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ENVIRONMENTAL INDUCEMENT OF DIFFERENT COLOURATION IN ADULTS OF *EARIAS INSULANA* BOISDUVAL (LEPIDOPTERA NOCTUIDAE)*

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ABSTRACT

Six distinct colour groups were obtained in adults of *Earias insulana* by the application of winter photo periods and temperatures to a laboratory culture of larvae throughout their developmental period. The colour groups were: greyed brown, greyed orange, greyed yellow, greenish-yellow, green, and greenish blue. The last two groups are typical of summer forms in nature. Clones of pure greyed brown, greyed yellow and green groups could be separated after several generations of intermating of pairs within the groups.
KEY WORDS: colour groups, *Earias insulana*, Lepidoptera, morph induction, Noctuidae, spiny bollworm.

INTRODUCTION

The adults of the spiny bollworm (SBW), *Earias insulana* Boisd. (Lepidoptera: Noctuidae) demonstrate green-brown changes in nature throughout the year (Avidov and Harpaz, 1969). Variation of colours of this type is considered to be adaptation of the insect to its surroundings (Fuzeau-Braesch, 1972). Cotton is planted in Israel from the beginning of April and harvested in September and October. Green colours of SBW predominate in the main growing season of cotton and change to greyish forms when the cotton plants start to dry up (Avidov and Harpaz, 1969; Rivnay, 1962). In the tropics colours vary according to the wet and dry seasons (Couilloud, 1983; Pearson and Maxwell-Darling, 1958).

The critical conditions for colour changes in SBW were investigated in the laboratory (Klein, in press). The possibility of the existence of several genetic colour clones of this species, in response to different temperatures and photoperiods, was tested in this study. Such clones may give some clues on the involvement of internal factors in the production of colours in Lepidoptera.

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MATERIALS AND METHODS

Culturing techniques

The SBW was raised in the laboratory on a semiartificial media (Klein *et al.*, 1981, 1983). In this technique, pupae 3-6 days old were put in ten 500 ml volume glass jars, 50 pupae per jar. Sucrose solution (10% in water) was pipetted on cotton and supplied as adult food just before emergence. The adults mated in these jars and the females laid their eggs on the piece of cheesecloth which covered each jar. The covers were replaced each day to obtain 1-day-old eggs. Covers were immersed in 0.04% formaldehyde solution for 30 min to protect larvae from early infectivity by pathogens. After a short drying period these covers were placed on new, similar volume jars supplied with 250g of larval diet (diet C2MS, Klein *et al.*, 1983) adequate for feeding the SBW throughout the larval stage. After most of the larvae had pupated, pupae were transferred individually into test tubes (10.0 cm in height and 1.2 cm in diam) for observations on the colouration of the adults. Stocks of insects were held in a growing room with a constant temperature of 25°C and 16 h photoperiod, where all adults were green.

Induction of various colours in adults

The description of colour induction is given elsewhere (Klein, in press). Larvae of SBW were grown throughout their entire larval period in an incubator at 30°C for 10 h in the light and at 17°C for 14 h in the dark. Their larval period was extended by more than 10 days by these conditions as compared with the controls held at 25°C. After pupation the insects were transferred to 25°C to shorten the pupal period. Emergence of adults and their colour were recorded almost daily.

Description of colours

The definition of colours is according to the Colour Chart of the Royal Horticultural Society, London.

Colour morphs in the second generation

The effects of different temperature and photoperiod on the coming generations were studied on adults of defined colour form. Five pairs from the same colour group were allowed to mate in each container (500 ml in volume), with three replicates. The neonates of these groups were transferred to the 10L/14D and 30/17°C incubator with enough food for their larval period. Each test was followed by a control from the stock insects. Samples of pupae were treated as above and the colours of the adults were recorded.

RESULTS AND DISCUSSION

Description of the induced colour morphs

The adults which emerged from larvae conditioned at 10L/14D and 30/17°C were of six distinct colour groups:

1) Greyed Brown (199 B, RHS Colour Chart) – Moths had a dark brown appearance due to the brown scales covering their forewings, mesoscutum and head. Greyed brown, darker than that of most of the moth's background, distinguished four transverse lines on the forewings from the remaining areas.

TABLE 1. INDUCTION OF COLOUR MORPHS IN ADULTS OF *EARIAS INSULANA* BY DIFFERENT TEMPERATURES AND 10L/14D PHOTOPERIOD

Test No.	Parent source		Resultant colours, (in parentheses, %)					
	Generation	Colour	Greyish-brown	Greyed orange	Greyed yellow	Greenish-yellow	Green	Greenish-blue
1	I	Green	69 (79.2)	10 (11.5)	8 (9.1)	2 (2.2)	0 (0)	0 (0)
2	I	Green	24 (23.7)	43 (42.6)	12 (11.9)	18 (17.8)	4 (3.9)	0 (0)
3	I	Green	60 (67.4)	15 (16.7)	14 (15.9)	0	0	0
4	I	Green	46 (49.5)	21 (21.5)	15 (16.2)	11 (11.8)	0	0
5	II	Green*	0 (0)	4 (8)	10 (20)	2 (4)	32 (64)	2 (4)
6	II	Green	0 (0)	2 (4)	13 (25.5)	14 (27.5)	22 (43)	1 (2)
7	II	Green	9 (18)	17 (14)	6 (12)	9 (18)	19 (38)	0 (0)
8	II	Greenish-yellow	19 (25)	21 (27.6)	20 (26.3)	7 (9.2)	9 (11.9)	0 (0)
9	II	Greyed yellow	68 (77.3)	14 (15.9)	6 (6.8)	---	---	---
10	II	Greyed brown	58 (100)	---	---	---	---	---
11	II	Greyed brown	54 (95)	2 (3.3)	1 (1.7)	---	---	---

*Some of the color groups are missing because not enough insects of this group were available for the tests.

