THE ANALYSIS OF EXCHANGES IN TRITIUM-LABELLED MEIOTIC CHROMOSOMES

1. SCHISTOCERCA GREGARIA

T. CRAIG-CAMERON and G. H. JONES
Department of Genetics, University of Birmingham

Received 19.v.69

1. Introduction

Autoradiography following tritiated thymidine incorporation offers a new technique for the analysis of meiotic recombination. This method depends on the fact that germ line chromosomes, like those of somatic cells, show a semi-conservative segregation of labelled DNA in successive cell generations. Chromosomes which incorporate label in the pre-meiotic S phase enter meiosis with both chromatids labelled, while chromosomes labelled one cell cycle earlier, in the S phase of the last pre-meiotic cell cycle, enter meiosis having one labelled and one unlabelled chromatid. This pattern of partial labelling becomes modified due to the occurrence of recombinational exchange events in meiotic prophase, and these determine the final labelling pattern of Anaphase 1 and Metaphase II chromosomes. This much has been established from earlier autoradiographic studies of the replication and recombination of meiotic chromosomes (Taylor, 1965; Callan and Taylor, 1968; Peacock, 1968; Church and Wimber, 1969). Evidently this method can be applied to the search for possible sister chromatid exchange (SCE) in meiotic cells as well as to the analysis of non-sister crossovers, and these were the two foremost aims of the present work. The potential of tritium autoradiography for the study of meiotic SCE has been explored in an earlier paper (Jones and Craig-Cameron, 1969). In the present report we present a more thorough analysis of the label segregation patterns in *Schistocerca gregaria* germ line chromosomes.

2. Material and methods

Several young adult males of *Schistocerca gregaria* (the desert locust) were given a single abdominal injection containing 20 μC H^3-TdR in a volume of 0·05 ml. (concentration = 400 μC/ml.). These insects were maintained in an aluminium cage at approximately 32° C. and individual insects were sampled at half-daily intervals and their testes dissected and fixed in 3 : 1 acetic alcohol. Autoradiographs were then prepared (Kodak AR 10 stripping film) from Feulgen-stained squash preparations of testicular material and these were allowed to expose for 7 weeks before processing. The timing of meiotic and spermatogonial events was then assessed from the labelling status of meiotic stages at various fixation times. The dose of H^3-TdR administered to each insect was adequate to give complete and uniform labelling of chromosomes, while at the same time avoiding any carry-over of label from one S phase to the next.

The meiotic chromosomes of one individual sampled on day 11½ showed a pattern of partial labelling such that the equivalent of one chromatid per

chromosome was labelled at Anaphase I and Metaphase II. This means that under these experimental conditions, cells take roughly 11½ days to progress from the S phase of the last spermatogonial cycle to Anaphase I of meiosis. The remaining testicular follicles of this individual were prepared for autoradiography as described above and the labelling pattern of every available Anaphase I and Metaphase II cell was studied and typed for exchanges. For practical reasons attention was confined to the three longest (L) autosome pairs and the univalent X chromosome; weakly labelled or overlapped chromosomes were excluded. In all, 55 complete L pairs from Anaphase I cells and 192 individual L chromosomes from Metaphase II cells were recorded.

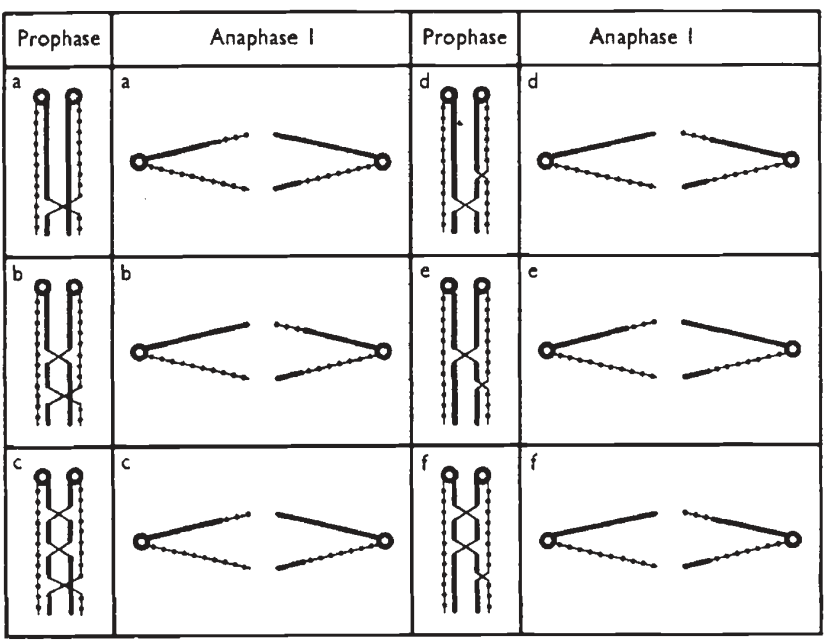


FIG. 1.—The patterns of label exchange generated in meiotic chromosomes by various combinations of non-sister crossovers and sister chromatid exchanges (SCE).

