Further Investigations On Colour Change In Orthoptera

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Özet: Bu travay, müellifin daha evvel neşrettiği (1951,1953) araştırmaların bir devamıdır. Tecrübeler 1952 ve 1953 yaz ve sonbahar mevsimlerinde *Acrida anatolica* Dirsh denilen çekirge türü üzerinde yapılmıştır. Metod evvelki araştırmalrda olduğu gibidir.

Birinci ve ikinci larva safhasından itibaren karanlıkta beslenen yeşil ve sarı (veya gri) *Acrida*'lar renk değiştirir. Renk değişmesi besine bağlıdır. Aydınlık ve karanlıkta elde edilen yüzde nispetleri yekdiğerine çok yakındır.

Renkli kafeslerde büyütülen larvalar arasında bir fark görülmemiştir. Bu kafeslerdeki yeşil ve sarı fert nisbetleri aydınlık ve karanlıkta elde edilen nisbetlerin takriben aynıdır.

Hararetin renk değişmesi üzerine yaptığı tesir araştırılmıştır. 21° ve daha düşük ısıda kırmızı-menekşe renkte bir pigment teşekkül ederek, kısmî bir renk değişikliği görülür. Buna mukabil 28° ve daha yüksek hararette bu pigment meydana gelmez. Ayni pigment larvalar erginleştikten sonrada görülür. Bu pigmetin teşekkülü tabiatta da incelenmiştir.

Deri histo-kimyasal olarak araştırılmıştır. Dondurucu mikrotomla yapılan kesitlerlerde, epidermisi dolduran kahve rengi pigment granüllerinin insektorubin-protein kompleksinden teşekkül ettiği tespit edilmiştir.

Kırmızımtrak ve menekşe rengi endokutikulada bulunan ayni renkte diffuz bir pigment meydana getirir. Kimyasal bakımdan bu pigmentin indirgenmiş serbest (yani proteine bağlı olmayan) insektorubin olduğu gösterilmiştir. Erginde veya alçak hararette büyütülen larvada, epidermiste bulunan insektorubin proteinden ayrılarak endokutikulaya geçer ve kısmî renk değişikliğini meydana getirir.

Acrida'nın pigment metabolizması *Schistocerca* ile muukayese edilmiştir.

It was shown by the author in earlier papers that the appearance and disappearance of green pigment, or more exactly those of its blue component, in a certain number of species of Orthroptera depend on the food[18),19)]. The phytophagous hoppers reared on growing green grass become green, while those bred on more or less dry grass do not develop this pigment. Green background is without effect on the formation of blue component. Hoppers of *Locusta* may become green in darkness on fresh grass.

It has been reported by a number of authors that Orthoptera hoppers may adapt themselves to yellow, orange, brown, grey, reddish and violet backgrounds [4], [6], [7], [8].

These observations suggest that it may be a double adaptation in Orthoptera : (i) a nutritional adaptation to green colour, (ii) a background adaptation to non-green colours. Experiments were performed during the summers 1952 and 1953 with *Acrida anatolica* Dirsh (*) to study this problem.

1) Material and Method

Although it has yet not been possible to hatch the eggs of this species in our laboratory, the number of its nymphal instars is studied by rearing it from the first stage. It is found that this species has five nymphal insars. These insars are rather similar to those of *Locusta* wich are described by Dirsh[5]. The stage of a hopper collected in the field may easily be determined according to the subgential plate if it is a male, or to the ovipositor valves if it is a female.

First and second instar hoppers which are found at the end of June or early in July, were used for the experiments. They were followed until the adult stage. The hoppers who died before the pre-adult instar were not taken into account. Breeding experiments in darkness and in coloured cages were effected out of doors. The cages were exactly similar to those used in the previous experiments [19]. The food was only *Triticum repens.*

2) Experiments in darkness.

It is easy to breed *Acrida* in darkness. The experiment lasted about two and a half months. All the hoppers were kept until the adult instars under 20 × 20 × 20 cm cages on which were put metal cases to assure absolute darkness. At the end of the experiments they were looking quite normal and eggs were obtained later from the females.

