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ASYNAPSIS AND POLYPLOIDY IN SCHISTOCERCA PARANENSIS

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With 61 Figures in the Text

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A. Introduction

Acridoid chromosomes are unique in a number of respects. First, they are sufficiently large in size, few in number and of such a size range as to permit an unambiguous and consistent classification of the members of the complement. Secondly, diplotene in male meiosis offers an unparalleled opportunity for the study of chiasma frequency and distribution. The genus *Schistocerca* combines both these advantages with an additional marker: the positions of the centromeres at diplotene are revealed by precocious pro-centric condensation. In this paper we are concerned with showing how these several chromosome markers can be used to clarify two main controversial issues, namely the problem of chiasma localisation and that of multivalent formation in autotetraploid cells.

B. Materials and Methods

The South American locusts used in this study were all supplied by courtesy of the Anti-Locust Research Centre, London. They originate from Nicaragua and have been provisionally identified by Sir BORIS P. UVAROV as *S. paranensis*.

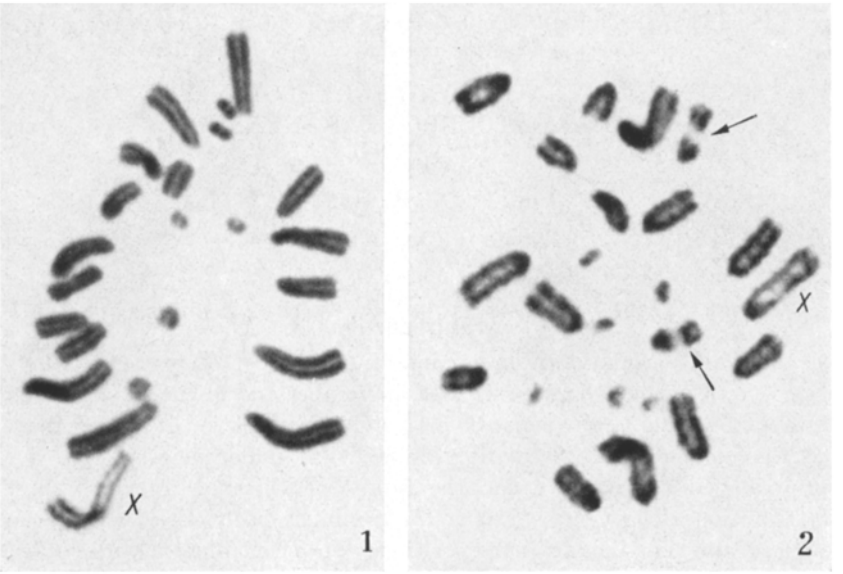
The testes of male imagines were removed by vivisection under insect saline and fixed in 1:3 acetic-alcohol. Squash preparations were made in acetic orcein and aceto-carmine.

C. Observations

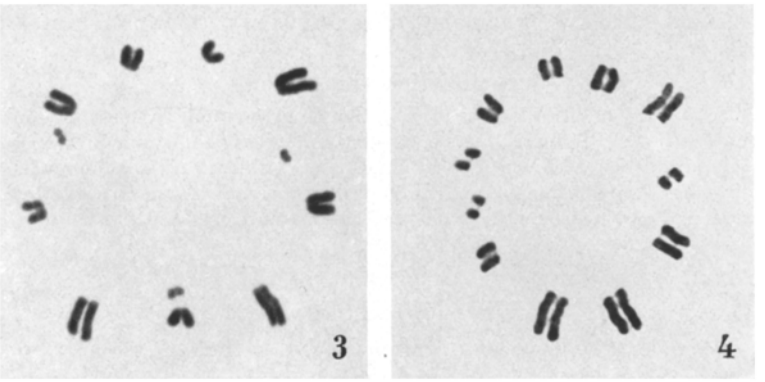
I. The Normal Complement

The male karyotype of *S. paranensis*, like that of its sister species *S. gregaria*, consists of 23 acrocentric chromosomes. It agrees with *S. gregaria* also in the number of size groups present (3 L, 5 M and 3 S pairs) and in the relative sizes of the members of each group (Table 1). But it differs in one clear respect. The S-chromosomes in *S. paranensis* are considerably smaller than those of *S. gregaria* (Figs. 1—4, Table 1). Chromosome size is sometimes a difficult character on which to base comparisons, since it can be influenced by a number of variables. But this difference between the small chromosomes of *S. gregaria* and

S. paranensis is consistent both at mitosis and at meiosis. Indeed, the two species can usually be distinguished on this character alone.



Figs. 1 and 2 (both 1750 ×). 1: Male mitotic complement ($2n = 23$), *X* negatively heteropycnotic. 2: Mitotic metaphase showing secondary constrictions in the M_6 chromosomes



Figs. 3 and 4. Second meiotic metaphase (both 1000 ×). 3: *Schistocerca paranensis*. 4: *Schistocerca gregaria*. Note difference in size of the three small chromosomes in the two species

There is a further difference in their chromosome phenotype which involves nucleolar organisation. In the mitotic complement of *S. paranensis* the M 6 chromosomes sometimes exhibit submedian secondary constrictions (Fig. 2). At first prophase of meiosis, aceto-carmine pre-

parations reveal a nucleolus attached at this locus. This persists until diplotene when it becomes detached. Thereafter it either gradually diminishes in size or else breaks up into a number of smaller nucleoli. At zygotene-pachytene it is possible to show that the nucleolus is in fact attached to a prominent chromomere, which we have called the nucleolar chromomere (Fig. 5). Indeed, this chromomere can be recognised even in acetic-orcein preparations where, of course, the nucleolus does not stain (Fig. 6). Phase -contrast examination of such preparations, however, reveals the presence of a nucleolus both at these early stages and until diplotene (Fig. 11).

At early prophase of the first meiotic division only one nucleolus is usually found; one presumes that nucleolar fusion is a common occurrence during the premeiotic interphase. Sometimes, though rarely, the nucleolus is associated with the X-chromosome (Fig. 14). At diplotene the locus of attachment frequently appears as a non-staining chromosome segment (Fig. 9). These achromatic gaps presumably represent localised regions which remain unspiralised, though their inability to stain may be enhanced by the accumulation of the products of their own activity.

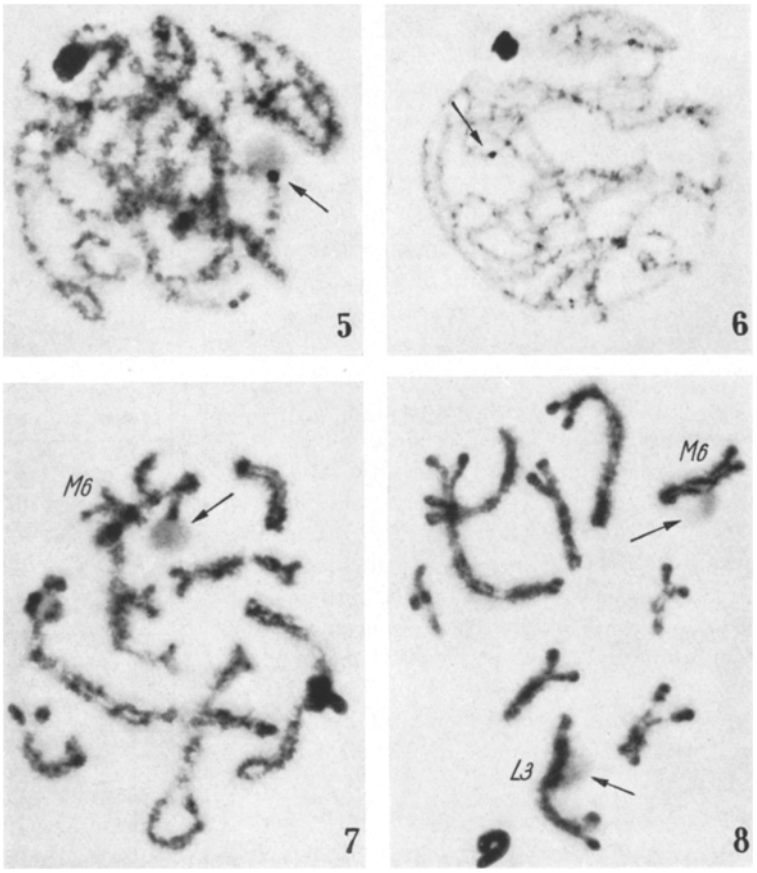
The nucleolar gaps are usually heteromorphic in expression in the sense that they are found on only one side of a bivalent. Such heteromorphism may indeed be evident as early as pachytene. Homomorphism is rare (Fig. 16). In some heteromorphic cases two disproportionate nucleoli may in fact be formed. Here the larger nucleolus is associated with the achromatic segment. Comparable heteromorphism of non-staining segments can also be found at first or even second anaphase, though this phenomenon is not regular in occurrence (Fig. 20). We have

Table 1. *Comparison of chromosome length in S. paranensis and S. gregaria*

Species	Mean mitotic length in micra with standard deviations											
	L 1	L 2	L 3	M 4	M 5	M 6	M 7	M 8	S 9	S 10	S 11	X
S. paranensis (mean of 10 nuclei)	8.5 ±0.33	7.5 ±0.26	6.5 ±0.26	5.7 ±0.18	5.1 ±0.18	4.7 ±0.17	4.3 ±0.18	3.8 ±0.13	1.7 ±0.05	1.5 ±0.04	1.3 ±0.03	7.8 ±0.41
S. gregaria[1] (mean of 16 nuclei)	7.4 ±0.1	6.7 ±0.2	5.8 ±0.1	4.9 ±0.1	4.3 ±0.1	3.9 ±0.1	3.6 ±0.1	3.3 ±0.1	2.4 ±0.1	2.1 ±0.1	1.9 ±0.1	6.7 ±0.2

[1] Data from JOHN and NAYLOR (1961).

also seen such heteromorphism in a number of other orthopterans, for example *Tetrix* (HENDERSON 1961, Fig. 14 and 15), *Pyrgomorpha* (LEWIS and JOHN 1960, Fig. 13) and *Chorthippus*. OHNO *et al.* (1961)

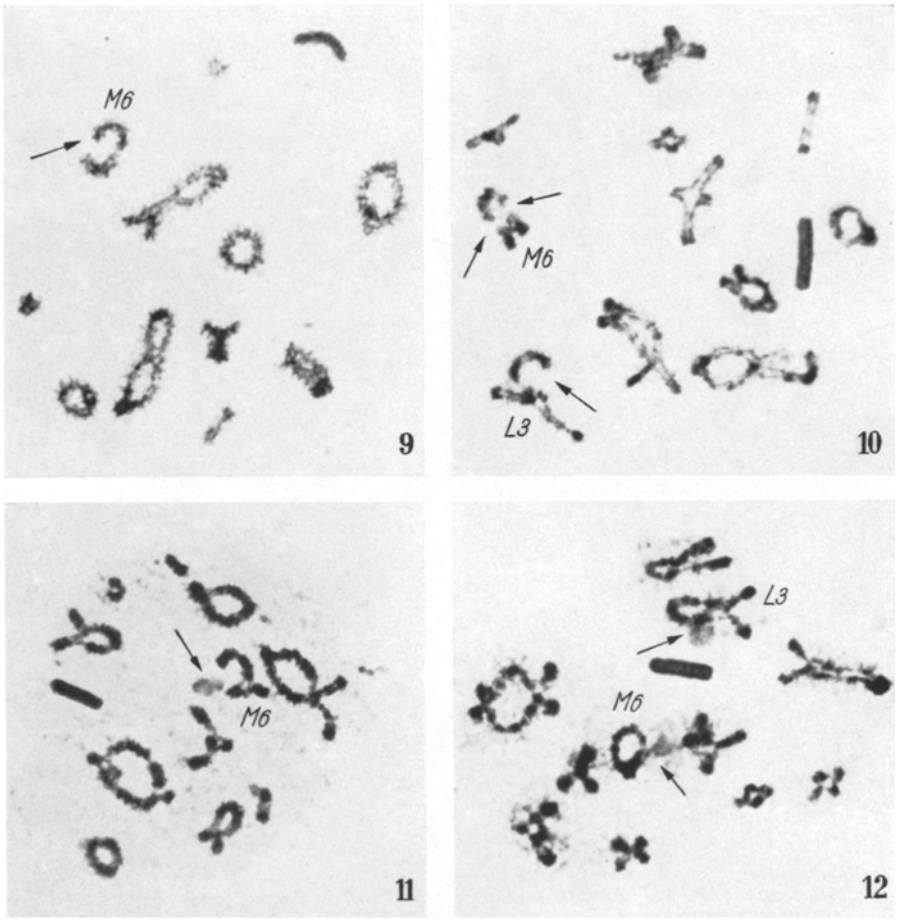


Figs. 5—8 (all 1250 ×). 5: Zygotene in *S. paranensis* with nucleolus attached to prominent chromomere (aceto-carmine). 6: Early zygotene in *S. paranensis* showing prominent nucleolar chromomere (acetic orcein). 7: Pachytene in *S. paranensis* with single nucleolus attached to M_6 (aceto-carmine). 8: Pachytene in *S. gregaria* with two nucleoli, attached to L_3 and M_6 respectively (aceto-carmine)

have recently described a more consistent heteromorphism for nucleolar organisation in a pair of autosomes of *Cavia cobaya*. This is expressed in somatic and germinal cells of both sexes. A similar consistency had been reported earlier in *Disporum sessile* (KAYANO 1960) where two different pairs of autosomes were involved.

