J. comp. Physiol. 115, 185–193 (1977)

Journal of Comparative Physiology · B

Morphological Colour Change: Stage Independent, Optically Induced Ommochrome Synthesis in Larvae of the Stick Insect, *Carausius morosus* Br.

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Received November 17, 1976

Summary. The eyes of 4th, 5th, and 6th instar larvae of the stick insect, *Carausius morosus*, were partially covered with black varnish, and the ommochrome content of treated and untreated animals was determined throughout the instar. In untreated animals kept at 20 °C in 16:8 LD the ommochrome content of the integument remains at the level found in newly hatched larvae (10 µg/animal), up to the third moult, and increases only slightly (to 30 µg) until the adult moult. Darkening the dorsal halves of the eyes does not influence the ommochrome content. Darkening the ventral halves, thus imitating the visual situation of an animal in light on a dark ground, causes after a lag of 3–4 days, a continuous increase of ommochrome content by about 5 µg per day. The increase is equal at the beginning and during the second half of the 4th instar, and is also equal in the three instars investigated. Ommine and xanthommatine equally contribute to the increase.

Introduction

In *Carausius morosus* the body pigmentation is affected by visual stimulation of the compound eyes. A certain light and dark distribution, imitated by darkening the ventral halves of the eyes, causes an increase of ommochrome content in the integument (Bückmann and Dustmann, 1962; Dustmann, 1964). This situation resembles that of an animal in light on a dark background. It is assumed that this ommochrome synthesis is under hormonal control (Berthold, 1973; Bückmann, 1974).

In order to explore the physiological mechanism by which the eyes can influence pigment synthesis, the time course of ommochrome increase after the beginning of the effective visual situation, the amount of ommochrome formed, its individual variation and its relation to instar and to the phase of the moulting cycle are investigated.

In the previous studies the effective visual situation was continued through

several instars to achieve a clearly defined difference in ommochrome content between experimental and control animals. The total amount of ommochrome formed during this time was extracted from large batches of animals. Thus it remained unclear, during which period the ommochrome had really been synthesized, how soon after darkening the ventral halves of the eyes the ommochrome synthesis started, how long it lasted, and to which extent it varied between individual animals.

It is expecially interesting to know whether ommochrome synthesis can be evoked at any time and in any instar, or only directly after a moult, and whether the response becomes weaker in the later instars of larval development, as suspected by Dustmann (1964).

A dependence of the optically controlled ommochrome formation on the number of the instar or on the phase of the moulting cycle would imply that the hormones controlling moulting and development, ecdysone and juvenile hormone, are also involved directly or indirectly in the control of ommochrome synthesis. Independence, on the other hand, would mean that this process is a true colour change comparable to a physiological colour change, and not simply a colour adaptation which is determined during a short sensible period and which cannot be reversed. Such colour adaptation occurs in several lepidopterous pupae (cf. Bückmann, 1974).

Dustmann (1964) observed a considerable variation of body colour in individual animals grouped under identical conditions. Therefore, an estimate of individual variation in ommochrome synthesis is a prerequisite for further physiological studies in which the number of experimental individuals is limited. In preliminary experiments on the time course of optically controlled ommochrome synthesis (Bückmann, 1974) the results indicated large variations and possibly transient decreases of ommochrome content. A degradation of ommochrome is, however, unlikely in *Carausius* (Dustmann, 1964). Most probably such apparent decrease of ommochrome content during development is a result of large individual variation within the samples.

In the present study the ommochrome contents of samples taken at regular intervals after treatment, are compared with each other and with untreated animals in the following sets of experiments: a) darkening the dorsal halves vs. darkening the ventral halves of the eyes; b) darkening the ventral halves of the eyes at the beginning vs. the middle of the 4th instar; c) darkening the ventral halves of the eyes in the 4th, vs. the 5th, and 6th larval instar. In the latter experiment an improved method allowed the measurement of ommochrome content in individual animals.