A) Growing green grass.

The grass was moistened before putting the cages on it and their place was changed at least once a weak, because the grass was no longer fresh. The changing of place is effected within one or two minutes.

(*) Its ancient name is *Acrida turitta* L.

a) Green hoppers.

Hoppers tested	60
» becoming yellow (or grey) ...	6
» remaining green	54
Percentage remaining green	90

b) Yellow or grey hoppers.

Hoppers tested	66
» becoming green	49
» remaining yellow (or grey) ...	17
Percentage becoming green	74.2

B) Cut grass.

The hoppers received cut grass which was changed every three or four days.

a) Green hoppers.

Hoppers tested	52
» becoming yellow (or grey) ...	32
» remaining green	20
Percentage becoming yellow..	61.5

These results indicate that the colour change from green to yellow or grey is perfectly possible in darkness. It is noteworthy that the hoppers which lost their green pigment in darkness were not identical among themselves. As in nature, some were yellowish and some greyish. It gave the impression that they were returning to their original colour

b) Yellow or grey hoppers.

Hoppers tested	56
» becoming green	0
» remaining yellow	56
Percentage becoming green	nil

These results obtained in darkness do not differ much from those obtained previously in light, [18], [19], as tabulated below.

Table I

Growing green grass		Percentage
Green Hoppers	Remaining green in light	78 (Okay 1953)
	» » in darkness	90
Yellow Hoppers	Becoming green in light	69.8 (» »)
	» » in darkness...	74.2
Cut grass		**Percentage**
Green Hoppers	Becoming yellow (or grey) in light ...	71.1 (Okay 1953)
	» » » in darkness	61.5
Yellow Hoppers	Becoming green in light	nil (» »)
	» » in darkness	nil

3) Experiments on orange, red and violet backgraunds.

The cages used for this purpose were similar to those used for the previous experiments. Orange, red and violet-coloured nettings were put around the cages and their bottoms were covered with a corresponding crêpe-paper. In each case half of the cages were put directly on growing grass. As usual, the place was changed when the grass was no longer fresh. The other half received cut grass which was changed every three or four days. The experiments were continued until the adult stage. As the hoppers reared on these three backgrounds had no differences between themselves, the results will be given together, as shown below (Table II).

These results do not differ much from those obtained previously on growing green and cut grasses in light (Okay 1953) and in darkness (present paper). The examination of the Tables I and II suggests the following conclusions: (a) The majority of the hoppers — ranging from 69.8 to 90 % — remain or become green when they are reared on growing green grass. (b) On more or less dry grass the majority of the hoppers — ranging from 61.5 to 83.3 % — remain or become yellow or grey. (c) Similar results are obtained in darkness or on coloured backgraunds (orange, red and violet).

Table II.

Growing green grass (orange, red and violet backgrounds)		
Hoppers tested	Hoppers remaining or becoming green	Percentage
69 (green)	51 (remaining green)	81.1
51 (yellow or grey)	45 (becoming green)	88.2
Cut grass (orange, red and violet backgrounds)		
Hoppers tested	Hoppers becoming yellow or green	Percentage
54 (green)	45 (becoming yellow)	83.3
51 (yellow or grey)	51 (becoming green)	nil

In addition to the main colour change from green to yellow or grey and *vice versa*, a partial colour change occurs especially in pre-adult and adult individuals. During breeding experiments reddish or violet areas appear especially on the underparts of the abdomen and thorax in many fifth-stage and also in some younger male or female non-green hoppers. The upper part of the body may become partly violet in some fifth-stage green hoppers, especially in darkness. In a few cases the antennae, the supra-anal plate and femora of the hind legs may also be violet in green and yellow individuals. It is noteworthy that the fifth-stage hoppers with reddish or violat pigmentation cast off sometimes partly reddish coloured exuviae.