S. gregaria, like *S. paranensis*, also organises nucleolar material, and with similar consequences. As in *S. paranensis* the M 6 bivalent produces

one of the nucleoli, while an additional nucleolus is organised by the L_3 bivalent (Figs. 8, 10 and 12). This clarifies our earlier report (JOHN and NAYLOR 1961, p. 189) of achromatic gaps in the long and medium



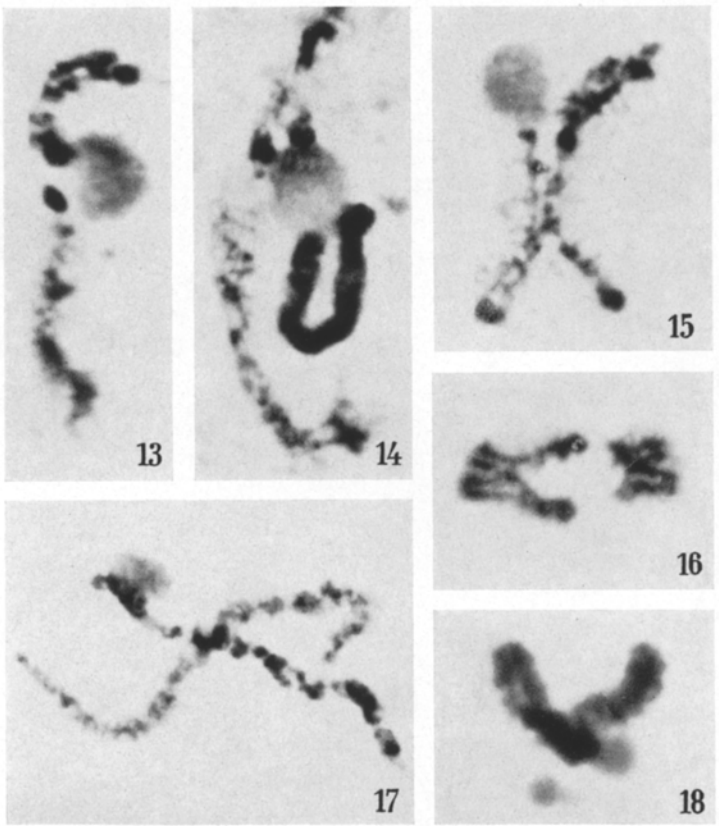
Figs. 9—12 (all 1000 ×). 9: Diplotene in *S. paranensis* showing heteromorphism for an achromatic gap in the M_6 (acetic orcein). 10: Diplotene in *S. gregaria* showing heteromorphism for achromatic gaps in L_3 and M_6 (acetic orcein). 11: Diplotene in *S. paranensis* with nucleolus attached at achromatic gap in M_6 (acetic orcein, phase contrast). 12: Diplotene in *S. gregaria* with nucleoli attached at achromatic gaps in L_3 and M_6 (acetic orcein, phase contrast)

chromosomes of this species. We have re-examined these preparations and confirm that these consistently involve the L_3 and M_6.

These observations on nucleolar development in *Schistocerca spp.* are instructive in three respects:

1. It has been claimed (DARLINGTON 1947; JOHN and LEWIS 1959) that in male orthopterans nucleolar formation and heteropycnosity

of the X chromosome are mutually exclusive states. Our observations here show that this is clearly not the case. Indeed, we are not the first to notice this concurrence (COREY 1940; SINOTO 1944). We have also found a nucleolus and a positively heteropycnotic X chromosome to



Figs. 13—18. 13: Pachytene in *S. paranensis*, M_6 chromosome with attached nucleolus (aceto-carmine, 3000 ×). 14: Pachytene in *S. paranensis* showing non-specific association of nucleolus with the ends of the X-chromosome (aceto-carmine, 3500 ×). 15: Early, diplotene in M_6 chromosome of *S. paranensis* (acetocarmine, 3500 ×). 16: M_6 chromosome of *S. paranensis* homomorphic for the achromatic gaps (acetic orcein, 2250 ×). 17: Pachytene in *Tylotropidius gracilipes* showing nucleolus attached to terminal region of the precocious bivalent (aceto-carmine, 2000 ×). 18: Diplotene in *T. gracilipes* with fragmenting nucleolus still attached to the precocious bivalent (aceto-carmine, 2000 ×)

be present simultaneously in the male of *Tylotropidius gracilipes*. Furthermore, in this species the nucleolus is organised by a positively heteropycnotic precocious bivalent (Figs. 17 and 18). In *Blaberus discoidalis* (JOHN and LEWIS 1959) the reduced heteropycnosity of the X chromosome is presumably not causally related to the nucleolus which is present in the male of this species.

A further orthopteran in which a nucleolus has been described is *Mantis religiosa* (CALLAN and JACOBS 1957), though in this species the sex chromosomes lose their positive heteropycnosity by zygotene. Finally, DARLINGTON (1936) has described the presence of a small nucleolus organised by the L_3 chromosomes in *Chorthippus brunneus* (= *C. bicolor*). He concluded that the organisers were located near the ends of the chromosomes, but from our experience they are submedian in location.

2. These findings in *S. paranensis* and *S. gregaria* parallel those of BEERMAN (1960). He reported a difference in the number and distribution of nucleolar organisers in the sibling species *Chironomus tentans* and *C. pallividivatus*. *C. tentans* has two nucleoli, one on chromosome 2 and one on chromosome 3. The other sibling species possesses only one nucleolus, and this is found on chromosome 2. Moreover, salivary gland chromosome analysis in F_1 hybrids reveals that the site of attachment of nucleoli on the second chromosome differs in these two sibling species.

On the basis of the banding patterns in the hybrids BEERMAN concluded that these differences were not due to structural rearrangement of the chromosomes. In hybrids the nucleolar organisers behave as Mendelian loci.

That nucleolar organisers can be lost by mutation is clear from the findings of FISHBERG and WALLACE (1960) in *Xenopus laevis*. But here it is not possible to decide unambiguously whether the mutation is chromosomal or genic in character. Nucleolar organisers must also be capable of increasing in number since in man, for example, five pairs of chromosomes are satellited (FERGUSON-SMITH and HANDMAKER 1961; OHNO et al. 1961) and all of these appear to be involved in nucleolar production. We cannot say whether loss or gain is involved in the case of these two species of *Schistocerca*. Similarly it is impossible to decide whether the organisers are at exactly the same loci on the M_6 chromosome in both species, though they certainly occupy comparable positions.

3. The presence of such sites of nucleolar attachment might be expected to influence chiasma formation in their proximity. Indeed, it has been shown that the presence of a nucleolus in *Fritillaria* (DARLINGTON 1935) and *Eremurus* (UPCOTT 1935) can interfere with chiasma distribution. Significantly, the organisers in the *Schistocerca spp.* occur at positions where chiasma formation takes place infrequently. This siting is, however, consequential, not causal. The distribution of chiasmata in the nucleolar organising chromosomes is not modified, but is in fact similar to that of other members of the complement in the same size range (HENDERSON, in preparation).

These differences we have mentioned between the two species of *Schistocerca*, namely, the difference in the size of the S-chromosomes and the difference in the number and distribution of nucleolar organisers, are the only ones we can detect. They do not differ significantly in mean or variance of chiasma frequency, or in chiasma distribution (Table 2; Fig. 19). And this, despite the fact that *S. paranensis* is confined to the new world, while *S. gregaria* is present only in the old.

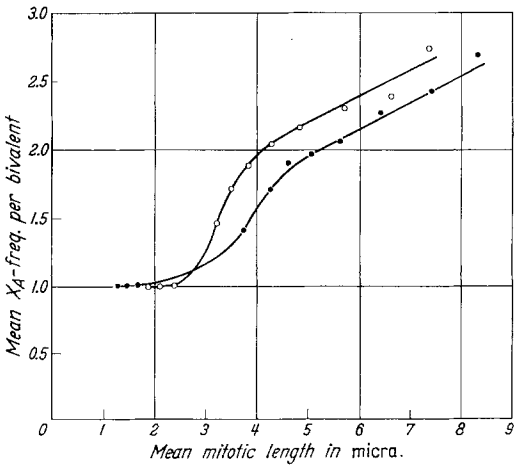


Fig. 19. Relationship between the mean mitotic chromosome length and mean chiasma frequency per bivalent in *Schistocerca gregaria* (——o——) and *S. paranensis* (——•——)

However, chromosome morphology is not always a good criterion of chromosome homology. In the grasshopper genus *Eyprepocnemis*, for instance, two of the so-called subspecies which have been recognised, *E. plorans ornatipes* and *E. p. meridionalis*, possess complements which are indistinguishable numerically and morphologically, though they do differ significantly in chiasma frequency. Experimentally produced hybrids between these two sub-species reveal that the complements have undergone considerable structural alteration (JOHN and LEWIS, in preparation).

Table 2. *Comparison between the chiasma frequencies of S. paranensis and S. gregaria.* 25 first metaphases were scored in each of 12 individuals from the two species, making a total of 300 observations in each case

Species	Chiasma frequency per bivalent $\left(\frac{\text{Mean}}{\text{Variance}}\right)$											Cell total $\left(\frac{\text{Mean}}{\text{Variance}}\right)$
	L 1	L 2	L 3	M 4	M 5	M 6	M 7	M 8	S 9	S 10	S 11	
S. paranensis	2.67 / 0.02	2.40 / 0.02	2.25 / 0.03	2.05 / 0.01	1.96 / 0.01	1.89 / 0.01	1.70 / 0.02	1.40 / 0.05	1.00 / 0.00	1.00 / 0.00	1.00 / 0.00	19.33 / 0.64
S. gregaria	2.71 / 0.03	2.36 / 0.03	2.29 / 0.03	2.13 / 0.02	2.01 / 0.03	1.87 / 0.03	1.69 / 0.04	1.43 / 0.02	1.00 / 0.00	1.00 / 0.00	1.00 / 0.00	19.43 / 1.02

Evidently, the outward appearance of chromosomes may conceal their own change. Attempts to hybridise the two species of *Schistocerca* have unfortunately failed.

II. The Anomalies

Several departures from the standard behaviour were found. Some of these were present in only occasional cells. For example, in this category we have obtained:

(i) An extra centric fragment of unknown origin.

(ii) Univalent production involving the S_{11} chromosomes.

(iii) Delayed separation of the negatively heteropycnotic X chromosome at second anaphase (Fig. 20) and

(iv) The production of at least two types of autosomal chromatid bridge at first anaphase. One of these types (Figs. 21 and 22) we have described earlier in *Chorthippus* (JOHN, LEWIS and HENDERSON 1960). The other (Figs. 23 and 24) is of a kind which has only been previously reported in rye (REES and THOMPSON 1955).

