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The Genetic Analysis of Meiosis in Female Drosophila melanogaster [and Discussion]

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The genetic analysis of meiosis in female *Drosophila melanogaster*

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The three major features of meiosis are first synapsis, then exchange, and finally, disjunction of homologous chromosomes; these phenomena occur before pachytene, during pachytene, and after pachytene respectively. The effects of meiotic mutants, or other perturbations, either endogenous or exogenous, on the meiotic process may be assigned tentatively to one of these intervals, based on the earliest discernible abnormality. Thus mutants exhibiting abnormal disjunction and normal exchange affect post-pachytene functions; mutants exhibiting abnormal disjunction and exchange but with ultrastructurally normal appearing synaptonemal complex affect pachytene functions; and mutants with abnormal disjunction, exchange, and synaptonemal complex affect prepachytene functions. This rationale is applied to the temporal seriation of effects of meiotic mutants and chromosomal abnormalities on the meiotic programme.

The genetic constitution is designed for meiosis in two different ways. In the first place, the sequence of meiotic events depends upon the normal functioning of an array of structural genes, controlled by some number of regulatory genes, that together insure that the proteins required for meiosis are present in the proper cells, sequence, and quantities. These genes control steps in a complicated net of reactions, which it is the ultimate task of investigators of meiosis to unravel. At another level, the chromosomes must be organized in such a way that their behaviour is affected by these gene products; that is, there must be receptor sites distributed along the chromosome upon which gene-mediated control is exerted. For example, particular sequences may be involved in chromosome condensation, initiation of synapsis, recombination, and segregation. Parts of this system are doubtless common to both meiotic and mitotic cell divisions, whereas others are uniquely meiotic.

We know that the genetic control of meiosis in *Drosophila* involves largely independent constellations of genes in males and females because the majority of meiotic mutants in *Drosophila* are sex specific in their effects; only a small minority affect functions common to males and females and thus exhibit meiotic abnormalities in both sexes. This discussion will be limited to a consideration of meiosis in females for two reasons: first, genetic exchange, which is a central feature of meiosis in most species, is confined to females in *Drosophila melanogaster* and second, most thoroughly investigated meiotic mutants are female specific. The genetic control of meiosis in *Drosophila* has been discussed by Sandler, Lindsley, Nicoletti & Trippa (1968), Lindsley, Sandler, Nicoletti & Trippa (1968), Baker & Carpenter (1972), Sandler & Lindsley (1974), Baker & Hall (1976).

The preceding paper in this volume (Stern & Hotta 1977) contains an account of the sequence of metabolic events characterizing meiotic prophase in lilies in which the interaction of gene products and specific DNA sequences is beautifully demonstrated – a trenchant example of the power of a suitable experimental system in providing insights into a biological

phenomenon. Just as lily is well suited to a molecular investigation of meiosis, *Drosophila melanogaster* is the organism *par excellence* for the genetic investigation of this process. This is so largely because this organism has been the object of intense genetic investigation during the past sixty-five years. These efforts have yielded a vast array of mutant genes that allow us to monitor chromosomes region by region, as well as a large collection of abnormal or rearranged chromosome constitutions which make it possible to determine how the meiotic system responds to abnormal chromosomal situations. Also, techniques are available for screening the entire genetic complement for mutations in genes whose products are required for the orderly progression of meiosis, and the tools for characterizing such mutants are many and varied; thus (1) exchange can be monitored on all chromosomes in females and, if indicated, in males as well; (2) non-disjunction can be monitored for all chromosomes in both sexes, and it can be determined whether non-disjunction occurs at the first or second meiotic division; (3) the effects of mutants on diverse meiotic phenomena such as chromosome loss, meiotic drive, non-random disjunction, preferential segregation, non-homologous disjunction, the interchromosomal effect on recombination, first and second anaphase bridge behaviour, ring chromosome stability, univalent behaviour, etc., can be monitored; and (4) the mitotic effects of meiotic mutants can be investigated by observing chromosome stability either in the early cleavage divisions or in gonial or imaginal disk cells and by measuring mitotic recombination.

In this account we consider the behaviour of such meiotic mutants. We assume that the molecular features of meiotic organization demonstrated in the lily obtain in *Drosophila* as well. Inasmuch as the lily genome has three orders of magnitude more DNA than *Drosophila*, there may be dangers in such a course; nevertheless, the many features of meiosis that are conserved over a wide range of organisms suggest that in general such an assumption is likely to be valid. Thus this presentation is divided into considerations of prepachytene, pachytene, and post-pachytene stages.

For the purposes of this discussion, prepachytene functions are defined by the normal alignment of homologous chromosomes and synaptonemal complex formation; meiotic mutants which interfere with normal synaptonemal complex formation are defined as affecting genes with prepachytene functions. Prepachytene mutations, then, result in abnormal synaptonemal complex, and may be accompanied by reduced recombination and increased non-disjunction. In practice, difficulty in recognizing abnormal synaptonemal complex may interfere with the identification of prepachytene mutations. Pachytene mutations are defined as those in which the synatonemal complex is normal but recombination is reduced and non-disjunction is increased. Post-pachytene mutations exhibit normal synaptonemal complex and normal recombination, but abnormal disjunction.

A word of caution is perhaps in order about assigning times of action of genes on the basis of the earliest detectable meiotic abnormality. Whereas it is legitimate to say that a gene product is required at least as early as the earliest observable effect of a mutant allele of that gene, it does not follow that the product is required no earlier than that. For example, when a mutant exhibits normal recombination and abnormal disjunction then we infer that it acts between the times of genetic exchange and disjunction of homologous chromosomes. This is a heuristic inference, but it is not without ambiguity, for it is possible that the mutant defect occurs before, but is without effect on, genetic exchange.

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Prepachytene

It seems reasonable to assume that the normal sequence of zygotene and pachytene functions is a prerequisite of normal genetic exchange. However, when the distribution of recombinants among the progeny of a cross is abnormal, indirect and sometimes questionable inferences are required to implicate any specific abnormality in either synapsis or exchange. The most promising development for obviating this difficulty is the cytological analysis of synapsed homologues by means of reconstruction of the spatial disposition of the synaptonemal complex in the pachytene nucleus employing electron-microscopic analysis of serial sections, as pioneered by Wettstein & Sotelo (1967) and subsequently exploited by Moens (1969), Gillies (1973), Rasmussen (1974) and Carpenter (1975*a*).

