REPLICATION, RECOMBINATION, AND CHIASMATA IN *GONIAEA AUSTRALASIAE* (ORTHOPTERA: ACRIDIDAE)

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PROPONENTS of the chiasmatype theory of crossing over (JANSSENS 1909, 1924; DARLINGTON 1937) have usually made the assumption that the recombination event occurs at the time when chiasmata first become visible, i.e., at pachytene in prophase I of meiosis. Suggestions that this was not the case and that recombination occurs at the time of chromosome duplication (premeiotic interphase) followed from work on nonreciprocal crossing over and negative interference, principally in fungi (PRITCHARD 1960). Recent experiments have yielded apparently contradictory evidence on both the time and mode of crossing over in higher organisms. TAYLOR (1965) has shown, by autoradiographic methods, that at least some recombination events involve breakage and exchange of chromosome segments, an essential feature of the chiasmatype theory of crossing over. Also, MOENS (1966) found that in diplotene bivalents "chiasmata do not show crossing over of labeled material as is expected if the chiasmata are the result of breakage and rejoining."

Conflicting conclusions as to the time of crossing over have arisen from essentially similar studies. In each case a temperature-induced change in recombination frequency was used to delimit the time of crossing over in relation to the meiotic sequence. ABEL (1965) found, in Sphaerocarpus, that a high temperature treatment increased recombination if applied on the fifteenth day after fertilization, a time he had earlier claimed was from 1½–2 days subsequent to chromosome duplication (ABEL 1963). GRELL and CHANDLEY (1965), under more rigorous experimental conditions, found that a temperature effect on recombination in *Drosophila melanogaster* could be evoked only at the time of DNA synthesis in the premeiotic interphase. They concluded that replication and recombination were coincident, or nearly so. This conclusion is compatible with a copy-choice mechanism of recombination, a possibility which would be excluded if crossing over were to occur after the completion of DNA synthesis (GRELL 1965). HENDERSON (1966) using chiasmata as an index of crossing over, rather than measuring recombination directly as did ABEL, and GRELL and CHANDLEY, found that high temperature treatment in zygotene or early pachytene resulted in a significant decrease in chiasma frequency. HENDERSON concluded that recombination must be a postreplication event. This sequence is also demanded by ROSSEN and WESTERGAARD's (1966) findings in the ascomy-

cete, Neottiella, where their photometric measurements of DNA content showed that DNA replication of the two "gamete" nuclei precedes their fusion which in turn precedes meiosis. Similarly, detailed analyses of DNA replication and the life history of a strain of *Chlamydomonas reinhardi* have indicated that recombination and chromosomal DNA replication are separable events (CHIANG and SUEOKA 1967). Further support to the suggestion that crossing over may occur in prophase is given in the work of LAWRENCE who has shown that chiasma formation in Tradescantia and Lilium (1961 a, b), and both genic and allelic recombination in Chlamydomonas (1965, 1967) are sensitive to gamma radiation in the meiotic prophase as well as in the premeiotic interphase.

Emphasis to the lack of agreement between the various experiments discussed above is provided by MAGUIRE's (1966) considerations of crossing over in maize occurring prior to the premeiotic DNA replication.

The experiments described in this paper were addressed to the question of the time of recombination in relation to the sequence of stages in meiosis, to the relationship of chiasmata and crossing over and to the mechanism of recombination.

MATERIALS AND METHODS

Spermatocytes of *Goniaea australasiae* (Leach), (Orthoptera; Acrididae), were used as the experimental system.

The reasons for the choice of a grasshopper were principally that HENDERSON (1963) had demonstrated that high temperature might be expected to produce a significant change in chiasma frequency, and that TAYLOR (1965) had shown that an autoradiographic analysis of meiotic chromosomes was possible in grasshoppers. This particular species was chosen, following advice from Professor M. J. D. WHITE, because of its favorable cytological characteristics and its relatively slow development. Stocks of nymphs and adults were maintained in large cages but during experiments males were placed in plastic bags (2–4 males per bag). Fresh Eucalyptus leaves, the sole diet, were provided at two-day intervals. Control experiments were carried out at a constant 26°C and after preliminary investigation, 37°C was chosen as the high temperature regime. At this temperature there was a marked reduction in chiasma frequency with no other visible side effects on the meiotic chromosomes. Three experimental designs were used:

A—Males, either last nymphal instar or adults, were injected with tritiated thymidine and were then maintained at a constant 37°C.

B—Males were injected with tritiated thymidine, placed at 26°C for 4 days and then transferred to a constant 37°C.

C—Males were injected with tritiated thymidine, placed at 37°C for 24 hours and then transferred to a constant 26°C.

Tritiated thymidine (5-methyl T), 1 mc/1 ml sterile distilled water, specific activity 3.0 c/mmole, was administered by microsyringe into the abdomen in two injections of 10 μl each, the injections being 3 hr apart. Fixations were made in 1:3 acetic ethanol, the tubes being shaken initially to separate the testis tubules from the surrounding fatty sheath. After a minimum of 2 hr fixation, the testes were transferred to absolute alcohol for storage at 4°C. Squash preparations were made on subbed slides using standard Feulgen or aceto-orcein procedures. Autoradiograms were prepared with Kodak NTB emulsion, exposure varying from two weeks to four months with development in Dektol 1:1 for 1 min at 5°C.

For electron microscopy, testes were fixed in 2.5% glutaraldehyde in 0.05M phosphate buffer (pH = 7.2) and 0.005M calcium chloride for 6 hr at room temperature, washed in phosphate buffer and post-fixed in 2% osmium tetroxide in 0.05M phosphate buffer (pH = 7.2) for 2 hr at 4°C. The tubules were then dehydrated in ethanol, embedded in epon, sectioned and post-stained with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION

Chiasmata formation and chromosome replication

Karyotype: Goniaea australasiae has an X/0 sex determining mechanism, the male having 11 pairs of acrocentric autosomes and a single acrocentric X chromosome. In the present experiment, chromosomes were scored in four groups (see Figure 1), no attempt being made to distinguish individual chromosomes within groups. One autosome (group 3) (Figure 2b) is easily identified in prophase I since it is positively heteropycnotic, but from metaphase I onwards it is not distinguishable from group 2 chromosomes. The X is positively heteropycnotic during prophase I (Figure 2a, b) and is negatively heteropycnotic at metaphase–anaphase I (Figure 2d, e).

Chiasma frequency: At the control temperature of 26°C chiasma frequency and distribution are similar to those found in the natural populations sampled. The mean chiasma frequency at 26°C is 16.68 ± 0.47; chiasma frequency distributions for the different chromosome groups are presented in Table 1. Chiasma determinations were routinely made on diplotene cells (Figure 2a, b): scores from metaphase I cells yielded similar results indicating that terminalization is not a significant factor in this species.

Temperature response: Meiosis appears normal in individuals reared at a constant 37°C (experiment A) until the 9th day after transfer to this tempera-

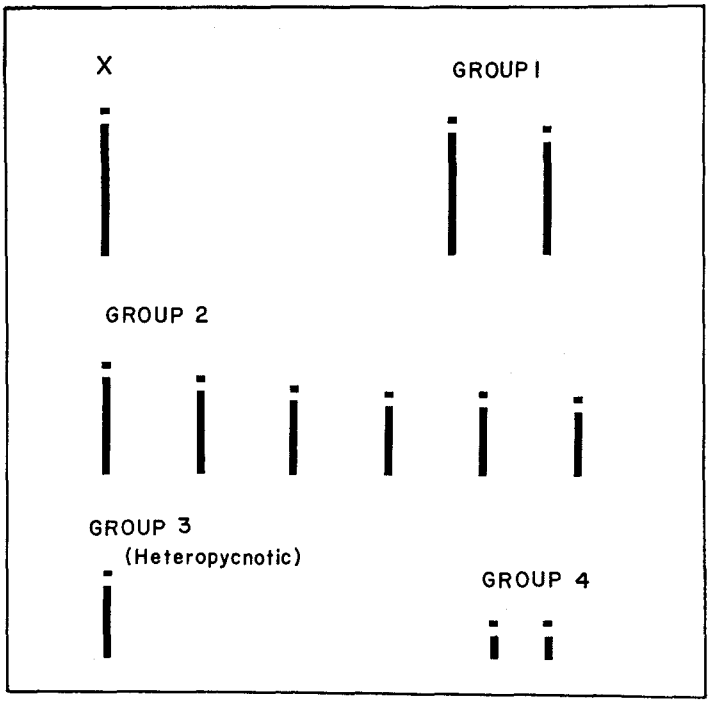

FIGURE 1.—Karyotype of the haploid chromosome complement of *Goniaea australasiae*.