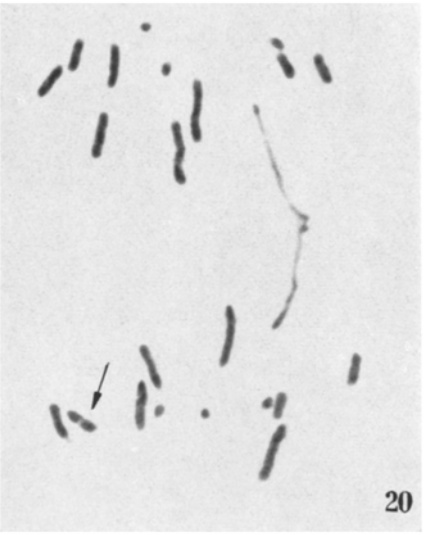
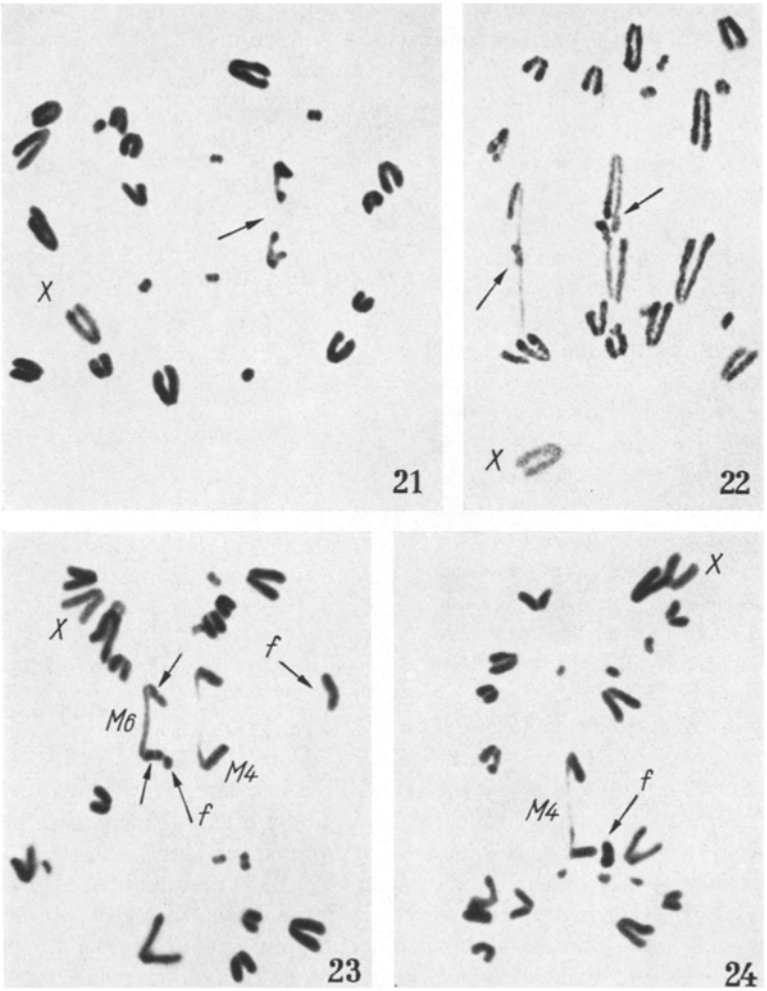


Fig. 20. Delayed separation of the *X*-chromatids at second anaphase in *S. paranensis*. Note also the nucleolar constriction (arrow) in the M_6 chromosome (1000 ×)

The former type is not, strictly speaking, a dicentric bridge, for chromatid arms are associated sub-terminally. In all probability this association involves subchromatid fractions. The latter type is a true dicentric bridge, and is associated with one or two free acentric fragments. Bridges similar to these have undoubtedly been erroneously described on many occasions as the products of single crossovers in relatively inverted chromosome segments. But our unpublished observations on other orthopterans leave us in no doubt that they originate following sister chromatid breakage and reunion during meiotic prophase. Indeed, we have found one diplotene cell showing this type of breakage in *S. paranensis*.

In addition to these isolated errors two anomalies were of much wider distribution. One of these was in fact restricted to a single individual, but affected all the meiotic cells. This was an individual possessing a much reduced chiasma frequency, a modified pattern of chiasma distribution and partial asynapsis. The second anomaly was found in several individuals, though in any one case only a relatively small number of meiotic cells were affected. These cells were tetraploid.

There have been relatively few adequate analyses of either situation in complements where all the chromosomes can be individually distinguished. For this reason they will be considered in detail.



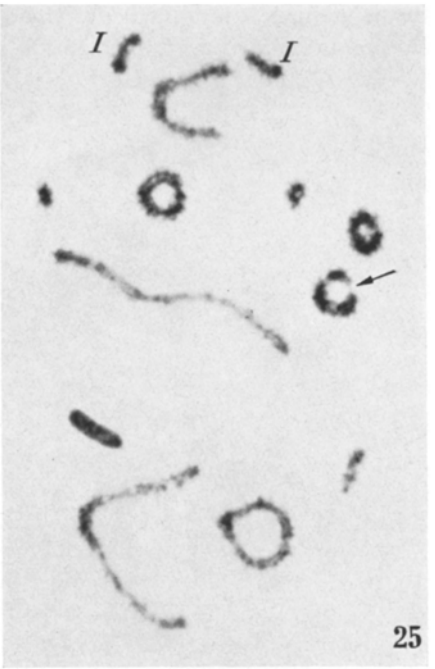
Figs. 21—24. Bridge formation at first anaphase in *S. paranensis* (all 1000 ×). 21: Single, broken, sub-chromatid bridge in M_7 homologues. 22: Sub-chromatid bridges in L_2 and M_6 bivalents. 23: Two dicentric bridges with free acentric fragments, resulting from chromosome breakage with sister chromatid reunion in the M_4 and M_6 chromosomes. Note secondary constrictions in both the arms of the M_6 bivalent (arrows). 24: Single dicentric bridge with acentric fragment involving the M_4 chromosomes

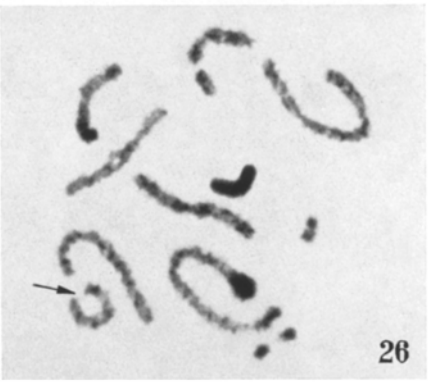
a) The Asynaptic Individual

Under normal circumstances in *S. paranensis* the number of chiasmata present in a chromosome is related to its length (Fig. 19). The

large chromosomes usually form 3 or 2 chiasmata (Figs. 9, 11 and 39), rarely 4 or 1; the mediums 2 or 1 (Figs. 9, 11 and 39), rarely 3 (Fig. 9); and the smalls invariably 1 (Fig. 9, 11 and 39). When more than one chiasma is present in a bivalent, they tend to be distributed equidistantly along the length of the chromosome. In cases where only one chiasma is present, this is either interstitial or distal in location — rarely proximal (Fig. 39). In this it is comparable to *S. gregaria* (HENDERSON, in preparation).

Twenty-five cells of the asynaptic individual were analysed in detail. These were all scored at first metaphase, since the centromeric marker was surprisingly absent from diplotene cells of this individual (Figs. 25 and 26). In fifteen cells univalent chromosomes were present. Eight of these possessed 2 univalents per cell and in the remaining seven there were 4 univalents per cell. This gave an overall mean univalent frequency for the individual as a whole of 1.76 univalents per cell. In all cells, however, the pattern of chiasma distribution was considerably modified. This redistribution involved two main changes:

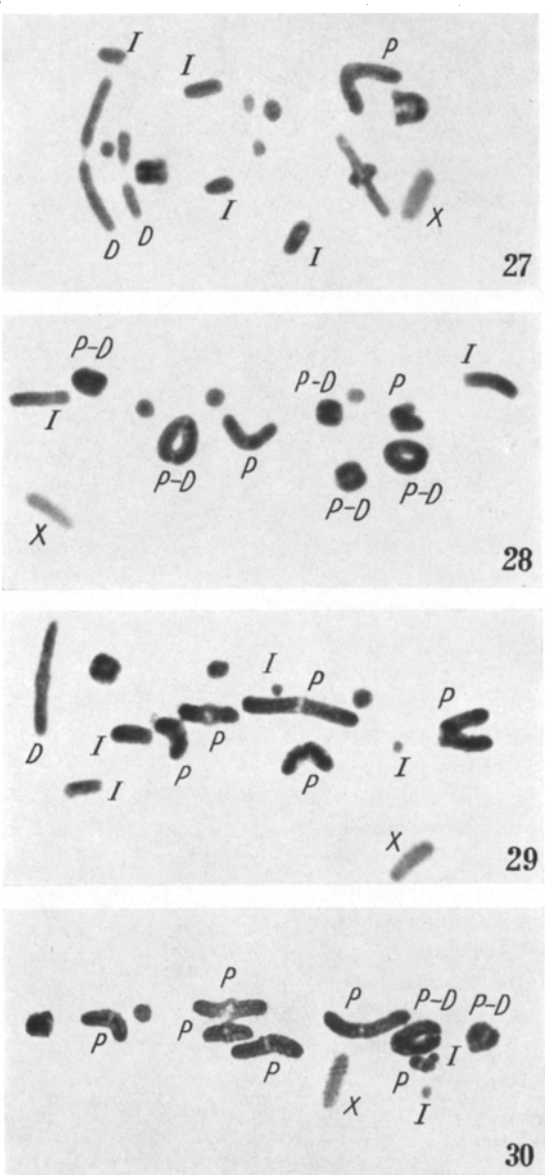




Figs. 25 and 26. Diplotene in the partially asynaptic individual of *S. paranensis* (acetic orcein; both 1250 ×). 25: Asynapsis in the M_7 pair, with accompanying reduction in chiasma frequency and localisation. 26: Localisation without asynapsis. With the exception of the M_6 (arrow) all other bivalents have only one chiasma. In both cells, nucleolar gaps are present in the M_6 (arrow)

(i) An overall reduction in the number of chiasmata formed by all members of the complement, the longs being affected the most. Thus the mean chiasma frequency of the long chromosomes was reduced from ca. 2.4 per

bivalent to 1.3 per bivalent, the longs forming either one or two chiasmata. Only one L bivalent in all twenty-five cells was observed



Figs. 27—30 (all 1250 ×). Asynapsis and localisation at metaphase I in *S. paranensis*. 27: 4 M-univalents, one bivalent with a proximally localised chiasma (*P*-bivalent) and two showing distal localisation (*D*-bivalent). 28: 2 *L*-univalents, two *P*-bivalents and 5 *P*—*D* rings. 29: 2 *M* and 2 *S*-univalents, 5 *P*- and 1 *D*-bivalent. 30: 2 *S*-univalents, 6 *P*- and 2 *P*—*D* bivalents

to form 3 chiasmata. In the medium chromosomes the frequency was reduced from ca. 1.8 per bivalent to 1.3 per bivalent. Here again only one or two chiasmata were present in those bivalents which did form. Finally, in the small class there was relatively little reduction from the control mean of 1.0 per bivalent to a value of 0.9 per bivalent. These changes reduced the overall mean chiasma frequency per individual from the 19.32 per cell normally found to a value of 13.08 per cell.

(ii) The presence of a completely novel pattern of chiasma distribution. This depended on a restriction of chiasmata to the chromosome ends. As a result, when two chiasmata were present, these were proximally-distally localised (Figs. 25—30). Single chiasmata were predominantly located proximal to the centromere (Table 3; Figs. 27—30).

Table 3. *Analysis of chiasma and univalent frequency and distribution in 25 cells of the partially asynaptic individual of S. paranensis at first metaphase*

Chromosome character			Chromosome type			
			Long	Medium	Short	
Total number univalents			12	24	8	
Xa frequency	Total		98	158	71	
	Mean/bivalent		1.3	1.3	0.9	
Chiasma distribution[1]	1 Xa	P . . .	29	51	80 (72.7%)	Totals
		D . . .	11	16	27 (24.6%)	
		I . . .	1	2	3 (2.7%)	
	2 Xta. P—D .		27	43	70 (97,2%)	
	3 Xta. P—I—D		1	1	2 (2,8%)	

[1] N. B. It is impossible to consistently score the positions of chiasmata in the small chromosomes at MI, they have therefore been excluded from this section of the analysis.

When they did not occupy a proximal position they were almost invariably distal. In only 3 cases were markedly interstitial chiasmata present.

A study of zygotene and pachytene stages helped to clarify the nature of these two categories of change. In some zygotene cells there was complete failure of pairing of certain homologues: clearly therefore, the formation of univalents here must result from true asynapsis. In other cells, pairing was incomplete (Fig. 31), chromosome ends pairing in preference to interstitial regions. Since the centromere was still marked by its pronounced heteropycnosity in these early zygotene/pachytene nuclei, it was possible to distinguish the two ends of the chromosome. With this marker it was apparent that such restricted pairing involved

either both proximal and distal ends or else, and more commonly, proximal ends alone. Distal localisation of pairing was not common. This incompleteness of pairing commonly gave pachytene an atypical "diplotene-like" appearance (Fig. 31). But this stage is distinguishable from true diplotene, particularly in those cells where some of the homologues succeed in pairing fully while others do not.