3. RESULTS

Two distinct types of label exchange can be recognised in Anaphase I and Metaphase II chromosomes; non-sister label exchanges which are reciprocal between non-sister chromatids (NSLE) and sister label exchanges which are reciprocal between sister chromatids (SLE) (see plate I). Unfortunately there is no direct relationship between these patterns of label exchange and the original exchange event. For instance a non-sister crossover can produce NSLE or SLE (fig. 1, a and b), and likewise a SCE can produce either type of label exchange (fig. 1, d-f) depending on the precise combination of crossovers in the bivalent. This means that SCEs cannot be readily distinguished from non-sister crossovers on the basis of label exchange and there is no simple method of detecting SCE in chiasmate bivalents. However, the expected proportions of NSLE and SLE can be

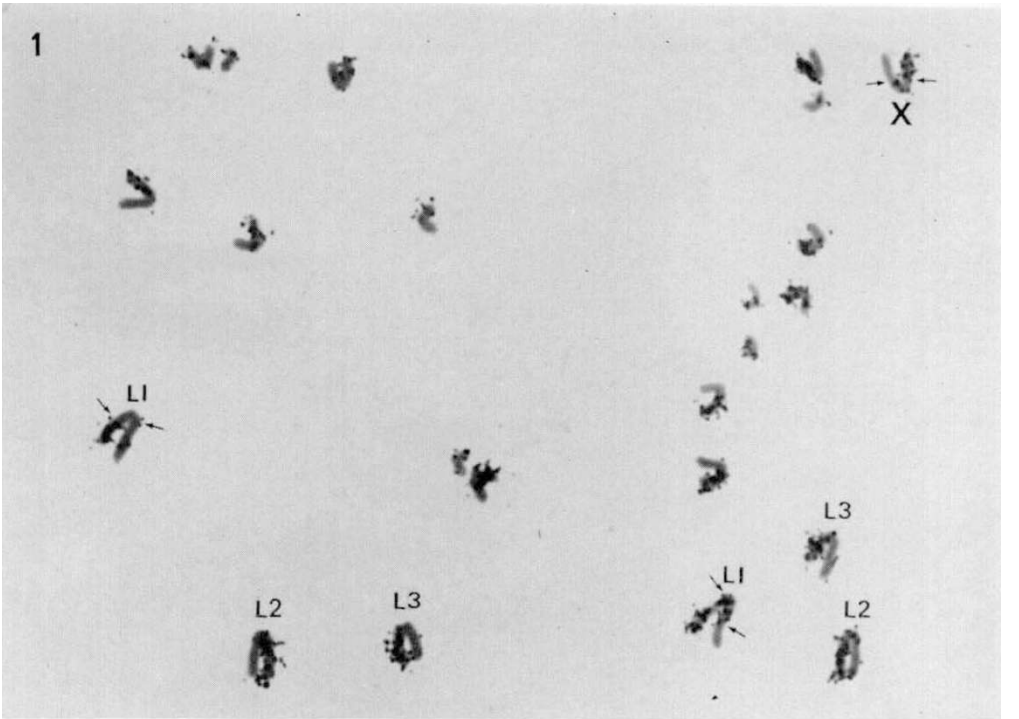
Plate I

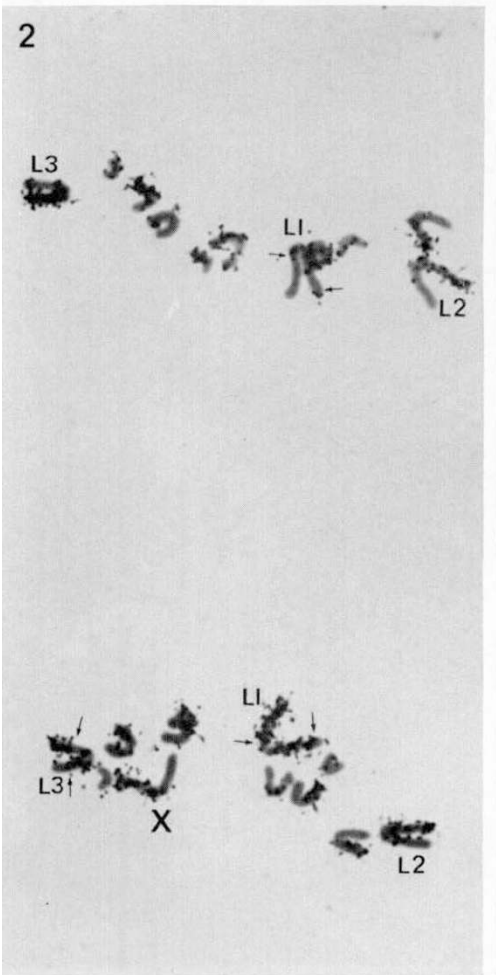
Autoradiographs of 3 *Schistocerca gregaria* spermatocytes at Anaphase I, showing label segregation and various patterns of label exchange. The **X** and L chromosomes in each cell are identified and the L chromosomes are arranged in pairs based on considerations of size, position and labelling pattern. Points of label exchange are indicated by arrows. The label exchange patterns of these cells were recorded as follows:

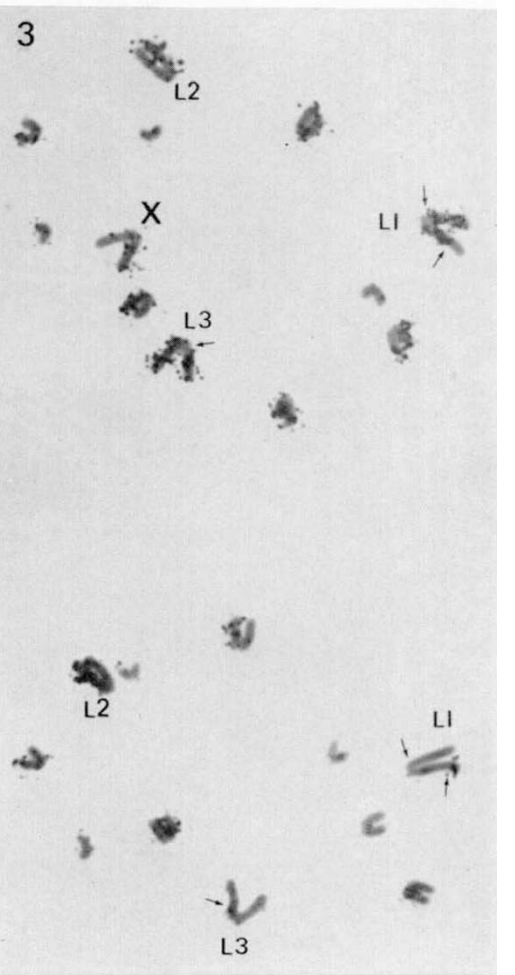
FIG. 1.—L1, 2NS; L2, 0; L3, 0; **X**, 1S.

FIG. 2.—L1, 2NS; L2, 0; L3, 1S; **X**, 0.

FIG. 3.—L1, 2NS; L2, 0; L3, 1NS; **X**, 0.


1
X
L1
L3
L1
L2
L3
L2


2
L3
L1
L2
L1
L3
X
L2


3
L2
X
L1
L3
L2
L1
L3

computed for a given crossover frequency and the effect of SCE on this distribution can be assessed in a similar manner.

These assessments are made subject to the following rules:

(1) Only one-half of all crossovers produce a label exchange, namely those involving chromatids of different labelling status (visible crossovers). Other crossovers involve chromatids of the same labelling status and do not give label exchanges (hidden crossovers).
(2) Visible crossovers produce NSLEs provided they are not accompanied by proximal crossovers (fig. 1, a).
(3) A hidden proximal crossover will convert one or more distal and adjacent visible crossovers to give SLE (fig. 1, b). Proximal visible crossovers will not carry out this conversion but will themselves generate NSLEs.
(4) Any single crossover proximal to a converting hidden crossover will cancel the conversion (fig. 1, c).

This set of rules determines that an additional proximal crossover converts all existing SLEs to NSLEs, but on average only one-half of all NSLEs are converted to SLEs by an additional proximal crossover. From this latter principle it follows that the expected proportion of SLEs generated by a distal visible crossover with a given number of proximal crossovers is half the proportion of NSLEs with one fewer proximal crossovers. Thus if Sx and $\mathcal{N}Sx$ are the proportions of sister and non-sister label exchanges with a given number of proximal crossovers x, then,

$$Sx = \tfrac{1}{2}(\mathcal{N}Sx-1)$$

Since $Sx+\mathcal{N}Sx = Sx-1+\mathcal{N}Sx-1 = 1$, it follows that

$$Sx = \tfrac{1}{2}(1-Sx-1)$$

of which the general formula is

$$Sx = \tfrac{1}{3}[1-(-\tfrac{1}{2})]$$

This gives the series for expected SLEs, 0, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{3}{8}$, $\frac{5}{16}$, $\frac{11}{32}$ etc., of which the infinite term is $\frac{1}{3}$, which agrees with the series found by Mather (1935) for the proportion of reductional separations at a locus and by Upcott (1937) for the proportions of 2nd division inversion bridges with different numbers of proximal crossovers.