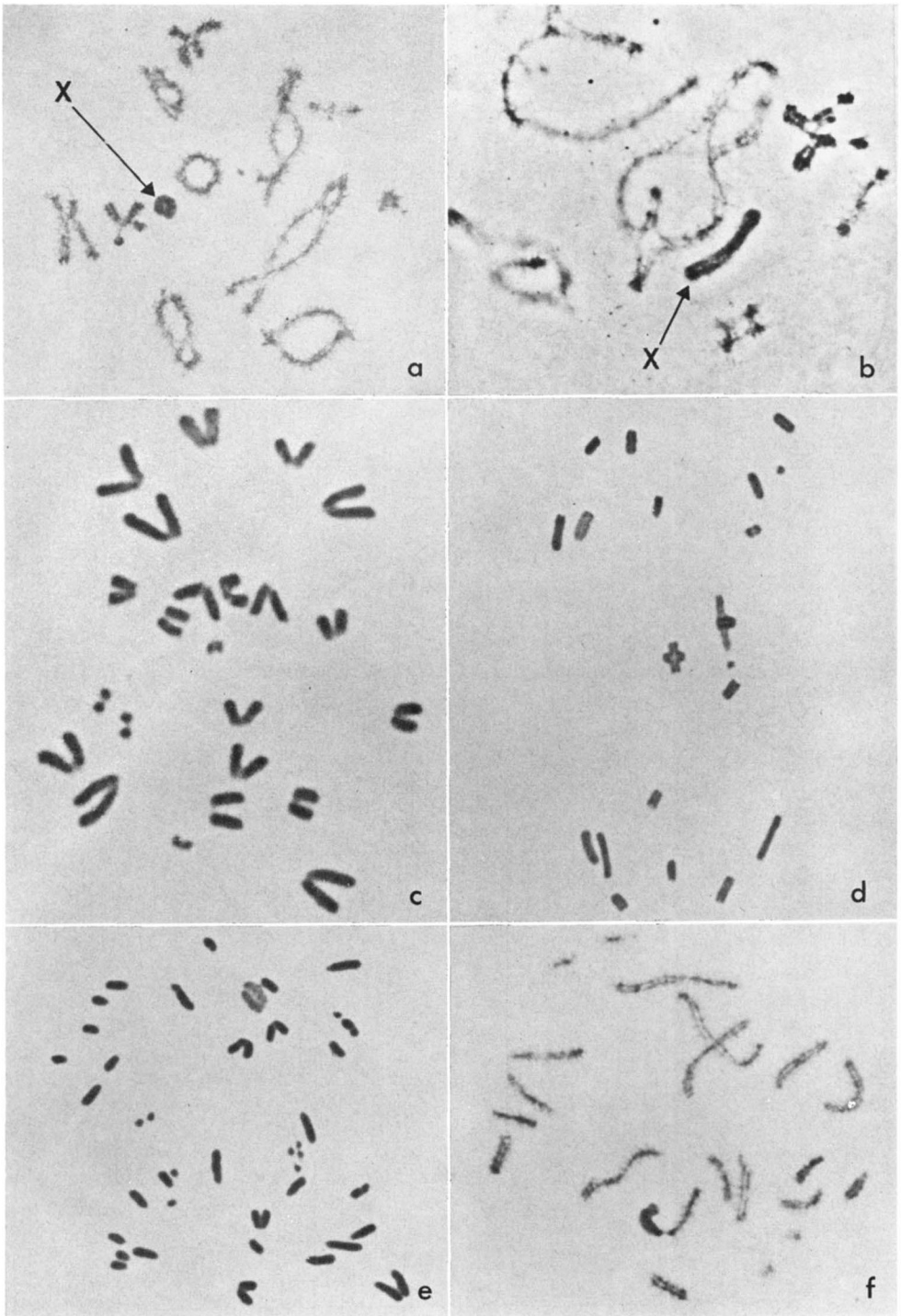
X
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TABLE 1

Chiasma frequency distributions (26°C)

Chromosome group	Number of chiasmata per bivalent				Number of bivalents scored
	1	2	3	4	
1	0.002	0.533	0.434	0.031	918
2	0.573	0.407	0.020	0	2808
3 (heteropycnotic autosome)	0.965	0.035	0	0	430
4	0.994	0.006	0	0	845

ture. At this time univalents (usually of groups 3 or 4) are seen at diplotene, diakinesis and metaphase I in some cells (Figure 2c). On day 10 chiasma frequency is significantly lower than in controls (Figure 2d, e) and it continues to drop until on day 14 the chiasma frequency may be less than 1 chiasma/cell. This level is maintained with continued high temperature at least until day 28. The results of a representative experiment are given in Table 2 and Figure 3: two individuals were scored at each fixation time. A similar response, both in time and magnitude, occurred in experiments in which the tritiated thymidine injection was omitted.

In diplotene cells having many univalents it is obvious that the univalents are not randomly distributed in the nucleus (Figure 2f)–sister univalents tend to be associated as if they had separated from a previously paired condition. In fact, pachytene in cells treated with high temperature is not distinguishable from that in control cells. The close homologous pairing of the heteropycnotic autosome is particularly evident. The univalents must result, then, from *desynapsis* rather than *asynapsis*, whereas in *Schistocerca gregaria* HENDERSON (1962) reports the occurrence of *asynapsis*. The criticism may be offered that the desynapsis results from induced terminalization and subsequent loss of existing chiasmata, rather than from failure of chiasma formation. An analysis of chiasma localization throughout the course of the experiment described above has shown that the reduction in chiasma frequency results from actual reduction in chiasma formation and not from terminalization (Table 3). The

FIGURE 2.—(a) Late diplotene cell from individual cultured at 26°C, showing eleven bivalents and the heteropycnotic X chromosome; (b) Portion of a diplotene cell showing a group 1 bivalent with 3 chiasmata and heterochromatic centromere regions, the group 3 heteropycnotic bivalent with a single chiasma, and the unpaired, heteropycnotic X chromosome; (c) Anaphase I on the ninth day of 37°C culture showing nondisjunction of a group 2 chromosome pair to the upper pole and nondisjunction of a group 4 chromosome pair to the lower pole. Presumably this cell would have shown 4 univalents at metaphase I; (d) Metaphase I on the tenth day of 37°C culture with only 3 bivalents; (e) Anaphase I on the tenth day of 37° C culture showing 4 dyads, 36 single chromatids from divided univalents (2 group 4 univalents in process of chromatid separation) and the X chromosomes. This cell would have shown 2 bivalents at metaphase I. Univalent division at the first division does not occur in most individuals; (f) Diplotene showing complete univalence and "paired" association of sister univalents.