Reconstruction of the entire germarium of an ovariole from a wild-type *Drosophila* ovary (Carpenter 1975*a*) indicates that pachytene, as judged by the presence of fully formed synaptonemal complex, is achieved early in the germarial development of each cyst and persists for several days until after cysts leave the germarium and enter the vitellarium. Zygotene, as defined by the presence of nascent synaptonemal complex, on the other hand, appears to be a relatively abbreviated stage, lasting probably less than twelve hours. Three mutants, $c3G^{17}$ (Gowen & Gowen 1922), $c3G^{68}$ (Hall 1972), and *mei-W68* (Baker, unpublished), which eliminate genetic recombination also fail to form synaptonemal complex between homologous chromosomes [$c3G^{17}$ (Meyer 1964; Smith & King 1968), $c3G^{68}$ and *mei-W68* (Carpenter, unpublished)]; an intermediate allele, $mei\text{-}W68^{L1}$, which does not completely abolish exchange, has not yet been examined microscopically. *ord* (Mason, 1976) is a mutant at another locus with an early onset of action. It reduces recombination to but 10 % its normal level and according to unpublished observations by Carpenter, synaptonemal complex is incompletely and abnormally formed.

The correlated elimination of recombination and synaptonemal complex suggests a dependence of one upon the other. Peacock's (1970) demonstration in the Australian grasshopper, *Goniaea australasiae*, that chiasma formation can be eliminated by a temperature shock in early pachytene without affecting the synaptonemal complex suggests that it is meiotic recombination that depends on the formation of synaptonemal complex and not the converse. Furthermore, in lily we see that transient arrest of protein synthesis during late zygotene, when synaptonemal complex is nearly fully formed, can lead to partially or entirely achiasmatic cells, again suggesting that synaptonemal complex formation precedes chiasma generation.

The mutations, *c3G*, *mei-W68*, and *ord*, define the only three loci for which a reasonable case for prepachytene function can be made. It should be added that the fertility of females homozygous for these mutants shows that the normal functions of the loci are not required for the completion of meiosis. In this respect their effects parallel that of colchicine in lily.

In addition to effects of meiotic mutants, there are two general categories of effects of abnormal chromosome constitution on recombination that are likely attributable to defects in alignment of homologous chromosomes. One is a reduction in recombination between two normal homologues caused by the duplication for a region under study, and the other is the reduction in recombination between mutually rearranged chromosomes compared to isosequential homologues. A duplication for the proximal 20 % of the X chromosome causes a reduction in recombination between normal homologues in that region. E. H. Grell (1964) observed

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that the magnitude of the reduction depends on the position of the duplication in the genome. When the duplication is present as a free centric fragment or appended as a second arm to the centromere of one of the two Xs, recombination is reduced by 75 and 95 % respectively; when the duplication is appended terminally to either the *X* [*Dp(1;1)B^{S}TAG*] or *3L* [*Dp(1;3)B^{S3t}*], the observed reductions are 30 %. These results might be interpreted to indicate that association between the free duplication and the base of the X is facilitated by the aggregation of centromere regions into a chromocentre, whereas a distally disposed duplication is constrained from proximal association. However, contrary to expectations from this interpretation, the reciprocal constraint does not apply; a free centric duplication for the terminal 15 % of the X is capable of associating efficiently with the normal Xs as evidenced by a 67 % reduction in distal recombination (R. F. Grell 1967). It appears that duplications which are not tied to major heterologous regions are able to associate with homologous regions more readily than those that are.

The reduction in recombination caused by heterozygosity for inversions has been extensively investigated by Sturtevant & Beadle (1936) and Novitski & Braver (1954). Although much of the effect of heterozygous inversions is attributable to the selective recovery of non-recombinant strands (Sturtevant & Beadle 1936), there is also a sizeable reduction in genetic exchange (Novitski & Braver 1954). Serial reconstructions of oocyte nuclei from inversion heterozygotes have yet to be reported in *Drosophila*; however, Meyer (1964) reported a reduction in synaptonemal complex content in such oocytes.

Worthy of special mention are Roberts's (1972) observations on the extraordinary effectiveness of heterozygous reciprocal translocations in eliminating exchange in the translocated chromosome arm. Females heterozygous for translocations between either of the large metacentric autosomes and the minute fourth chromosome exhibit reduced recombination throughout the translocated arm of the metacentric. Maximum reduction (1–20 % of control depending on the autosomal arm) is achieved by translocations with breakpoints in the subterminal quarter of the arm. Translocations with more distal or more proximal breakpoints are less effective. Roberts postulates an important role of a region distal to the midpoint of the chromosome arm in the initiation of synapsis.

Furthermore, a translocation with a distal break in the left arm of chromosome *2* and a proximal break in *3R* is much more effective in reducing recombination in *2L* than is a translocation with the same distal break in *2L* and a fourth-chromosome break [*T(2;3)22C-D;86E* heterozygotes exhibit 0.6 % control recombination between *dp* and *pr*, whereas *T(2;4)22A;101* heterozygotes exhibit 29 % the control value]. These observations also suggest that chromosome elements that are rearranged to major heterologous regions [i.e. *2L* in *T(2;3)22C-D;86E*] are less able to associate with their normal homologues than those that are not [i.e. *2L* in *T(2;4)22A;101*]. *T(2;3)22CD;86E* homozygotes on the other hand exhibit nearly normal levels of *dp-pr* recombination indicating that heterozygosity for the translocation, rather than the translocation *per se*, is responsible for the observed effect.

These structural effects probably do not relate directly to metabolic features of zygotene; rather they suggest topological constraints on the chromosome complement's capacity to respond to the biochemical signals controlling alignment of homologues. As pointed out by Stern & Hotta (1977, this volume), completion of synaptonemal complex is not required for the initiation of pachytene. Furthermore, once pachytene is initiated, continued formation of synaptonemal complex appears to be precluded. Thus a delay in the completion of

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synaptonemal complex until prevented by the onset of pachytene could account for the reduced recombination observed in certain rearrangement heterozygotes.