Material and Methods

Experimental and control animals were reared ab ovo and kept at 20 °C in 16:8 LD. The ventral halves of the eyes were darkened by applying black varnish (Zaponlack) on the first day after a moult, except of one experimental group in which the dorsal halves of the eyes were darkened, and one group which was treated on the 10th day of the instar (see below). Samples were taken daily until the 10th day after treatment, and thereafter every second day until the end of the instar.

In the experiments a) to c) batches of 10 animals were extracted. Usually three such samples were analysed each day (for exceptions, see Figs. 1–3). In series d) six individual animals, both in the experimental and control groups, were analysed per day. In treated animals we made sure that the black varnishing of the eyes was still in position.

Animals were decapitated and the guts removed. The remaining carcass was lyophilized. In order to remove carotenoids and pteridines the material was extracted exhaustively with acetone and methanol. Ommochromes were extracted with HCl-methanol (0.05 N). Ommine and xanthommatine were separated on columns of SE-Sephadex (Umebachi and Uchida, 1970). In later experiments the ommochromes of single animals were separated on columns of SP-Sephadex (Stratakis, 1976a). Specific absorbance of xanthommatin in 0.5 N HCl solution was found to be $E^{1\%}_{1cm}=292$ at $\lambda_{max}=460$–462 nm. Ommine yielded a value of $E^{1\%}_{1cm}=139$ in 0.5 N NaOH solution at $\lambda_{max}=505$ nm (Stratakis, 1976b).

Statistical significance was tested by Student's *t*-test.

Results

1. The Effects of Dorsal and of Ventral Darkening of the Eyes during the 4th Instar

The eyes were covered with black varnish on the ventral or on the dorsal halves one day after the third moult. The change of ommochrome content is shown in Figure 1. Animals with the dorsal halves of their eyes darkened, did not differ from untreated animals at any time. Darkening of the ventral halves of the eyes, however, caused the ommochrome content to increase. The difference becomes apparent 5 days after treatment (Fig. 1), however, the difference between animals with ventrally masked eyes and those with dorsally masked eyes as well as untreated controls is significant ($p<0.05$) only from the 12th day onwards (except on days on which only one sample had been taken).

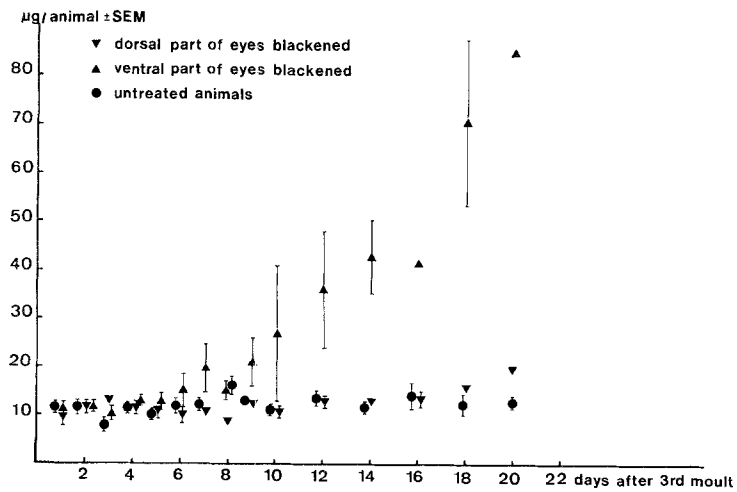

Fig. 1. *Carausius morosus*, 4th larval instar. Ommochrome content of the integument after darkening the dorsal or the ventral halves of the eyes on the 1st day of the instar, and of untreated control animals. Mean values of 1–3 samples in the experimental animals, of 3–5 samples in the untreated controls (each sample consisting of 10 larvae) ± S.E.M.