This series can be used to compute the expected proportion of SLEs in *Schistocerca gregaria* L chromosomes since the chiasma frequencies of L bivalents are known from diplotene observations. These bivalents most frequently have 2 or 3 chiasmata in the stock, used for this experiment. Very occasionally bivalents with 1 or with 4 chiasmata are found, but so infrequently that they can be disregarded in these computions. Crossovers in different positions in these bivalents will generate varying proportions of SLEs depending on the number of proximal crossovers, and the overall proportion of SLEs generated in this way is obtained by averaging over the various situations. Thus the predicted overall proportion of SLEs is $\frac{1}{4}$ both for bivalents with 2 chiasmata $\frac{1}{2}(0+\frac{1}{2})$ and for bivalents with 3 chiasmata $\frac{1}{3}(0+\frac{1}{2}+\frac{1}{4})$. Therefore, in the absence of both SCE and chromatid interference the L chromosomes of *Schistocerca* should contain $\frac{1}{4}$ SLEs to $\frac{3}{4}$ NSLEs.

The rules governing the types of exchange generated by SCE are rather different from those governing exchange production by non-sister crossovers, and can be summarised as follows:

(1) SCEs produce SLEs provided they are unaccompanied by proximal crossovers (fig. 1, d).
(2) Any single proximal crossover will convert a SCE to give NSLE instead of SLE (fig. 1, e).
(3) One SCE plus one proximal crossover are equivalent to one visible crossover when considering the effects of further proximal crossovers on the LEs generated by the SCE (see, for example, fig. 1, f).

Evidently the effects of proximal crossovers on LEs generated by SCE follow the same pattern as established previously for label exchanges generated by visible crossovers, with the qualification that the intial exchange involves sister chromatids. It follows that the proportions of SLEs generated by SCE will vary with different numbers of proximal crossovers to give the series 1, 0, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{3}{8}$, $\frac{5}{16}$, etc. Provided that SCEs occur independently of crossover positions in bivalents, the average proportion of SLEs generated by SCE will be $\frac{1}{2}$ in bivalents with 2 chiasmata $\frac{1}{3}(1+0+\frac{1}{2})$ and $\frac{7}{16}$ in bivalents with 3 chiasmata $\frac{1}{4}(1+0+\frac{1}{2}+\frac{1}{4})$, which approximates overall to $\frac{1}{2}$.

In addition to the exchanges actually generated by SCEs we must also consider the influence of SCE on the segregation of label exchanges generated by visible crossovers. Fortunately this does not affect the overall pattern of label exchange as proximal SCEs convert equal numbers of crossover-generated SLEs and NSLEs, and thus the original ratio of exchanges is restored.*

Thus in L bivalents of *Schistocerca gregaria*, non-sister crossovers on their own generate NSLEs and SLEs in the ratio 3 : 1, whereas the contribution made by SCE if it occurs, is in the ratio 1 : 1. Frequent meiotic SCE should therefore lead to an excess of SLEs and a departure from the 3 : 1 ratio expected with crossovers alone. However, a low frequency of SCE is not likely to have a detectable influence on this ratio. The present observations seem to provide a clear answer on this point. Among Anaphase I L chromosome pairs, 53 NSLEs were observed as compared to 14 SLEs (see table 2); this ratio does not depart significantly from 3 : 1 ($\chi^2 = 0{\cdot}566$; $P > 0{\cdot}3$). The expectation for Metaphase II chromosomes is rather different since the reciprocal products of exchange are not recognisable and hence

* SCEs only convert distal visible crossovers when the sister chromatids involved segregate to different homologous centromeres. Thus the proportion of visible crossovers which are converted by proximal SCEs varies with different numbers of proximal crossovers to give the reciprocal of the series for SLEs generated by SCE; *i.e.* the reciprocal of 1, 0, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{3}{8}$, etc., which is 0, 1, $\frac{1}{2}$, $\frac{3}{4}$, $\frac{5}{8}$, etc.

Visible crossovers distal to non-converting SCEs always generate NSLEs (as sister chromatids are attached to the same centromere and therefore non-sister chromatids must go to different centromeres). It follows that all SLEs generated by crossovers must be converted by proximal SCE. The proportion of convertible visible crossovers which initially generate SLEs is found by comparing the two series:

(1) Visible crossovers which are converted by proximal SCE (includes all SLEs) = 0, 1, $\frac{1}{2}$, $\frac{3}{4}$, $\frac{5}{8}$, etc.
(2) SLEs generated by crossovers = 0, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{3}{8}$, $\frac{5}{16}$, etc.