Pachytene

We now turn our attention to the pachytene stage which ordinarily begins with the completion of the synaptonemal complex. The germarium of the *Drosophila* ovariole presents a quasi-linear array of 6–12 16-cell cysts whose spatial order roughly reflects the temporal sequence of development with approximately 12 h separating adjacent cysts (King 1970). Each cyst comprises 2 pro-oocytes and 14 nurse cells until rather late in germarial development, when 1 pro-oocyte becomes the definitive oocyte and the other becomes a fifteenth nurse cell. Thus a single germarium contains a good deal of information about the progression of bivalents through pachytene. The two large autosomal bivalents and the smaller X-chromosome bivalent can be followed throughout their entire length in serially reconstructed nuclei (Carpenter 1975*a*). Two landmarks can be identified: the nucleolus organizer near the base of the X chromosome and a fuzzy accumulation of chromatin, termed the blob, which surrounds the complex near the middle of one of the autosomes. In addition, the complex in the middle of the autosomes and at one end of the X is thinner and more difficult to follow – because it is more tortuous – than that in other regions of the bivalent. The distribution of this attenuated region of the complex corresponds to that of the pericentric heterochromatin.

Serial reconstruction of entire germaria reveals that synaptonemal complex is usually fully formed by the third cyst and that cysts with nascent complex are rare (Carpenter 1975*a*, but see Rasmussen 1974). When first formed, bivalents, as measured by synaptonemal complex length, are relatively long. As pachytene proceeds, the complexes shorten and thicken until the length is approximately half that measured initially. Maximal contraction is attained by the seventh cyst, after which there is a partial relaxation as evidenced by a lengthening of the complex.

Of special interest are a low number of electron-dense spherical structures about 100 nm in diameter, first observed in several species of fungi, that are transiently associated with the synaptonemal complex in pachytene nuclei (Gillies 1972; Zickler 1973; Radu, Steinlauf & Koltin 1974; Carpenter 1975*b*; Byers & Goetch 1975). On the basis of serial reconstructions of *Drosophila* pachytene nuclei in which these structures were enumerated and their positions relative to the bivalents determined, Carpenter (1975*b*) postulated that they are related to genetic exchange and termed them *recombination nodules*. In a sample of 13 pachytene nuclei, the maximum number of recombination nodules (5) corresponds well to the mean number of genetic exchanges per nucleus (5.6) as determined from the total length of the Drosophila genetic map of 2.8 Morgan units. Furthermore, the distribution of these structures among arms is not Poisson; it is underdispersed. In two nuclei with five nodules, and of course five bivalent arms, apiece, the mean number of nodules per arm is one, and the expected number of arms with 0, 1, and 2 nodules is roughly 4:4:2 based on Poisson; the observed numbers were 1:8:1. The ratio of arms with 0, 1 and 2 genetic exchanges as estimated by the method of Weinstein (1936) from single strand data is 1.4:7.3:1.3. Finally, within arms, the recombination nodules are confined to euchromatic regions. They tend to be centrally located in single-nodule bivalent arms and at opposite ends of two-nodule bivalent arms (i.e. they exhibit chiasma interference characteristic of genetic exchange). This distribution of nodules corresponds to the distribution of exchanges as determined by Charles (1938).

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More recently, recombination nodules have been scored in reconstructed nuclei in yeast (Byers & Goetch 1975). Once again the nodules have a frequency and distribution consistent with estimates of recombination. Particularly striking is the fact that this correspondence obtains even though yeast has over 200 times as many exchanges per length of DNA as does *Drosophila*.

These correspondences between the number and distribution of nodules and exchanges within the meiotic complement provide strong circumstantial evidence that the two are causally related. Thus recombination nodules provide a powerful tool for the cytological investigation of genetic exchange during pachytene.

Another approach to the investigation of the organization of the chromosome for pachytene function (i.e. for genetic exchange) is to examine the distribution of exchanges throughout the complement. It has been known since the early investigations of recombination in *Drosophila* that the distribution of exchanges is not a uniform function of chromosome length; some regions have more exchanges per unit of cytological length than others. The most striking example of this inequality is the virtual absence of meiotic recombination in the pericentric heterochromatin. Thus, the X-chromosome pericentric heterochromatin, which comprises between one-third and one-half of the total X-chromosome DNA, exhibits 0.04 % recombination in comparison with 65 % for the remainder of the chromosome (Roberts 1965). This reduction in recombination may be related to the atypical structure of the synaptonemal complex in that region (Carpenter & Baker 1974; Carpenter 1975*a*) and both may ultimately be explicable in terms of the sequential monotony of the satellite-rich pericentric heterochromatin (Brutlag & Peacock 1975; Endow, Polan & Gall 1975), if, as Stern & Hotta's (this volume) observations on the DNA synthesized during zygotene and pachytene suggest, specific sequences are important for the initiation of synapsis and genetic exchange.

Non-uniformity of exchange is also evident in euchromatic regions. To estimate the 'coefficient of exchange' (exchange per unit of DNA), it is necessary to estimate both the frequency of exchange and the amount of DNA between loci. Standard map positions (Lindsley & Grell 1968) are used in estimating levels of exchange between loci in spite of the known approximate nature of this map. The loci used are those whose cytological positions are known to within a few bands; the quantity of DNA between loci is estimated by enumerating the intervening bands in polytene chromosomes. Although the DNA content of bands is known to vary over a 10-fold range (Beerman 1972), it is assumed that the mean quantity of DNA per band is nearly constant when reasonably long chromosome segments are considered. That such estimates are reasonable can be seen in figure 1 for the X chromosome, where the coefficients of exchange are computed using both the number of bands and Rudkin's (1965) spectrophotometric estimates of the DNA content of the different regions.

The distribution of coefficients of exchange over the chromosome complement is presented in figure 1; these coefficients differ by a factor of ten in different parts of the complement. Both autosomes exhibit reduced recombination per unit of euchromatic length in the regions around the centromeres with recombination increasing as the regions monitored approach the ends of the arms. The X chromosome is different; the coefficient of exchange is lowest at the terminus, increases abruptly to a maximum 10–20 % of the way toward the centromere, levels off over the middle of the arm (see also Lefevre 1971), and finally decreases modestly over the proximal 30 %. Each point on the curves in figure 1 provides a measure of the average amount of exchange per band over the region represented by the horizontal line through the point. Values

within subdivisions of the regions will fluctuate considerably about this mean because sampling error in the estimate of the quantity of DNA from the number of bands increases as the number of bands sampled decreases; this is illustrated by the departures of some of the points based on short regions from the general contour of the curve, especially in the vicinity of the white locus on the X chromosome where a run of DNA-rich bands creates a segment of a few bands with relatively very high recombination. The error in the estimated quantity of DNA associated with short regions is compounded by the inaccuracies in the estimates of genetic length in such regions.