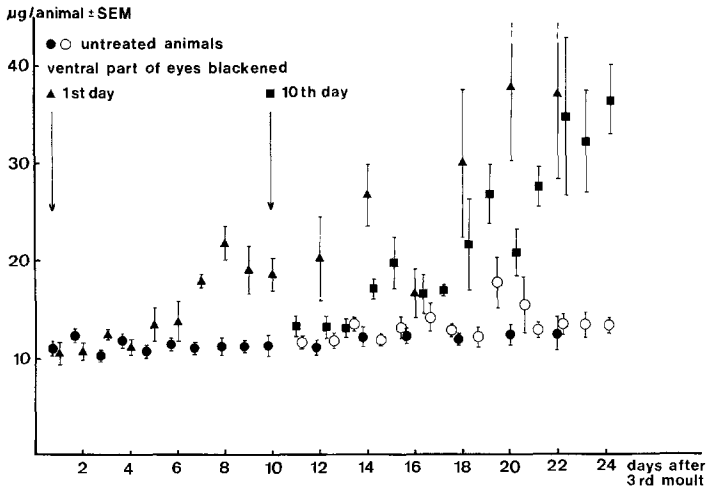

Fig. 2. *Carausius morosus*, 4th larval instar. Ommochrome content of the integument after covering the ventral halves of the eyes on the 1st, and on the 10th day, and of untreated controls. Mean values of 3 samples (10 animals each) ± S.E.M.

2. The Effect of Ventral Darkening of the Eyes at the Beginning and in the Second Half of the 4th Instar

Under our rearing conditions the 4th instar lasted between 16 and 26 days, the average being about 20 days.

Larvae with eyes blackened ventrally on the first day after the 3rd moult, were compared to larvae treated on the 10th day, which is about the middle of the instar. Figure 2 shows that the ommochrome content in both groups increases in a similar way and at the same rate.

The difference between control animals and animals treated on the first day is significant ($p < 0.05$) at the 3rd day and from the 6th day onwards (except days 12 and 16). On 5 of these 9 days the differences are even highly significant ($p < 0.01$). In larvae treated 10 days after the moult, $p < 0.05$ on the 4th, 7th, 8th, 9th, 10th day after the treatment, and on 2 of these days $p < 0.01$.

Calculating ommochrome content on the basis of fresh weight leads to essentially the same result. The number of days in which the difference to the untreated animals is significant or highly significant is one day more in the first group, and 2 days more in the second group, compared to the data of the absolute ommochrome content. This is due to the slower increase of body weight in the experimental animals (s. p. 191).

3. The Effect of Ventral Masking of the Eyes in Different Larval Instars, and an Estimate of Individual Variation in Ommochrome Synthesis

In this part of the study, the 4th, 5th and 6th instars were compared. These are the three last larval instars preceding the imaginal moult. In this series

