FURTHER OBSERVATIONS ON DIRECTIONAL CHANGES IN LOCUSTS AND OTHER SHORT-HORNED GRASSHOPPERS (INSECTA: ORTHOPTERA: ACRIDIDAE), AND THE IMPORTANCE OF THE THIRD INSTAR.

(With one Text-figure.)

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Contents.

I—Introduction.

It was shown some years ago (Roonwal, 1938, 1940) that locusts and other short-horned grasshoppers (Orthoptera: family Acrididae) undergo in or about the third instar a series of extraordinary changes in respect of morphological, behaviouristic and physiological conditions, often resulting in a peculiar 'directional reversal'. As these changes are centred around the third instar hopper in the 5-moult species, the exceptional importance of that instar in the life-history of these grasshoppers was emphasised. The number of moults (excluding the 'intermediate moult') and the normal number of nymphal instars in the Acrididae varies from 4 to 7 depending upon the species (see Roonwal, 1946), but 5 is the most common number.

In the present paper I have discussed the new material assembled during the last 10 years, which has a bearing on the subject of directional reversal and the importance of the third or middle instar. In particular, I have discussed the data of Webley (1951) on the blood-cell counts, and of myself (Roonwal, 1946, 1947) on eye-stripes and extra-moulting in relation to the reversal of the elytron-wing complex. Some new data on the number of antennal segments are also discussed.

As a result of this new information, the importance of the third instar has been confirmed. As Webley (1951, p. 35) recently stated in discussing his work on blood-cell counts:

'Roonwal (1940) has shown the importance of the third instar in the Acrididae. The blood-cell counts of the present work give a further physiological indication of its importance.'

While it is now clear that great metabolic changes are taking place in the third instar, we are still far from understanding their biological significance, especially in regard to the directional changes.

II—Examples of Changes.

1. *Blood-cell counts.*

Recently, Webley (1951), from a study of the blood-cell counts in the African Migratory Locust, *Locusta migratoria migratorioides* R. & F., which is normally a 5-moult species, has confirmed the importance of the third instar. He counted the number of blood-cells per cubic millimetre in the various instars and in the adults of varying ages. He found that in both males and females the number of cells in the hopper stages showed a sudden and great increase in the middle of the third instar from an initial figure of the order of about 6,000–7,000 to about 10,000–13,000 per cu. mm., and thereafter dropped rapidly to approximately the initial figure (Table I).

TABLE I.

Average blood-cell counts and weights of nymphal stages and adults of Locusta migratoria migratorioides *R. & F.*

(*From* the data of Webley, 1951.)

Abbreviations.—N, newly emerged; M, middle of instar; E, end of instar.

Instar.	Age.	Males.		Females.	
		Weight (gms.).	No. of blood-cells per cu. mm.	Weight (gms.).	No. of blood-cells per cu. mm.
2nd ..	....	.....	6,834 cells (sex not known)		
3rd ..	N	0·074–0·097	6,336	0·83–0·144	7,029
	M	0·098–0·122	13,352	0·145–0·206	10,420
	E	0·123–0·148	11,091	....	....
4th ..	N	0·118–0·219	6,861	0·125–0·287	7,112
	M	0·220–0·321	6,992	0·288–0·450	7,670
	E	0·322–0·426	7,768	0·451–0·616	9,359
5th ..	N	0·331–0·583	5,675	0·362–0·650	7,597
	M	0·584–0·836	8,208	0·651–0·939	5,491
	E	0·837–1·09	8,729	0·940–1·23	7,202
Imago (adult).	Newly eclosed.	0·77	4,682	1,343	6,044
	25-26 days old.	1·31	21,378	2·6	14,760
	60–70 days old.	1·41	22,312	2·2	9,445

These results show first, that the third instar is physiologically very active, and secondly, that there is a directional reversal in this activity. Discussing these results, Webley wrote (p. 29):

'The third instar was peculiar in showing a very large increase in all counts. This may be associated with the profound metabolic changes taking place at that time, including the great growth and turning of the wing rudiments (Roonwal, 1940)'.

Mathur and Soni (1937) studied the blood-cell counts in the Desert Locust, *Schistocerca gregaria* (Forskål). Although their data (Table II) are less complete than those of Webley (1951), it is clear that the greatest increase occurs, as in *Locusta*, in the third instar, the blood-cell count in that instar being 203% higher than in the immediately preceding or second instar hopper. Between the other consecutive instars the differences vary from 77% to 167%.