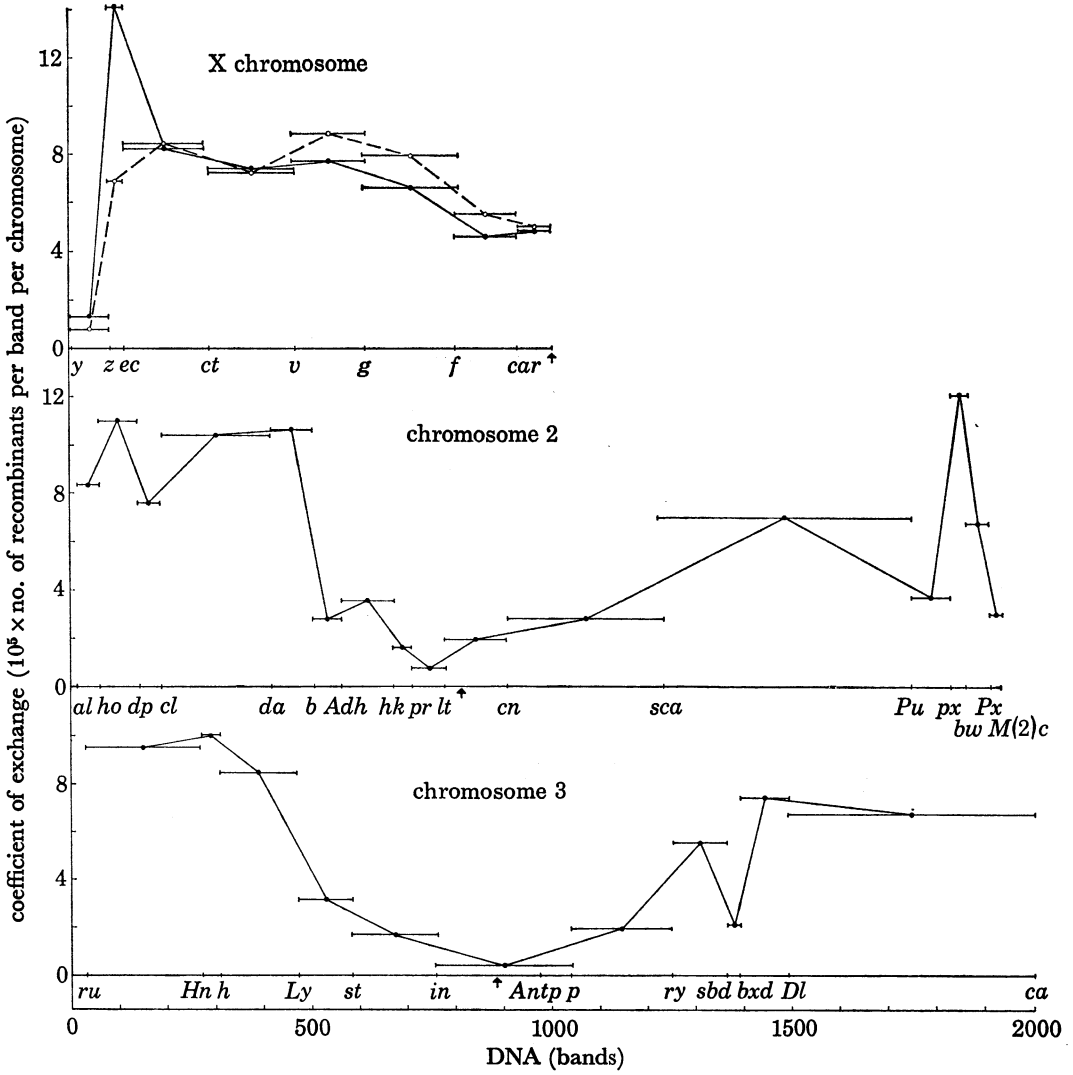

FIGURE 1. The distribution of the coefficients of exchange within the chromosome complement of *Drosophila melanogaster*. The abscissae represent the amount of DNA estimated by the number of polytene bands for the solid curves and for the broken line in tenths of a percent of the total DNA content of the euchromatic portion of the X chromosome, as measured by Rudkin (1965). As the pericentric heterochromatin is virtually unbanded and of zero genetic length it is not represented. Also indicated along the abscissae are the relative positions of the loci used in estimating the coefficients of exchange and of the centromeres (arrows). The ordinates represent the coefficient of exchange which is the map distance in Morgan units between two markers divided by the number of bands (——) or tenths of a percent of euchromatic DNA (– – –) separating them. Points represent the mean coefficient of exchange over chromosomal segments indicated by the horizontal lines.

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The striking non-uniformity of exchange per unit length of DNA illustrated in figure 1 reflects a linear differentiation in the degree to which recombination is inhibited by chromosomal organization. In order to ask whether the coefficient of exchange is an autonomous feature of a region, and thus possibly attributable to its sequential organization (e.g. to the distribution of sequences repaired during pachytene), it is possible to compare coefficients of exchange of regions in their normal positions with those when the same regions are in abnormal positions. Figure 2 plots data collected by Beadle (1932) in which he compared the amount of recombination in the right arm of the third chromosome in normal females with that in females homozygous for a translocation between the third and the fourth chromosomes in which the third chromosome is broken in the proximal fourth of *3R*. It can be seen that the translocation reduces the coefficient of exchange in *3R* distal to the breakpoint; Beadle attributed this effect to the juxtaposition of the fourth chromosome centromere to the region monitored for recombination; shifting the centromere 280 bands to the right results in a shift of the exchange coefficient curve 280 bands to the right. The conclusion from this type of observation is that a higher level of control of genetic exchange exists than is attributable to local DNA sequence organization.

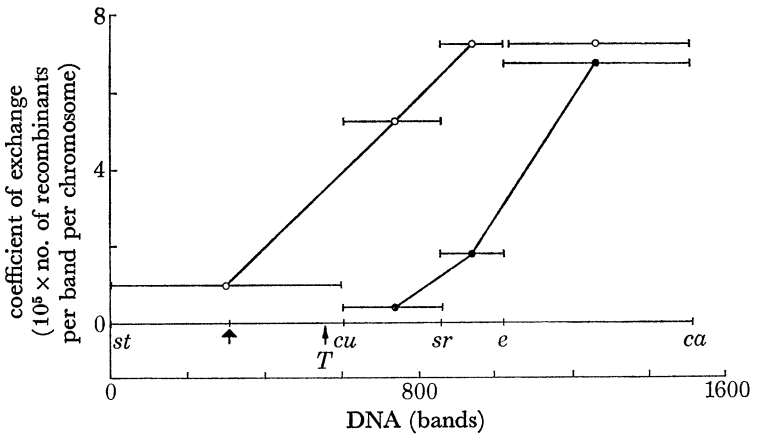

FIGURE 2. Coefficients of exchange over a portion of the third chromosome including the base of *3L* and all of *3R* in normal females (○) and in females homozygous for *T(3;4)86C* whose third-chromosome breakpoint is indicated by the arrow marked *T*. From data of Beadle (1932). Other conventions described under figure 1.

