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The Effect of Colchicine on Meiosis in *Lilium speciosum* cv. "Rosemede"

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Abstract. Chromosome pairing and chiasma frequency were studied in meiocytes at diakinesis of *Lilium speciosum* cv. "Rosemede" fixed up to 21 days after the start of either continuous or 3 day pulse colchicine treatment. The two treatments gave similar results. In pulse treated pollen mother cells (PMCs) the mean chiasma frequency per cell fell from 26.4 in controls to 8.5 after fourteen days while the mean number of univalents per cell increased from 0.05 to 17.58. There was a negative correlation between mean chiasma frequency per bivalent and per PMC in colchicine treated buds; univalents were preferentially induced in bivalents with one chiasma, and preferentially excluded in bivalents with 4 chiasmata. Some chiasmata were redistributed to surviving bivalents despite the concurrent reduction in chiasma frequency per meiocyte. – Colchicine sensitivity began in premeiotic interphase and extended to mid or late zygotene in PMCs; ongoing synapsis was unaffected. However, susceptibility to univalency was asynchronous between bivalents occurring at zygotene in short chromosomes but at late premeiotic interphase in the longest chromosomes. The number of chiasmata per bivalent could be altered by colchicine without inducing univalents, but the ultimate effect was to reduce the number of chiasmata per bivalent (or per chromosome arm) directly to zero. The major factors determining the order and extent of reduced pairing and chiasma number were total chromosome length and arm length. Pairing and chiasma formation in embryo sac mother cells were less sensitive to colchicine than in PMCs, but their behavior was otherwise similar.

Introduction

Colchicine is known to suppress chiasma formation when applied to undoubled cells in several higher plant species (Toledo et al., 1979). However, attempts to identify the sensitive stage for chiasma formation have been made in only two groups of plants, namely, *Lilium* (Shepard et al., 1974) and the wheat-rye-

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Triticale complex (Dover and Riley, 1973; Thomas and Kaltsikes, 1977; Bowman and Rajhathy, 1977). Shepard et al. (1974) showed that microsporocytes of *Lilium speciosum* cv. "Lucy Wilson" exposed to colchicine during leptotene and early zygotene subsequently showed univalents and reduced chiasma frequencies at division I. By comparison, Dover and Riley (1973) showed that colchicine has a similar effect on chiasma formation in *Triticum aestivum* only if it is applied during the first stage of premeiotic interphase; colchicine applied during later stages of premeiotic interphase or at any stage of prophase I of meiosis had no effect on pairing scored at subsequent division I (Dover and Riley, 1973). Similar experiments using *Secale cereale* (Bowman and Rajhathy, 1977) also showed that early premeiotic interphase was the most sensitive phase of development for colchicine-induced univalency, but indicated that the colchicine-sensitive stage extended to late meiotic interphase and perhaps into leptotene, but not later. Using pentaploid *T. durum* × *Triticosecale* hybrids, Thomas and Kaltsikes (1977) showed that the colchicine-sensitive stage began in premeiotic interphase and ended "no earlier than the end of leptotene and no later than the middle of zygotene".

Comparing the respective results for *Lilium* and *Triticum* led Shepard et al. (1974) to conclude that the interval of colchicine-sensitivity is different in the two species. While this conclusion is probably correct for the end of the interval (during early-mid premeiosis in bread wheat, but during zygotene in *Lilium*), the question as to whether they differ with respect to the time of onset of colchicine sensitivity has remained open because of the inability of *Lilium* PMCs to proceed beyond prophase when exposed to colchicine before leptotene either in bud or microsporocyte culture (Shepard et al., 1974). The development of successful methods for sustaining meiotic development to division in *Lilium* buds treated with colchicine as early as premeiotic mitosis (Toledo et al., 1979) opened the way for a re-examination of the duration and nature of colchicine sensitivity in this material. The present work describes the results of such an experiment.

Materials and Methods

The present work used *Lilium speciosum* cv. "Rosemede" ($2n = 2\times = 24$) – a chiasmate horticultural variety.

During 1977 batches of about 200 bulbs were planted one per pot in a glasshouse during April-May and reached meiosis in June and July. Colchicine treatment was by the cut-shoot method (Toledo et al., 1979). For studies of male meiosis 85 cut shoots with buds 3–24 mm long at stages of anther development from premeiotic mitosis to tetrads were placed 5 or 6 per 1-litre beaker and given one or other of two treatments. The first treatment (pulse) involved 3 days exposure to 1×10^{-3} M colchicine solution followed by transfer to distilled water for up to another 18 days. The second treatment (continuous) involved 3 days exposure to 1×10^{-3} M colchicine followed by transfer to 1×10^{-4} M colchicine for up to another 18 days.

For studies of female meiosis, 24 shoots with buds 15–41 mm long, containing ovaries with meiocytes at stages from premeiotic interphase to tetrads were given a 3-day pulse colchicine treatment, as described above. The length of each treated bud was measured using calipers (Erickson, 1948) at the onset of colchicine treatment and at excision and fixing up to 18 days later. Bud length was measured at suitable intervals of 1–3 days, between 9 and 12 a.m., on control spikes either still attached to the bulb growing in a pot, or cut and stood in a beaker of distilled water for up to 25 days. There was no significant difference between the rates of bud elongation in

uncut shoots and in cut shoots administered distilled water alone for up to 21 days after excision at which time they reached the tetrad stage. However, the rate of increase in length of colchicine treated buds was significantly lower than in these controls; given stages of meiosis occurred in progressively shorter buds with increasing intervals of colchicine treatment. There was no significant difference in the rate of increase in bud length between buds given a pulse or a continuous colchicine treatment.