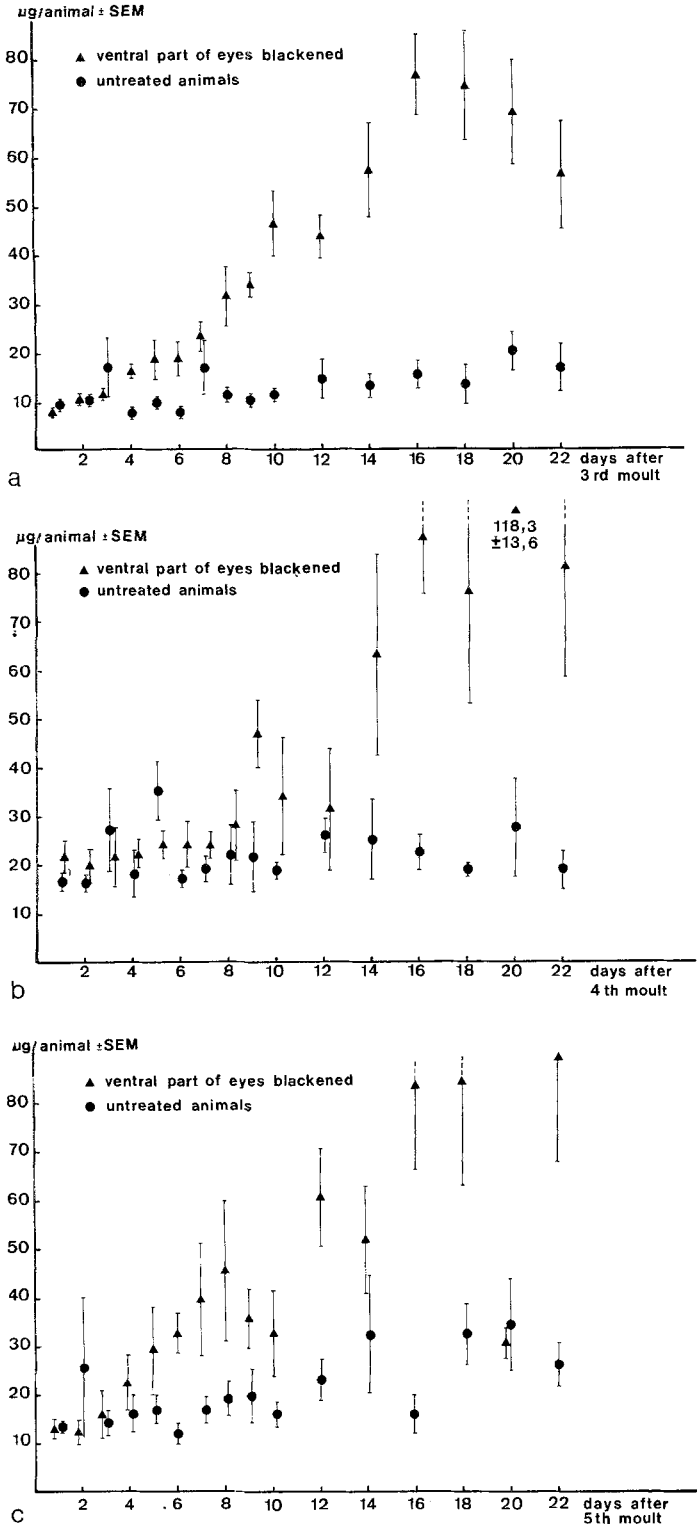

Fig. 3a–c. *Carausius morosus*, 4th, 5th and 6th larval instar. Ommochrome content of the integument after darkening the ventral halves of the eyes on the first day of the instar, and of untreated controls. Mean values of 6 animals assayed individually, ±S.E.M. Level of significance: **a** 4th instar: $p < 0.01$ from day 4 onwards, except day 5 and 22 ($p < 0.05$) and day 7 ($p > 0.05$). **b** 5th instar: $p < 0.01$ on days 16, 20 and 22, $p < 0.05$ on days 9, 17 and 18. **c** 6th instar $p < 0.01$ on days 6, 12 to 18, $p < 0.05$ on day 22

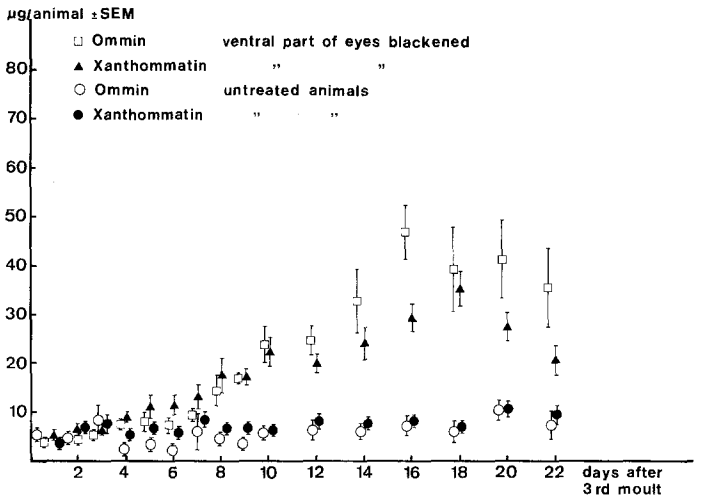

Fig. 4. *Carausius morosus*, 4th larval instar. Content of xanthommatine and of ommine in the integument after darkening the ventral halves of the eyes, and of untreated controls. Each point represents the mean of 6 animals (same as in Fig. 3a), assayed individually, ±S.E.M.

the ommochrome content of individual larvae could be determined (Fig. 3). At the beginning of the 4th instar, one day after the 3rd moult, the integument of an untreated animal contains about 10 µg of ommochrome. This is the same amount as in young larvae just hatched (Stratakis, 1976b). During the last three larval instars this amount increases only slightly in untreated animals to about 30 µg per animal at the end of the 6th (final) larval instar.