Insights into the nature of this control must await further investigation. The synaptonemal complex reveals a gradual change from the ill-defined and attenuated morphology of the proximal heterochromatin to the more robust structure characteristic of euchromatic regions. It may be that the region of transition includes proximal euchromatin, and the synaptonemal complex plays a role in preferentially reducing pericentric exchange.

The few meiotic mutations examined to date that result in a reduction in recombination do not have any obvious effect on the structure of the synaptonemal complex. Therefore, according to the convention established here, these are defined as mutations of genes normally functioning during pachytene. It is of interest to examine the effects of such mutations on the coefficients of exchange over a chromosome arm. For this purpose we choose a sample of EMS-induced, sex-linked, recombination-defective mutants recovered and analysed by Baker & Carpenter (1972). The effects on recombination in the left arm of chromosome *2* of five mutants at four loci are compared in figure 3. With the exception of *mei-9^b*, all mutants show a more

nearly uniform distribution of recombinational events per unit of chromosomal DNA than wild type, and all but *mei-352* show an overall reduction in recombination. Because the distribution of recombination in the mutants is more nearly uniform, it has been suggested that the mutations result in a relaxation of spatial constraints on genetic exchange (Baker & Carpenter 1972). A further characteristic of these mutations is that they decrease the amount of interference compared to normal to a degree that is proportional to the severity of the reduction in exchange (Carpenter & Sandler 1974). Thus, it is possible by a single point mutation to relax the control of meiosis which restricts the positions of exchanges with respect to the linear differentiation of the chromosome and with respect to each other, and at the same time to reduce the level of exchange. This observation implies that a mechanism that functions in the production of genetic exchange is at the same time acting to restrict exchange levels in some parts of chromosomes more than others (Baker & Carpenter 1972) and to promote interference between adjacent exchanges.

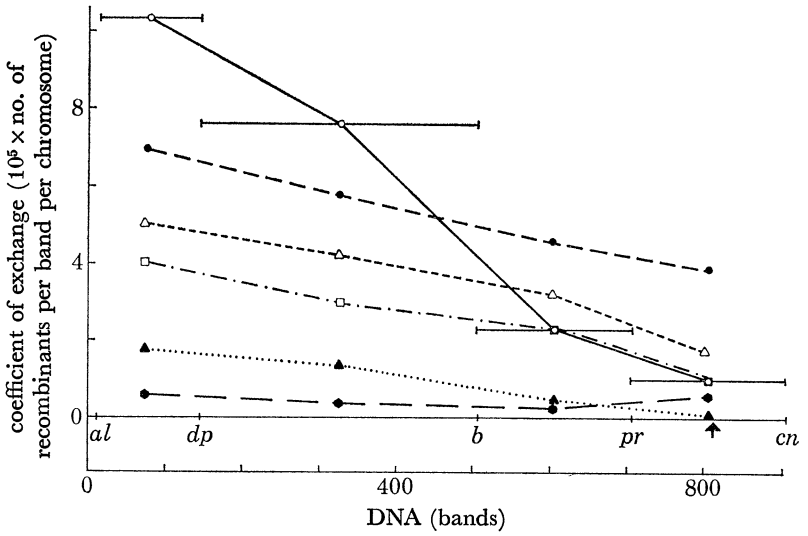

FIGURE 3. Coefficients of exchange in the left arm of the second chromosome of normal females (○) compared with those of females homozygous for various sex-linked meiotic mutants. ●, *mei-352*; △, *mei-41*^{195}; □, *mei-41*; ▲, *mei-9*^{b}; ⬢, *mei-218*. Data of Baker & Carpenter (1972). Other conventions the same as in figure 1.

mei-9, unlike the other mutants plotted in figure 3, causes a proportional reduction in exchange throughout the chromosome and no decrease in the level of interference; it appears to interfere with a function that promotes exchange but is independent of the mechanism restricting the distribution of exchanges. The coordinate response of the spatial constraints on genetic exchange and chiasma interference in all mutants so far examined suggests that these two phenomena may be causally related; indeed, they may be different manifestations of the same phenomenon such that both are either altered or unaffected.

It may be recalled that *mei-W68* is a mutant which is classified as affecting a prepachytene function on the grounds that it eliminates synaptonemal complex and genetic exchange; a less severe allele, *mei-W68*^{L1}, permits a subnormal level of recombination. The pattern of reduction resembles that of the pachytene mutants described above in that the coefficient of exchange is more nearly uniformly distributed than normal; there are insufficient double recombinants for an estimate of interference. The similarity in phenotype between mutations postulated to

interfere with prepachytene and pachytene functions may simply mean that the classification is fallacious; on the other hand, it may indicate that while each normal allele functions at a different time in meiosis, all are in a common pathway leading to genetic exchange.

Preliminary reconstructions of pachytene nuclei from females homozygous for *mei-41* and *mei-218* (Carpenter, unpublished) indicate that both of these mutants result in an inaccurate assembly and abnormal distribution of recombination nodules. A fraction of the nodules are abnormal in either size or shape, and in both mutants one or more nodules has been found associated with synaptonemal complex of the type identified as heterochromatic, where no control nodule has been observed. These observations suggest that the recombination nodule may be a sensitive indicator of, and perhaps a sensitive discriminator among, recombination-defective meiotic mutants.