At first anaphase the univalent chromosomes lag on the spindle and undergo a delayed separation (Figs. 32 and 33). The presence of such dividing univalents at AI affects spindle elongation and cell cleavage. As a result, both these processes fail and a restitution cell is formed (Fig. 34). Such cells may contain a diploid number of half bivalents. But when univalent separation has been achieved at first anaphase, one or more pairs of half-univalents may be present. All orientate on a common spindle and go through an apparently normal second anaphase separation. But again cell cleavage fails and tetraploid spermatids result. These are distinguishable from the normal haploid spermatids by the presence of two heteropycnotic X's (Fig. 35). Haploid spermatids have either a single X (Fig. 35) or none at all. There is also a marked difference in size between the haploid and tetraploid spermatids. Indeed, this difference persists during spermiogenesis (Figs. 36 and 37).

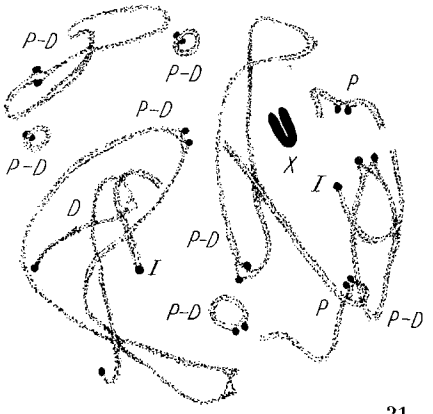


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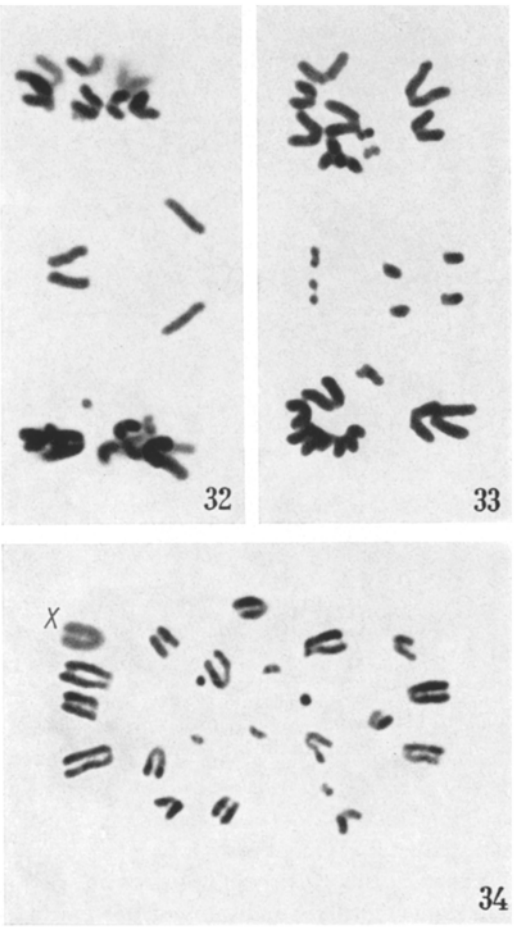
Fig. 31 (1000 ×). Pachytene in the partially asynaptic individual showing failure of pairing in one of the *M*-chromosomes (2 *I*) and in complete pairing with varying kinds of localisation in the remainder of the complement (2 *P*, 1 *D* and 7 *P*—*D* bivalents)

That the presence of autosomal or sex chromosomal univalents can affect the function of the spindle is clear from the work of CALLAN and JACOBS (1957) on *Mantis religiosa*. But in their case there was a complete suppression of separation: anaphase never even began. Such cells remained indefinitely at first metaphase and ultimately degenerated. There is, as CALLAN and JACOBS point out, no obvious explanation why the presence of univalents in *Mantis* should completely inhibit bivalent separation. But the situation in *S. paranensis* is more readily understood. The univalents do not divide until bivalent separation has already been achieved (Figs. 32 and 33). The presence of such dividing univalents maintains the spindle in its anaphase condition and precludes the process of mid-spindle elongation. This in turn leads to a failure

of furrowing. The failure of cleavage which must occur at second division cannot be explained so readily. Certainly it does not depend on the lagging of half-univalents, for this has never been observed. Perhaps the presence of such a large number of chromosomes (4×) on the spindle may be involved.

This case is in marked contrast to the two other known cases of spontaneous univalent formation in locusts (see JOHN and NAYLOR 1961). It differs in two important respects:

a) As in *Locusta* (REES 1957) all chromosome classes show univalent formation. But unlike *Locusta*, where the longer members of the complement are predominantly affected, in *S. paranensis* the effect is distributed more uniformly throughout the complement. In *S. gregaria*, on the other hand, the effect is confined to the small members of the complement. Indeed, *S. paranensis*, where the medium members are slightly more affected than either the longs or shorts, can be conveniently considered as an intermediary in the nature of the response shown. REES makes no comment on any localisation of chiasma present in his case.

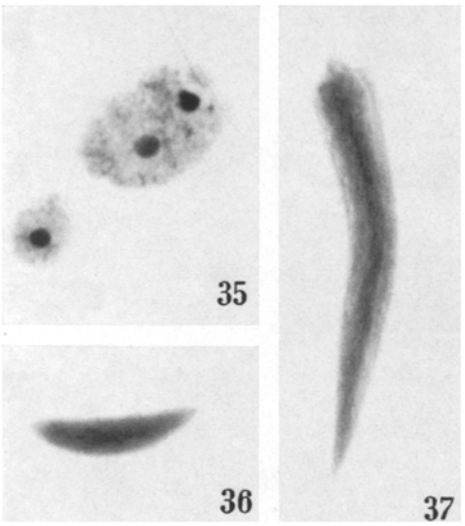


Figs. 32—34. The consequences of univalent formation in the partially asynaptic individual of *S. paranensis* (all 1250×). 32: Division of two lagging *L*-univalents at late AI. 33: Two S- and two M-laggards dividing at AI. 34: Restitution second metaphase with 22 autosomes and a single *X*-chromosome

b) Re-examination of the *S. gregaria* data (JOHN and NAYLOR 1961) reveals the significant fact that the reduction in chiasma frequency in those chromosomes which succeeded in forming bivalents was in

fact also occasionally associated with localisation. But this localisation, unlike that in *S. paranensis*, was distal, not proximal. Asynaptic individuals of *S. gregaria* induced by heat shock show the same pattern of predominantly distal localisation (HENDERSON, in preparation).

Genetic control of chiasma localisation has been established in *Allium* (DARLINGTON 1958). Moreover in *Allium nutans* (DARLINGTON 1937) and *Secale cereale* (REES 1955), proximal localisation has arisen following inbreeding. It may be significant that all three locusts under discussion were obtained from relatively inbred cultures. Certainly we have no reason to doubt that the asynapsis and localisation found in *S. paranensis* and *S. gregaria* are genotypic in origin. Even so, there are two possible ways in which these marked differences in localisation observed in two such closely related species could have arisen.



Figs. 35—37 (all 2000 ×). 35: Diploid and tetraploid X-containing spermatid nuclei in the partially asynaptic *S. paranensis* individual. 36 and 37: Diploid and tetraploid spermatids maintaining size differences during spermiogenesis

Either:

i) The localisation is a comparatively novel phenomenon, or

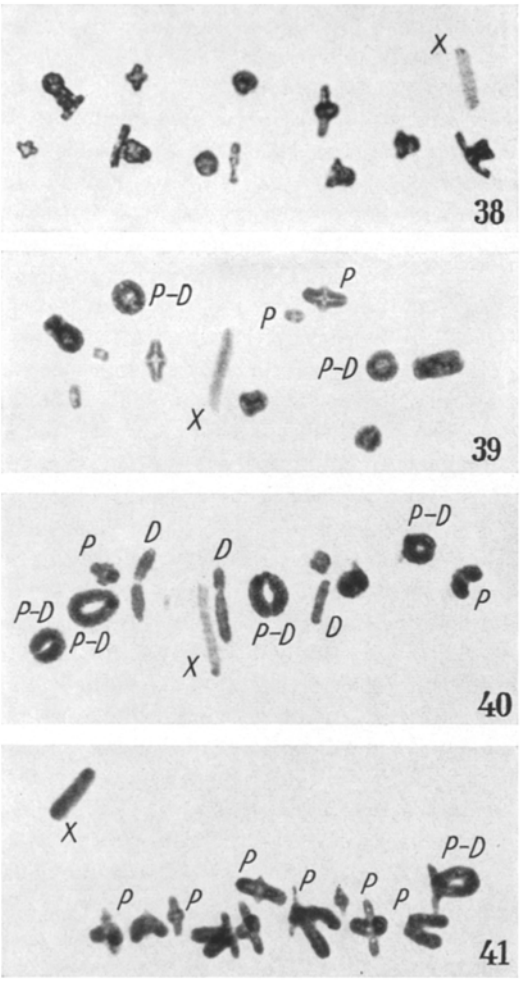
ii) It owes its origin to a mechanism already obtaining in the species.

Thus, in the latter case, pairing may usually be initiated at the proximal or distal segments, with or without subsequent interstitial regions of pairing. But this sequence may not be reflected in chiasma distribution under normal conditions of full pairing. However, with restriction of pairing, chiasmata would then be localised to regions which pair first.

The fact that proximally localised chiasmata, though rare in both species, are more commonly found in *S. paranensis* than in *S. gregaria* under normal circumstances (Fig. 39), suggests the latter possibility is involved. Heat induced asynapsis supports this suggestion: distal localisation is most commonly induced in *S. gregaria*, proximal is more commonly induced in *S. paranensis* (HENDERSON, in preparation). The differences are species specific.

WHITE (1954b, pp. 96—97) has suggested that "In the *Acrididae* and *Tettigoniidae* there is a combination of proximal and distal localisa-

tion ... thus in many grasshoppers localisation is of the proximal-distal type in the larger bivalents, proximal only in the smaller ones". We know of two common kinds of exception to this rather broad generalisation:



Figs. 38—41 (all first metaphases, 1000 ×). 38: *S. gregaria.* 39: *S. paranensis,* this cell shows the most marked localisation we have observed in a normal individual. 40: *Duronia tricolor* (2n = 23 ♂). 41: *Tylotropidius gracilipes* (2n = 23 ♂). These latter two species show localisation as a regular feature

i) Species in which single interstitial chiasmata occur in one or more size classes. For example, single interstitial chiasmata occur in all classes in *Locusta migratoria*, *Pyrgomorpha kraussi (Acrididae)*, *Leptophyes punctatissima* and *Pholidoptera griseoaptera (Tettigoniidae)*. The

same is true of the medium and small chromosomes of both species of *Schistocerca, Chorthippus spp.* and *Omocestus viridulis (Acrididae)*.

ii) Species in which the larger members of the complement form more than two chiasmata. Here additional chiasmata occupy the interstitial region. Such species include the *Schistocerca spp.*, the *Chorthippus spp.*, *Omocestus viridulis (Acrididae)*, *Pholidoptera griseoaptera* and *Tettigonia viridissima (Tettigoniidae)*.

On the other hand, there are some clear instances which support WHITE's contention. We have direct experience of two of these. One of them is *Duronia tricolor* ($2n = 23\ \delta$) where chiasmata are commonly localised at the ends of the chromosomes (Fig. 40). In the other, *Tylotropidius gracilipes* ($2n = 23\ \delta$) chiasmata are more strictly localised. Single chiasmata are proximal, two chiasmata are proximal-distal in distribution (Fig. 41). Such cases are, however, in our experience, uncommon. Indeed, it is perhaps significant that only two species, *Stethophyma grossus* and *Bryodema tuberculatum bavaricum* (WHITE, 1954a) have been described in which chiasma localisation is more extreme than in *Tylotropidius*. For this reason we regard WHITE's statement as an over-generalisation. As GEORGE BERNARD SHAW reminds us, "*The golden rule is, there is no golden rule*".

Our example in *S. paranensis* shows how such extreme patterns of localisation may be established relatively quickly in evolution. For while it is true that 15 of the 25 cells scored possessed univalents, and as a result would have no future in heredity, the remaining 10 (ca. 40% of the cell population) had no univalents but showed an equally marked degree of localisation. These cells will produce normal, functional gametes, and given a selective advantage could, within a few generations, establish a completely novel pattern of localisation. Unlike many evolutionary changes, this need not affect both sexes. Indeed, in some cases of localisation the two sexes are known to differ in the nature of the localisation present. In newts of the genus *Triturus* the male shows distal localisation (CALLAN and SPURWAY 1951) but in the lampbrush chromosomes of the female, chiasmata can be seen to be proximally localised (CALLAN and LLOYD 1961).