Clearly one-half of all crossovers which are converted by proximal SCE are initially SLE and are therefore converted to NSLE. Likewise, the remaining convertible crossovers initially generate NSLEs and are converted to SLEs.

NSLEs are recorded twice on average while SLEs are only recorded once. Consequently NSLEs and SLEs should occur in a 6 : 1 ratio among Metaphase II chromosomes if all exchanges are the outcome of non-sister crossovers. In all 215 Metaphase II LEs were observed and of these 183 were NSLEs and 32 were SLEs which is very close to a 6 : 1 ratio. These comparisons therefore suggest that meiotic SCE is infrequent or else it does not occur at all. A comparison of mean chiasma frequency and mean label exchange frequency leads to a similar conclusion. As each visible crossover produces two label exchanges in chromatids, the mean chiasma frequency of L bivalents can be used to predict the frequency of label exchanges, and any excess of label exchanges would suggest the occurrence of SCE. In fact, the frequency of chromatid label exchanges per chromosome, in the absence of SCE, should be half the frequency of chiasmata per bivalent, and the present data are in agreement with this expectation (mean bivalent chiasma frequency = 2·65; mean label exchange frequency per chromosome = 1·34). This agrees with Taylor's finding in *Romalea*, where again there was a close parallel between observed and expected label exchange frequencies. But in contrast, Church and Wimber (1969) claim an excess of label exchanges over the predicted frequency based on chiasma scores in the plant species, *Ornithogalum virens*.

Further information on the recombinational exchange events of meiotic chromosomes comes from a detailed analysis of the variation in label exchange pattern among L chromosomes. This pattern is varied and individual chromosomes may contain no label exchanges or various numbers of SLEs or NSLEs (see plate I). The pattern of exchanges shown by individual chromosomes must reflect the precise combination of exchange events in prophase, and an attempt has been made to predict label exchange patterns from the chiasma frequencies of diplotene bivalents. The only assumptions made are that chromatid interference does not operate, and initially that only non-sister crossovers occur. The expected proportions of the various LE classes were derived by considering the LE patterns generated by all possible combinations of 2 and 3 crossovers where one chromatid per chromosome is labelled (table 1). The resulting LE class frequencies derived from all combinations of 2 crossovers and all combinations of 3 crossovers were weighted in proportion to the frequencies of 2 and 3 chiasmata in L bivalents, and these weighted values were then combined to give the expected frequencies of the various LE classes. This procedure was followed separately for Anaphase I (table 2) and Metaphase II (table 3) expectations. Tables 2 and 3 give the expected frequencies and the expected numbers of the various LE classes, along with the numbers actually observed. The Anaphase I data show close agreement with the expected numbers in different LE classes ($\chi^2_{[6]} = 5{\cdot}741$; P = 0·5—0·3) which means that crossovers can account entirely for the LE patterns seen in Anaphase I L chromosomes. The close agreement of observed and expected values in this comparison is further evidence against widespread SCE in meiotic chromosomes of *Schistocerca*. We saw earlier that SLE generates approximately equal numbers of NSLEs and SLEs in chiasmate bivalents, and frequent meiotic SCE would therefore lead to a disturbance of the LE class proportions; in fact the SLE classes would be inflated relative to NSLE classes. Once again, however, this method is not sufficiently sensitive to detect low frequencies of SCE.

TABLE 1

Label exchange patterns generated by all possible combinations of 2 and 3 crossovers (h = *hidden crossover*, v = *visible crossover; proximal to distal reads from left to right*)

Crossover combinations	Label exchanges	
	Anaphase I	Metaphase II
hh	O	O/O
hv	1S	1S/O
vh	1NS	1NS/1NS
vv	2NS	2NS/2NS
hhh	O	O/O
hhv	1NS	1NS/1NS
hvh	1S	1S/O
hvv	2S	1S/1S 2S/O
vhh	1NS	1NS/1NS
vhv	2NS	2NS/2NS
vvh	2NS	2NS/2NS
vvv	3NS	3NS/3NS

TABLE 2

The expected frequencies and expected numbers of label exchange classes among Anaphase I L chromosome pairs showing their derivation, together with the observed numbers in the various classes