TABLE II.

Blood-cell counts of the Desert Locust, Schistocerca gregaria (*Forsk.*).
(*From* the data of Mathur and Soni, 1937.)

Stage.	Average number of blood-cells per cu. mm.	Percentage increase.
1st stage hopper	220	....
2nd ,, ,, ...	390	77%
3rd ,, ,, ...	1,180	203%
4th ,, ,, ...	3,000	154%
5th ,, ,, ...	5,000	167%
Young adults (sex not stated) ..	6,300	126%

It will thus be seen that in both *Locusta* and *Schistocerca* the relative increase in the number of blood-cells per unit volume is the greatest in the third stage. However, in the absolute number of blood-cells per unit volume, the results in the two species differ from each other. Whereas in *Locusta* the absolute number among hoppers is the highest in the third stage (Table I), in *Schistocerca* there is a progressive increase up to the adult stage.

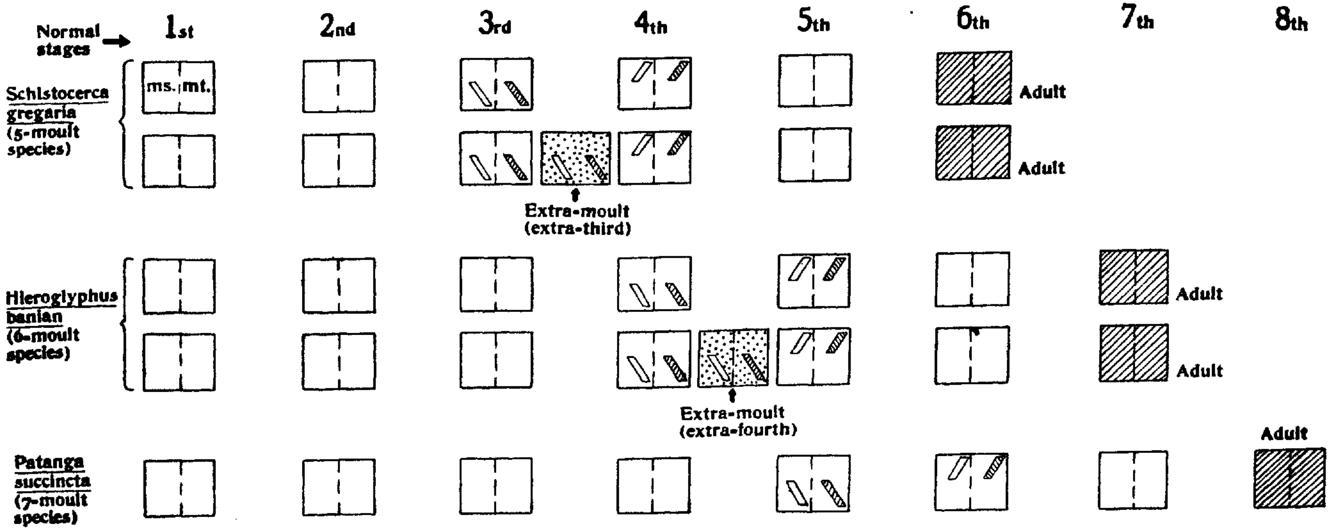
2. *Extra-moulting, and its correlation with elytron-wing complex and eye-stripes* (Text-fig. 1).

As already mentioned above in the Introduction, the normal number of moults in the Acrididae varies with the species from 4 to 7. Within a species itself, the number may vary, and in addition to the number normal to that species frequently one, or more rarely two, extra-moults may occur. More rarely still, under-moulting may occur, as in the 5-eye-striped individuals of *Schistocerca gregaria* and in *Poecilocerus pictus* (*vide* Roonwal, 1946).

The occurrence of the extra-moult in the third stage in *Schistocerca gregaria*, where the normal number of moults is 5, was already emphasised by Mathur (1938) and by Roonwal (1940, p. 142). More detailed studies (Roonwal, 1946, pp. 79-80; and 1947, p. 254) in that same insect showed that out of 215 hoppers, each reared in isolation, 43 or 20% extra-moulted. Of these 43 extra-moulting hoppers, 37 (or 86%) extra-moulted in the third stage (*i.e.*, at the third chronological moult), 2 (or 4·7%) in the fourth stage, 3 (or 7%) in the third and fourth stages (2 extra-moults), and 1 (or 2·3%) in the second and fourth stages (2 extra-moults). In several other 5-moult species, the extra-moult generally occurs in the third stage. It will thus be seen that, from the point of view of extra-moulting, the third instar is the most important. Data for species in which the normal number of moults is more than 5 are scanty; but in the 6-moult *Hieroglyphus banian* the extra-moult occurs in the fourth stage.