The presence of such a difference between the sexes is commonly found in cases where an adjustment in crossing over has been made during evolution. And this adjustment follows HALDANE's Rule (DARLINGTON and MATHER 1949, p. 229): the reduction always appears to be more pronounced in the heterogametic sex. In *Drosophila* it is in the male (XY) that meiosis has become achiasmate. In silkworms, the male sex is homozygous and crossing over is absent in the heterogametic female (TANAKA 1953). Significantly, chiasmata are distally localised in the female (MAEDA 1939) but more distributed in the male where a

moderate amount of crossing over occurs. Finally in the hermaphrodite *Fritillaria meleagris* the same degree of localisation occurs in both embryo-sac and pollen mother cells (DARLINGTON and LACOUR 1941).

Such genotypically controlled changes in chromosome behaviour as we have here described in *Schistocerca* show how a change in the heredity of the individual may control the variation of the population of which it is a member. And the selection of this variation may provide the material for speciation. The heredity and variation which control evolution are therefore themselves subject to evolution. Their mechanism varies, is selected and adapted in just the same way as are the more conventional morphological characters (DARLINGTON and MATHER 1949).

b) The Polyploid Cells

Polyploid cells were present in several of the specimens examined but their frequency varied in different individuals. Two types of polyploid cells were found: those containing and those which did not contain

Table 4. *Analysis of 25 tetraploid cells in S. paranensis, summarising the frequencies and distributions of the different chromosome associations throughout the 3 size classes of the complement*

Cell no.	IV				III + I				II				2 I			
	L	M	S	Total	L	M	S	Total	L	M	S	Total	L	M	S	Total
1	3	5	1	9	—	—	—	—	—	—	4	4	—	—	—	—
2	3	5	1	9	—	—	—	—	—	—	4	4	—	—	—	—
3	3	3	1	7	—	—	—	—	—	4	4	8	—	—	—	—
4	3	3	—	6	—	—	—	—	—	4	6	10	—	—	—	—
5	3	2	—	5	—	—	—	—	—	6	6	12	—	—	—	—
6	3	3	—	6	—	1	—	1	—	2	6	8	—	—	—	—
7	3	4	1	8	—	—	—	—	—	2	4	6	—	—	—	—
8	3	3	2	8	—	—	1	1	—	4	—	4	—	—	—	—
9	3	5	—	8	—	—	—	—	—	—	6	6	—	—	—	—
10	1	3	2	6	—	—	—	—	4	4	2	10	—	—	—	—
11	3	4	1	8	—	—	—	—	—	2	4	6	—	—	—	—
12	3	5	—	8	—	—	—	—	—	—	5	5	—	—	1	1
13	3	4	—	7	—	—	—	—	—	2	6	8	—	—	—	—
14	3	4	—	7	—	—	—	—	—	2	6	8	—	—	—	—
15	3	5	—	8	—	—	—	—	—	—	6	6	—	—	—	—
16	3	5	1	9	—	—	—	—	—	—	3	3	—	—	1	1
17	3	2	—	5	—	—	—	—	—	6	6	12	—	—	—	—
18	1	3	1	5	—	—	1	1	2	4	4	10	—	—	—	—
19	3	4	—	7	—	—	—	—	—	2	5	7	—	—	1	1
20	3	5	1	9	—	—	—	—	—	—	4	4	—	—	—	—
21	3	3	1	7	—	—	1	1	—	4	2	6	—	—	—	—
22	3	3	—	6	—	—	—	—	—	4	6	10	—	—	—	—
23	3	3	—	6	—	—	—	—	—	4	6	10	—	—	—	—
24	2	4	—	6	—	—	—	—	2	2	6	10	—	—	—	—
25	3	2	—	5	—	—	1	1	—	6	4	10	—	—	—	—
Total	70	92	13	175	—	1	4	5	8	64	115	187	—	—	3	3
Mean	2.8	3.7	0.5	7.0	—			0.2	0.3	2.6	4.6	7.5	—	—	0.1	0.1

multivalents. Like their counterparts in *Pyrgomorpha* (LEWIS and JOHN 1960), the latter, which were more common, originate in multinucleate cells produced following a failure of cytoplasmic division. Multivalent containing cells were all tetraploid. Though less common they were much more instructive. Twenty-five such cells were analysed in detail with regard to the formation and distribution of multivalents throughout the complement (Tables 4, 5). This analysis revealed the following points of interest.

Table 5. *Chiasma frequency analysis in 16 tetraploid cells of S. paranensis*

Total chiasmata per chromosome group				Cell total	
	L (3)	M (5)	S (3)		
	19	18	6	43	
	20	21	7	48	
	18	20	6	44	
	16	16	5	37	
	16	18	7	41	
	16	19	6	41	
	17	24	6	47	
	20	20	8	48	
	24	21	7	52	
	17	19	5	42	
	14	16	6	36	
	18	22	6	46	
	16	20	9	45	
	24	21	6	51	
	19	22	6	47	
	14	20	9	43	
Cell mean	18.00	19.81	6.56	44.44	$4x$ Mean
Mean per chromosome pair					
$4x$	3.00	1.98	1.09		
$2x$	2.44	1.80	1.00	19.33	$2x$ Mean

1. In all cases the two X chromosomes do not form a chiasmate association (Figs. 42—47).

2. All size classes can form multivalents.

3. The L-chromosomes almost invariably form multivalents (93.3%), the M-chromosomes do so very commonly (73.6%) but the S-chromosomes rarely form them (17.3%).

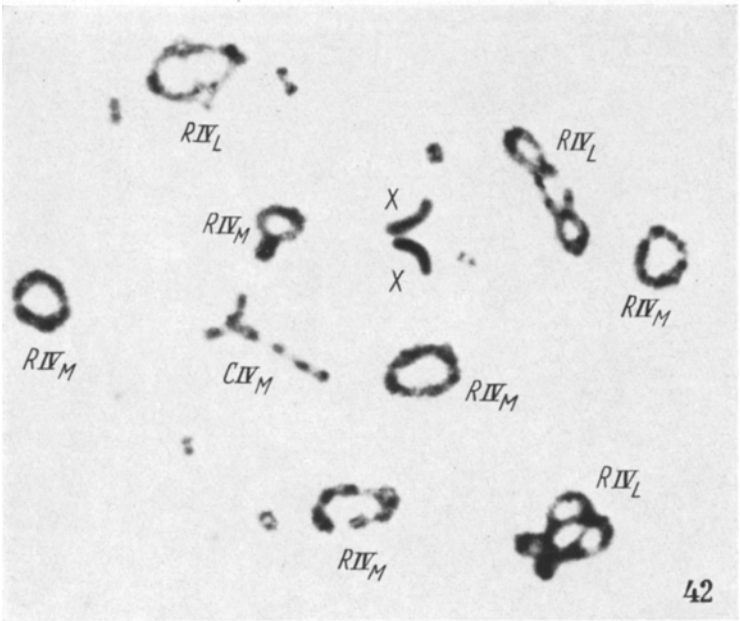
4. Occasionally trivalents and univalents were formed, but in the medium and small classes only (Table 5, Fig. 45).

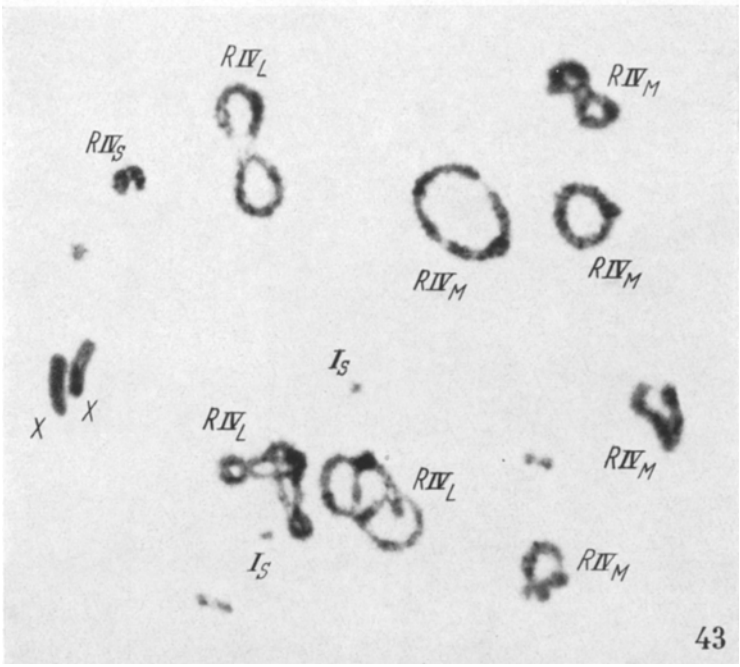
5. Univalent production in the small chromosomes, unaccompanied by trivalents, also occurred in three cells (Fig. 43).

6. Chain quadrivalents were never observed in the long chromosomes. They were sometimes present in the medium class (Figs. 42 and 45) while almost all quadrivalents involving the small chromosomes were of this type (Figs. 45 and 46).

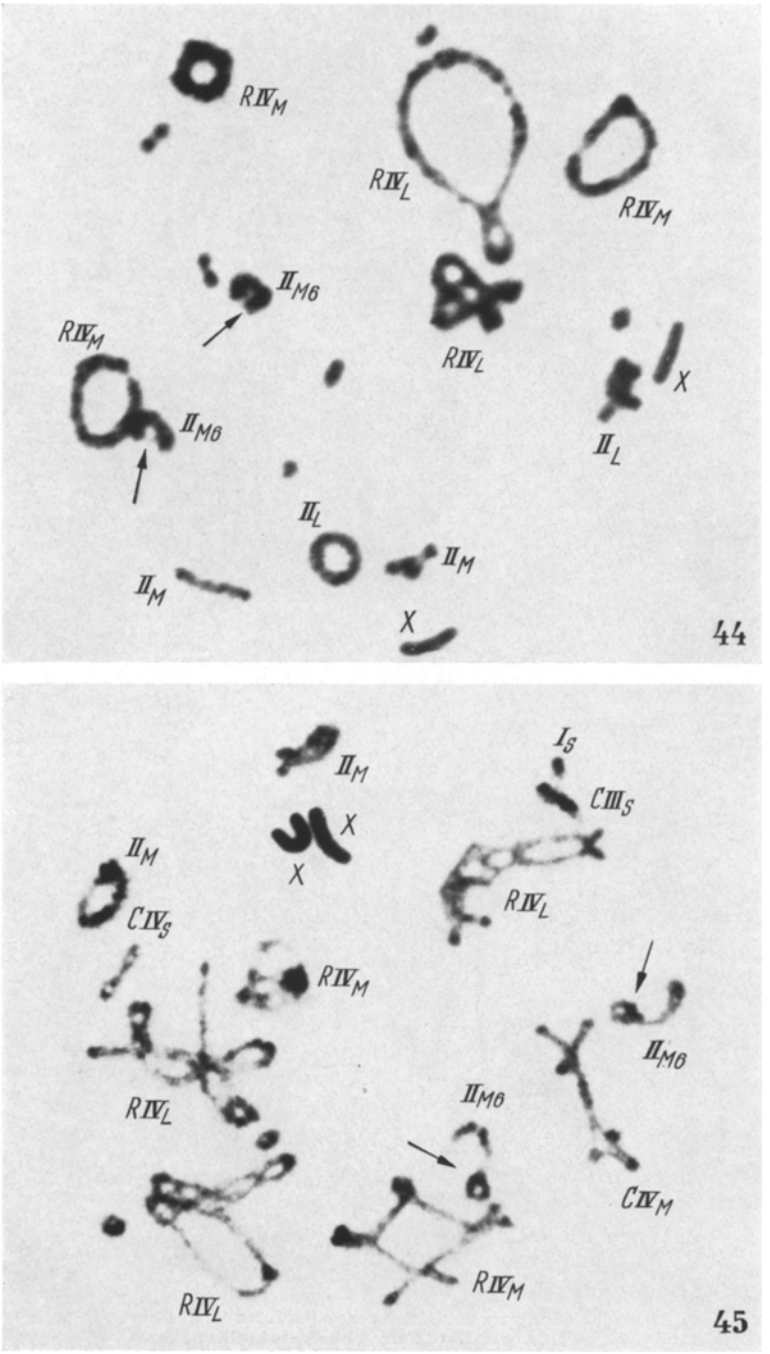
7. Many of the large quadrivalents had a complex structure, with a large number of chiasmata involved (Figs. 42—47).