TABLE 2

Chiasma temperature response (continuous 37°C)

Day of fixation	Chiasma frequency ± SE	Day of fixation	Chiasma frequency ± SE
	19.89 ± 0.25		13.29 ± 0.38**
1	15.51 ± 0.18	10	8.32 ± 0.25**
	18.21 ± 1.44		9.41 ± 1.92
	15.82 ± 0.18		7.01 ± 0.38**
4	15.17 ± 0.22	11	5.29 ± 0.30**
	15.57 ± 0.95		5.99 ± 1.90
	17.96 ± 0.12		3.19 ± 0.21**
7	14.46 ± 0.17	12	4.51 ± 0.17**
	16.85 ± 0.91		3.81 ± 1.11
	17.78 ± 0.25		0.01 ± 0.01**
9	15.86 ± 0.30*	14	0.16 ± 0.03**
	16.67 ± 1.82		0.08 ± 0.08

Each entry consists of the mean chiasma frequency for each of two individuals, together with their weighted mean.
* Univalents present in some cells.
** Univalents present in most or all cells.

high contingency χ^2 values for the data of both chromosome groups 1 and 2 do not seem to result from any consistent change in chiasma localization patterns over time (i.e., from 26°C to 37°C). Figure 4 shows that if any trend exists, it is primarily an increase in medially placed chiasmata with a corresponding decrease in proximal chiasmata and to a lesser extent in distal chiasmata. This change in distribution does not conform to that of a substantial increase in the

TABLE 3

Localization pattern of chiasmata (continuous 37°C)

Day of fixation	Mean chiasma frequency	Chromosome group 1 Distribution of chiasmata			Number of chiasmata scored	Chromosome group 2 Distribution of chiasmata			Number of chiasmata scored
		Proximal	Medial	Distal		Proximal	Medial	Distal	
1	18.21	.37	.25	.38	775	.34	.27	.39	1522
4	15.57	.38	.24	.38	518	.18	.47	.34	769
7	16.85	.36	.25	.39	518	.31	.27	.42	992
9	16.67	.35	.32	.33	475	.29	.33	.38	761
10	9.41	.37	.29	.34	524	.17	.52	.31	603
11	5.99	.35	.30	.35	574	.18	.47	.35	443
12	3.81	.22	.45	.33	280	.17	.47	.36	196
		Contingency $\chi^2_{12} = 59.7$				Contingency $\chi^2_{12} = 262.6$			

proportion of distal chiasmata expected if the temperature effect were induced by terminalization of existing chiasmata. The increase in the medial class can largely be ascribed to an increasing predominance of bivalents with a single chiasma (the distribution pattern for a single chiasma is proximal 0.16, medial 0.64, distal 0.20; for two chiasmata it is 0.42, 0.13, 0.45; and for 3 chiasmata 0.44, 0.22, 0.34).

DNA synthesis and the meiotic cycle: Autoradiograms from individuals used in experiment A (i.e., under 37°C regime) showed that the latest meiotic stage labeled ten days after injection was late pachytene (Figure 5a). Labeled diplotene and first and second metaphases did not appear until day 14 (Figure 5b) at which time both sister chromatids of each dyad were labeled. By the 18th day after injection, metaphase chromosomes showed segregation of label over sister chromatids (Figure 5c). These results are interpreted to mean that the DNA synthesis prior to meiotic prophase precedes metaphase I by 14 days at 37°C (diplotene, first and second meiotic divisions have not been separated by the technique and must all occur within a 24 hour period). The DNA synthesis occurring in the final premeiotic mitosis (gonial) occurs about 18 days prior to metaphase I.

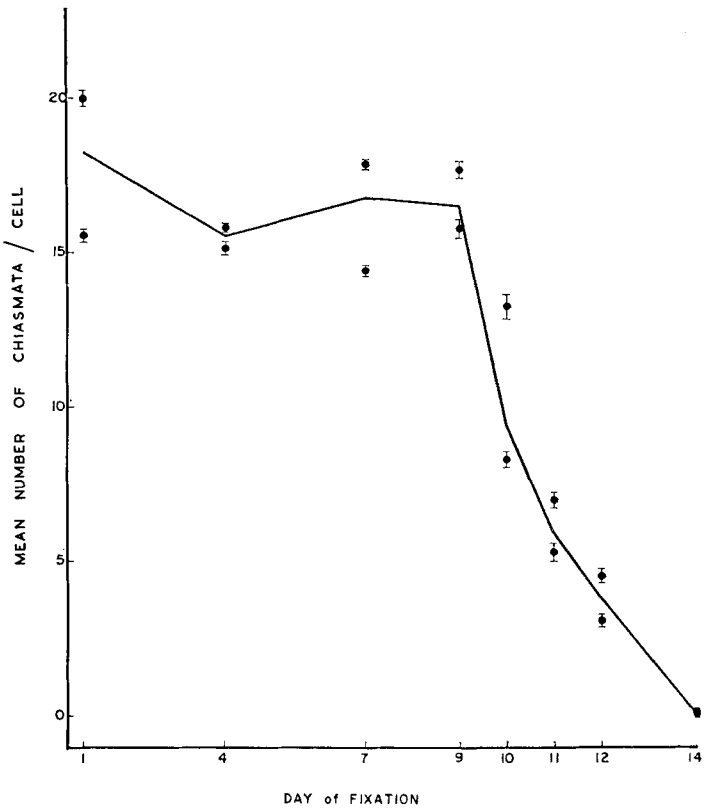

FIGURE 3.—Chiasmata frequencies for individuals fixed at various times after initiation, at day 0, of culture at 37°C. Vertical lines represent standard errors.

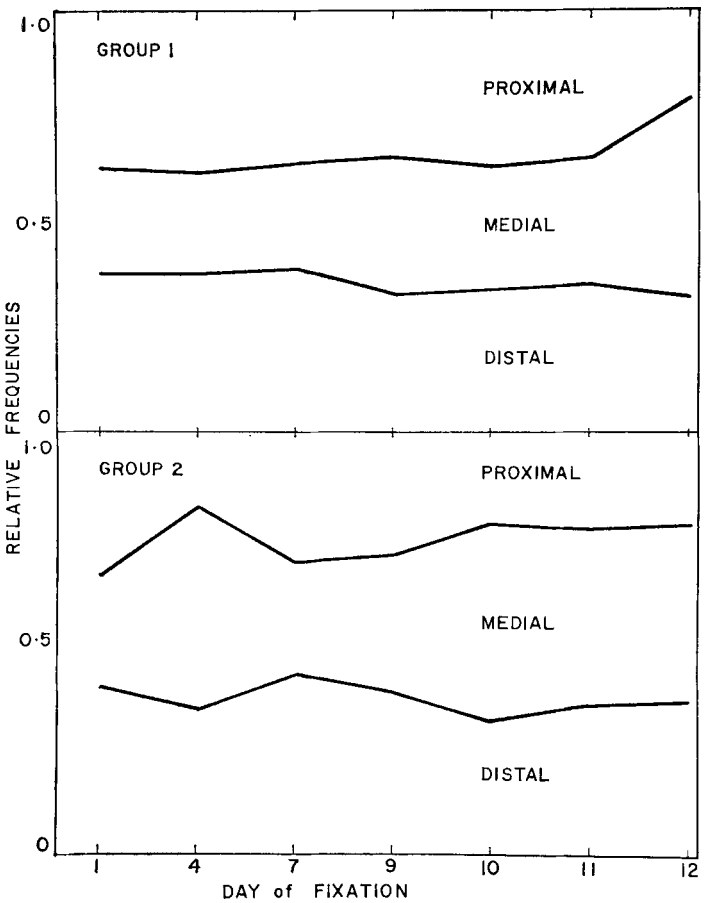

FIGURE 4.—Relative frequencies of chiasmata positions in successive fixations at 37°C; plots of data given in Table 3.

Isotope distribution confirmed TAYLOR's (1965) finding of semiconservative segregation of chromosomal DNA in the premeiotic interphase (i.e., in the replication period preceding the onset of meiotic prophase).

These experiments have shown that the time of chiasma formation, marked by the latest possible induction of a temperature response in chiasma frequency, occurs 4 days (day 10) after the completion of chromosomal replication in the premeiotic interphase (day 14). This sequence of events is in agreement with that reported by HENDERSON (1966) in Schistocerca.