		Label exchange classes					
		O	1S	2S	1NS	2NS	3NS
Expected frequencies	2 Chiasmata	0·2500	0·2500	—	0·2500	0·2500	—
	3 Chiasmata	0·1250	0·1250	0·1250	0·2500	0·2500	0·1250
Weighted expected frequencies	2 Chiasmata (×0·3786)	0·0947	0·0947	—	0·0947	0·0947	—
	3 Chiasmata (×0·6214)	0·0777	0·0777	0·0777	0·1554	0·1554	0·0777
Combined expected frequencies		0·1724	0·1724	0·0777	0·2500	0·2500	0·0777
Expected numbers		8·7924	8·7924	3·9627	12·7500	12·7500	3·9627
Observed numbers		8	4	5	17	15	2
χ^2		0·0714	2·6122	0·2715	1·4167	0·3971	0·9721

TABLE 3

The expected frequencies and expected numbers of label exchange classes among Metaphase II L chromosomes showing their derivation, together with the observed numbers in the various classes

		Label exchange classes					
		0	1S	2S	1NS	2NS	3NS
Expected frequencies	2 Chiasmata	0·3750	0·1250	—	0·2500	0·2500	—
	3 Chiasmata	0·2180	0·1250	0·0320	0·2500	0·2500	0·1250
Weighted expected frequencies	2 Chiasmata (× 0·3786)	0·1420	0·0473	—	0·0947	0·0947	—
	3 Chiasmata (× 0·6214)	0·1355	0·0777	0·0199	0·1554	0·1554	0·0777
Combined expected frequencies		0·2775	0·1250	0·0199	0·2500	0·2500	0·0777
Expected numbers		53·2800	24·0000	3·8208	48·0000	48·0000	14·9184
Observed numbers		40	26	3	73	40	10
χ^2		3·3100	0·1666	0·1763	13·0208	1·3333	1·6215

The Metaphase II data show a poor agreement with expectation ($\chi^2_{[6]} = 19{\cdot}628$; $P < 0{\cdot}01$), but only the 0 and 1NS classes show large departures from expectation. The reason for this poor agreement is obscure but may well be due to the inherent unreliability of Metaphase II classifications where the reciprocal products of exchange are not available for comparison. This argues for the desirability of Anaphase I classification where the reciprocal products of exchange can be compared, thus minimising misclassification.

4. Discussion

Several previous studies have established the principle of semi-conservative label segregation in germ line chromosomes and the modification of this pattern due to exchange of labelled and unlabelled chromatid segments. Taylor's (1965) analysis of label segregation in the meiotic chromosomes of *Romalea* first revealed the possibilities of this technique for recombination analysis. He found that label exchanges or " switch points " appeared in part labelled Metaphase II chromosomes and these were interpreted as the manifestation of crossing over by breakage-reunion between homologous non-sister chromatids. This view was supported by a close correlation between the number of observed switch points and chiasma frequency. More recently Peacock (1968) has investigated meiotic exchanges in autoradiographs of spermatocytes from another grasshopper species, *Goniaea australasiae*. This study establishes a more direct connection between chiasmata and label exchanges in meiotic chromosomes, and depends in part on tracing the pattern of labelling in the chromatids of chiasmate bivalents. In addition Peacock showed that a heat induced reduction in chiasma frequency is accompanied by a corresponding reduction in label exchanges.

In this present study patterns of label segregation were analysed in meiotic chromosomes of *Schistocerca gregaria* and these observations were related to predictions based on diplotene chiasma scores. One such prediction relates to the proportion of label exchanges which are reciprocal between sister chromatids, and a good agreement of observed and predicted values was obtained both for Anaphase I and Metaphase II data. In further comparisons both the mean label exchange frequency, and the frequencies of the various LE classes seen in Anaphase I chromosomes agreed closely with predictions based on chiasma scores.

These comparisons establish clearly that diplotene chiasmata correspond to points of crossing-over by breakage-reunion of homologous non-sister chromatids. Indeed, as Peacock (1968) points out, this technique provides the only direct demonstration of breakage and exchange in the production of recombinant chromatids during meiosis in higher organisms. Despite repeated claims to the contrary, the several cytological and cytogenetic correlations which relate chiasmata and crossing-over to the production of structurally recombinant chromosomes, have no bearing on the mechanism of recombination. The comparisons also show that non-sister crossovers can account for all, or nearly all the LEs seen in Anaphase I and Metaphase II chromosomes and that SCEs are therefore absent from meiotic cells of *Schistocerca*, or else they occur at a low frequency. These comparisons do not, however, allow a final decision on this point as they are not sensitive to low frequencies of SCE.