In all the Acrididae the elytron- and wing-rudiments undergo an upturning or directional reversal in one of the hopper stages. This stage is the moult between the third and fourth stages in individuals of those species which undergo a 5-moult or 6-stage (including the adult) development, as in *Schistocerca gregaria*. The correlation between this directional change on the one hand and normal- and extra-moulting on the other, has been discussed in detail by Roonwal (1946), and two generalised rules (Rules 1 and 2) which govern this correlation formulated. The rules are as follows:—

Rule 1.—If in a species the directional reversal of the elytron-wing complex normally takes place in the nth stage, then the morphological stage which usually represents the extra-instar (in individuals in which only one such instar occurs) is $n-1$,



TEXT-FIG. 1.

Diagrammatic representation of the meso- and metathoracic regions, in side-view, of the various post-embryonic developmental stages in the Acrididae, to show the chronological sequence of the upturning of the elytron- and wing-rudiments (directional reversal of the elytron-wing complex) and its relationship to the normal number of moults (5–7) in the species on the one hand, and to extra-moulting on the other. For the sake of simplicity, the elytron- and wing-rudiments are shown only in the stages when the upturning of the elytron-wing complex occurs. In the earlier stages the eltyron- and wing-rudiments (which first make their appearance in the second stage) point downwards, and in the later stages upwards.

Rule 2.—If in a species the total number of normal stages (including the adult, but excluding the vermiform larva) is t, then the chronological stage in which the directional reversal of the elytron-wing complex usually occurs is $t-2$; and, in accordance with Rule 1, the stage which usually represents the extra-instars (in individuals in which only one such instar occurs) is $[(t-2)-1]$.

Data available later have supported these rules. Thus, in the Moroccan Locust, *Dociostaurus maroccanus* (Thunb.), Jannone (1939) has reported 5 moults, and the reversal or upturning of the elytron-wing complex occurs at the third moult, *i.e.*, when hoppers moult from the third into the fourth stage, as in *Schistocerca gregaria*. Again, in *Hieroglyphus nigrorepletus* (Bol.) recently studied by me (Roonwal, 1952, in press), the number of moults is 6, and the directional reversal of the elytron-wing complex occurs in the fifth stage.

A generalised diagram illustrating Rule 2 is given in Text-fig. 1. The bearing of these rules on the importance of the third instar is discussed below (*vide* Discussion and Conclusions).

The limited correlation which exists between extra-moulting and the number of eye-stripes added during post-embryonic development has been discussed below. Here it needs only to be emphasised that the third instar is exceptional both in normal and extra-moulting forms in the 5-moult species. The same is true, to a certain extent, with regard to the antennal segments (*vide* below).

3. *Eye-stripes, dorsal spot of eye and antennal segments.*

(i) *Eye-stripes.*

It is now well known that the Desert Locust, *Schistocerca gregaria* (Forskål), possesses brownish, vertical eye-stripes which in the *gregaria* phase individuals are partly obscured owing to the heavy development of dark pigment in the inter-stripe region. Roonwal (1936) has shown that the *solitaria* phase individuals are of two types, *viz.*, 6- and 7-eye-striped. A study of the post-embryonic development of the eye-stripes has shown (Roonwal, 1937, 1947) the existence of features which are of interest for our present discussion. Basically, the development runs along the following pattern:—There is no stripe in the freshly emerged first instar hopper, but one develops later in that instar. With every subsequent moult a stripe is added so that there are 2 stripes in the second, 3 in the third, 4 in the fourth, 5 in the fifth, and 6 in the sixth (or adult) stages. This pattern occurs in the 6-eye-striped individuals which undergo the normal number of 5 moults. In the 7-eye-striped individuals, the one-moult-one-stripe relationship holds good in most stages, but the extra seventh stripe is produced in two ways (Roonwal, 1937, 1947): (i) by the addition of *two* stripes at the second moult (*i.e.*, the third stage hopper has *four* stripes instead of the normal three); and (ii) by the interposition of an extra-moult in the third stage (and rarely in the fourth) during which a new eye-stripe is added (stripe-positive extra-moult). But extra-moulting does not necessarily lead to the addition of a stripe; stripe-neutral extra-moults occasionally take place.