At adult emergence a violet colour appears on the upper parts of the abdomen and thorax in all green and yellow or grey *Acrida* (Pl. I, *Fig. 1*). The pigmentation of the underpart of the body becomes more distinct at this stage in many yellow or grey individuals, but it is always less dark than on the upper part of the body. In green adults the underparts of the abdo-

men and thorax become partly reddish-violet. All the pigmentation described above becomes darker and wider at maturity.

4) Observations from nature

During cage experiments, observations were carried out on more than two hundreds youg and old male or female hoppers and adults collected in the field.

Except for the violet colour which is seen in some reared fifth-stage green hoppers on the upper parts of the abdomen and thorax, especially in darkness, the green hoppers and adults collected in the field were similar to those bred in coloured cages or in darkness. The violet colour which appears at adult emergence on the upper part of the body, darkens and widens at maturity whether the individual is cage bred or naturel (Pl. I, *Fig. 1*).

The reddish or violet areas were not conspicuous in non-green hoppers, even in fifth-stage, collected in the field. The adults, also, had very little reddish or violet pigment on the underparts of their body. The upper parts of the abdomen and thorax were similar to those of green adults.

5) Influence of temperature on colour change in *Acrida*.

The reason for appearance of a reddish or violet colour in both sexes, especially on underpart of the body, in cage reared yellow or grey hoppers and adults was not understood by the author during the experiments 1952.

The following year some of the hoppers were bred in cages heated with a bulb. It was soon observed that the pigmentation described above does not appear in these hoppers.

The following results were obtained by rearing the yellow or grey hoppers and adults in different temperatures :

(I) The hoppers bred at 21° and below develop reddish or violet areas, especially, on the underpart of the body. The general aspect of these hoppers is slightly darker than that of those reared at high temperature. The darker hoppers possess more melanin spots in the cuticle (see below).

(II) The hoppers reared at 28° and over do not develop the pigmentation mentioned above. Their general aspect is slightly paler than that of those reared at lower temperature. The paler hoppers have less melanin spots than the darker ones.

(III) Individuals, which have developed reddish or violet

areas at lower temperature lose a large part of this pigmentation within a few days after they are moved to a higher temperature This may occur at any stage including the adult instar. The adult obtained at higher temperature possess the reddish-violet pigment which is seen on upper part of the body, although in a reduced area and at much less intensity than in those bred at lower temperature (Pl. I, *Fig. 2*).

6) Histochemical study of the integument

(I) Transverse sections were carried out with a freezing microtome through the reddish or violet areas of the integument in green and yellow or grey individuals. The epidermis was filled with brown granules and often contained relatively big globules of the same colour *(Fig. 3)*. But wherever the integument

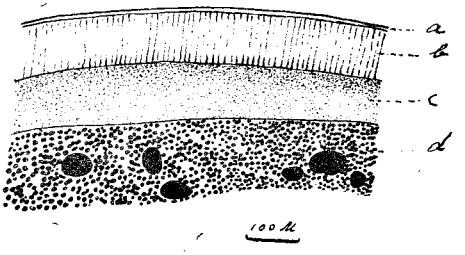

Fig. 3. Section through the violet integument of a mature female *Acrida*.

a) epicuticle; *b*) exocuticle; *c*) endocuticle, containing diffure violet pigment; *d*) epidermis, containing granules and globules of insectorubin

is reddish or violet the cuticle has a very unusual aspect. Whereas the exocuticle is free of pigment, the endocuticle is filled with a reddish-violet continucus or diffuse pigment. The pigment appears dark violet on the limit of the endo-and exocuticle, due probably to a higher concentration of pigment. There is no doubt that the colour of reddish or violet areas is due to an endocuticular pigment. Where the integument has not this colour the endocuticle is free of pigment. But its underlying epidermis is, as usual, filled with brown granules. It is noteworthy that the brown pigment does not much affect the colour of the integument. However the paler individuals which are obtained at higher temperature have clearly less pigment in the epidermis than the darker ones gained at lower temperature.