In order to check whether any of the four-day differential between DNA replication and time of chiasma formation resulted from a tritium-induced delay in the meiotic cycle, some individuals were maintained at 26°C for 4 days after administration of isotope before being transferred to 37°C (experiment B). In

each of three separate experiments it was found that the first cells to show a marked temperature response (10 days after transfer to 37°C) were labeled. From this we may conclude that incorporated tritium does not result in any cell cycle delay detectable under the present experimental conditions.

Period of temperature sensitivity: Sequential fixations of individuals maintained at 26°C, but having been subjected to a 24 hour "pulse" treatment at 37°C immediately following injection of thymidine (experiment C), have established that chiasma frequency can be reduced only at a discrete stage of the cell cycle (Table 4). Univalents were first noted in fixations on day 22. On day 24, chiasma frequencies were significantly reduced below the control level and a reduced mean frequency was also evident on day 26. In one individual on day 28 univalents were present in some cells but on this and the subsequent fixations chiasma frequencies did not differ significantly from the control level. The fact that chiasma frequencies in individuals fixed on days 30–34, in which the meiotic chromosomes are labeled, do not differ from control levels shows that incorporated tritium does not alter the probability of chiasma formation. The pattern of chiasma frequencies in the experiment indicates that the temperature-sensitive stage in chiasma formation precedes metaphase I by about 25 days at 26°C and that treatment either subsequent to, or some time prior to this stage does not affect chiasma formation. Meiosis is about 2½ times faster at 37°C than it is at 26°C. However, it can be inferred from experiment B that the various meiotic stages do not have a uniform temperature coefficient and at least the initial stages (4 days) of meiosis following chromosome replication appear to progress similarly at 26° and 37°C.

Cytological stage of chiasma formation: To determine the cytological stage at which chiasma formation may be suppressed, autoradiograms were examined from individuals fixed 4 days after injection of tritium and transfer to 37°C. The latest labeled meiotic stage was found to be a stage which could not clearly be defined as being either zygotene or pachytene (Figure 5d). However, the heteropycnotic autosome had a characteristic appearance and clearly showed a paired condition of its two homologues. The remainder of the autosomes were diffuse (indistinct) at this stage but where detail was discernible homologues appeared to be closely paired. Soon after this stage the nucleus has a typical pachytene appearance. For these reasons the stage will be termed "early pachytene."

Chiasma formation and chromosome replication: To briefly recapitulate: chiasma frequency is drastically reduced in spermatocytes of Goniaea by high temperature treatment at a particular stage in the meiotic cycle. This temperature-sensitive stage is subsequent to the completion of DNA synthesis in the premeiotic interphase and has been identified as "early pachytene." The reduction in chiasma frequency appears to reflect a reduced probability of chiasma formation rather than an increased probability of their loss by terminalization. It has been established that the sensitive stage is discrete and that there is no later sensitive stage. A temperature-stable period precedes the sensitive stage but the possibility of another earlier sensitive stage has not been excluded.

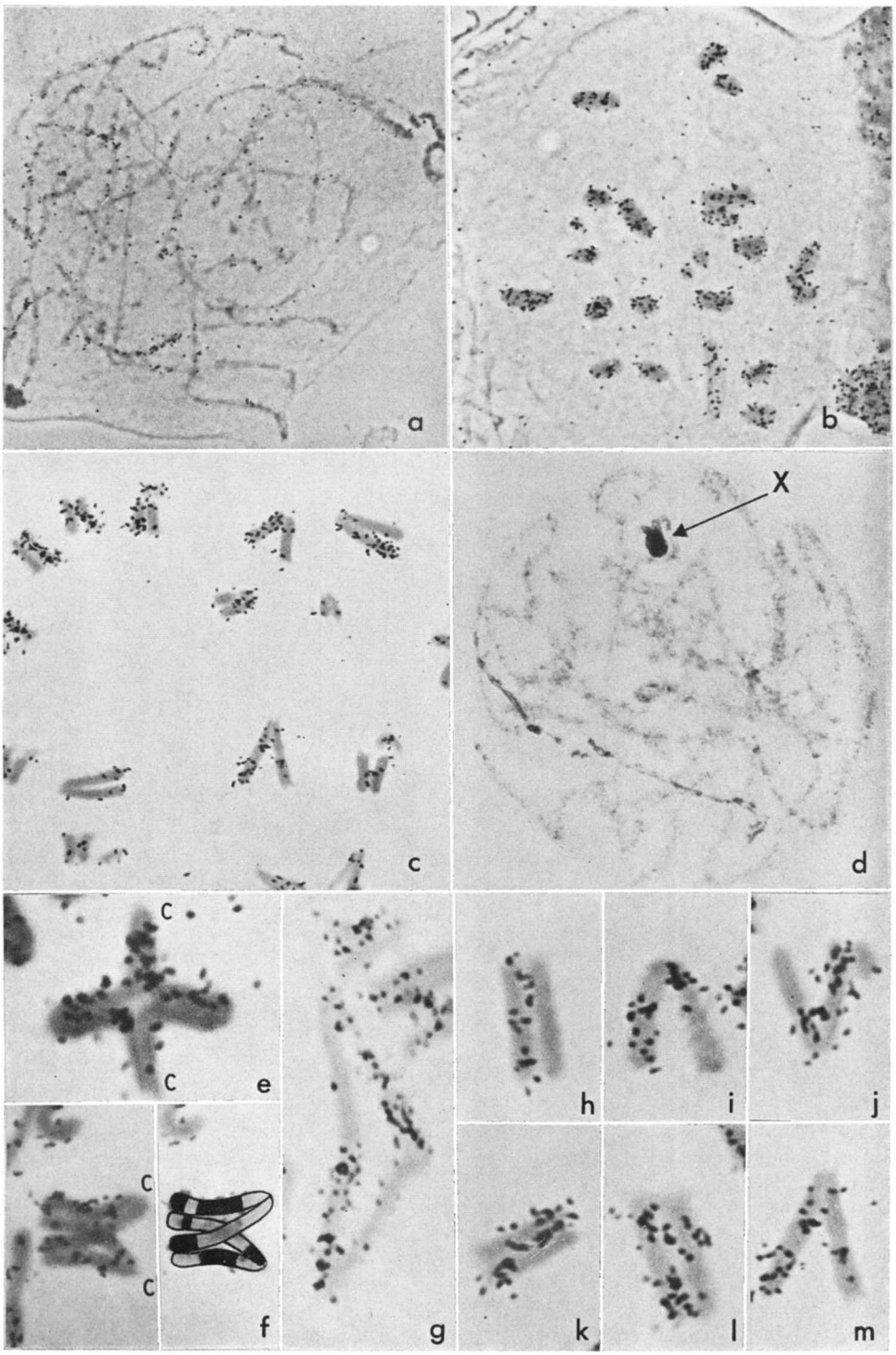
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TABLE 4

Chiasma temperature response (24 hrs at 37°C; continuous 26°C)

Day of fixation	Chiasma frequency	Day of fixation	Chiasma frequency
	16.88 ± 0.11		14.45 ± 0.32**
1	14.33 ± 0.12	26	11.05 ± 0.58**
	16.15 ± 0.94		13.62 ± 1.84
	15.84 ± 0.23		16.44 ± 0.21
10	16.97 ± 0.20	28	17.02 ± 0.19
	16.37 ± 1.00		16.72 ± 1.10
	19.76 ± 0.30		16.85 ± 0.25
18	16.67 ± 0.26	30	17.25 ± 0.24
	18.95 ± 1.27		17.12 ± 1.20
	16.33 ± 0.14		15.81 ± 0.22
22	15.19 ± 0.14*	32	16.94 ± 0.18
	15.83 ± 0.86		16.62 ± 1.04
	12.31 ± 0.21**		16.77 ± 0.15
24	13.78 ± 0.25**	34	16.12 ± 0.14
	12.79 ± 1.18		16.48 ± 0.98

Each entry consists of the mean chiasma frequency for each of two individuals, together with their weighted mean.

