Seasonal Polymorphism of the Adult Rice-plant Skipper, *Parnara guttata guttata* (Lepidoptera : Hesperiiidae)¹ and Its Control

Minoru ISHII and Toshitaka HIDAKA

*Department of Zoology, Faculty of Science, Kyoto University,
Sakyo, Kyoto 606, Japan*

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Larvae and pupae of *Parnara guttata guttata* were reared under various temperature and photoperiodic conditions. The adult morphology varied manifoldly with temperature and photoperiodic conditions during larval period. Longer photoperiod induced paler wing coloration and smaller size but independent of each other. Temperature exerted threefold effect: lower temperature inducing larger size, stronger melanization and more conspicuous silver spots on wings. Higher density combined with shorter day seemed to equalize adult size. Because of such complicated control of morphological features, the polymorphism in this species seemed to have different implications, including its role in connection with migration, from well known seasonal forms in other Lepidoptera.

INTRODUCTION

It has already been described by EVANS (1949) that there are two types of the adult rice-plant skipper, *Parnara guttata guttata* BREMER et GREY, with respect to the wing coloration (pale and dark). Recently, HASEGAWA (1975) reported that the present species has two seasonal forms of adult: the yellowish-brown "summer form" and the dark-brown "autumn form" which are supposed to be determined by the daylength during larval stage. As it is well known that adults of the present species migrate in early autumn, the presence of the seasonal dimorphism of adults is an intriguing fact. Larvae and pupae were reared under various temperature and photoperiodic conditions and adults obtained from the rearing were examined morphologically.

MATERIALS AND METHODS

Experiment I. Eggs laid by a female *Parnara guttata guttata* captured near Kyoto University in June, 1976 were divided into 10 groups and reared under the following conditions: 25±1.5°C–24L0D, 16L8D, 15L9D, 14L10D, 13L11D, 12L12D; 20±0.5°C–16L8D, 12L12D; and 16±0.5°C–16L8D, 12L12D. Larvae were reared in

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200 ml plastic cups with 10–12 individuals each and given fresh leaves of Susuki, *Miscanthus sinensis*.

Experiment II. Eggs were obtained from females captured on the campus of Kyoto University in July, 1977. Hatching 1st instar larvae were reared under the following 11 conditions: $28 \pm 0.5^\circ\text{C}$ –16L8D, 12L12D; $24.5 \pm 1.5^\circ\text{C}$ –16L8D, 15L9D, 14L10D, 13L11D, 12L12D; $20 \pm 0.5^\circ\text{C}$ –16L8D, 12L12D; and $16 \pm 0.5^\circ\text{C}$ –16L8D, 12L12D. The light source was a 10-Watt fluorescent lamp. Rearing was done both under solitary (an individual per 200 ml cup) and crowded (12 individuals per cup) conditions. In the crowded rearing, as well as in Experiment I, no larvae were added when some larvae died in the course of rearing. Two species of Enokorogusa, *Setaria* spp., or Inumugi, *Bormus catharticus* (in winter season) were given as the diet.

Experiment III. Photoperiodic conditions were changed from 16L8D to 12L12D or from 12L12D to 16L8D in the course of rearing at 28°C . The change of photoperiod was made on the day of the 1st, 2nd, 3rd and 4th larval ecdysis. Five to ten individuals were kept in each 200 ml cup. Enokorogusa, *Setaria* sp. was given as the diet.

Morphological characters, especially coloration and wing size (expanse and forewing length) of the adults obtained from these rearings were examined. Duration of larval and pupal period and pupal wet weight were also measured to know the relationship to the adult morphology.

Field survey: Feral adults were collected near Kyoto University from May to September in 1977, and their morphological features were examined.

RESULTS

Experiment I

Adults obtained from rearing under various photoperiodic conditions at 25°C seemed to be divided in two groups. One was 12L–13L–14L group and the other

Fig. 1. Adult *Parnara guttata guttata* obtained from rearing under various photoperiodic conditions at 25°C . Top row : female, middle row : male and bottom row : undersurface.

Fig. 2. Relationship between photoperiodic conditions during larval period and adult wing expanse in *Parnara guttata guttata*. Means and 95% confidence limit are indicated.

Fig. 3. Relationship between duration of larval and pupal periods and adult wing expanse in *Parnara guttata guttata*. ○: individuals reared under a photoperiod of 24L or 16L; ●: those under 14L, 13L or 12L; △: those under 15L.

was 15L-16L-24L group. The adults of the former group had darker and larger wings in comparison with those of the latter (Fig. 1).