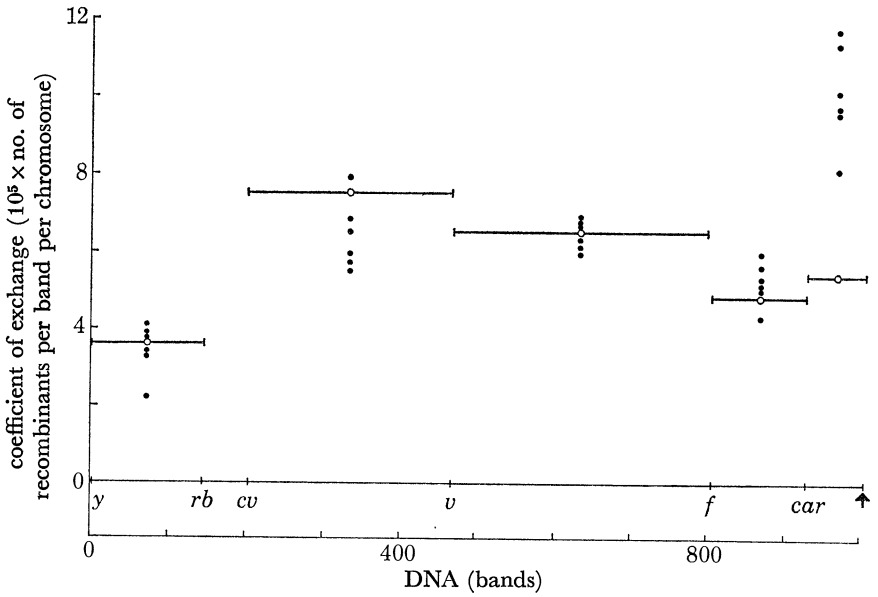

FIGURE 4. Coefficients of exchange within the X chromosome of normal females (○) compared with those from two-day broods of females irradiated with 4000 R of X rays (●). The relative positions of solid circles are unrelated to brood number. Data taken from Roberts (1969). Other conventions the same as in figure 1.

A final attribute of recombination-defective mutants is that several have been shown to be also defective in the repair of u.v. damaged DNA (Boyd *et al.* 1976). *mei-9* was shown to be ineffective in removing pyrimidine dimers, and others appear to be defective in postreplication repair processes, either caffein-insensitive, which characterizes recombination independent repair, or caffein-sensitive, which characterizes recombination dependent repair (Boyd & Setlow 1976). These observations imply that the same gene functions that in meiosis govern recombination, in mitosis are required for the repair of DNA damage.

Stern & Hotta provide evidence that the formation of single strand nicks is programmed in pachytene of lily microsporocytes in response to a meiotic endonuclease acting specifically at certain moderately repeated DNA sequences which are distributed at relatively regular intervals along the chromosomal DNA molecules. Radiation-induced nicks, on the other hand, occur randomly in the chromosomal DNA. Roberts (1969) provides evidence that *Drosophila* oocytes are unable to convert X-ray induced nicks into genetic exchanges. He irradiated adult females with 4000 R of X-rays and scored recombination in six successive two-day broods;

the first two comprise cells that were treated in post-pachytene stages; the next two include cells treated in pachytene; and the last two were most likely irradiated as oogonia. His results are summarized in figure 4.

Experiments utilizing exogenous perturbations of meiosis in *Drosophila* are fraught with difficulty because the lag between the time that a cell is treated and the time that it is sampled at oviposition must be used to infer the meiotic stage treated. The precision with which the lag period is usually measured in adult females and the meiotic time table determined is only moderate.

Roberts's results, however, are unambiguous since the only increase in recombination as a consequence of irradiation is found in the right-most region of the chromosome, which includes 73 euchromatic bands and the pericentric heterochromatin in its entirety. Roberts argues on three grounds that this increase is probably not due to exchange, but is rather the result of induced translocations between homologous heterochromatic regions: (1) no appreciable increase is seen in any of the four wholly euchromatic regions; (2) the increase is observed in all broods and not just those derived from cells irradiated in meiotic prophase; and (3) the increase is observed in females homozygous for *c3G*, a mutant in which meiotic recombination is obliterated. These observations are consistent with the view that meiotic exchange is confined to endogenously produced nicks whose production is part of the meiotic programme. By way of contrast, mitotic crossing over is not so controlled. It is a common observation that X irradiation causes striking increases in mitotic exchange (see, for example, Garcia-Bellido 1972); here, however, the events are distributed in proportion to chromosome length (Garcia-Bellido 1972).

One of the puzzles that has perplexed *Drosophila* geneticists for many years is the increase in recombination in one chromosome effected by heterozygosity for chromosome aberrations, specifically for inversions, in other chromosomes in the complement (the interchromosomal effect; see Lucchesi & Suzuki 1968, for review). The effects of heterozygous inversions in both major autosomes on the coefficients of exchange for the X chromosome are presented in figure 5. All regions measured show an increase, but the increase is especially great in the proximal-most region, which includes the pericentric heterochromatin. Roberts (1965) showed that this increase is confined to the proximal euchromatin and that the interchromosomal effect is inoperative in the centric X heterochromatin. In general, abnormal pairing of rearranged homologues is invoked in models seeking to explain the interchromosomal effect. One class of models invokes non-homologous pairing, either as a normal constraint to exchange that is removed by a rearranged pairing configuration (Oksala 1958) or occurring as a consequence of the rearrangement in such a way as (1) to relax a normal constraint on exchange (i.e. the centromere effect (Thompson 1964)), (2) to promote breakage and reunion of non-rearranged homologues (Schultz & Redfield 1951), or (3) to eliminate, as inviable exceptions, no-exchange tetrads (Cooper, Zimmering & Krivshenko 1955). All but the last model require non-homologous associations at the time of exchange for which there is no evidence. The model of Cooper, Zimmering & Krivshenko involves non-homologous associations at the time of disjunction, and their data demonstrate the existence of such associations; the magnitude of this effect is not sufficient to account for observed interchromosomal effects (Redfield 1957). The second major class of models does not involve the physical interaction of non-homologous chromosomes; rather pairing difficulties of rearranged homologues are considered to release the other chromosomes from either molecular (Mather 1936) or temporal (Lucchesi & Suzuki 1968)

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constraints that limit exchange. Restating Mather's argument in current terms, it seems reasonable to suppose that rearrangement heterozygotes are often prevented from completing synaptonemal complex by the punctual onset of pachytene which, according to Stern & Hotta (this volume), takes place irrespective of the state of the complex. As the exchange mechanism is unaltered, a normal number of exchanges (e.g. recombination nodules) may then be concentrated into a smaller fraction of the genome. This model, however, predicts an inverse proportionality between the level of exchange between rearranged homologues and the magnitude of the interchromosomal effect. The data do not support such a relation. Lucchesi & Suzuki,