The hoppers and adults of *Acrida* possess little melanin pigment in the cuticle. This pigment exists generally at the basis of the bristles and gives a spotted appearance to the integument *(Fig. 4)*. The grey individuals contain more melanin

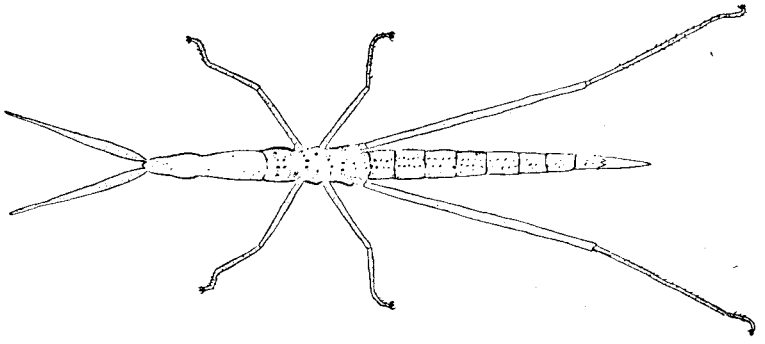

Fig. 4. Fifth-instar grey female *Acrida* seen from ventral side showing the melanin spots. (The reddish-violet areas are not designed)

spots than the yellow ones. The number of these spots is greater, as already mentioned, in the hoppers and adults reared at lower temperature and gives a slightly darker aspect to them.

(II) Pieces of integument containing reddish or violet areas in adults or fifth-stage hoppers are separated carefully from the surrounding tissues and then treated with ether to remove carotenoids. The pieces are then extracted either with methanol containing 3 % (*v*/*v*) of conc. HCl or dilute alkali (0.1-n NaOH). The whole pigment, including the cuticular one, dissolves rather quickly in these solvents. The extracts in both solvents have an orange colour. If they are reduced by sodium hydrosulphite ($Na_2S_2O_4$), they become wine-red. These properties are those of the redox pigment present in insects (acridioxanthin, ommochrome, insectorubin) [1, 2, 3, 4, 9]. The presence of the redox pigment has already been described in *Acrida* by the present author[17].

Tha sodium hydrosulphite may be used on section of integument for the recognition of the redox pigment. A tiny crystal of this salt, after being dissolved in the water surrounding the section, reduces the brown granules in the epidermis and gives a wine-red colour. The reddish-violet diffuse pigment in the endocuticle remains unchanged.

If the sections are treated with acid-ethanol or methanol the reddish or violet pigment of the endocuticle dissolves quickly, whereas most of the brown granules in the epidermis dissappear, producing an orange diffuse pigment. So the insectorubin-protein complex, which forms the brown granules, is disrupted by both solvents. The free insectorubin turns wine-red when it is reduced by $Na_2S_2O_4$. The granules may first be reduced and then distrupted. The same diffuse wine-red pigment is obtained.

If a green or yellow mature adult or fifth-stage hopper is extracted with acid-methanol after removal of its internal organs and carotenoids, the whole insect becomes wine-red with a violet tinge (In green *Acrida*, the green pigment is destroyed before it turns wine-red). The wine-red pigment dissolves completely producing an orange coloured solution. The solvent removes all colour, including that of the areas which were originally reddish-violet.

If an insect is taken out from the solvent just after it becomes red with a violet tinge and sections are made with a freezing microtome through an area which originally was not reddish or violet, one can see that the epidermis still contains some brown granules whereas the endocuticle is filled everywhere with a continuous pink-violet pigment like that in the reddish-violet areas of the living insect. The colour change is thus due in the treated insect to the liberation of the insectorubin from its protein carrier. Once detached, the pigment may penetrate into the endocuticle in its reduced form. It then dissolves in the solvent in its oxidized form with an orange colour.

It may be concluded from this histochemical study that the pigment of the reddish or violet areas in the living insect and the red pigment with a violet tinge of the treated animal is a reduced free insectorubin which penetrates into the endocuticle.

7) Discussion

The author has already shown that when hoppers of *Locusta* are reared on growing grass from hatching in darkness they may grow up as green individuals, whereas blackish, buff and brown individuals were obtained in darkness on cut grass [19].