The main obstacle to the detection of meiotic SCE by autoradiography is the lack of correspondence between the final pattern of label segregation and the original exchange event, so that SLEs cannot be taken as reliable indicators of previous SCE. But there is some evidence for CSE in meiotic chromosomes of *Schistocerca* from two other sources. One indirect source of evidence derives from the occurrence of LE patterns involving one or more proximal SLEs combined with one or more distal NSLEs. We have shown previously that a single proximal SLE combined with a single distal NSLE cannot be derived from any combination of 3 crossovers (Jones and Craig-Cameron, 1969), and the argument can be extended to other combinations of proximal SLEs and distal NSLEs. These label exchange patterns can however be derived from certain rare combinations of 4 or more crossovers, but as bivalents with 4 chiasmata were very infrequently observed in this material (2 in a sample of 105 L bivalents), the most likely origin of these exchange patterns is through proximal SCE combined with distal non-sister crossovers. Exchange patterns of this type were recorded in 4 out of 55 Anaphase I L chromosome pairs (not included in table 2), and Taylor (1965) records similar cases in *Romalea*.

Secondly, the single X chromosome has no homologous partner and cannot thus engage in non-sister crossovers. A reciprocal label exchange within the X chromosome must therefore indicate previous SCE. In all, 21 X chromsomes were identified during this study and among these 6 contained one or more SCEs (5 single and one double) giving a mean exchange frequency of 0·33 for this chromosome.

These observations establish the possibility that SCE occurs regularly in meiotic cells of *Schistocerca*, albeit at a low frequency. In this respect these observations are at variance with those of Peacock (1968) on label segregation patterns in *Goniaea australasiae*, where SCEs occurred at a much higher frequency. Indeed, in Peacock's data SLEs appear far more frequently than NSLEs. This disparity cannot be readily explained but the following suggestions are offered:

(1) This represents an inherent species-specific difference in SCE frequency.

(2) The higher frequency of SCE in *Goniaea* may have been induced by a higher radiation dose from endogenous tritiated thymidine. This explanation is not very likely in view of the apparent dose-independence of mitotic SCEs (Marin and Prescott, 1964).

(3) The resolution of label exchanges in *Schistocerca* is hindered to some extent by the rather low precision of the autoradiographic technique in relation to chromosome size in this species. Thus it is possible that some, though not many, exchanges were overlooked. However, it is unlikely that this lack of precision would discriminate markedly against SLEs rather than NSLEs, although SLEs arising very near the centromere are difficult to resolve due to the convergence of chromatids at this point.

(4) In Peacock's study, high temperature treatments were applied to reduce chiasma frequency and also to mark the heat sensitive period of meiosis. It is possible that this high temperature treatment induced some of the SCEs in this experiment.

The detection and estimation of meiotic SCE by autoradiography is also hindered by the fact that a proportion of SCEs arise in the penultimate interphase prior to their observation, as for instance in the case of the twin

exchanges of polyploid and endoreduplicated mitotic nuclei (Taylor, 1958; Geard and Peacock, 1969). Thus SCEs in meiotic chromosomes may not be directly meiotic in origin but could originate instead during the last premeiotic cell cycle. Indeed, there is evidence from other organisms for rather frequent SCE during the mitotic cycles preceding meiosis (Moens, 1966; Church and Wimber, 1969). A further difficulty stems from the possibility that SCEs are induced by irradiation from the endogenous tritium which is incorporated into chromosomal DNA. Thus it is not clear whether the label exchanges we observe and attribute to SCE are manifestations of a real meiotic phenomenon, or whether they are simply artefacts of the method used to detect them. However, there is evidence from other genetic and cytological studies (Schwartz, 1953; Michaelis, 1959; Green, 1968) to suggest that SCE does occur as a regular feature of meiotic cells.

Traditionally, the study of meiotic recombination has relied on genetic methods of analysis or on the direct cytological examination of meiotic division stages. The autoradiographic method of recombination analysis, as described here, shares some of the advantages and disadvantages of these other methods. Thus label exchanges provide more direct and reliable information about certain aspects of recombination than do chiasmata, which although generally accepted as indicators of previous crossing-over do not necessarily reflect faithfully the original frequency and distribution of exchanges. In addition, the pattern of label segregation in meiotic chromosomes provides a means of studying SCE, although the situation regarding these exchanges is rather confused at present due to the limited application of this technique. On the debit side, the autoradiographic method only permits a partial analysis of recombination since on average only a half of all crossovers give visible label exchanges. Nevertheless, label exchange studies, like chiasma studies, do have a bearing on the total recombination potential of meiotic cells and its distribution through the genome, whereas genetic recombination studies are usually restricted to selected regions of the genome. Evidently this autoradiographic method can serve as a useful complement to other methods of recombination analysis and should serve to reduce the gap between conventional genetical and cytological techniques.

5. Summary

1. The DNA of *Schistocerca gregaria* germ line chromosomes was labelled at the last spermatogonial S phase so that the equivalent of one chromatid per chromosome appeared labelled in autoradiographs of meiotic Anaphase I and Metaphase II stages.