The third instar is exceptional in two respects, *viz.*, (i) that in about one-half to three-quarters of the 7-eye-striped individuals, the extra or seventh stripe (as compared to 6-striped individuals) is produced by the addition of a stripe-positive extra-moult usually in the third stage; (ii) in the remainder of the 7-striped individuals, the third stage hopper possesses four, instead of the usual three eye-stripes, the extra stripe being added without the intervention of an extra-moult.

(ii) *Dorsal spot of eye.*

At the dorsal end of the compound eyes in the Desert Locust, *Schistocerca gregaria* (Forskål), and several other (probably all) Acrididae, there lies, nearer the posterior than the anterior side, a small dark chocolate-coloured area shaped somewhat like the head of a hammer, with the base pointing towards the anterior side and the narrower end towards the posterior (Roonwal, 1947, pp. 248-249, Figs. 1 and 2). This has been termed the *dorsal spot* by Roonwal (1936). Its post-embryonic development has been studied by Roonwal (1947, p. 256). In the freshly-hatched first stage hopper the base of this sub-triangular hammer-shaped dorsal spot points posteriorly, a condition which is maintained in the second and third stages. When the hopper moults from the third into the fourth stage, there is a *directional reversal* of the base which now points antero-dorsally. The new orientation is maintained in the fifth stage and the adult.

This directional reversal of the dorsal spot of the eye is comparable to the similar reversal of the elytron-wing complex occurring in the same stage, and emphasises the importance of the third instar.

(iii) *Antennal segments.*

It is well known that the number of antennal segments in the Acrididae increases during post-embryonic development from about 8–14 in the first stage hopper to about 25–30 in the adult.

In *Schistocerca gregaria* (Rao, 1938; Mukerji and Batra, 1938) there are 13 antennal segments in the first stage hopper, 19 in the second stage, 20-21 in the third, 22-23 in the fourth, 24-25 in the fifth, and finally 26-27 in the sixth or adult stage with 6 eye-stripes. In the 7-eye-striped adults the number is 27–29, in the 8-striped adults 30, and in the 5-striped adults 25, the increase or decrease in the number being explicable to a certain extent by extra-moulting and under-moulting respectively. It will be noticed, as has already been pointed out by me (Roonwal, 1947, p. 255, foot-note), that, as in the case of the eye-stripes, the second moult and the resulting third stage here also are exceptional. Two kinds of individuals are produced in the third stage: one with 20 and another with 21 antennal segments. This condition applies alike to the 6- and 7-eye-striped individuals, although ultimately the 7-eye-striped adults possess more antennal segments than 6-striped ones, this excess resulting from additions made later as a result of an extra-moult.

In *Hieroglyphus nigrorepletus* Bol. (Roonwal, 1952, in press) the increase in the number of antennal segments in the various stages occurs as follows:—I, 13; II, 14; III, 18–20 (rarely 16–20); IV, 21–23 (mostly 21, and rarely 16); V, 23–25 (mostly 23, rarely 20); VI, 26-27 (rarely 14–29); and VII (adult), 27-28 (rarely 20–29). Here again, as in *Schistocerca gregaria*, the first intra-instar difference appears in the third instar. Another feature noticed is the occurrence of bilateral asymmetry in the right and left antennae of the same individual, the difference in the number of antennal segments being as high as 14 in some cases. It is interesting that the bilateral asymmetry also is first evident in the third stage and continues thereafter.

It should, however, be pointed out that not in all the Acrididae is the intra-instar difference first discernible in the third instar, for, according to Uvarov (1928, p. 45, Table), this difference may arise even in the second instar and rarely in the first (see discussion in Roonwal, 1952).

III.—Discussion and Conclusions.

It will be seen from the data on extra-moulting and the directional reversal of the elytron-wing complex (E-W complex, for brevity) that, so far as the 5-moult individuals of all the species thus far studied are concerned, the extra-moulting occurs in the stage immediately following the normal third. As the majority of the Acrididae so far studied are of the 5-moult kind, we may state that in these forms the extra-moulting and the directional reversal of the E-W complex occurs in the *middle* hopper instar, a description, however, which does not apply to the remaining Acrididae. It will be seen from my Rule 2 discussed above and Text-fig. 1 that the numerical factor of two instars always following the instar in which the directional reversal of the E-W complex occurs would appear to represent the position more generally. Both the present writer (Roonwal, 1938, 1940, 1946, 1947, and the present paper) and Webley (1951) have discussed the importance of the third instar, and this is quite clear in the 5-moult species. In the other species, however, Rules 1 and 2, which were formulated to generalise the position regarding extra-moulting and the direction reversal of the E-W complex, represent the true position. No comparative data are yet available to enable us to decide whether these two rules also apply to other metabolic activities, *e.g.*, variation in blood-cell counts, respiratory rates, etc.; data regarding these activities are hitherto available for the 5-moult species only.

