THE GENETICS OF GRASSHOPPERS: *CHORTHIPPUS PARALLELUS.*

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(With Plates XVII and XVIII.)

The chromosomes of *Stenobothrus lineatus*, *Chorthippus parallelus*, *C. albomarginatus*, *C. bicolor* and *Mecostethus grossus* among other Acrididae have been extensively studied by cytologists (Janssens, 1924; McClung, 1914; Belar, 1926; Darlington and Dark, 1932; etc.). The chromosomes are large, and the prophase stages of meiosis are exceptionally clear (see Plate XVII, fig. 1).

While collecting material for cytological purposes in 1931 we were struck by the large amount of variation in the colours of the British species (*Chorthippus* species and *Stenobothrus lineatus*). Later several species from the continent of Europe were also found to have different colour forms.

It seemed that if these insects could be bred they would provide the long-desired experimental material suitable for both cytological and genetical investigation.

This paper describes the technique of breeding, the life history of *Chorthippus parallelus*, and the procedure which is being used in the analysis of wild populations. Much of the information will be well known to entomologists, but it is given here for the use of others who propose to breed these insects. Further information will be found in Uvarov (1928), Parker (1930) and Rubtzov (1934).

Technique.

No information was available to us regarding the possibility of raising several generations of grasshoppers under laboratory conditions, and during the first attempts we met with considerable mortality of the insects. The method finally adopted has reduced the mortality to 1 per cent., but has not yet completely overcome the incidence of parasites which slightly reduce the fertility. Nevertheless the average progeny from a single mating is 35 individuals, which is quite useful for a genetic analysis.

The container (Plate XVII, fig. 2) is a hurricane-lamp globe with a fine muslin top held in place by a rubber band. The soil used is old soil taken from flower pots which have been one season in a warm greenhouse. Other materials such as sand or sterilised soil have not been satisfactory, since the moisture content is not easily controlled and these substances tend to form a hard surface which is unsuitable for egg laying.

The grasshoppers in captivity thrive best on a limited number of grasses. *Agrostis* spp. and *Agropyron repens* were found most suitable. Other grasses had to be used with caution, since any excess moisture or succulence caused death of the insects in a short time. The grass was placed in test-tubes containing water and was changed every second day.

There was considerable mortality until it was found that the insects were killed by excess moisture. When no water is supplied to the containers other than with the food the death-rate is negligible. The insects can stand a large range of temperature, but seem to prefer a temperature of 70–75° F.

The most dangerous period in the life cycle is between the first and penultimate instar. During this period the insects are highly susceptible to external conditions and cannot even be moved from one container to another without lethal results. It is for this reason perhaps that there is more mortality among wild insects brought into the cultures than there is among insects which are born in captivity. Adult insects caught in the wild may be kept some time in culture, but insects caught in the juvenile stage often succumb.

Anaesthesia with ether is difficult, therefore the insects are caught and examined in test-tubes.

The insects have to be protected from birds and spiders, both of which have caused havoc.

Life history of *Chorthippus parallelus*.

The eggs are laid about half an inch below the surface of the soil. About sixteen eggs are laid together in one pod, and one female lays on an average four pods. Rubtzov finds that in Russian material the potential number of eggs in the egg tubes is 120–140, and Maltsev (cf. Rubtzov, 1934) has found that a female of *C. albomarginatus* gives an average of 100 actual eggs. It is probable that parasites of various orders are reducing our egg numbers, since these have been observed in the insect bodies and females of a particular family have died at the time of egg laying. Death is probably caused by blockage of the ovipositor by the parasites.

The eggs remain dormant in the soil over the winter. In the following April the first of the young insects make their appearance. In two consecutive years the first emergence occurred on 26 April. It would appear that all the eggs of one pod hatch at the same time, but there is no relation between the time of hatching and time of egg laying. The hatching may continue until the end of June from different egg pods. Emergence usually takes place when the soil is warmed by the sun and can be encouraged by placing the soil in a warm greenhouse. Attempts to raise more than one generation in a year have so far proved unsuccessful. Alternate freezing and warming the eggs, or keeping the insects at a higher temperature, have not influenced the normal periodicity of the life cycle.

The young insects on emergence are colourless, but within an hour have taken on the characteristic green or other colour of the more advanced stages. It has been suggested that some of the colours were derived from the food. That the chlorophyll of the plant does not influence the colour development is shown by feeding the insects with albino oats, and that other plant ingredients do not influence the colour development was shown by starvation experiments. In both cases neither time nor degree of coloration was different from normal.

There are usually five moults with 14 days' interval between each moult, but in some strains the number may be increased to six and will extend over a much longer period than the usual 10 weeks. Whether the greater number of moults is of genetic origin is unknown, but we have found such forms as stragglers in wild habitats unusual to *C. parallelus*. These forms appear to have defective gonads; the male may have one abortive testis and few divisions in the other.