* Univalents present in some cells.

** Univalents present in most cells.

Chiasmata and crossing over

Since a temperature-sensitive phase in the process of chiasma formation exists at early pachytene, we may now ask whether genetic recombination is similarly

FIGURE 5.—(a) Late pachytene, being the most advanced meiotic stage labeled 10 days, at 37°C, after injection of tritiated thymidine; (b) Metaphase I, labeled 14 days after isotope incorporation. The cell shows complete univalence; (c) Metaphase II showing label segregation over sister chromatids of dyads. This cell, from an individual fixed 18 days after thymidine application, incorporated the isotope during chromosome replication in the last premeiotic mitosis; (d) Early pachytene, the latest stage to show label 4 days, at 37°C, subsequent to isotope incorporation in the premeiotic interphase. This stage, which is most easily recognized by the appearance of the heteropycnotic autosome, marks the time of operation of a process, or processes, involved in the exchange of genetic material; (e) Bivalent at metaphase I, upper dyad showing terminal isolabeling. The position of the centromeres is indicated by "C"; (f) Bivalent just beginning anaphase movement. The chromatids attached to the upper centromere show a terminal isolabel pattern generated by crossing over; (g) Bivalent in anaphase separation showing dyads without any terminal isolabeling; (h) Dyad with label segregation; (i,j,k) Dyads with a sister chromatid exchange; (l,m) Dyads with two sister chromatid exchanges.

sensitive. Does the reduction in chiasma frequency reflect a reduction in crossing over?

Correlation between chiasmata and crossing over: A number of lines of cytological evidence lend support to the thesis that a 1:1 correlation exists between chiasmata and crossover events. Most critical are the experiments involving heteromorphic homologues (e.g., BROWN and ZOHARY 1955; KAYANO 1960) and the autoradiographic studies of TAYLOR (1965). TAYLOR showed that certain patterns of label distribution in meiotic dyads were explicable only on the basis of breakage and exchange of segments of homologous chromosomes. He found that the frequency of switch points of label over dyads corresponded to the frequency of chiasmata in bivalents, although the frequency distributions seemed not to be homogeneous.

Mrs. ALICE SCHROEDER of Stanford University has shown that if the distributions are analyzed on a tetrad strand basis, they are found to be homogeneous (Dr. D. D. PERKINS, personal communication).

Given that a correlation does exist between chiasmata and crossing over we have yet to establish that each chiasma is actually produced by a crossover event. It could be proposed, for example, that the occurrence of recombination between a pair of chromosomes predisposes those chromosomes to form a chiasma subsequently. If this were the case then chiasma formation might well be suppressed independently of crossing over.

Label pattern in bivalents: Autoradiographic data bearing on this problem have been obtained from individuals fixed on the 17th and 18th day of incubation at 37°C following injection of tritiated thymidine (experimental design A). These cells show segregation of label over sister chromatids and thus would have incorporated isotope during the mitosis preceding the meiotic divisions. The chiasma frequency in these cells was 1.4. Forty-six metaphase I bivalents were scored for label patterns, the data being summarized in Tables 5 and 6. The first point to be made from these data is that some bivalents have dyads with terminal isolabeling (cf. PEACOCK 1963), i.e., both chromatids being labeled over homologous segments (Figures 5e–g). Where a dyad is isolabeled, the sister dyad is unlabeled for that same segment in both chromatids. This label pattern in bivalents corresponds to the "nonreciprocal switch points" described by TAYLOR and reinforces his conclusion that actual breakage and exchange of chromosome segments is a feature of recombination in higher organisms.

TABLE 5

Label patterns over metaphase I bivalents

Chromosome group	One dyad with terminal isolabeling	Neither dyad with terminal isolabeling	Number of bivalents scored
1	9(1)	11(5)	20
2	11(3)	15(1)	26

Numbers in parentheses represent the number of dyads having an intercalary isolabeled segment.

TABLE 6

Frequency distribution of exchanges in dyads of bivalents scored for Table 4

Number of exchanges per dyad (other than terminal isolabel exchange)					Number of dyads scored
0	1	2	3	4	
32	26	15	2	1	76

Since each bivalent scored had a single chiasma it is predicted on the basis of 1 chiasma: 1 crossover, that half of the bivalents should contain a terminally isolabeled dyad, the sister dyad having the reciprocal label pattern (Figure 6). The data for both chromosome groups are consistent with this expectation (Table 5). *It should be noted that this expectation is independent of sister chromatid exchange.* In Romalea, TAYLOR found a low frequency of sister chromatid exchange but it is evident from Table 6 that such exchanges are not uncommon in Goniaea (mean number of exchanges per bivalent = 1.74). The presence of sister chromatid exchanges excludes any meaningful comparison of chiasma position and length of isolabeled segment, since the latter is dependent on the position of sister chromatid exchanges as well as of actual crossovers. Some chiasmata do coincide with the initiation point of a terminal isolabeled segment as would be expected if a chiasma resulted from an exchange event but others are either proximal or distal to such points.

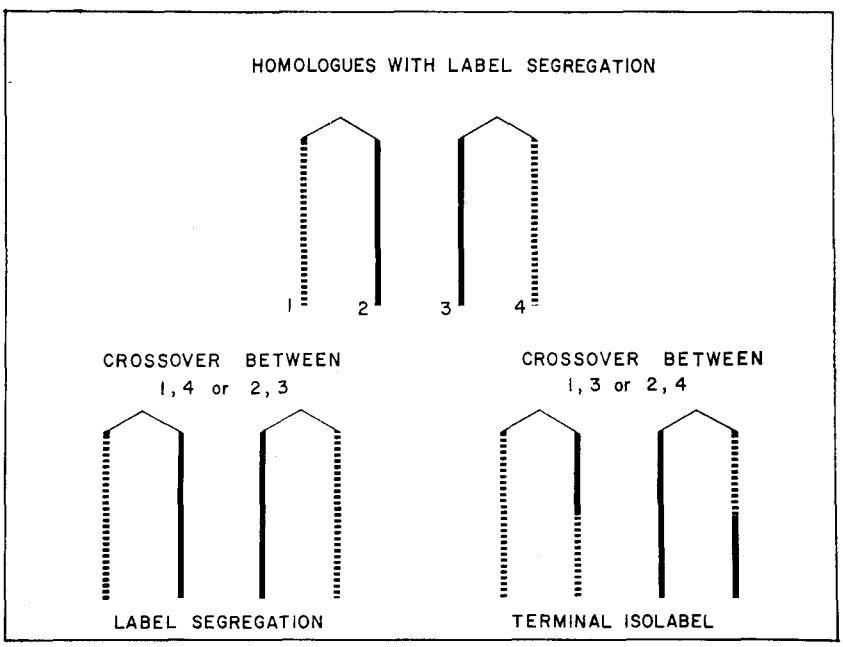


FIGURE 6.—Diagram showing the two patterns of label distribution among the dyads of a bivalent having a single crossover. It is assumed that the crossover is achieved by breakage and reunion of nonsister chromatids. Presence of label is indicated by a broken line.

Two conclusions arising from the above data are (1) that label distribution yields information on recombination both as to mechanism and frequency and (2) that a 1:1 correlation exists between a chiasma and a nonsister chromatid exchange event.

Label patterns over dyads: With these points established we may now examine the relationship of chiasmata and crossing over utilizing label patterns over dyads (Figure 5h–m), most of which are univalents resulting from the reduction of chiasma frequency induced by the 37°C regime. Only those dyads which were labeled along their whole length were scored, e.g., of the dyads shown in Figure 6 the sister dyad of the one with terminal isolabeling would not have been scored. By scoring only "positive" patterns, confusion from segmental labeling was avoided. The data are presented in Table 7.