8. Because of this last feature it was not possible to score chiasma frequency accurately in the multivalents of all 25 cells. But 16 cells were analysed in this way (Table 5) and in these the total chiasma frequency per cell varied from 36 to 49, with a mean of 44.44. The corresponding mean in diploid cells is 19.33. The average number of





Figs. 42 and 43. Tetraploid cells from *S. paranensis* (both 1000 ×). 42: 8 *RIV* + 1 *CIV* + 6 *II* + 2 *X*, all the S-chromosomes have formed bivalents in this cell. 43: 9 *RIV* + 3 *II* + 2 *I* + 2 *X*, two of the S-chromosomes are unpaired, there are 3 S-bivalents and one S-quadrivalent



Figs. 44 and 45 (Legend p. 133)

chiasmata present in the tetraploid cells is thus usually well in excess of twice the average diploid number.

9. Not only the number of quadrivalents, but also the number of chiasmata they contain, and hence their complexity, is a reflection of the diploid chiasma frequencies of the bivalents concerned.

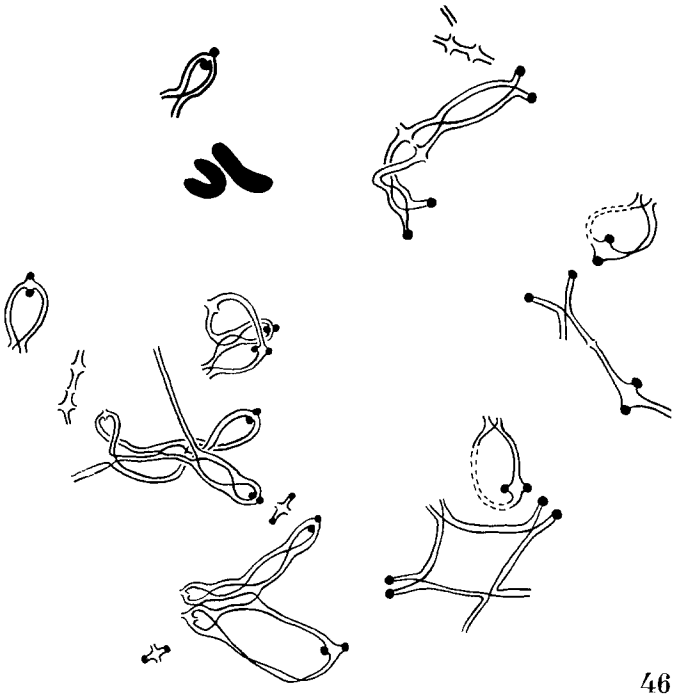


Fig. 46. Tetraploid cell from *S. paranensis* drawn to show chromatid structure — 5 *RIV* + 2 *CIV* + (*CIII* + *I*) + *bII* + 2 *X* (compare Fig. 45: 1000 ×)

These observations on *S. paranensis* are sufficiently instructive to merit a more general consideration of the properties of multivalent formation. There are six relevant points to deal with:

1. White (1934) has studied tetraploid spermatocytes in *S. gregaria*, though unfortunately he only obtained two such cells. In these a total of 6 quadrivalents were found, which were confined exclusively to the larger chromosomes. Since *S. gregaria* and *S. paranensis* do not differ in either their mean chiasma frequency or its variance, and since the chromosomes have a similar size range, one might expect comparable behaviour to obtain in tetraploid cells of both species. To date we have

Figs. 44 and 45. Tetraploid cells from *S. paranensis* (both 1000 ×). 44: 5 *RIV* + 12 *II* + 2 *X*, all the S-chromosomes have formed bivalents, the two M_6-bivalents both show a nucleolar gap (arrow). 45: 5 *RIV* + 2 *CIV* + (*CIII* + *I*) + 6 *II* + 2 *X*, one of the chain quadrivalents is composed of S-chromosomes as is the (*CIV* + *I*) and the 2 M_6-bivalents are again heteromorphic for pronounced nucleolar gaps (arrows)

found only one complete and fully analysable tetraploid cell in *S. gregaria*. This had 6 quadrivalents, one of which was a chain of four S-chromosomes (Fig. 57). The cell as a whole was similar to the tetraploid cells found in *S. paranensis*, both with regard to the high total chiasma frequency (53) and with regard to the number of chiasmata present in the complex L-multivalents. On of these, involving the L_1 chromosome, possessed a total of 10 chiasmata (Figs. 56 and 58). We would conclude, therefore that the quadrivalent and chiasma frequency in *S. gregaria* would not appear to be as low as WHITE'S use of microtomy would lead us to believe.

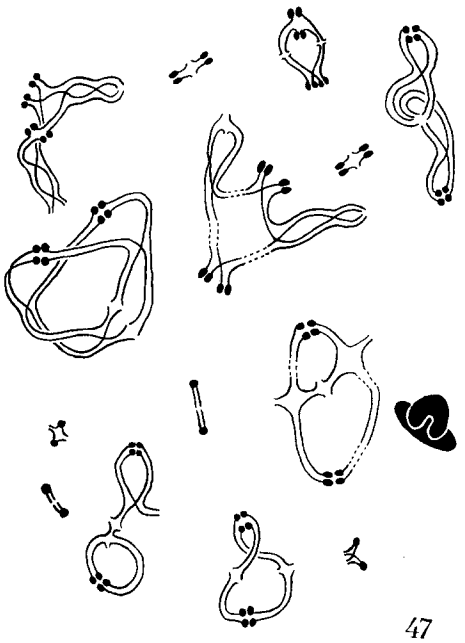


Fig. 47. Tetraploid cell from *S. paranensis* drawn to show chromatid structure — 8 *RIV* + 6 *II* + 2*X* (1000 ×)

2. All the evidence we have obtained from tetraploid orthopteran cells supports the contention that, within the limits imposed by the genotype, quadrivalent formation is predominantly a function of relative chromosome length and of chiasma frequency.

In *Pyrgomorpha kraussi* (LEWIS and JOHN 1960) there are 19 chromosomes in the diploid male complement, one of which is an X chromosome. This species has a mean diploid chiasma frequency of 9.8 per cell. The L-chromosomes often form rings, but these are uncommon in M's and unknown in S's. Single chiasmata are either interstitial or distal in position. In tetraploid cells, multivalents are rarely formed and those that are possess few chiasmata. For this reason chains are more common than rings, though most are full quadrivalents: trivalents are rare. Never more than five quadrivalents have been found in any cell at one time and usually only one or two are present. These invariably involve the larger autosomes (Table 6). The low chiasma and multivalent frequency of the tetraploid cells in this species are reflections of the low diploid chiasma frequency. However, here again (Table 7), the mean chiasma frequency per tetraploid cell (22.3) is in excess of twice the mean diploid value (9.8 per cell).

We have found isolated polyploid cells in other orthopteran species (Table 8), for example *Chorthippus brunneus* (Fig. 59) and *Locusta*

Table 6

Analysis of 10 tetraploid cells in Pyrgomorpha kraussi, summarising the frequencies and distributions of the different chromosome associations throughout the 3 size groups

Cell no.	IV				III + I				II				2I			
	L	M	S	Total	L	M	S	Total	L	M	S	Total	L	M	S	Total
1	2	3	—	5	—	—	—	—	—	4	4	8	—	—	—	—
2	1	3	—	4	—	—	—	—	2	4	4	10	—	—	—	—
3	—	1	—	1	—	—	—	—	4	7	4	15	—	1	—	1
4	1	1	—	2	—	—	—	—	2	8	4	14	—	—	—	—
5	1	1	—	2	—	—	—	—	2	8	4	14	—	—	—	—
6	—	—	—	—	—	—	—	—	4	10	4	18	—	—	—	—
7	1	—	—	1	—	—	—	—	2	10	4	16	—	—	—	—
8	1	—	—	1	—	—	—	—	2	9	4	15	—	1	—	1
9	1	—	—	1	—	—	—	—	2	10	4	16	—	—	—	—
10	2	—	—	2	—	—	—	—	—	10	4	14	—	—	—	—
Total	10	9	—	19	—	—	—	—	20	80	40	140	—	2	—	2
Mean	1.0	0.9	—	1.9	—	—	—	—	2.0	8.0	4.0	14.0	—	0.2	—	0.2

migratoria (Figs. 51 and 52). These two cases further support our above findings. Thus in *Chorthippus* (2n = 17 ♂) the two longest pairs of chromosomes only were involved in quadrivalent formation. One of these possessed 6, the other 10 chiasmata, raising the chiasma frequency of the tetraploid cell as a whole to 35. The mean diploid chiasma frequency in this species is 13.05 per cell (LEWIS and JOHN, in preparation) and the long chromosomes usually possess 2 or 3 chiasmata.

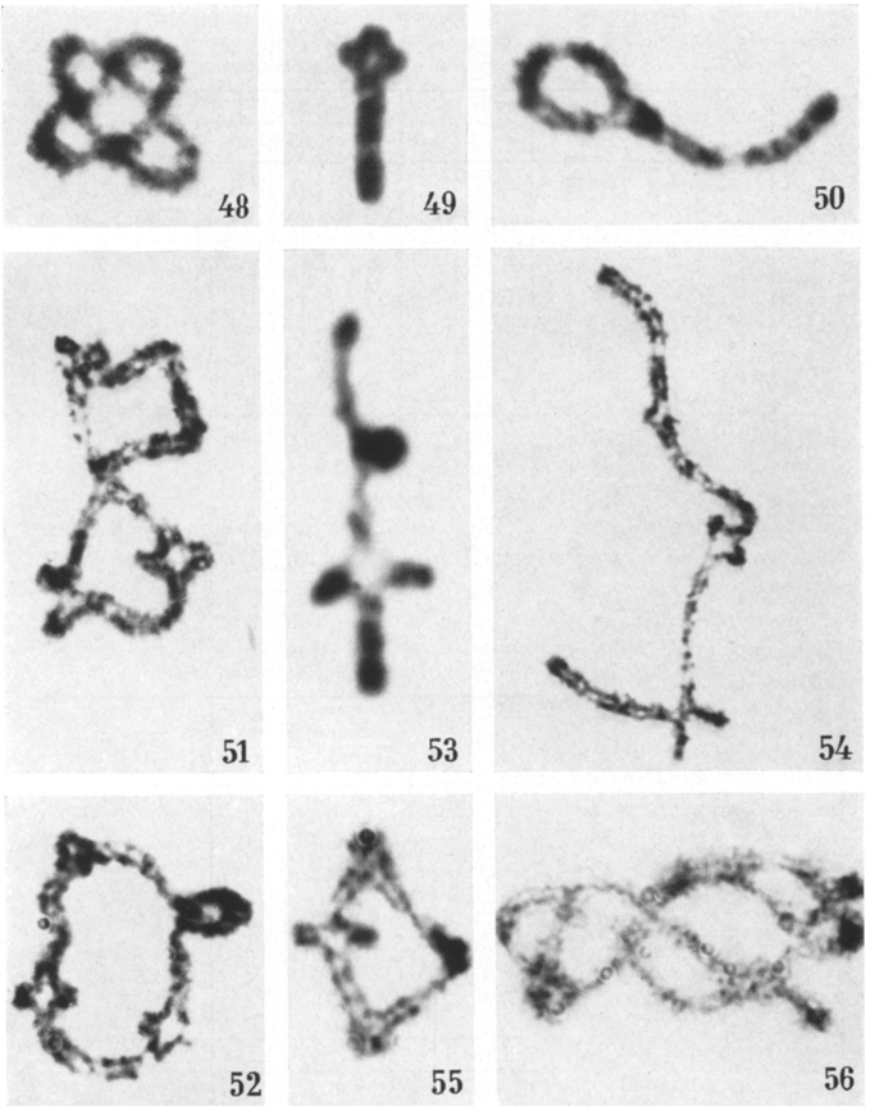
Table 7

Chiasma frequency analysis of the 10 tetraploid cells from Pyrgomorpha kraussi listed in Table 6

Cell no.	Total Xta. per chromosome group			Cell total	
	L (2)	M (5)	S (2)		
1	10	16	4	30	
2	7	15	4	26	
3	7	10	4	21	
4	8	11	4	23	
5	6	12	4	22	
6	5	10	4	19	
7	7	10	4	21	
8	6	9	4	19	
9	7	10	4	21	
10	6	11	4	21	
Cell Mean	6.9	11.4	4.0	22.3	4*x* Mean
Mean per chromosome pair					
4*x*	1.73	1.14	1.00		
2*x*[1]	1.42	1.02	1.00	9.8	2*x* Mean

[1] Scored in 25 cells taken from the individual in which all 10 tetraploid cells were obtained.