2) Greyed Orange (163 B, RHS Colour Chart) – Background colouration was uniformly bronze. Two transverse lines and most of the mesoscutum were greyed brown. The margin of the forewing at the posterior side was usually distinct, with a brighter colour than the bronze.
3) Greyed Yellow (161 A, RHS Colour Chart) – Most of the dorsal side of the moth was greyed yellow. Two greenish yellow areas could be observed on the forewing, one situated between the two first transversal lines and the other on the margin.
4) Greenish Yellow (144 B, RHS Colour Chart) – Most of the dorsum area was coloured with a mixture of green and yellow. The mesoscutum was greyed yellow. Usually the whole margin of the forewings could be observed easily. Thin grey or yellow lines generally connected the last transversal line with the margin.
5) Green (143 A, RHS Colour Chart) – Background was deep green. The margin of the forewings close to the mesoscutum was bright green or yellow. The transverse lines were deeper in colour than the surroundings and could be only with difficulty observed. The central area in the mesoscutum of most moths in this group had a darker green colour than the wings.
6) Greenish Blue (124 A, RHS Colour Chart) – Most of the naturally bred individuals in August and September had this typical colour too. The forewings were light blue, while the crossing lines and the mesoscutum were dark blue. The upper margins of the forewings could be observed easily, having a bright blue colour in most of the moths in this group raised in the laboratory.

Previously, only the typical green form and the brown winter form of SBW were observed in biological studies (Avidov and Harpaz, 1969; Couilloud, 1983; Pearson and Maxwell Darling, 1958; Sorey, 1914; Yathom, 1956). The green adults are prevalent during the cotton growing season, while the brown ones start to appear at the end of the season, and in Israel can be called the winter form. This situation was not dependent on the food consumed by the larvae [*e.g.* cotton (Storey, 1914), *Hibiscus mutabilis* (Yathom, 1956), semi-artificial media (Klein, unpublished data)]. Mostly brown males were caught in Israel by pheromone traps installed in winter in 1981 and 1982 (Klein, unpublished data). The catch of several green males by this trap indicates that the natural population is not genetically homogeneous for colour. The source of insects for the laboratory cultures was a batch of puparium collected in heavy infested cotton fields in several different areas in Israel more than 10 years ago. It was therefore expected that the laboratory population would not react uniformly to "winter" temperatures and photoperiod. Also during the past 10 years of rearing SBW on semi-artificial media in the laboratory, several individuals underwent colour mutations (Klein, unpublished data) and this may add to the variation of the colour groups.

Table 1 summarizes the results of colour induction tests. While tests No. 1-4 show the distribution of colour groups following the first induction, those of No. 5-11 describe the results in the second generation of intermating in the same colour groups.

Unfortunately, only very few adults of the greenish blue group could be obtained at one time and we therefore had to neglect this group for further investigations. In order to supply enough material of the green group, it was necessary to conduct a series of similar tests at the same time and collect from different batches as test No. 2 (Table 1). We did not test further the greyed orange due to its proximity to the greyed yellow group. The easiest clone to be obtained was the greyed brown "winter form" and in the F_2 almost 100% of the offspring were of this colour (tests no. 10 and 11, Table 1). Adults of the fourth generation of pure greyed brown parents all returned to the summer form of green and greenish blue colours when maintained as larvae at a constant temperature of 25°C. At the tenth generation, when raised at 30/17°C, 10L/14D daily, all the emerging adults were greyed brown. The greyed yellow group was inclined to produce at the beginning more of the greyed brown (77.3% test No. 9) and it took five generations until a pure line of this colour group was obtained and maintained for several additional generations in the same phase. The three greyed forms dominated in the greenish yellow group in the second generation (test No. 8). The greenish yellow group was problematic because we could not fully separate it from the green and greyed yellow groups (test No. 8) even after five generations, at which time we obtained more than 50% of the parental group (data not given). The green groups seemed to be more resistant to the winter conditions. More dramatic induction could not change it into the grey groups (Klein, unpublished data) as the 17°C was critical for the development of the larvae.

Fuzeau-Braesch (1972) emphasized that: "Change of forms from green to brown or *vice versa* are always associated directly or indirectly with the life cycle of the animals which harmonize their colours with those of their surroundings." We believe that the same applies to the SBW. This insect is oligophagous; in summer in Israel its larvae feed mainly on malvaceous plants. It does not enter a larval diapause and survives in nature as greyed brown adults in winter. The relatively low temperatures prevailing at night in winter in Israel do not allow its mating (Klein, unpublished data). The exact hiding places in winter are not known. It is only an assumption that it is easier for the grey forms to camouflage themselves in cracks of bared trees or on posts in the fields than for the green forms, which are at an advantage in summer when many leaves of their feeding host plants, having colours similar to their bodies are available. Similar behavior was noted also in the moth *E. biplaga* (Couilloud, 1983) and in only one tortricid, *Acleris minuta* (Weatherby and Hart, 1984). It is therefore still very difficult to draw any conclusions as to the significance of the colour morphs in these insects.

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