Green and non-green *Tryphaena* larvae (Lepidoptera) (*) were already obtained in darkness [21]. The nymphs of *Dixippus* which are kept from hatching in complete darkness may become pure green individuals [16]. Recently Jovancic obtained colour change from brown to green or *vice versa* with *Mantis* in complete darkness varying the temperature and humidity of the environment [14].

Rearing experiments in the present paper with *Acrida* indicate that a colour change from green to yellow and grey or *vice versa* is easily effected in darkness according to the freshness of the grass. This observation completes the author's earlier investigations [19] and shows, moreover, that not only the green but the yellow or grey colours are not due to background.

Ergene's claim [6, 7] that the appearance of a reddish or violet pigmentation is due to background cannot be accepted. Rearing experiments in orange, red and violet cages of the present author gave exactly similar results to those obtained earlier [18, 19] and in darkness. The colour change from green to yellow and grey or *vice versa* occurs everywhere according to the freshness of the grass.

The appearance of reddish or violet areas, especially on underpart of the yellow or grey hoppers (and in the antannae, femora of hind legs and supra-anal plate of green hoppers) is due to a low breeding temperature. If the hoppers are reared at 28° and above they do not develop any reddish or violet pigment. Adults emerging from these hoppers possess few reddish—violet pigment on the upper part of the body.

The hoppers collected in the field do not possess this colour which appears after emergence. If the hoppers are reared at 21° or below they develop a reddish-violet colour, especially on underpart of the body. The appearence of the same pigment in hoppers reared in cages covered with a red or violet netting or in darkness, cannot be attributed to another factor than to a difference of temperature which must exist between that in the field under the sun and that in a continuous shadow.

The number of melanin spots is greater at lower temperature, and is less at higher temperature. But this has only a slight effect

(*) According to Junge [15], Becker [2], Goodwin and Srisukh [9] the green and brown pigments are identical in Orthopera and Lepidoptera.

on the colorotion. The influence of temperature on melanin formation is especially observed in *Melanoplus* [20] in *Schistocerca* and *Locusta* [12, 13]. These species become dark at low temperature and light coloured at high temperature.

The histochemical study of the reddish and violet areas in the integument of the hoppers and adults shows that the epidermis is filled with brown granules of insectorubin-protein complex. The colour of the reddish and violet areas is due to a liberated insectorubin from its protein carrier. The reduced free insectorubin penetrates into the endocuticle and forms a continuous reddish or violet pigment. The overall red colour with a violet tinge of the treated insect with acid-methanol is due to the detached insectorubin which penetrates into the endocuticle as in the living insect.

Goodwin and Srisukh found by solvent treatment in *Schistocerca* phase *gregaria* that the overall pink colour is due to the presence of free insectorubin [10]. *Acrida* and *Schistocerca* are, thus, similar in regard to their insectorubin metabolism. In both species the insectorubin-protein complex is disrupted after adult emergence. But while the reddish-violet areas are held until the death in *Acrida*, the pink colour disappears on reaching sexual maturity in *Schistocerca*. It would be interesting to know whether the free pigment penetrates into the endocuticle in the last species.

What is particularly interesting in *Acrida* is that the insectorubin—protein complex may be disrupted in hoppers, especially on underpart of the body, when they are reared at low temperature. The penetration of the reduced free insectorubin in the endocuticle causes a partial colour change.

It is known in *Locusta* and *Schistocerca* hoppers that the amount of insectorubin-protein complex increases when they are bred at low temperature [11]. No quantitative study is made in *Acrida* at different temperatures. But judged solely by the examination of the sections in paler and darker hoppers, one may think that the amount of brown pigment increases at low temperature.

A propable increased production of insectrubin-protein complex from one hand and its disruption from other at low temperature may perhaps be considered as an adaptation of the hoppers to the environmental temperature.

8) Summary

1 — *Acrida* hoppers may change themselves from green to yellow and grey or *vice versa* in complete darkness according to the freshness of the grass.

2 — Hoppers bred in orange, red and violet cages are identical among themselves. They may either be green or yellow (and grey) according to the food.

3 — The appearence of reddish and violet areas in the hoppers is due to low temperature. The same pigment appears after emergence in all individuals.