If the eyes are masked ventrally on the first day after a moult, about the same absolute amount of ommochrome is formed in each instar. Ommochrome content increases to about 80–110 µg per animal. Contrary to a suggestion by Dustmann (1964), the increase in ommochrome is somewhat stronger in the later instars. However, since individual variation is larger in the later instars, the differences to untreated animals are more frequently significant in the 4th instar than in the 5th and 6th instars (Fig. 3).

Occasional transient decreases of ommochrome content (Fig. 3) are not significant. This is true even for the apparently similar decrease at the end of the three instars.

The data indicate an almost linear increase in ommochrome content from the 3rd to the 16th day after masking the ventral halves of the eyes. During this time the animals synthesized about 5 µg of ommochrome per day, on the average. The similar increase in terms of absolute ommochrome content during the three successive instars implies that increase in relation to body weight is less in the later instars.

4. The Contribution of Ommine and Xanthommatine

Figure 4 shows the time course of ommine and xanthommatine content, respectively, in 4th instar experimental and control animals. Both pigments appear

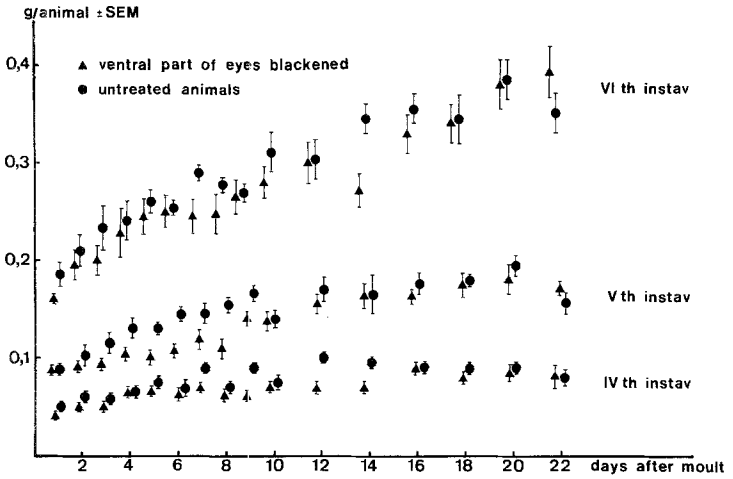

Fig. 5. *Carausius morosus*, 4th, 5th and 6th larval instar. Fresh weight after darkening the ventral halves of the eyes. Each point represents the mean of 6 animals (g/animal) ± S.E.M.

in about the same amounts and increase at the same rate. There is no difference in the other instars investigated. An evaluation of all other series of experiments for ommine and xanthommatine separately, yields the same result. Since a clean separation of xanthommatine and ommine is not routinely achieved, occasionally some xanthommatine will be measured along with the ommine fraction, or vice versa. For this reason, a comparison of total ommochrome in different series should give the most reliable picture of the actual development of ommochrome content.

The increase of xanthommatine seems to start a little earlier than that of the ommine (Fig. 4). Later on, however, ommine increases slightly faster than xanthommatine. A similar result was obtained in all other experiments. It causes the quotient ommine/xanthommatine, in the experimental animals, to rise towards the end of the instar. Such a situation was already suspected by Dustmann (1964).

5. Growth of Treated and Untreated Animals

In untreated animals the increase of body weight (Fig. 5) is surprisingly low during the 4th instar, and only little larger in the following instar. On about the 16th day after the moult the weight decreases again. At this time evidently more and more animals stop feeding to prepare for the following moult. At the same time the increase in ommochrome content ceases. In all three instars studied, the body weight of the experimental animals is generally (between days 4 and 14) a little lower than in the controls.

This difference, though small, is statistically significant ($p < 0.05$) on 4 days of the 4th instar, 5 days of the 5th instar, and 3 days of the 6th instar, and highly significant ($p < 0.01$) in a few cases.