2. Some chromosomes at these stages show complete chromatid label segregation, but the majority show characteristic label exchanges which may be reciprocal between sister chromatids or between non-sister chromatids.

3. Diplotene chiasma counts were used to predict (*a*) mean label exchange frequency, (*b*) the proportion of sister chromatid label exchanges, (*c*) the frequencies of various label exchange classes, and the effects of sister chromatid exchanges on these predictions was also assessed. These predictions were then compared with actual label exchange patterns from Anaphase I and Metaphase II chromosomes.

4. It was found that non-sister crossovers could account entirely, or almost entirely, for observed label exchange patterns. However, meiotic sister chromatid exchange is not entirely precluded as these tests are not sensitive to low frequencies of sister chromatid exchange.

5. The possibility that sister chromatid exchange occurs at a low frequency in germ line chromosomes is supported by the occurrence of certain classes of label exchanges, both in autosomes and in the univalent X chromosome, which are unattributable to non-sister crossovers. The meiotic origin of these exchanges is uncertain however as they may be a legacy of spermatogonial exchanges.

Acknowledgments.—We are indebted to Miss Patricia Wilkinson for valuable technical assistance in the preparation of plates and figures, T. C. C. acknowledges support from the Science Research Council.

Note added in proof

Since the sensitivity of the comparisons applied to our data is clearly a limiting factor for the detection of meiotic SCE, we have assessed their sensitivity by simulated comparisons over a wide range of conditions. These simulations reveal that the various comparisons differ in sensitivity; the comparison of mean chiasma frequency and mean LE frequency, for example, is considerably more sensitive than the comparison of NSLE/SLE ratios. In our experimental material, the most sensitive of these tests, the comparison of means, can detect SCEs (0·05 probability) if they occur at or exceed a frequency of 0·35 per chromosome pair (AI data) or 0·25 per chromosome pair (combined AI and MII data). We conclude that meiotic SCE probably does not occur at a frequency exceeding 0·25-0·35 per chromosome pair of *Schistocerca*, but this does not preclude a lower frequency of SCE.

6. References

CALLAN, H. G., AND TAYLOR, J. H. 1968. A radioautographic study of the time course of male meiosis in the newt *Triturus vulgaris*. *J. Cell Sci.*, *3*, 615-626.

CHURCH, K., AND WIMBER, D. E. 1969. Meiosis in *Ornithogalum virens* (Liliaceae): Meiotic timing and segregation of H^3— thymidine labelled chromosomes. *Canad. J. Genet. Cytol.*, *11*, 573-581.

GEARD, C. R., AND PEACOCK, W. J. 1969. Sister chromatid exchanges in *Vicia faba*. *Mutation Res.*, *7*, 215-223.

GREEN, M. M. 1968. Some genetic properties of intrachromosomal recombination. *Molec. Gen. Genetics*, *103*, 209-217.

JONES, G. H., AND CRAIG-CAMERON, T. 1969. Analysis of meiotic exchange by tritium autoradiography. *Nature*, *223*, 946-947.

MARIN, G., AND PRESCOTT, D. M. 1964. The frequency of sister chromatid exchanges following exposure to varying doses of H^3— thymidine or X-rays. *J. Cell Biol.*, *21*, 159-167.

MATHER, K. 1935. Reductional and equational separation of the chromosomes in bivalents and multivalents. *J. genetics*, *30*, 53-78.

MOENS, P. B. 1966. Segregation of tritum-labelled DNA at meiosis in *Chorthippus*. *Chromosoma* (*Berl.*), *19*, 277-285.

MICHAELIS, A. 1959. Über das Verhalten eines Ringchromosoms in der Mitose und Meiose von *Antirrhimun majus* L. *Chromosoma* (*Berl.*), *10*, 144-162.

PEACOCK, W. J. 1968. Chiasmata and crossing-over. In, *Replication and Recombination of Genetic Material*, pp. 242-252 (ed. W. J. Peacock and R. D. Brock). Australian Academy of Science, Canberra.

SCHWARTZ, D. 1953. Evidence for sister-strand crossing over in maize. *Genetics*, *38*, 251-260.

TAYLOR, J. H. 1958. Sister chromatid exchanges in tritium-labelled chromosomes. *Genetics*, *43*, 515-529.

TAYLOR, J. H. 1965. Distribution of tritium-labelled DNA among chromosomes during meiosis. 1. Spermatogenesis in the grasshopper. *J. Cell Biol.*, *25*, 57-67.

UPCOTT, M. 1937. The genetic structure of *Tulipa*. 11. Structural hybridity. *J. Genetics*, *34*, 339-398.