We may now conclude, on the evidence discussed above, that at any rate in the 5-moulting species of Acrididae—which is by far the most common condition—the third instar is of exceptional importance from the point of view of metabolic activity, as some striking changes occur either during that instar or in the immediately preceding or immediately following periods. In many of these changes a directional reversal occurs either in a morphological structure or a physiological process or a behaviouristic pattern. The hitherto known processes of this kind are listed below under three categories. (For fuller details of these examples, see Roonwal, 1940, and the preceding pages of the present account. Except where otherwise stated, the data apply, as a rule, to the 5-moult species.)

List of the hitherto known changes.

(*a*) *Morphological changes.*

1. The upturning of the elytron- and wing-rudiments (which has been termed as the directional reversal of the elytron-wing complex) occurs immediately after the third stage (*i.e.*, at the third moult). This applies to the 5-moult species only; in the rarer 6- and 7-moult species, the chronological position of this reversal is governed by Rule 2 of Roonwal (1946), already discussed above.
2. The growth coefficients in the 5-moult species increase towards the third stage when they are highest, and thereafter decline. In the 4-moult species also this reversal occurs in the third stage, but in the opposite direction.
3. The number of eye-stripes added is normally one in each stage, but *two* are added in the third stage in some forms.
4. The dorsal spot of the eyes undergoes directional reversal in its disposition immediately after the third stage, a feature which is closely comparable to the directional reversal of the elytron-wing complex.
5. The number of antennal segments increases from 8–13 in the first stage to 25–30 in the adult. In the first two hopper stages there is usually a constant number for all the individuals of a species, and intra-instar difference in numbers first arises in the third stage although sometimes also in the second and even in the first.

(*b*) *Behaviouristic changes.*

6. In the reaction to humidity, as regards locomotory activities, hoppers of the third and following stages behave differently from those of the first and second stages.
7. The 'activity figure' of the hoppers shows a directional reversal. It falls up to the third stage, but rises again in the fourth, the rise being maintained in the fifth.

(c) *Physiological changes.*

8. The respiratory metabolism decreases up to the third instar, and increases from the fourth instar onwards.

9. In the 5-moult species, an extra-moult occurs mostly in the third stage and only rarely in the second and fourth. In the rarer 6-moult species, Rule 2 of Roonwal (1946), as already discussed above, holds good.

10. In the 5-moult species, the relative increase in the number of blood-cells (as well as their absolute numbers in one case) per unit volume of blood in the hopper stages is the greatest in the third stage, and declines thereafter.

IV—Summary.

1. Since the subject of directional reversal of certain morphological, behaviouristic and physiological processes in the Acrididae and the importance of the third instar was first opened by the writer (Roonwal, 1938, 1940), many new facts which have a bearing on the subject have been assembled. These new facts are discussed here.

2. In *Locusta migratoria migratorioides* there is, in the third instar hopper, a great increase, both relatively and in absolute numbers, in the number of blood-cells per unit volume (Webley, 1951). A similar increase in relative numbers also occurs in *Schistocerca gregaria* (Mathur and Soni, 1937).

3. In the 5-moult species, extra-moulting occurs as a rule in the third stage (Roonwal, 1946, 1947). The chronological relationship in the rarer 6- and 7-moult species is somewhat different and Rules 1 and 2 of Roonwal (1946) are obeyed.

4. In *Schistocerca gregaria* the third instar is exceptional in the addition of two eye-stripes, instead of the normal one, in some of the 7-eye-striped forms. In that same species, the directional reversal of the dorsal spot of the eyes occurs immediately after the third stage.

5. In the increase in the number of antennal segments during post-embryonic growth, the third instar is exceptional in the fact that the intra-instar differences as well as bilateral asymmetry are first evident most commonly in the third instar.

6. The correlation between extra-moulting and the upturning of the elytron-wing complex is discussed.

7. A list is given of the hitherto known instances of directional reversal and the importance of the third instar.

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