Finally in *Locusta* (2n = 23 ♂), where a chiasma frequency between that of *Pyrgomorpha* and *Schistocerca* obtains (mean diploid value of 14.3 per cell), the only cell so far found had 3 quadrivalents, all of which involved the long chromosomes and which possessed either 4 or 5 chiasmata (Figs. 51 and 52). In diploid cells such chromosomes have only one or two chiasmata. The



Figs. 48—56. Isolated multivalents from tetraploid cells of *S. paranensis* (48—50 2000 ×); *L. migratoria* (51—52, 2500 ×); *P. kraussi* (53—54, 1500 ×) and *S. gregaria* (55—56, 2000 ×). 48: Ring quadrivalent with 8 chiasmata. 49: Chain quadrivalent. 50: "Frying pan" quadrivalent. 51 and 52: Ring quadrivalents with 5 chiasmata. 53: Trivalent. 54: Chain quadrivalent. 55: Ring quadrivalent with 4 chiasmata. 56: Complex ring quadrivalent with 10 chiasmata. (See also Fig. 58)

total chiasma frequency of this tetraploid cell was 35, again more than twice the diploid value.

Essentially the same correlations can be drawn from the studies of ROTHFELS (1950) on *Neopodismopsis abdominalis*, WHITE (1954) on *Tettigonia viridissima*, and KLINGSTEDT (1939) on *Chrysochraon dispar*. The latter author tabulates information which had, to that date, been

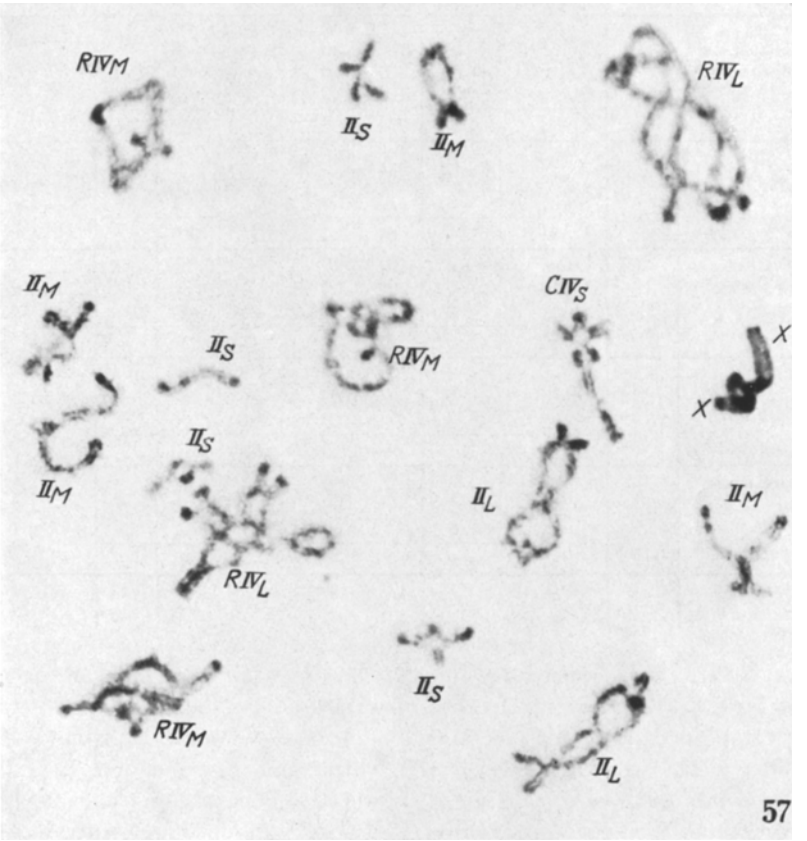


Fig. 57. Tetraploid cell from *S. gregaria* possessing 5 *RIV* + 1 *CIV* + 10 *II* + 2 *X*. In this cell two of the bivalents involve L-chromosomes and the single chain quadrivalent is composed of S-chromosomes (1000 ×)

obtained on the occurrence of multivalents in tetraploid cells in species with chromosomes of different types (His Table 2, p. 199). This table clearly demonstrates a correlation between relative length, chiasma frequency and the occurrence of multivalents.

3. CALLAN (1949) has made a study of tetraploid cells at first meiotic metaphase in the dermapteran *Forficula auricularia*. In this species the eleven pairs of metacentric autosomes normally possess a single chiasma in one arm only, though ring bivalents having a single chiasma

Table 8. *Frequency and distribution of chromosome associations within the complements of 6 different species of insect*

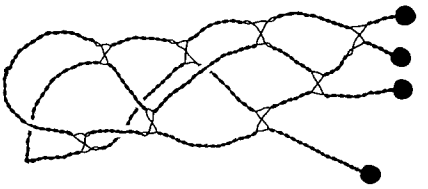
Species	$2x$	Autosomal complement		Observed numbers of				Total cells analysed
		Type	haploid number	IV	III + I	II	2I	
Schistocerca paranensis	23	Long	3	70 (93.3%)	—	8	—	25
		Medium	5	92 (73.6%)	1	64	—	
		Short	3	13 (17.3%)	4	115	3	
		Total	11	175 (63.6%)	5	187	3	
Schistocerca gregaria	23	Long	3	2 (66.6%)	—	2	—	1
		Medium	5	3 (60.0%)	—	4	—	
		Short	3	1 (33.3%)	—	4	—	
		Total	11	6 (54.5%)	—	10	—	
Pyrgomorpha kraussi	19	Long	2	10 (50.0%)	—	20	—	10
		Medium	5	9 (18.0%)	—	80	2	
		Short	2	— (0.0%)	—	40	—	
		Total	9	19 (21.1%)	—	140	2	
Locusta migratoria	23	Long	3	3 (100.0%)	—	—	—	1
		Medium	5	— (0.0%)	—	10	—	
		Short	3	— (0.0%)	—	6	—	
		Total	11	3 (27.2%)	—	16	—	
Chorthippus brunneus	17	Long	3	2 (66.6%)	—	2	—	1
		Medium	4	— (0.0%)	—	8	—	
		Short	1	— (0.0%)	—	2	—	
		Total	8	2 (25.0%)	—	12	—	
Forficula auricularia	24	All small	11	37 (24.0%)	12	206	4	14

in each arm are occasionally found. This restriction of chiasmata he attributes to chiasma interference operating across the centromere. In 14 tetraploid cells of this species (Table 8) CALLAN found that from 0—6 quadrivalents were present per cell, with a mean value of 2.6. Trivalents and univalents, as in our cases, were far less common (range 0—3; mean value 0.85 per cell). Only rarely were independent univalents produced (range 0—2 pairs per cell; mean value 0.28 per cell). Most of the quadrivalents were chains. The mean chiasma frequency per nucleus in these tetraploid cells was 24.71 which, as CALLAN points out, is significantly greater than twice the comparable diploid mean value of 11.1 chiasmata per nucleus. As might be expected, the variance of these tetraploid cells, like those we have studied, was greater than that of the diploid. He attributes this disproportionality between tetraploid and diploid chiasma frequency values to a breakdown of chiasma interference across the centromere.

In all the cases we have reported here, the average chiasma frequency in the tetraploid cells was also higher than twice the average

diploid value. This excess can invariably be accounted for in terms of the particularly high chiasma frequencies of some of the longer quadrivalents.

Since at any one site only two homologues can pair, one might have expected pairing to be less efficient in the multivalent, where it is of necessity interrupted at one or more points. Far from reducing the efficiency of the process of chiasma formation, however, such an interruption obviously increases the chiasma potential of a system of homologues. And the reason for this is clear: where pairing partners change there must be a marked drop in chiasma interference (see Fig. 58). There is a notable difference between our cases and the one studied by CALLAN. In the earwig, chiasma interference is operative across the centromere i.e. it is inter-arm. But where, as in most of our examples, all the chromosomes are acrocentric, the interference which breaks down in tetraploid cells is of necessity intra-arm. The same is true for the long metacentrics of *Chorthippus*, where there is no pronounced chiasma interference across the centromere.



58

Fig. 58. Wire model showing chromatid structure of the complex ring quadrivalent of *S. gregaria* shown in Figs. 56 and 57. For clarity some chromatids have been displaced. (2000 ×)

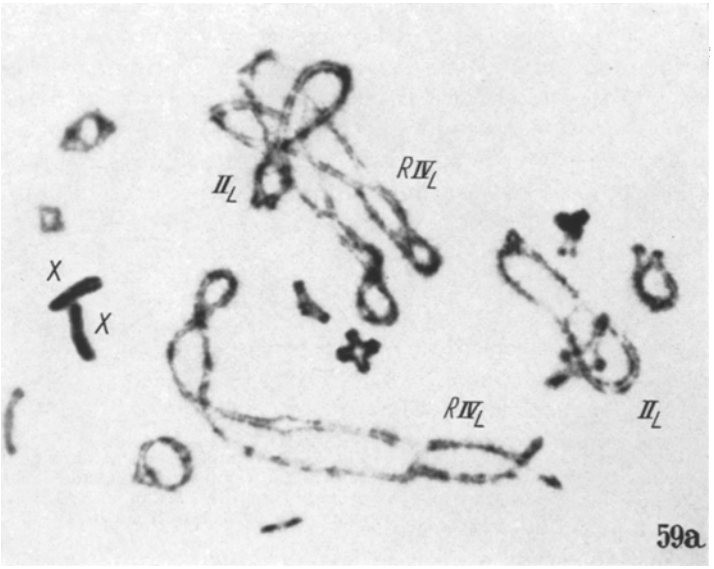
4. It is necessary to distinguish between two kinds of tetraploid meiotic cells capable of forming multivalents. First, those which originate spontaneously, either in a newly established tetraploid or as occasional cells within an otherwise diploid reproductive tissue. Secondly, those present in established tetraploids.

In the former, meiotic behaviour necessarily reflects the potentialities inherent in the initial diploid. But this may not be true of the latter. Here meiotic behaviour may have been adjusted by natural or artificial selection. Furthermore it is not always clear with what diploid form the comparison should in fact be made. With these qualifications in mind, the available data on established tetraploids suggests three possible relationships with related diploids. In these cases the tetraploid chiasma frequency is:

i) Less than twice the diploid. Such a situation has been claimed to obtain in *Tulipa* (UPCOTT 1939).

ii) Exactly double that of the diploid. This has been shown to hold in some subspecies of the *Dactylis glomerata* complex by MCCOLLUM (1958). He has also found the same relationship in colchicine-induced tetraploids of this species complex.

iii) Higher than twice the diploid value. This occurs, for example, in *Agrostis canina* (JONES 1956), which exists in both diploid (*A. c. canina*, 2n = 14) and tetraploid (*A. c. montana*, 2n = 28) subspecies.



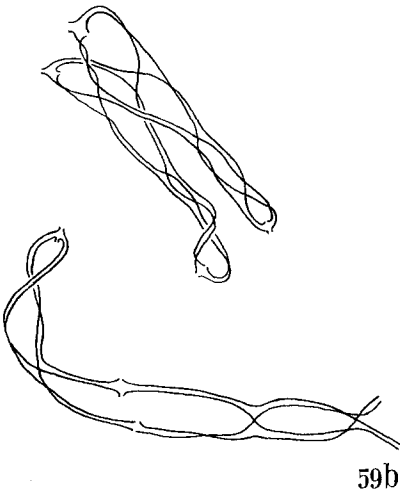


Fig. 59a. Tetraploid cell from *Chorthippus brunneus*, with 2 *RIV* + 12 *II* + 2 *X*. Of the 12 long chromosomes present in this cell, 4 form 2 bivalents and the remainder are associated as 2 quadrivalents. One of these has 6 chiasmata, the other 10 chiasmata. Fig. 59b. The two quadrivalents drawn to show chromatid structure. (Both 1000 ×)

Though selection would appear to have produced a reduction in chiasma frequency in polyploid tulips, it does not seem to have had any

marked effect in *Dactylis glomerata*. This may also be true for *Agrostis canina montana* since, as we have demonstrated, an increase in chiasma frequency is usually found in spontaneous polyploidy.