4 — The histochemical study of the integument shows that the pigment of the reddish-violet areas is a reduced free insectorubin which is produced by the disruption of insectorubin—protein complex granules in the epidermis. The free insectorubin penetrates into the endocuticle producing a partial colour change.

5 — This partial colour change is compared with that in *Schistocerca*.

Acknowledgments

The author wishes to thank Dr. B. P. Uvarov, D. Sc., F.R.S., for reading the manuscript and giving his advice and criticism.

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(Manuscript received on November 24, 1953)

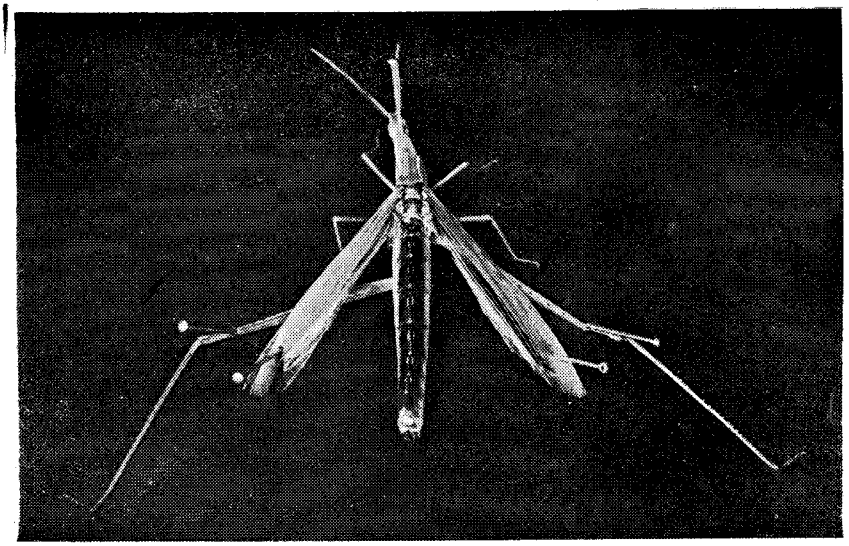

Fig. I. A mature green female *Acrida* schowing the reddish-violet area on the upper part of the body.

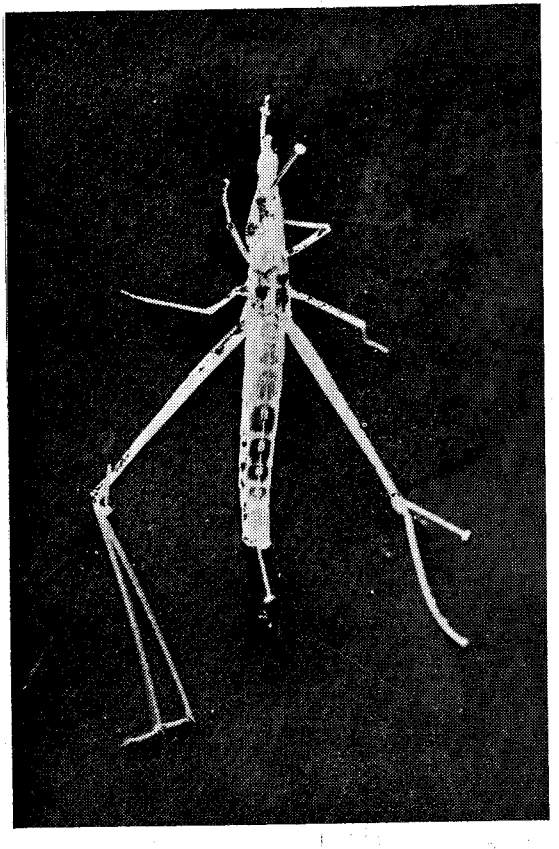

Fig. 2. A mature yellow female *Acrida* bred at 30°. The reddish-violet area on the upper part of the body is greatly reduced. The insect is paler than those reared at lower temperature.
(Fore and hind wings are removed after emergence)

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Table des Matières