Wing size (at 25°C). Fig. 2 shows the relationship between photoperiod and the wing size of adults shown in expanse. Although females were always larger than males, the wing size decreased gradually in proportion to the increase in photoperiod within the range between 14L and 16L. Adults from 12L, 13L and 14L rearings seemed to have wings of almost the same size. Wing size of 16L- and 24L-females was nearly equal. Adults from 15L rearing had wing size intermediate of 12L-13L-14L adults and 16L adults. Such differences in wing size were not correlated with the length of larval period (Fig. 3).

Under 12L or 13L condition, some larvae were extremely delayed in growth. Those larvae died without pupation.

Wing coloration (at 25°C). The adults from 15L, 16L and 24L ("long-day=LD") rearings were different from those from 12L, 13L and 14L ("short-day=SD") rearings in their wing coloration (Fig. 1). Although both are brown in ground color,

Fig. 4. The "pale form" (top) and the "dark form" (bottom) adults of *Parnara guttata guttata*. left: upper surface; right: undersurface.

adults from "LD" rearings are paler than those from "SD" rearings. Thus, they can be called "pale form (LD form)" and "dark form (SD form)" respectively. The distinction between these two forms was as follows (Fig. 4):

- i) Ground color of the upper side of the wings: dark-brown in the "dark form" and pale-brown in the "pale form".
- ii) Ground color of the underside of the wings: brown in the "dark form" and yellowish-brown in the "pale form".
- iii) Color of hair on body and wings: dark-brown in the "dark form" and yellowish-brown and more conspicuous in the "pale form".
- iv) Color of folds on the underside of the hind wings: dark-brown in the "dark form" and gray in the "pale form".
- v) Color of marginal cilia scales: in the "dark form", cilia scales of the upper row are pale-yellow while most of the under row are pigmented, while in the "pale form", scales of both upper and under rows are equally pale-yellow. Because of this difference, the cilia on the marginal line appear to be longer and more conspicuous in the "pale form" than in the "dark form".
- vi) Color of the ventral side of the abdomen: dark-brown in the "dark form" and yellowish-brown in the "pale form".

Influence of temperature. The wing size of adults had a tendency to increase with the lowering of rearing temperature. Lower temperature also caused the melanization of ground color of wings and augmented the size and conspicuousness of silver spot patterns on the wings (Fig. 5).

Experiment II

Both in solitary and crowded rearings, effect of photoperiodic conditions on wing

Fig. 5. Adult *Parnara guttata guttata* obtained from rearing at various temperature conditions under a 16L8D photoperiod.

color was similar to that observed in Experiment I: adults from 12L, 13L and 14L rearings had darker wings than those from 15L and 16L rearings and the former and the latter were regarded as the "dark form" and the "pale form" respectively from the morphological characters enumerated above.

Wing size measured as forewing length decreased with increasing daylength in the range of 14 to 16 hours in 24.5°C rearing (Fig. 6). Adults from crowded rearing had shorter wings than those from solitary rearing under every photoperiodic condition (see Fig. 7).

Under 12L or 13L photoperiod, many "retarded larvae" appeared as in Experiment I. The incidence of these "retarded larvae" was about 95% in 12L- and about 60% in 13L- solitary rearings. Some of them reached adults after 3 to 4 months of the larval period. Forewing of these adults was shorter than that of adults from

Fig. 6. Relationship between photoperiodic conditions during larval period and adult forewing length in *Parnara guttata guttata*. Rearing was made at 24.5°C. Means and 95% confidence limit are shown for 14L-, 15L- and 16L-rearings, and raw data are given for 12L- and 13L-rearings. NDL: adults from normally developing larvae; RL: adults from retarded larvae (see text).

Fig. 7. Relationship between pupal wet weight and adult forewing length in *Parnara guttata guttata* in solitary and crowded rearings. ○: individuals reared under a photoperiod of 16L; ●: those under 14L; △: those under 15L.

Fig. 8. Relationship between temperature conditions during larval period and adult forewing length in *Parnara guttata guttata*. Means and 95% confidence limit are shown for 16L rearing and only means for 12L rearing (see text).

Fig. 9. Adult *Parnara guttata guttata* obtained from rearing at various temperature conditions under 16L8D (top row) and 12L12D (bottom row).

normally developing larvae (Fig. 6). This delay in larval development was regarded as a diapause, and will be discussed elsewhere (ISHII and HIDAOKA, unpublished).

Fig. 7 shows the relation between the forewing length and the pupal wet weight as measured within two days after pupation. Both forewing length and pupal weight fluctuated much in the solitary rearings irrespective of photoperiod. In crowded cultures, however, much less fluctuation was observed in rearings except under 16L. Males from 15L rearing were uniform but females were not.