This shows that growth is somewhat retarded in the animals with partially blackened eyes. As a result the differences in ommochrome content per unit of body weight, between experimental and control animals, are more clearly expressed than the differences in absolute values.

Discussion

A true physiological colour change caused by pigment movements is rare among insects. Changes or variations of pigment content, controlled by external conditions are found more frequently. They are, however, in most species restricted to certain developmental stages. This may be related to the notion that in insects most developmental changes are matched to gross changes in environmental conditions (cf. Bückmann, 1974). E.g. the pigmentation of certain lepidopterous pupae is irreversibly determined by environmental factors, such as optical conditions, during a certain sensible period in the larva. This should be termed a colour adaptation, since no change of colouration can occur during the pupal stage itself. A true morphological colour change, as a true physiological colour change (for nomenclature, cf. Bückmann, 1974), should be inducible by external factors independently of the phase of the moulting cycle, and should be reversible. The suggestion that optically controlled ommochrome synthesis in *Carausius* is restricted to the period immediately after a moult, and that it is dependent on the number of larval instar (Dustmann, 1964) has caused doubts whether a true colour change could occur in this species.

The results of this study show, however, that ommochrome synthesis as a consequence of visual stimuli is feasible during the second half of any larval instar as well as during the first half, and that it takes the same course during the successive larval instars studied. Therefore, this is a case of a genuine, stage independent morphological colour change, controlled by external factors. According to Dustmann (1964) it is, however, reversible only in so far as the animals can turn green again only by "dilution" of integumental ommochromes, the ommochrome content remaining constant during further growth.

The conclusion drawn by Dustmann (1964), that ommochromes once formed in the integument of *Carausius* are never removed again, is supported by the finding that larvae at the beginning of the 4th instar still contain the same amount of ommochrome as after hatching.

Contrary to the embryo within the egg shells, the feeding larva can dispose of excess tryptophan in the form of cynurenic acid in the faeces (Berthold and Bückmann, 1975). – On the other hand tryptophan intake evidently does not determine the magnitude of ommochrome synthesis. Otherwise one would expect the larger 5th and 6th instar animals with a higher food intake to form greater amounts of ommochrome. Relative ommochrome content (per unit body weight) should then be the same in all three instars, rather than absolute ommochrome content. Unexpectedly, the latter is the case.

Due to the great individual variation of ommochrome content, conclusions pertaining to the time course of ommochrome synthesis may be drawn only cautiously. Preliminary experiments (Bückmann, 1974) indicated that ommo-

chrome deposition in the integument begins after a lag period of about 5 days, following exposure to visual contrast stimulation. The present results indicate a shorter lag period, ommochrome synthesis starting only 3–4 days after treatment (Fig. 3a, c). Xanthommatine increases slightly faster than ommine during the first three days (Fig. 4) but is surpassed by ommine content at a later stage. For about 14 days, ommochrome content increases at a constant rate of 5 µg per day. The period of increase ends when feeding is stopped, about three days before the next moult. If the visual contrast stimulation is continued during the following instar, the animals are evidently able to continue ommochrome synthesis. This is shown by the large amounts of ommochrome formed in animals which are treated anew after each moult through several instars (Bückmann and Dustmann, 1962; Dustmann, 1964).

The independence of optically induced ommochrome synthesis of moulting rhythm would indicate that it is also independent of the ecdysone/juvenile hormone system, which controls moulting and metamorphosis. Histological investigations and transplantation experiments indicate that pigmentation is under neurosecretory control (Raabe, 1966). – Whether the retarded growth of the treated animals is caused by the effect of increased ommochrome synthesis or is caused in another way awaits further study.

The availability of new methods for estimation of ommochromes (Stratakis, 1976a) makes it possible to analyse individual specimens and to detect changes of ommochrome content in relatively small experimental groups and after a short period of optical stimulation. This is prerequisite to further studies of morphological colour change and optical control of pigment synthesis.

This investigation was supported by the Deutsche Forschungsgemeinschaft, SFB 87, Projekt A1. The author wishes to thank Ms. Ute Möschler for her assistance, and Miss E. Schreiber and Miss G. Frank for help in rearing the animals and in the ommochrome measurements.

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