Sex can be detected at an early stage in *C. parallelus* by the slender short antennae of the female and the long baton-like antennae of the male.

Copulation does not take place until after the final moult. After copulation the female lays an egg pod and copulation can again take place in two or three days. By using two males with one female at an interval of a fortnight two distinct progenies may be obtained. This is sometimes of great use in genetical analysis.

The stridulation note of each species is characteristic. *Mecostethus grossus* has a type of stridulation quite distinct from that of the other species studied. It is a high-pitched scraping sound of short duration as compared with the incessant lower note of the other species. *M. grossus* also differs from the remaining species in that the young insects do not

have the colours of the adult. Indeed the young insects of this species may be mistaken for *Stenobothrus lineatus* unless the carinae are examined.

The colours of the adult are found in young *Chorthippus parallelus* and *Stenobothrus lineatus* and these cannot be changed by environmental conditions, as in *Locusta*. The rosy and red colours of *Chorthippus parallelus*, however, increase in intensity with age and the pale yellow and white colours on the dorsal surface of the pronotum may become greenish in old adults. About twelve families of *C. bicolor* have been raised from eggs. These contained nearly black, sandy and grey fawn individuals. Except for a degree of mottling these individuals did not change in colour during development, under the laboratory conditions. Reports of colour changes in grasshoppers due to environmental conditions have been given at various times, but we are of opinion that the basic colours of the above species, which are dealt with later, do not interchange in one individual. It will be seen however that one colour may fluctuate round a mean value.

Of distinct interest is the fact that copulation has been observed between species of different genera and the females have been observed performing the normal processes of egg laying. It remains to be seen whether fertilisation has taken place.

So far, parthenogenesis has not been found in *C. parallelus*. Unfertilised females are sterile, and no exceptional recessive factor has been found in the progenies of recessive females crossed to a male homozygous for the dominant allelomorph.

Genetics.

At the start of the investigation, *Chorthippus parallelus, C. bicolor* and *Stenobothrus lineatus* were bred in order to discover which species would be most useful for our purpose. It was soon found that all three species were promising material. *Chorthippus parallelus* was selected because it is normally wingless and more easily handled. It is also possible that the colours on this species are more distinct and that patterns of colour are less frequent. Nevertheless the remaining species deserve attention by geneticists. *Mecostethus grossus* would be valuable as a contrast to *Chorthippus parallelus*, since the former species has localised chiasmata while the latter has chiasmata distributed at random. Through the kindness of Mr M. J. D. White of University College, London, we obtained six individuals of this species and were able to raise a progeny. Variation unfortunately is much less in degree than in *C. parallelus*.

Although a wild population of *C. parallelus* appeared uniform in type

it was found that no population was homozygous. Rarely have we found a wild individual which is homozygous for all the genes we have isolated.

By breeding like types together and crossing unlike types information regarding dominance, segregation and factor interaction was obtained and material has been created which will prove useful for the later more intensive genetical work. Generally one female and one male were used for one cross, which was replicated as much as possible. The sex ratio in the laboratory cultures is 887 males to 963 females, which is not significantly different from the expected 1:1.

So far, fourteen genes have been isolated and several others are indicated. The segregation of ten of these genes is given in Table I. The remaining four (**p**, **Y**, **n**, **v**) exhibit interactions with other genes which are not sufficiently understood to enable them at present to be dealt with in the same way.

TABLE I.

Factors	Phenotype of recessive	Ratios (Dominant/Recessive) *Back-cross*	Ratios (Dominant/Recessive) F_2
o	Olive genae	263 : 264	259 : 79
b	Brown pronotum	290 : 261	118 : 42
g	Grey pronotum	76 : 80	41 : 15
d	Dark apex	132 : 106	90 : 22
l	Light-coloured legs	16 : 20	29 : 8
s	Salmon legs	16 : 16	93 : 25
r	Rosy body colour	18 : 21	125 : 20
e	Post-ocular region differentiated	37 : 39	256 : 96
sp	Spangled eye	13 : 23	107 : 30
C	Lines (dominant)	133 : 148	96 : 24

The action of the genes so far discovered is given below. Since there is no "wild type" in *C. parallelus* we have adopted the form with green on all dorsal parts of the body, except eyes, as the conventional type with which others can be compared.

o converts the green colour on genae, dorsal surface of pronotum and abdomen to olive or greenish brown.

b converts the green colour on the dorsal surface of pronotum and abdomen to brown.

g gives grey dorsal surface to pronotum and abdomen (in presence of **b**).

d darkens the whole of the apical region of the femora.

l converts the green dorsal carinae of the femora into light carinae.

s gives salmon colour to the dorsal carinae of the femora (in presence of **l**).

r gives rosy colour to genae, dorsal surface of pronotum and abdomen (in presence of **o** and **b**; possibly sex limited).

e causes the colour differentiation of the post-ocular region.

sp gives spangles of black and white on the post-ocular region (in presence of **e**).