Expectations can be formulated on the basis of two general models:

1. *The temperature-induced reduction in chiasma frequency does not reflect a similar reduction in recombination and crossover frequency should correspond to the 26° chiasma frequency.*

2. *The reduction in chiasma frequency reflects a reduction in recombination so that crossover frequency should correspond to the 37° chiasma frequency.*

In computing the expectations for these models only "positive" label patterns were considered since these were the only patterns included in the observed data. Figure 7 presents the relative probabilities of the four categories of "positive" dyads expected from a bivalent having one, 2 or 3 chiasmata, assuming a 1:1 relationship of chiasmata to crossovers. The derivation of expectations for a single crossover is readily seen by reference to Figure 6. (One of the dyad types generated by 2 or 3 crossovers, "sister chromatid exchange," is indistinguishable from that resulting from a reciprocal exchange between sister chromatids, cf. next section). When these probabilities are considered along with observed chiasma frequency distributions (see Table 1 for 26° data, Table 8 for 37° data) they generate the expectations of dyad types for Models 1 and 2 (Table 9). A summary of the observed data from Table 8 is also presented in Table 9. A comparison of the 2 sets of expectations with the actual data yields 2 points worthy of note:

1. The observed data patterns do not conform to the expectations for either model! The principal difference is that the observed proportions of dyads having sister chromatid exchanges are considerably greater than expected (the label segregation class is correspondingly low).

2. If the terminal isolabel class, which can result only from true crossing over, is considered it is seen that the observed data favor model 2. The distinction between models is particularly evident for chromosome group 2 (and 3).

Consequences of sister chromatid exchange: Thus far we have not considered the consequences of the sister chromatid exchanges which occur in considerable frequency in this material (see above). Demonstration that the majority of sister chromatid exchanges are comparable to those observed in mitotic chromosome systems and do not result from certain double crossover events is given both by the comparison of expectations and data made above, and in particular by the

TABLE 7

Distribution of label patterns over first and second division dyads

Meiotic division	Type of dyad	Dyads without isolabeling Number of exchanges per dyad 0	1	2	3	4	Dyads with terminal isolabeling	Dyads with intercalary isolabeling	Total dyads scored
First division dyads	Chromosome group 1	40	42	40	5	1	11	3	142
	Chromosome group 2 + heteropycnotic autosome	93	105	51	1	0	18	0	268
	X chromosome	5	10	13	3	0	1	0	32
Second division dyads	Chromosome group 1	6	14	13	0	1	5	1	40
	Chromosome group 2 + heteropycnotic autosome	23	33	6	0	0	5	0	67

TABLE 8

Chiasma frequency distributions (37°C)

Chromosome group	Number of chiasmata per bivalent			Number of bivalents scored
	0	1	2	
1	0.59	0.41	0	34
2	0.92	0.08	0	115

TABLE 9

Expected and observed relative frequencies of dyad label patterns

		Label segregation	Terminal isolabel	Intercalary isolabel	Sister chromatid exchange
Chromosome group 1 (n=180)	Expectations for model 1	0.42	0.20	0.18	0.20
	Expectations for model 2	0.87	0.13	0	0
	Observed proportions	0.25	0.09	0.02	0.64
Chromosome groups 2 and 3 (n=335)	Expectations for model 1	0.60	0.27	0.06	0.07
	Expectations for model 2	0.97	0.03	0	0
	Observed proportions	0.34	0.07	0	0.59

fact that the X chromosome, which does not have a homologue and which does not participate in any meiotic exchange, has a frequency of sister chromatid exchanges comparable to that of the autosomes. It is possible that most sister chromatid exchanges result from breakage and reunion events induced by the endogenous radiation from DNA-incorporated tritium (BREWEN and PEACOCK 1968).

In determining the effects of sister chromatid exchange on the dyad label patterns, factors of importance are (1) the number of crossovers, (2) the number of sister chromatid exchanges, (3) the relative position of the sister chromatid exchange, proximal or distal, to the crossover, and (4) the time of occurrence of the sister chromatid exchange. For the sake of simplicity we will consider only the effects of one sister chromatid exchange on a single crossover since additional exchanges and crossovers cause changes of small magnitude to the expectations. The consequence of exchange after the cessation of synapsis may be ignored since this represents such a small portion of the cell's postreplication history. Exchanges occurring during synapsis or prior to synapsis (including the premeiotic mitosis) have identical effects so no estimates as to their respective frequencies need be made. One assumption made is that the sister chromatid ex-

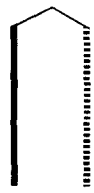
DYAD TYPE / No. of CHIASMATA in BIVALENT	 LABEL SEGREGATION	 TERMINAL ISOLABEL	 INTERCALARY ISOLABEL	 SISTER CHROMATID EXCHANGE
1	0·67	0·33	0	0
2	0·50	0·17	0·17	0·17
3	0·33	0·24*	0·19	0·24

FIGURE 7.—Diagram showing "positive" label patterns over dyads and their relative expectations from bivalents having one, two or three chiasmata assuming a chiasma results from a crossover event. Presence of label is indicated by a broken line. The asterisk denotes that the presence of a terminal isolabeled segment serves to characterize the dyad; some of this class have other exchanges proximal to the isolabeled segment.

change has equal prospects of being proximal or distal to any crossover event. This assumption is based on the symmetry of both the distribution pattern of chiasmata along the chromosome (see Table 3), and of the distribution of exchanges along the chromosome. The probabilities for the four dyad types are given in Table 10 for cases where both half-bivalents have 0 sister chromatid exchanges, where one has 0 and one has 1 (or more) exchange and where both have one (or more) exchanges. The method used in derivation of these probabilities is shown in Figure 8, for the case where only one of the half-bivalents has a sister chromatid exchange. This diagram relates to the second row of Table 10. The probability p of a half-bivalent having one or more exchanges is found from Table 7. Expectations for both chromosome groups on the basis of models 1 and 2, and using the values from Table 10, are given in Table 11 along with a summary of the observed data extracted from Table 7.

Inspection of Table 11 shows clearly for both chromosome groups that the actual distribution of label patterns does not conform to the expectations for model 1 but does approach those for model 2. In particular the distribution for chromosome group 2 (and 3) closely approximates the model 2 expectations. A statistical analysis yields a significant χ^2 value for chromosome group 1 against the model 2 expectation, however one-half of this χ^2 value is contributed by the intercalary isolabel class, a class which will tend to have been underestimated in

TABLE 10

Relative expectations of dyad label patterns from bivalents with a single chiasma, assuming sister chromatid exchange

Distribution of sister chromatid exchanges	Label segregation	Terminal isolabel*	Intercalary isolabel*	Sister chromatid exchange**
Both half-bivalents having 0 exchanges (q^2)	0.67	0.33	0	0
One half bivalent having 0 exchange the other 1 exchange ($2pq$)	0.20	0.40	0.20	0.20
Both half bivalents having 1 exchange (p^2)	0	0.33	0.33	0.33

* Some chromosomes in these categories have a sister chromatid exchange as well as an isolabeled segment.

** Some chromosomes in this category have more than one sister chromatid exchange.

TABLE 11

Expected and observed relative frequencies of dyad label patterns, assuming sister chromatid exchange

		Label segregation	Terminal isolabel	Intercalary isolabel	Sister chromatid exchange	Goodness-of-fit*
Chromosome group 1 (n = 180)	Expectations for model 1	0.14	0.36	0.25	0.25	$\chi^2_3 = 199.6$
	Expectations for model 2	0.23	0.15	0.10	0.52	$\chi^2_3 = 21.1$
	Observed proportions	0.25	0.09	0.02	0.64	
Chromosome group 2 & 3 (n = 335)	Expectations for model 1	0.18	0.37	0.23	0.23	$\chi^2_3 = 348.2$
	Expectations for model 2	0.36	0.03	0.02	0.60	$\chi^2_2 = 7.9$
	Observed proportions	0.34	0.07	0	0.59	

* The χ^2 value was obtained from the numerical results, not from the relative frequencies given in the table.