Influence of temperature. Under 16L condition, forewing length increased in accordance with the lowering of rearing temperature (Fig. 8). In 12L rearing, under every temperature condition "retarded larvae" appeared but in varying ratios (about 20% at 28°C, about 95% at 24.5°C, 100% at 20°C and 16°C). Mean forewing length of adults obtained from these 12L rearings are plotted in Fig. 8.

Influence of temperature on the wing color and the silver spot patterns was similar as was observed in Experiment I. Melanization proceeded with lower temperature in both "dark" and "pale" forms (Fig. 9).

Experiment III

When a photoperiodic regimen was switched into another during larval development at 28°C, adult wing coloration seemed to be influenced by the later condition irrespective of the time of switching: As Fig. 10 shows, all larvae which grew under 16L8D condition during later instars produced the "pale form" adults, while those which passed later instars under 12L12D condition developed into the "dark form" adults. Among normally developing larvae, there were some larvae which molted once more than the others and became 6th instar. Whether the final larval instar

s t a g e s						adults obtained	dark form	pale form
I	II	III	IV	V	pupa			
						7	7	0
						5	5	0
						6	6	0
						3	3	0
						3	3	0
						5	0	5
						8	0	8
						2	0	2
						4	0	4
						13	0	13

12 L
 16 L

Fig. 10. Number of "dark" and "pale" forms of *Parnara guttata guttata* obtained from 28°C rearings during which photoperiodic regimen was switched at various stages.

was 5th or 6th, however, was independent of determination of adult forms. The period sensitive to photoperiod for determining two forms is the stage after 4th larval ecdysis and will be the final larval instar.

DISCUSSION

Presence of seasonal dimorphism differing in the wing coloration in *Parnara guttata guttata* was reported by HASEGAWA (1975). He suggested that these two seasonal forms are determined by the daylength during larval period. Our experiments show, however, that dimorphism manifested itself not only in wing coloration but also in wing size, and is controlled by a combination of daylength and temperature conditions during larval period. The mode of determination of these characteristics proved to be complicated. Manifold combination of characteristics renders the species to be polymorphic rather than simply dimorphic.

When the temperature conditions are equal, size and coloration of adult wings are determined independently by the photoperiod during larval stage. The critical daylength at 25°C for the wing coloration seems to lie between 14 and 15 hours. The "pale (LD) form" is induced under daylengths above this, while the "dark (SD) form" is obtained under daylengths below this. The wing size is larger under a photoperiod less than 14 hours and smaller under a photoperiod longer than 16 hours. Under 15 hour-photoperiod, the wing size is intermediate of these two groups. At a temperature of about 25°C, for example, larger "dark form" is induced under the daylength of 14 hours, while under the daylengths exceeding 16 hours, smaller "pale form" appears. Under a daylength of about 15 hours, "pale form" of intermediate size occurs.

In areas of latitude lower than 30°N, maximum daylength does not reach the critical daylength even at the summer solstice. Therefore, populations with the same photoperiodic reaction pattern as in the Kyoto population will produce only the "dark form" throughout the year in such areas.

When the photoperiodic condition is equal, in turn, lower temperatures induce darker and larger wings and make the silver spot patterns on the wings larger and

clearer. Both "pale" and "dark" forms likewise darken with the fall of rearing temperature, and the color contrast between two forms becomes less remarkable under low temperatures. It is difficult to distinguish the two forms at a temperature as low as 16°C.

A low temperature also causes melanization of pupae in the present species as has been reported previously (ISHII, 1977). The adult morphology, however, seems to be independent of pupal coloration.

The rearing density does not seem to influence the wing coloration. Difference in adult wing size between 16L and 14L photoperiods, however, is more marked in crowded cultures than in solitary. In crowded cultures, variation of pupal weight and adult wing size is smaller under 14L condition than under 16L, while in solitary cultures range of variation is not so much different from 14L, 15L and 16L conditions.

Adults of the overwintering generation (spring adults) appear in the middle of May but are very few in number. These adults have dark wings, but careful examination of the color of the cilia seems to show that both "pale" and "dark form" adults are mixed.

Adults of the first generation (summer adults) are commonly encountered in every paddy fields and meadows from the beginning of July until early August. These adults are clearly identified as the "pale forms" by the bright yellowish undersurface of the wings. Near the middle of August, adults of the "dark form" are first found among the "pale form" adults. The "dark form" adults probably belong to the second generation and can be called autumn adults, because they are very fresh. Adults of this type became prevalent by the end of August and are commonly seen until middle September. As the adult morphology is supposed to be determined in the final larval instar (see Fig. 10), these observations of field survey can be well ex-

Fig. 11. Seasonal change of daylength^a and temperature in Kyoto and number of "pale" and "dark form" adults collected in the field. B: beginning; M: middle; E: end.