C (dominant gene) gives sub-median stripes extending to vertex (in presence of **b** and **g**).

p gives light outer pinnate area of femora.

Y (dominant gene) gives brilliant yellow dorsal surface to pronotum and abdomen (in presence of **b** and **g**).

n reduces breadth of light stripe on pronotum (in presence of **b** and **g**).

v gives no different intensity of colour on vertex to that on pronotum (in presence of **y** and **b**?, **b**, **g** and **y**?).

Recessive **o** inhibits the expression of the remaining genes except **Y**, **r** and possibly **g**. The colours associated with **o** and with **b** are somewhat variable, which may be due to genetic modifiers or to external conditions. For example the insects of constitution **ObG** may have light brown, pale biscuit, or dark brown pronotums. Insects of the constitution **oy** may have dark olive, olive, greenish brown or warm brown genae and pronotum.

With the exception of a relation between **Y** and **o**, and between **b** and **L**, no strong linkage has been found between these 14 genes. In the case of **Yo** and **bL** all individuals of some families which are **o** or **b** are also **Y** or **L** respectively. This may be due to strong repulsion or to interaction in a definite genotype which has not been isolated.

In a number of the families where olive genae are present the females may be rosy in colour, but the males are not affected. This phenomenon is not due to sex linkage, but may be due to sex limitation of the expression of **r**. The length of the elytra varies to a considerable extent. Normally the elytra of the female are a quarter of the length of that of the abdomen and those of the male are about three-quarters of the length of the abdomen (see Plate XVIII). Some individuals of both sexes in certain families have elytra as long as or longer than the abdomen. It is not known whether this is inherited or not. No clear segregation has been observed, but long elytra have been found in association with slow development (the individual taking a longer period than 10 weeks to reach the adult stage) and with abnormality of the gonads. Ramme (1931) reports a similar phenomenon in *Metrioptera* where long wings, abnormal gonads and wet conditions were associated together.

It has been mentioned that no individual of a wild population of *Chorthippus parallelus* has been found which is homozygous for all the genes we have investigated. Ninety-six single matings of wild individuals have been made during our work and only those genes which had been

recognised in wild conditions have segregated. Quite frequently a gene was segregated from an individual of one wild population which had been observed as an exclusive type of another wild population. Parallel variation in type between *C. parallelus* and *Stenobothrus lineatus* in one habitat has been frequently noticed. The problems of the distribution of these genes in wild populations are being followed up by (1) statistical analysis of wild populations, (2) breeding from samples of each population, and (3) populating habitats with individuals marked with paint and by genetic factors.

DISCUSSION.

The grasshopper material appears highly suitable for investigation by cytologists, geneticists and ecologists. Our primary object in starting the genetical work was to attempt to connect the cytological with the genetical data in the same organism, but the advantages of the material for ecological study were so pronounced that the work has been extended in that direction.

At this stage only one or two questions can be discussed. The contrast in genetical behaviour between *Drosophila* spp., *Paratettix* (cf. Nabours, 1929; Nabours and Kingsley, 1934) and *Chorthippus* is of considerable interest. In *Drosophila* there is a well-defined wild type which is found in different environments, in *Paratettix* and *Chorthippus* different genetical varieties are found in different environments. In *Chorthippus* the frequency of occurrence of varietal forms precludes the identification of any one type as the wild type. In *Paratettix* there is a series of closely linked dominant genes, in *Chorthippus* linkage is not so well marked but epistasy and factor interaction are pronounced, while in *Drosophila* the genes which control the wild type have neither strong linkage with one another nor strong factor interaction.

The polymorphism which occurs in *Chorthippus* and to a less extent (?) in *Paratettix* in the wild would appear to be associated with the mechanics and physiology of their gene behaviour. Of the fourteen genes in *Chorthippus parallelus* which have been isolated, **O**, **B**, **l**, **r**, **G** and **C** inhibit the expression of at least two non-allelomorphs. In addition the recessives **o** and **g** interfere with the expression of several other genes. One phenotype may therefore correspond to a comparatively large number of genotypes. By the presence of this epistasy the species is able to react readily to different habitats but yet remain fairly uniform in one habitat.

SUMMARY.

1. Several species of Acrididae provide valuable material for both cytological and genetical research.

2. The technique of breeding and the life history of *Chorthippus parallelus* are described.

3. The action of 14 genes in *C. parallelus*, and the segregation of 10 of them, are described.

4. The problems of genecology of grasshoppers are discussed and some preliminary results given.

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EXPLANATION OF PLATES XVII AND XVIII.