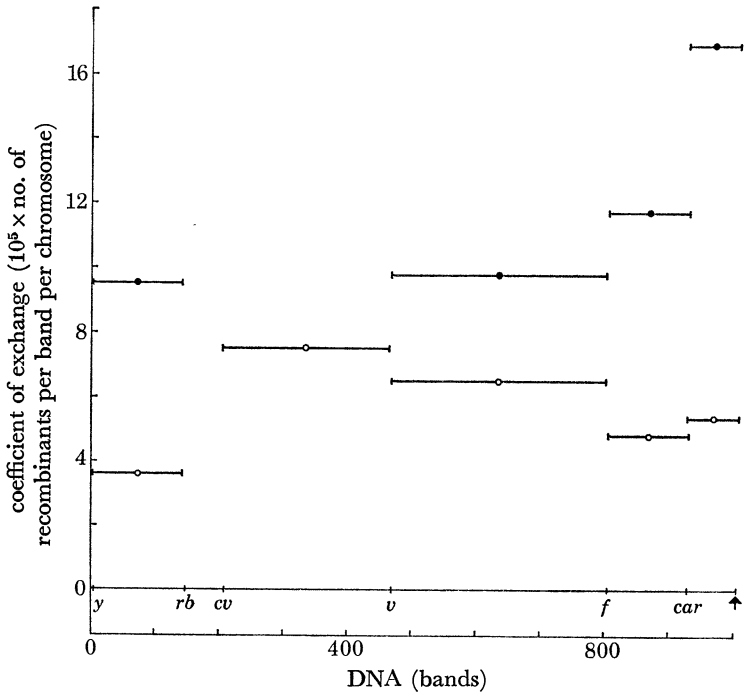

FIGURE 5. Coefficients of exchange within the X-chromosome of normal females (○) compared with those from females heterozygous for multiply inverted second and third chromosomes (*In*(2*L*+2*R*)*Cy*/+; *In*(3*LR*)*CxD*/+ (●). Data from Roberts (1969). Other conventions same as in figure 1.

alternatively, suggest that the oocyte contains a mechanism for monitoring the extent of pairing and that meiosis does not proceed until a critical level of pairing is achieved. They imagine that rearranged homologues encounter pairing difficulties that prolong the time required by the cell to attain complete pairing and thus allow more time than normal for isosequential homologues to undergo exchange. This model, therefore, presumes that pairing and exchange take place concurrently. Stern & Hotta (1976), however, provide evidence that in the lily pairing is confined to the zygotene stage whereas exchange is confined to pachytene. The data of Carpenter (1975*b*) indicate that the same is true in *Drosophila* inasmuch as approximately 36 h separate the completion of synaptonemal complex formation from the appearance of the first recombination nodules. A second point with respect to this model is that the meiotic mutants *c3G* and *mei-W68*, which eliminate synapsis, do not delay the meiotic timetable; on the contrary, if *c3G* has any effect on meiotic timing, it is to shorten the interval spent in early prophase (Smith & King 1968). Although these results militate against the Lucchesi & Suzuki (1968) view, it might be argued that *c3G* is defective in the block in meiosis that ordinarily

guarantees a critical level of pairing and thus allows meiosis to proceed before completion of pairing.

The inability to discern any attribute of inversions that is correlated with their interchromosomal effects has led some investigators (e.g. Steinberg & Frazier 1944) to the *ad hoc* hypothesis that the interchromosomal effect results from position effects on genes near the inversion breakpoints. Since virtually all inversions are able to elicit an interchromosomal effect, this hypothesis demands a large number of genes affecting recombination; furthermore, it is not simply reconciled with the absence of an interchromosomal effect in many inversion homozygotes.

Thus in spite of considerable attention to the problem of the interchromosomal effect there is still no wholly satisfactory model to account for it.

As stated in the discussion of prepachytene phenomena, the presence of a duplicated segment can reduce the level of recombination between normal homologues over their region of homology with the duplication. This observation is explained by postulating the alignment of the duplication with one of the normal homologues, thus displacing the other normal homologue. The reduction in recombination between normal homologues, however, is not accompanied by compensating recombination involving the duplication (e.g. Lindsley & Sandler 1965). This suggests that although the duplicated segment is capable of association with a normal homologue, this association is generally not competent for exchange. The reasons for this incompetence are obscure. One feature of such duplications that is shared by chromosome *4*, which is also exchange incompetent, is that they are short; perhaps a minimal length of uninterrupted complex is a prerequisite for exchange. If this is the case, however, it must be argued that the minimum length depends on the genetic constitution, inasmuch as fourth-chromosome recombination, which is virtually zero in diploid females, reaches levels as high as 3 % in triploids (Sturtevant 1951). Furthermore, fourth-chromosome recombination may be stimulated to occur by the application of a 24 h pulse of 35 °C to oocytes (Grell 1971).

Post-pachytene

The unique feature of post-pachytene meiosis is the reductional separation of homologous centromeres. In many species genetic exchange between homologues is essential to orderly segregation at the first meiotic division. *Drosophila*, on the contrary, has evolved fail-safe mechanisms to assure the disjunction of no-exchange bivalents. One such mechanism functions in the male where there is no meiotic exchange. A different mechanism, distributive pairing (Grell 1962), is operative in females where no-exchange bivalents are uncommon (except for chromosome *4*, see below). Cooper (1945) demonstrated that no-exchange X chromosome bivalents segregate regularly; he observed very low levels of X chromosome non-disjunction in females whose X chromosomes were rearranged such that at least 75 % of the tetrads had no exchanges. The same mechanism also assures the regular segregation of the fourth chromosomes, which normally fail to undergo exchange (Grell 1964). This mechanism is adequate for assuring the disjunction of one no-exchange chromosome pair (in addition to chromosome *4*) per cell; however, as Grell (e.g. 1967) has demonstrated in so many different constitutions, when two or more no-exchange tetrads coexist in the same oocyte, non-homologous chromosomes may segregate regularly from each other.

Virtually all recombination-defective meiotic mutants are characterized by elevated frequencies of non-disjunction; in fact, that is the basis upon which most were selected. This

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increased non-disjunction is very likely an indirect effect of the increased incidence of no-exchange tetrads caused by the mutants. This is so because chromosomes that non-disjoin are non-recombinants, because the patterns of non-disjunction follow the rules of distributive disjunction, and because the frequencies of non-disjunction are proportional to the incidence of no-exchange tetrads (Carpenter & Sandler 1974; Baker & Hall 1976).