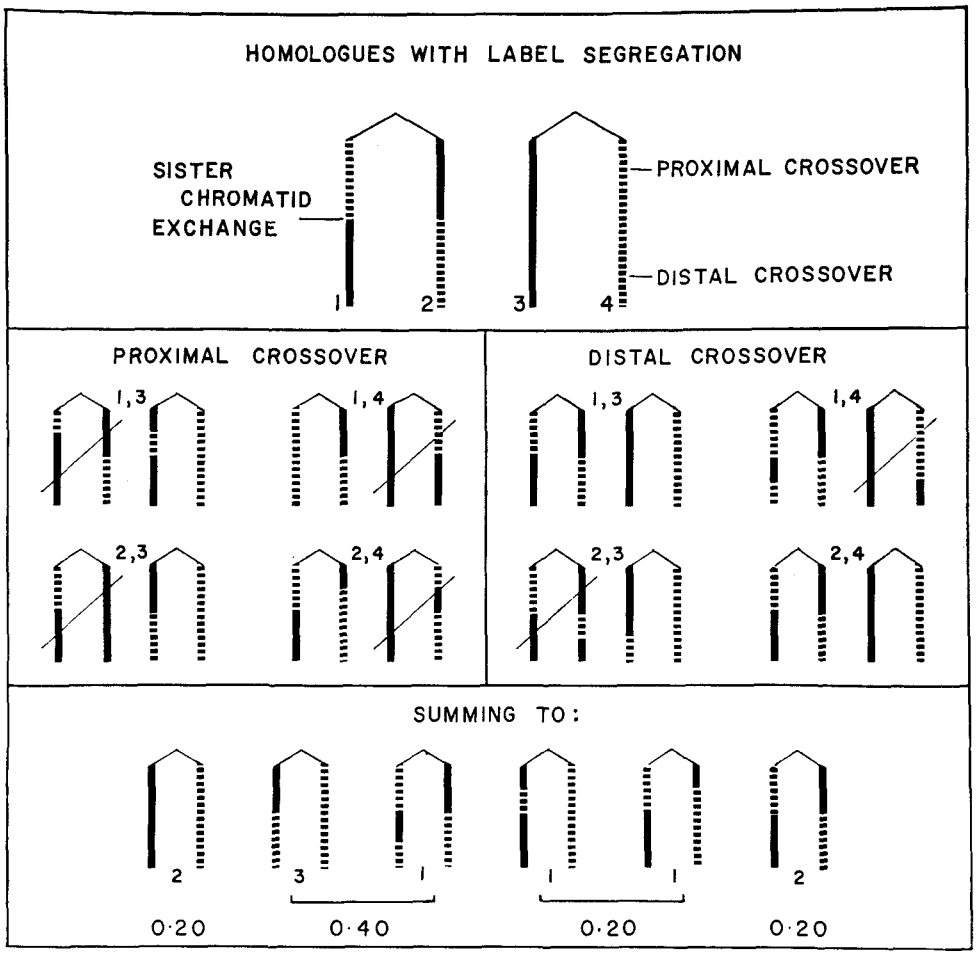


FIGURE 8.—Diagram showing the label patterns expected with a single crossover in a bivalent in which one homologue has a sister chromatid exchange. The relative frequencies of "positive" label patterns relate to Table 11. A line has been scored through the "negative" patterns which are excluded from the analysis. Presence of label is indicated by a broken line.

the collection of data. With this point considered it may be concluded that both sets of data distinguish between the two models in favor of model 2. The autoradiographic analysis shows that the reduction in chiasma frequency is associated with a comparable reduction in the exchange of genetic material.

Intercalary isolabeling in bivalents: A consideration of the frequency of intercalary isolabeling in bivalents, even in the small sample given, emphasizes that most of the dyads analyzed above were in fact drawn from a population in which crossing over did not occur. The interaction of sister chromatid exchanges and crossover exchanges leads to an expectation of segmental intercalary isolabeling, in bivalents, which is considerably higher than that found in the general dyad population (Table 12). From Table 12 it follows that the expected proportions of

bivalents having dyads with intercalary isolabeling is $2\,pq\ (0.25) + p^2\ (0.125 + 0.375)$ In the present experiments the expected values for chromosome groups 1 and 2 are 0.35 and 0.32, respectively: the data in Table 5 indicate that comparable frequencies do occur.

TABLE 12

Label patterns in bivalents, assuming sister chromatid exchange

		Dyads expected in bivalents with a single chiasma
Both half-bivalents with 0 sister chromatid exchange (q^2)	0.5	Both dyads with label segregation
	0.5	One dyad with terminal isolabel, the other with the reciprocal pattern
One half-bivalent with 0 sister chromatid exchange, the other with 1 sister chromatid exchange ($2\,pq$)	0.25	One dyad with label segregation, the other with sister chromatid exchange
	0.5	One dyad with terminal isolabel, the other with the reciprocal pattern*
	0.375	One dyad with terminal isolabel, the other with the reciprocal pattern*
Both half-bivalents with 1 sister chromatid exchange (p^2)	0.375	One dyad with terminal isolabel, the other with reciprocal pattern*
	0.125	Both dyads with sister chromatid exchange(s)
	0.125	One dyad with terminal isolabel, the other with intercalary isolabel*
	0.375	One dyad with intercalary isolabel, the other with the reciprocal pattern*

* Some chromosomes will have a sister chromatid exchange in addition to the diagnostic label pattern.

Isotope incorporation at the time of crossing over

A particular cytological stage termed "early pachytene" has been identified above as the time of genetic crossing over and chiasma formation. More correctly it should be said that a temperature-labile step in the recombination process occurs at this stage. Thus at least the completion of the process of recombination is quite distinct in time from DNA replication associated with chromosome duplication. There are, however, two reports of a discrete period of DNA synthesis during meiotic prophase. WIMBER and PRENSKY (1963) identified incorporation of tritiated thymidine in pachytene cells of the newt, *Triturus viridescens* and HOTTA, ITO and STERN (1966) have characterized DNA synthesis occurring in zygotene of some Liliaceous plants. In view of these reports and the implication of DNA turnover and repair in theories concerning the mechanisms of recombination (WHITEHOUSE 1963), an autoradiographic analysis was made on the "early pachytene" cells of Goniaea.

Individuals were fixed either one hour or three hours after injections of tritiated thymidine; autoradiograms were prepared of cells in the "early pachytene" stage associated with chiasma formation and crossing over. In all cases the re-

sults were negative, even when exposure times were such that as little as 1% of the synthesis occurring in interphase cells would have been detected. WIMBER and PRENSKY estimated the pachytene synthesis in the newt to be about 2% of the interphase synthesis but in Lilium, HOTTA, ITO and STERN detected a zygotene synthesis amounting to only 0.3% of the interphase level. It is possible then, in Goniaea, that DNA synthesis or turnover occurs in the meiotic prophase but at a level which is beyond the resolution of the autoradiographic technique employed.

The synaptonemal complex and crossing over

MOSES (1956, 1958) was one of the first to describe certain triaxial structures associated with paired homologues in electron micrographs of pachytene of meiosis. These "synaptonemal complexes" have since been recorded in a wide variety of plants and animals and generally can only be identified at pachytene. Detailed accounts of their form and occurrence may be found in MOSES and COLEMAN (1964) and SOTELO and WETTSTEIN (1965). Suggestions that the synaptonemal complex is in some way concerned with homologous pairing and with recombination have received direct support from the observations of MEYER (1964) in *Drosophila melanogaster*. MEYER showed that complexes were present in the first meiotic prophase of female Drosophila but were absent from the meiotic divisions of the male, which is known not to have any meiotic recombination. Furthermore, the complex was absent from females which were homozygous for a mutant *(c3G)* which inhibits meiotic recombination. Since in Goniaea cultured at 37°C, crossing over is largely suppressed but pachytene pairing appears to be normal, it was felt that an electron microscope comparison of pachytene cells of control and high temperature individuals was warranted.