This heterogeneity of established polyploids thus stands in marked contrast to the greater homogeneity of those newly originated. For this reason we would argue that meaningful generalisations concerning the initial relationship of a tetraploid to its diploid progenitor may best be obtained from the latter.

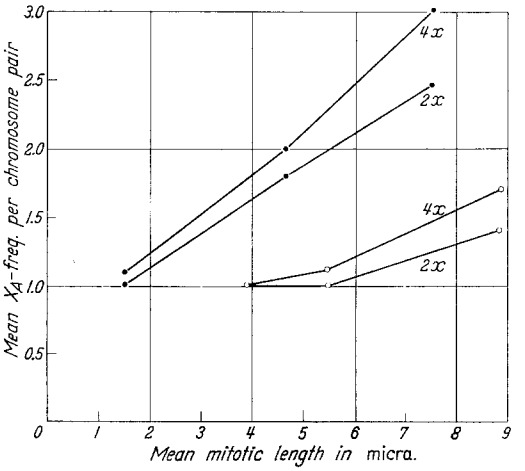


Fig. 60. Relationship between the mean mitotic chromosome length and mean chiasma frequency per chromosome pair in diploid and tetraploid cells of *S. paranensis* (——•——) and *P. kraussi* (——o——)

5. Morrison and Rajhathy (1960a, b) have compared quadrivalent frequencies in several unrelated autotetraploid plant species, some of which differed considerably in chromosome number and size. In one case, *Asparagus*, they also made a comparison between multivalent formation in the long and short chromosomes of the same complement. On the basis of these comparisons they imply that approximately two-thirds of a tetraploid complement will usually form multivalents, irrespective of chromosome size (within or between species), chiasma frequency or genotype.

As a generalisation there can be no doubt that this is incorrect. We have shown above, from our own and previous data, that there is an indisputable correlation between multivalent frequency and both (i) chiasma frequency between species, and (ii) relative length and chiasma frequency within the complement of a species.

Assuming that their figures are accurate and reliable, an assumption which may be incorrect (see pg. 143), one can reconcile the discrepancies between the species studied on the one hand by Morrison and Rajhathy, and on the other by other workers in this field in terms of a simple model (Fig. 61). Consider a metacentric chromosome where terminal initiation of pairing at both ends is more or less obligatory, i.e. there are two terminal pairing blocks. Such behaviour may normally characterise all those chromosomes within a complement which usually form two terminal chiasmata per bivalent and are about the same

relative size. In tetraploid cells where these conditions are satisfied, pairing can be defined, with statistical rigour. In homologues of the type A^1B^1—A^4B^4, let us assume that pairing is initiated between A^1A^2. This automatically means that A^3 can now only pair with A^4. Of the remaining B ends, any one may pair with any of the three others (e.g. B^1 with B^2, B^3 or B^4). But once it does so, pairing between the two remaining ends is again obligatory. The consequences of these various patterns of pairing are not uniform. Thus in this example, pairing between B^1 and B^2 would result in the production of two bivalents. But

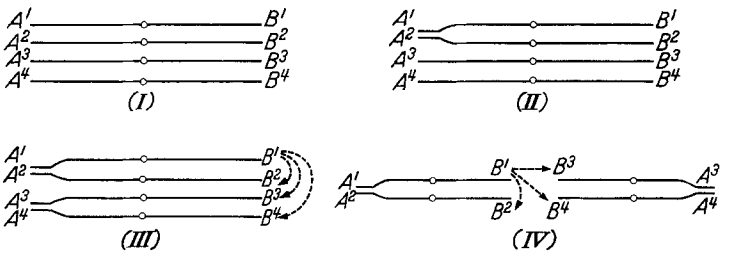


Fig. 61. Simplified model indicating how obligatory pairing for all chromosome ends in a system of four homologues in a tetraploid cell will give rise to only two types of chromosome association — quadrivalents and bivalents. The former will be formed twice as commonly as the latter in such a system. i) The four homologues, A^1B^1—A^4B^4. ii) Pairing between any two ends, e.g. A^1A^2, automatically makes pairing for the other two (A^3A^4) obligatory. iii) With the four A ends paired, any one B end, e.g. B^1 may pair with any of the other three ends (B^2—B^4). iv) The same as iii), opened out to clarify the pairing relationships. If B^1 pairs with B^2, then B^3 can only pair with B^4, and two bivalents will result. However, if B^1 pairs with either B^3 or B^4, a quadrivalent will be formed

pairing of B^1 with either B^3 or B^4 will produce a quadrivalent. This means that provided all ends pair and have an equal chance of doing so, quadrivalents should form twice as commonly as bivalents. And as a result, the total number of quadrivalents present will equal the total number of bivalents. For simplicity of description we have dealt with pairing in a stepwise manner. Exactly the same end result would be achieved if pairing took place simultaneously at more than one site. The model can also clearly be extended to include acrocentric chromosomes. Furthermore, this argument holds irrespective of the number of chromosomes in a complement and irrespective of their absolute size.

How this operates within a complement is demonstrated by our data on *S. paranensis*. Small chromosomes, which hardly ever form two chiasmata in the diploid state, rarely form multivalents. Long chromosomes have a mean chiasma frequency well in excess of two and, from the nature of the multivalents formed (Fig. 58), apparently possess several points where pairing may be initiated (pairing blocks). These chromosomes almost invariably form quadrivalents (93.3%). Just over two thirds of the M-chromosomes (73.6%) form quadrivalents and significantly their mean chiasma frequency in the diploid is only a little

below two. Furthermore, when two chiasmata are present in such bivalents they are located at the ends of the chromosomes.

One must therefore presume that if their quadrivalent scores were reliable, many of the plants with which MORRISON and RAJHATHY (1960a, b) were dealing may have fulfilled the conditions required by our model. The most obvious exception they present is that of *Lilium* which they have estimated as possessing three to five chiasmata per bivalent. The mean numbers of bivalents and quadrivalents per cell were 5.0 and 9.4 respectively. This is a significant departure from the equal frequencies expected. In six of the cereal and grass species which they studied (1960a) the same number of chromosomes was present ($2n = 4x = 28$). These were all metacentric and similar in relative size both within and between species. Their chiasma frequencies were also similar. Under these circumstances markedly different quadrivalent frequencies would not be expected.

It is regrettable that MORRISON and RAJHATHY were unable to score chiasma frequencies accurately in the plants they studied. But clearly they do not regard this as important in qualifying the relationship between chiasma and quadrivalent frequencies, since they believe that: "*... to be of any value ... a chiasma count would have to be made at the actual time of union or crossing over.*" Those workers who first equated cytological chiasmata so successfully with genetical crossovers were of course similarly handicapped! But this did not stop them making valuable conclusions which still apply. It is agreed that in many plants, particularly those possessing small chromosomes, chiasma recognition at diplotene is virtually impossible. But this is certainly not true for all organisms. In the Acridoid species we have dealt with in this paper, terminalization during diplotene and diakinesis is so slight that chiasma frequency scores made at first metaphase do not differ from those made at diplotene. Indeed, at early diplotene, one can score with confidence not only the number of chiasmata per bivalent but also the positions of these chiasmata along the chromosome and even, in most cases, the chromatids involved at each exchange. Observations made in species which meet these requirements must surely be of more value if generalisations are to be made, than those from species where the accurate detection of quadrivalents must be viewed with reserve (cf. Figs. 2 and 3, MORRISON and RAJHATHY 1960b), let alone the assessment of chiasma frequency.

Because those species with small chromosomes which they studied were held to form quadrivalents with a high frequency, they implied that size is unimportant, not only between, but also within species since: "*the shortest chromosomes are long enough to undergo the reactions necessary to produce quadrivalents*". In this they have missed the point. While

it is true that the absolute size of chromosomes in different species is not quite so important for chiasma or multivalent frequency, what is important within the complement of a given species is the relative size. Thus, within complements in which there is a distinct size range, there is unequivocal evidence for a direct correlation between chiasma frequency and chromosome length (cf. Fig. 19). And as we have shown above, this is also exhibited in the distribution of quadrivalents throughout the complement (Fig. 60). We defy even MORRISON and RAJHATHY to demonstrate how two-thirds of the complement are involved in quadrivalent formation in *Pyrgomorpha*, for example, where only one or two quadrivalents are usually formed in tetraploid cells (Table 5).

In their papers, MORRISON and RAJHATHY make a number of other rather naïve statements, two of which should not be allowed to pass without comment. One of these doubts the reality of chiasma localisation which they suggest may be "*an artefact arising from our* (i.e. their ?) *inability to trace chiasmata in cytological preparations*". As we have demonstrated in this paper, chiasma localisation is not "*difficult to detect by cytological methods*" in suitable material. And when present it will unquestionably be an extremely important factor in quadrivalent formation. This is particularly true when it is based on an incompleteness of pairing. Even where pairing is complete, however, it can still play a key role. An excellent example of this is provided by *Allium porrum*, where in all the metacentric chromosomes chiasmata are localised proximally: one usually forms on either side of the centromere (LEVAN 1940). During pachytene quadrivalents were found in most cells but at first metaphase quadrivalents are rarely found (10 IV's in 380 cells analysed). Clearly, as a result of chiasma localisation, homologues which have succeeded in pairing fail to maintain their association (LEVAN 1940, Fig. 1, p. 436). Where quadrivalents do persist localisation is invariably maintained, resulting in the development of a novel type of quadrivalent (LEVAN 1940, Fig. 2f—h).

MORRISON and RAJHATHY also doubt the role of the genotype in determining quadrivalent frequency. That chiasma frequency is under genotypic control is now beyond question (REES 1961). ROSEWEIR and REES (1961, 1962) have also shown that there is heritable variation in the frequency with which multivalents form among autotetraploids produced from F_2 families in rye. Genes control the chiasma frequency per cell and hence, in polyploids, the multivalent frequency per cell. Relative size then determines chiasma and multivalent distribution throughout the complement (Fig. 60).

6. DURRANT has, on a theoretical basis, derived the association frequencies expected in tetraploids on the assumption of random chiasma formation between the four homologues (Tables 2—4, DURRANT

1960). Our data show a significant departure from his expectations. Quadrivalents and pairs of bivalents are much more common than DURRANT'S predictions require. Conversely, trivalents and univalents, or associations of bivalents and two univalents, are extremely rare (Tables 4, 5). The same paucity of these latter two configuration types in tetraploid cells has also been commented on by CALLAN (1949), MORRISON and RAJHATHY (1960a), MCCOLLUM (1958) and ROSEWEIR and REES (1962). These discrepancies can be accounted for in terms of the simplified model we have outlined in section 5 (see pg. 142). If pairing, though random, is usually obligatory for all four homologues then the presence of one or two univalents will be rare.

Summary

1. *Schistocerca paranensis* is a South American locust which possesses the same number of chromosomes as the related, though allopatric, species, *S. gregaria* ($2n = 22\,AA + X$). The haploid complements also agree in the relative sizes of their members (3 L, 5 M and 3 S), in their mean chiasma frequency and in chiasma distribution throughout each size group. They differ, however, in two principal ways:

a) All three S-chromosomes of *S. paranensis* are consistently smaller than their counterparts in *S. gregaria*.

b) Both species organise nucleoli at first prophase of meiosis. But *S. paranensis* organises only one at a sub-median nucleolar chromomere on the M_6. *S. gregaria* has two such submedian organisers, one on the M_6, the other on the L_3. Sites of nucleolar production are often marked at diplotene by achromatic gaps.

2. One individual of *S. paranensis* proved to be partially asynaptic. In this individual, homologues which succeeded in forming bivalents showed a reduced chiasma frequency and a marked chiasma localisation. The pattern of localisation was characteristic. Single chiasmata were usually sited proximally; two chiasmata were proximal-distal in location. In this, *S. paranensis* differs from *S. gregaria* where, following partial asynapsis, single chiasmata are predominantly distally localised. The presence of univalents at first anaphase in *S. paranensis* led to the suppression of cleavage at telophase. Cleavage was also subsequently suppressed at second division in these restitution cells. This resulted in the formation of giant tetraploid spermatids.

3. An analysis was made of the number and distribution of multivalents throughout the complement of 25 tetraploid cells obtained from several individuals. The chiasma frequencies of 16 of these cells were also scored. This analysis revealed that:

i) Multivalent frequency in tetraploid cells was directly correlated with diploid chiasma frequency and relative chromosome size.

ii) The chiasma frequency of the multivalents, and hence their complexity, was directly related to the diploid chiasma frequency of the homologues involved in the multivalents.

These conclusions were supported by comparisons, both published and unpublished, with a number of other species.

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