Recombination-defective mutants also cause increased non-disjunction of the fourth chromosome, even though the distributive system ordinarily assures disjunction of the fourth chromosomes (Grell 1964; Carpenter 1973). This is a problem because distributive associations preferentially involve chromosomes of the same size, especially in the size range of chromosome *4* (see, for example, Grell 1967), so that even in cells with no-exchange tetrads, the fourth chromosomes are expected to associate distributively and thus exhibit regular segregation. How is it, then, that recombination-defective mutants greatly increase the incidence of fourth-chromosome exceptions? It has been argued (Baker & Carpenter 1972; Hall 1972; Parry 1973; Carpenter & Sandler 1974) that the fourth chromosomes participate in non-homologous distributive associations with chromosomes many times their size in cells in which there are two or three other no-exchange bivalents, even though regular patterns of distributive disjunction of the fourth chromosomes from other chromosomes are not discerned. This argument is based on three observations made in females homozygous for recombination-defective mutants: (1) fourth-chromosome non-disjunction tends to occur together in the same oocyte with X-chromosome non-disjunction (Sandler *et al.* 1968); (2) fourth-chromosome exceptions occur preferentially in ova which carry non-recombinant X chromosomes (Sandler *et al.* 1968); and (3) the incidence of fourth-chromosome exceptions is non-linearly related to the frequency of no-exchange tetrad arms as predicted if more than one no-exchange chromosome pair were required for fourth-chromosome non-disjunction (Baker & Hall 1976).

The foregoing provides ample indication of the importance of normal prepachytene and pachytene events to the regular disjunction of homologous chromosomes. We now turn our attention to post-pachytene functions as indicated by three different meiotic mutations.

The occurrence of distributive associations depends on the normal allele of a sex-linked meiotic mutant symbolized *nod* for 'no distributive disjunction'. An analysis of the progeny of females homozygous for *nod* (Carpenter 1973) indicates that genetic exchange is normal and the disjunction of homologues that have undergone exchange is normal under most conditions; the disjunction of no-exchange bivalents, however, is no longer assured. This is most strikingly seen for the fourth chromosomes, which appear to segregate irregularly in all meioses; furthermore, other genotypes which exhibit characteristic patterns of distributive disjunction no longer do so in the presence of *nod*.

The finding of a mutant that preferentially interferes with the disjunction of elements that have not undergone exchange provides powerful confirmation of Grell's (1962) hypothesis that two separable phenomena are involved in the disjunction of exchange and non-exchange chromosomes in Drosophila females. Studies of disjunction in XXY females homozygous for *nod* suggest, in addition, that distributive disjunction comprises at least two separable steps: (1) orientation with respect to the poles of the primary oocyte, and (2) disjunction. *nod* apparently does not interfere with the association of the Xs and the Y which assures that the two Xs will pass together to the same pole. Rather than always disjoining from the Xs, however, the Y chromosome appears to pass to one pole or the other independently of the Xs in *nod*/*nod*/Y females.

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A second meiotic mutant that is judged to affect a post-pachytene function on the grounds that it leads to irregular disjunction in association with normal levels of meiotic recombination is claret-nondisjunctional, *ca*nd. This mutant, which is homologous to *ca* described by Sturtevant (1929) in *Drosophila simulans*, was investigated by G. Davis (1969). The effects of this mutant are complex, and a completely satisfactory rationalization of the diverse observations is yet to emerge; thus the function of the normal allele cannot be inferred. Females homozygous for the mutant are characterized by increased rates of non-disjunction of exchange and non-exchange bivalents alike in the first meiotic division, as well as by chromosome loss in the first and presumably the second meiotic divisions and the first couple of post-meiotic mitoses in the zygotes produced by these females. There are more progeny that are exceptional for two different homologous pairs than expected were behaviour of the bivalents independent. In addition, double exceptions for X and *4*, for example, are much more likely to carry both Xs and both fourth chromosomes or none of them than two Xs or two *4*s. It seems that this mutant affects a function required for the separation and movement of centromeres of participants in all manner of first meiotic associations whether or not they are conjoined by chiasmata. Whether the defect involves the chromosomes themselves or some extrachromosomal aspect of the mitotic apparatus must remain for future investigations to reveal.

The final post-pachytene mutant that we wish to discuss results in the precocious separation of sister centromeres, beginning at anaphase I and continuing into the second meiotic division (Sandler *et al.* 1968; B. Davis 1971). This mutant, *mei-S332*, results in 30–40 % exceptions for every pair of homologues; different homologous pairs disjoin independently; the non-disjunction involves preponderantly sister centromeres at the second meiotic division. *mei-S332*, which has the latest effect in meiosis of any mutant so far investigated, and *ord* (Mason 1976), which is one of the three pre-pachytene mutants described earlier, are the only two mutations that also affect meiosis in males; two out of three pre-pachytene mutants, all pachytene mutants, and two of three post-pachytene mutants are female specific. Thus, the majority of meiotic prophase in female *Drosophila* appears to be under the control of a series of loci which are active in oocytes but not in spermatocytes.

We wish to express our appreciation for the thorough reading of the manuscript and the many constructive suggestions offered by Dr Adelaide T. C. Carpenter, Dr Bruce S. Baker and Dr Herbert Stern. We also thank Dr Carpenter for generously allowing us to cite her unpublished observations on mutant synaptonemal complexes.

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Discussion

S. J. Counce (*Duke University, Durham, North Carolina, U.S.A.*). Dr Montrose J. Moses at Duke University has observed structures which appear to be the equivalent of Carpenter's recombination nodules in physical association with synaptonemal complexes in spread preparations of mammalian pachytene chromosomes (Chinese hamster, golden hamster, laboratory mouse). Because the synaptonemal complexes of the entire chromosomal complement are often preserved in these spread mammalian cells, it should be possible to determine the number and location of these nodules per chromosome and per cell without resorting to serial section reconstructions. I have found similar structures in spread *Locusta* pachytene cells, but because the synaptonemal complex is rarely intact throughout its length in any chromosome, this identification is tentative. In any case, the recombination nodules seem to be structures, that, like the synaptonemal complex, withstand the rigours of cell disruption and surface spreading.

Ann C. Chandley (*M.R.C. Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh EH4 4XU, Scotland*). Have recombination nodules been found in heterochromatin of X-irradiated males or females of *Drosophila*, since it is in these regions of the chromosomes that induced crossing over mainly occurs ?

D. L. Lindsley. No irradiated flies have been examined.

K. R. Lewis (*School of Botany, University of Oxford*). I should like to put a joint question to Professor Stern and Dr Lindsley. Is there any indication of nodules or reassociation protein in mitotic cycles ? I have in mind a possible involvement in sister chromatid exchange or the chiasma-like aberrations which are so prevalent in Bloom's disease.

D. L. Lindsley. Only oocytes have been examined to date and recombination nodules are found only in association with synaptonemal complex which is confined to the pachytene stage of the primary oocyte.

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