PLATE XVII.

Fig. 1. Prophase stages of meiosis in *C. parallelus*.

Fig. 2. Breeding container for the culture of grasshoppers.

PLATE XVIII.

C. parallelus.

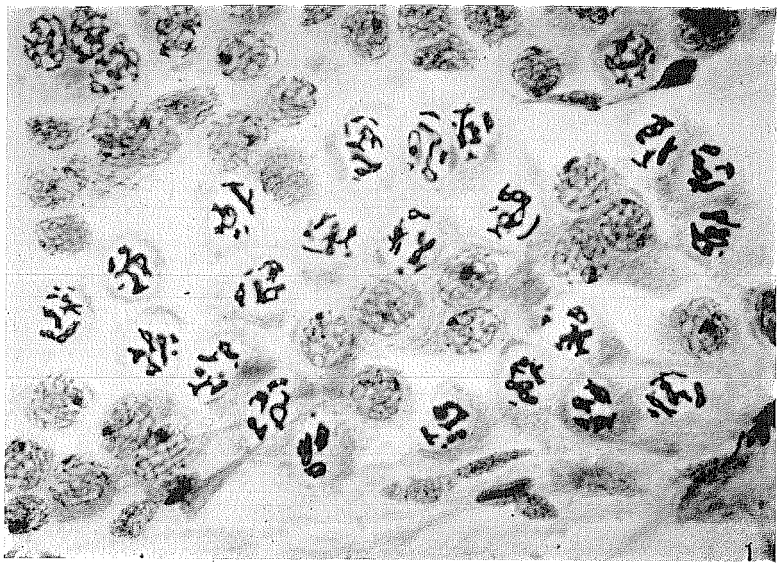
Fig. 1. Post-ocular region spangled (e, sp), narrow light stripe on pronotum (n).

Fig. 2. Brilliant yellow pronotum (Y), post-ocular region differentiated (e), vertex undifferentiated (v).

Fig. 3. Male, long elytra, vertex (V) and post-ocular regions (e) differentiated, brown pronotum (b).

Fig. 4. Female, long elytra, grey pronotum (gb).

Fig. 5. Female, green "conventional type".



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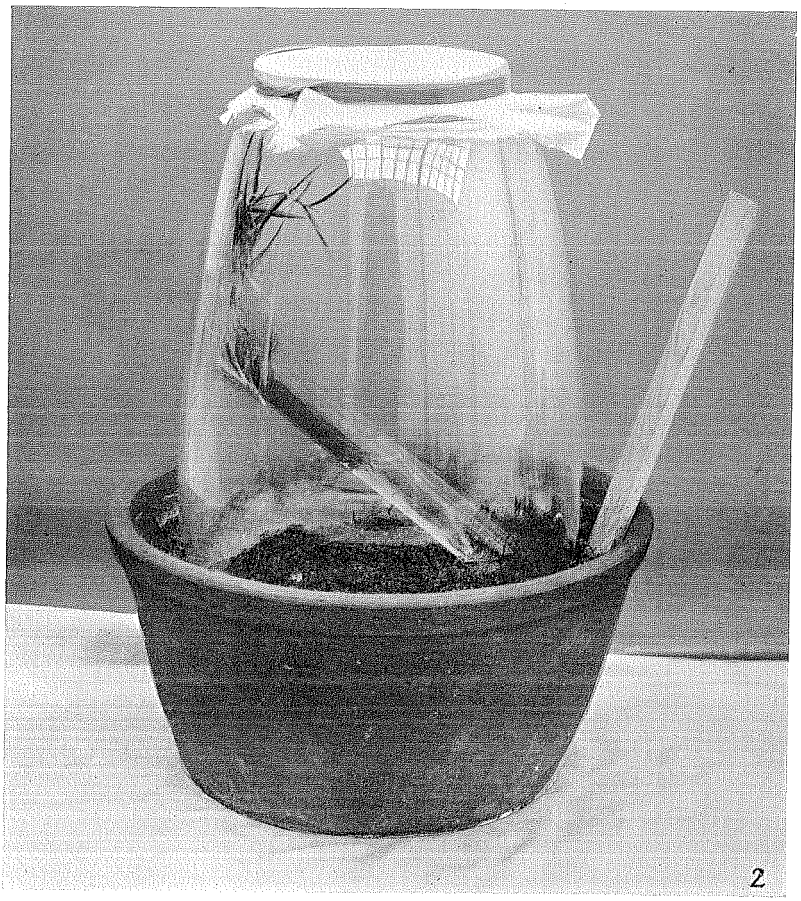

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