after 2 hours exposure, twelve more after 12 hours total exposure, and the rest after 36 hours total exposure. Results were (exposure time in hours, males expanded normally, females expanded normally, individuals with right wing crumpled, individuals with both wings crumpled): 2, 4, 2, 7, 1; 12, 3, 5, 6, 0; 36, 5, 2, 6, 1. (Of 45 controls, all eclosed, and only 2 had crumpled wings.) The adults eclosed 8–10 days from pupation (conditions: 70–81° F, 28–33% rel. hum.), with the exception of one female which eclosed about 25 days from pupation.

Three of the normally-expanded females had notably asymmetric

markings. Two (Nos. 12 and 13) are shown in Plate III. Examination of the wings under a 16× microscope showed that the scales on the upper surface of the forewing had a shriveled, narrowed appearance. Within the spot the scales were essentially white, the gray appearance of the spot being due to the presence of normally-pigmented black scales within the spot on the lower wing surface, beneath the transparent membrane. In these cases the effect of UV irradiation seemed to be to deform the scales and prevent those scales in the spot from undergoing black pigmentation (melanin formation), a process that begins approximately 25 hours before eclosion.

Summary

The European cabbage butterfly *Pieris rapae* (Linnaeus) has variable black markings which may be much reduced in the spring brood, even to the extent of disappearance of the forewing spot in the male ("immaculata" form). Attempts were made to influence these markings by various treatments of nondiapause pupae originating from larvae reared at 79°F under continuous light.

Refrigeration of 24–34 hour old pupae at 32–36° F for 5 months gave a high proportion (8 of 12) of males of the "immaculata" form and females with much reduced apical markings and weak spots (in one case virtually no markings at all). Reduction in markings also was achieved with 10–18 hour old pupae. However, pupae varying in age from 48 to 143 hours (white pigment appears at about 135 hours) failed to eclose with only one exception (a 133–143 hour old pupa, which yielded a male with unreduced markings), and the markings sometimes visible on the pupal wings were little reduced. This suggests that to affect markings refrigeration must precede outgrowth of the scales from the stem cells of the wing epithelium.

Heating of pupae under barely sublethal conditions failed to cause redistribution of melanin but produced structural deformities in one experiment (74–84 hour old pupae exposed to air at 41–48° C for 20 minutes); the wings were partly scaleless and wrinkled in several cases and were highly deformed and largely scaleless in one individual.

Exposure of 50–60 hour old pupae to UV light (largely 254 millimicrons) caused deformity of the scales in some individuals, with lack of black pigmentation of scales within the forewing spots on the upper, but not the under, surface of the exposed wing.

All these results seem consistent with final determination of the wing pattern early in the pupal stage, as reported for the meal moth *Ephestia kühniella* (Zeller) in intensive studies by Kühn and others.

Acknowledgments

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