Individual tubules were serially sectioned and the sections examined by both phase contrast and by electron microscopy. In controls, the synaptonemal complex occurred only in cells identified by phase microscopy to be pachytene. In general the dimensions and characteristics of the complex agreed with those given in MOSES and COLEMAN (details are given in GOODCHILD and PEACOCK, in preparation). The meiotic stage in which the complexes were most frequent was the "early pachytene" stage identified with chiasma formation and crossing over.

In individuals cultured at 37°C and having chiasma frequencies of approximately 1–2 chiasmata per cell, the complexes appear to have similar structure and occur in similar frequencies as in controls. Again the "early pachytene" stage, so easily identified by the characteristic appearance of the heteropycnotic autosome, was the time of their principal development.

CONCLUSIONS

The results of the present experiments may be conveniently considered in relation to three questions:

1. *What is the relation of chiasmata to crossing over?* The occurrence of bivalents in which one dyad has terminal isolabeling, with its sister dyad showing the reciprocal pattern, provides direct evidence of breakage and exchange of seg-

ments of nonsister chromatids. These exchange events represent normal meiotic recombination and are not induced by endogenous radiation from incorporated tritium. This conclusion is based upon the facts that (1) one-half of bivalents with a single chiasma have the isolabel pattern; (2) the frequency of terminal isolabeling parallels chiasma frequency in its temperature response with regard to both the time and magnitude of the effect; (3) the isotope does not induce any increase in chiasma frequency. Thus the exchange event bears an intimate relation to the chiasma and it seems obligatory to conclude that each chiasma is formed as a consequence of a process of recombination in which equivalent segments of two nonsister but homologous, chromatids are exchanged. The occurrence of one X chromosome with terminal isolabeling (Table 7) does not affect this conclusion. Since the X chromosome does not have a homologue and does not participate in recombination, the isolabeling must be comparable to that reported for mitotic chromosomes (PEACOCK 1963). A correction of the autosome data for this possibility does not affect the distinction between models 1 and 2.

2. *When does crossing over occur?* If primary spermatocytes in the cytological stage of "early pachytene" are subjected to high temperature shock, then the process of recombination is affected such that the probability of exchange between homologues is greatly reduced. This is evidenced by a reduction in chiasma frequency with a concomitant appearance of univalents, and by a reduction in the frequency of isolabeled dyads. The determination of the period between DNA synthesis in the premeiotic interphase and "early pachytene," viz., 4 days at 37°C, seems to be free from any error caused by the presence of isotope; no radiation-induced delay in the cell cycle was detectable. Thus at least the terminal step or steps of recombination in Goniaea occur during meiotic prophase at a stage well removed from the time of chromosomal duplication.

This is not in accord with GRELL and CHANDLEY's (1965) conclusion that, in Drosophila, recombination occurs at or about the time of DNA replication. The Goniaea results demand that recombination does not finally take place until "early pachytene" but do not exclude the possibility of other earlier component processes. It may be that in Drosophila temperature sensitivity is restricted to a process or processes occurring at a stage not coincident with the actual time of exchange. On the other hand it is conceivable that the 24-hour brood technique in Drosophila does not permit sufficient resolution between premeiotic interphase and pachytene, and even if the temperature-sensitive process occurred in prophase this would not be separable from the autoradiographic determination of time of DNA synthesis.

3. *What is the mechanism of recombination?* TAYLOR's (1965) autoradiographic analysis in Romalea demonstrated for the first time, in a higher organism, that at least some recombination involves actual breakage and reunion of existing chromosome segments. He noted a close relation between exchange and chiasmata frequencies. The present experiments have confirmed and extended this suggested relationship. The label pattern frequencies in Goniaea indicate that all meiotic recombination is the consequence of reciprocal exchange between two nonsister, homologous chromatids. However, we cannot exclude the possible co-

existence of other forms of recombination, reciprocal or noreciprocal, which involve only cytologically short regions of the chromosome and hence would not be detectable by autoradiography.

Recombination by breakage and reunion of chromosomes is thus common to higher organisms and bacteriophage (see MESELSON 1967). The similarity may extend to the molecular mechanisms involved. In phage some DNA synthesis has been identified in recombinant molecules (MESELSON 1964). Prophase DNA synthesis of importance in recombination has been identified in Chlamydomonas (DAVIES and LAWRENCE 1967), and in Lilium the DNA synthesis occurring at zygotene may be associated with crossing over (HOTTA, ITO and STERN 1966). In Goniaea the exchange event occurs long after interphase replication of chromosomal DNA, and incorporation of labeled thymidine has not been detected, autoradiographically, at the time of recombination, but it may well be that a small amount of replication or "repair" is involved in the reunion process.

Sister strand exchange has featured in some suggestions as to the mechanism of crossing over (LINDEGREN and LINDEGREN 1937; SCHWARTZ 1954). In Goniaea sister chromatid exchanges occur in comparable frequencies in bivalents (Table 6) and univalents (Table 7) and thus seem not to be associated with chiasma formation. That these exchanges are not involved in meiotic recombination is further implied by their occurrence in the X chromosome, where they are as frequent, per unit chromosome length, as in the autosomes. Sister chromatid exchanges may all be induced by the incorporated tritium. WOLFF's (1964) analysis of mitotic data favors this view and experimental support has recently been given by BREWEN and PEACOCK (1968) in studies with a ring chromosome. Thus tritium may be inducing sister chromatid exchanges to the extent of 0.5 exchanges/chromosome/division, but apparently does not contribute significantly to chiasma-like events (nonsister homologous chromatid exchanges). The difference may be due largely to spatial and temporal factors. Irrespective of whether sister chromatid exchanges are spontaneous or induced, it is not possible in the present experiment to conclude whether or not the exchanges observed occurred only in the premeiotic mitosis, or only in the meiotic division, or in both of these divisions.

The finding that the appearance of the synaptonemal complex is coincident with the time of recombination, at least in respect to identifiable meiotic stages, adds further weight to the suggestion that the complex is in some way involved in the recombination process (MOSES 1958; MEYER 1964). However, the temperature-induced cessation of recombination was not accompanied by any visible change in the complex, either in structure or frequency. These experiments, then, offer no direct information as to the role the complex may have in the mechanism of recombination, but since pachytene pairing also seems unaffected by the temperature shock, it is tempting to favor the view that the complex is involved in the achievement of synapsis which may well be a prerequisite to crossing over.

I would like to thank Dr. D. J. GOODCHILD for carrying out the electron microscopy reported in this article. I am most grateful to Miss K. L. FAULKNER who assisted in every phase of this work.

SUMMARY

Combined cytological and autoradiographic analyses of meiosis in spermatocytes of a grasshopper, *Goniaea australasiae*, have yielded information on the time of occurrence and mechanism of crossing over. Crossing over is achieved by breakage and exchange of segments of nonsister, homologous chromatids, and each such exchange event results in the formation of a cytologically visible chiasma. This conclusion, which amounts to a restatement of the chiasmatype theory of crossing over, is largely based on the identity of effects of temperature shock on chiasma frequency and the frequency of certain label patterns generated by crossing over. The meiotic stage at which this form of recombination takes place has been identified as "early pachytene" which precedes metaphase I by 10 days (in 37°C culture). This stage is well removed from premeiotic chromosome duplication which occurs 14 days prior to metaphase I. "Early pachytene" also marks the appearance of the synaptonemal complex in electron micrographs of the meiotic cells. Sister chromatid exchanges were frequent, but it is suggested that they do not have a direct role in the mechanism of crossing over.

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