^a from sunrise to sunset plus civil twilights (BECK, 1968)

plained by the seasonal change of daylength and temperature shown in Fig. 11. Larvae of the first generation grow under the longest daylength of the year, producing adults of the "pale form". That adults of the second generation appear near the middle of August means that the final larval instar has been passed in the beginning of August, when the daylength is a little below 14.5 hours. The adults of the second generation thus become "dark form".

In the case of the overwintering generation, the final larval instar grow at low temperature and under a photoperiod near the critical daylength. This will give rise to adults of either "pale form" or "dark form", but with similarly darkened wings because of the low temperature. The distinction of the two forms is possible only by the color of the cilia.

Determination of the seasonal forms have been studied in some Lepidoptera and the main factor is proved to be the daylength during larval stage. There are many cases where the seasonal polymorphism determination is directly or indirectly associated with diapause. In the European nymphalid, *Araschnia levana*, the daylength determines the seasonal dimorphism by determining diapause or nondiapause of pupae (DANILEVSKII, 1948 cited in 1965: MÜLLER, 1955). In the Japanese nymphalid, *Polygonia c-aureum* (HIDAKA and AIDA, 1963), and the pierid, *Eurema laeta bethesba* (YATA, 1974), the short-day form are regarded as diapausing form from its long life span and slow development of ovaries. There are, however, some instances where the seasonal polymorphism may not be connected with diapause, such as *Ascia monuste*, *Lycaena phlaeas daimio* and others.

The small copper, *Lycaena phlaeas daimio*, passes the winter as diapausing larvae. In this lycaenid, adult seasonal polymorphism determined by daylength is independent of larval diapause induced by a short daylength combined with a low temperature (SAKAI and MASAKI, 1965). In the American pierid, *Ascia monuste*, the adults which have been known to migrate (NIELSEN, 1961 cited in CHAPMAN, 1971), only the female shows seasonal polymorphism determined mainly by daylength during larval stage (PEASE, 1962).

The period sensitive to photoperiod for determining the seasonal polymorphism of adults is suggested to be later larval instars in *P. c-aureum* (HIDAKA and AIDA, 1963) and early larval instars in *L. phlaeas daimio* (SAKAI and MASAKI, 1965).

A high temperature acts in the sense of inhibiting the production of autumn form (HIDAKA and TAKAHASHI, 1967) and under a temperature as high as 30°C, even a short day of 8L16D can not produce the autumn form (FUKUDA and ENDÔ, 1966) in *P. c-aureum*. In *Eurema hecabe mandarina*, a short day of 11L13D fails to make all adults autumn form under a temperature as high as 25°C (YATA, 1974). As these examples indicate high temperatures may have the effect of decreasing the incidence of short-day form adults. In *L. phlaeas daimio*, a high temperature decreases diapause incidence of larvae (SAKAI and MASAKI, 1965). The instance where low temperature darkens adult wings is well known among Lepidoptera.

In the present species, two forms, "pale (LD) form" and "dark (SD) form", are determined by daylength during larval stage. The wing size, which is also subject to the effect of daylength, however, is determined independently of the wing coloration, while in other examples mentioned above, wing coloration, wing form, wing pattern and wing size seem to be determined as a set of features. The period sensitive to daylength for determining wing coloration seems to be the final larval instar.

Although a high temperature decreases incidence of larval diapause as in the

case of *L. phlaeas daimio*, it does not have the effect of decreasing the incidence of induction of the "dark (SD) form" adults. The seasonal form determination in this species is therefore independent of larval diapause induction. Effect of a low temperature on the adult morphology is threefold: it darkens adult wings, makes adult wings larger and silver spot patterns on wings more conspicuous.

Thus, in this species, determination of adult morphology is complicated: photoperiod controls the wing coloration and the wing size, temperature affects the wing size, the wing coloration and wing pattern. The adult morphology varies therefore with every combination of daylength and temperature. Because of this complexity of determination and of rather long critical daylength for determination of wing coloration, the adult of every generation may be morphologically different and the mode of difference may vary with localities. Seasonal polymorphism of adults in this species, therefore, appears not to be equivalent to seasonal forms known in other Lepidoptera.

It is well known that the adults of the present species migrate in early autumn. HIURA (1973) supposes that phase variation by crowd effect as reported in several insects would exist in this species, too, and adults of gregarious phase would migrate. Since the present experiments showed that higher density combined with shorter day seems to equalize adult size in the population, the presence of crowd effect can not quite be denied. As far as adult morphology is concerned, however, influence of rearing density appears to be less strong than that of photoperiod. The key to the solution of this question, therefore, lies most probably in the adult seasonal polymorphism determined by temperature and photoperiodic conditions during larval life.

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