The stage of development of pollen mother cells (PMCs) or embryo sac mother cells (EMCs) in control and colchicine treated buds was determined from Feulgen-stained anther or ovule squashes fixed and prepared as previously described (Bennett and Stern, 1975).

The correlation between bud length and stage of development in PMCs and EMCs was less marked in Rosemede than in two other *Lilium* cultivars (Bennett and Stern, 1975). Correlation between bud length and meiotic stage decreased with increasing bud length. However the following correlation was observed between bud length and cytological stage: 9.5 mm or less, premeiotic mitosis; 9.5–11 (all buds) and 12–15 (some buds), synchronous premeiotic interphase; 12–16, leptotene; 14–17, zygotene; 18–26, pachytene; 23–26 diplotene and diakinesis; 23–28, first metaphase and subsequent meiotic stages. These data are for buds fixed between 6–10th June. As the summer progressed the length of control buds containing each stage of meiosis decreased markedly, the most advanced showing reductions as great as 7 mm. Apart from the experiment to monitor the uptake of ^{3}H colchicine conducted in August these large changes do not apply to the present work which used cut shoots first sampled between June 10–23rd.

The duration of meiosis and its stages was estimated as described previously (Bennett and Stern, 1975) and the results are in agreement with previous estimates (Shepard et al., 1974; Bennett and Stern, 1975). The proportion of PMCs containing meiosis first reached 50% when the bud length was about 13 mm, and the proportion of cells at tetrad stage first reached 50% in buds about 27 mm long. Comparing these data with bud length growth curves for control shoots grown together with colchicine treated cut-shoots in the glasshouse showed that the mean duration of meiosis in PMCs was 10 ± 1 days. First prophase took 9.5 ± 1 days, and the respective durations of its stages were: leptotene, 1.5 days; zygotene, 2 days; pachytene, 5 days; diplotene and diakinesis inclusive, 1 day.

Similar comparisons showed that it took 6 or 7 days for buds to increase in length from 9.5 mm (the end of premeiotic mitosis) to 13 mm (the start of meiosis) in control spikes. Thus, in controls it took 17 ± 1 days for development to proceed from the end of synchronous premeiotic mitosis to the end of meiosis, and not less than 16 ± 1 days to reach diakinesis. The leading edge of colchicine-doubled tetraploid PMCs reached diakinesis 17–18 days after the start of colchicine treatment which, after subtracting the drug penetration time (1 day), shows that colchicine treatment had little or no effect in slowing PMC development prior to first metaphase in the sampled buds.

Meiosis in EMCs was initiated in buds 21–24 mm in length, and completed in buds 38–51 mm in length. The proportion of EMCs at meiosis first reached 50% in buds 21 mm in length, and the proportion of ovules containing quartet stage first reached 50% in buds 45 mm in length. Comparing these lengths with bud length growth curves showed that the mean duration of female meiosis was 14 ± 1 days. The stages of meiosis after diakinesis lasted about 1 day, and the stages of first prophase were estimated to have the following approximate durations: leptotene, 2 days; zygotene, 2.5 days; pachytene, 7 days; diplotene and diakinesis inclusive, 1.5 days. Thus, in Rosemede the duration of meiosis was longer im EMCs than in PMCs as previously shown in other *Lilium* cultivars (Bennett and Stern, 1975).

All six anthers and/or at least 20 EMCs were examined in each sampled bud. The univalent and chiasma frequency for a particular sampling time was estimated in 20 PMCs from one anther from each of two buds except in pulse treated material fixed on day 16 where 20 PMCs from only one anther were scored. Because cells may become arrested at first metaphase, but not at diakinesis (Toledo et al., 1979), only PMCs at diakinesis with well spread chromosomes were scored. However, the paucity of EMCs (only a single EMC per ovule) precluded such selection. The number of chiasmata and/or univalents was scored in all suitable EMCs (between 1 and 24 cells) at diakinesis or first metaphase. To do so, 20–100 ovules were fixed at each sample time up to 18 days after the start of pulse colchicine treatment. In the interest of brevity, several extensive tables giving chiasma and univalent frequency per cell and per bivalent are omitted from the present work, such data being presented as Figures. However, mimeographed copies of the original data are available on request to the first author.

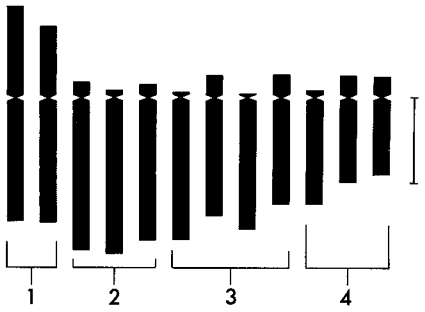

Fig. 1. The haploid complement of "Rosemede" divided into four arbitrary groups on the basis of total chromosome length. The bar corresponds to 5 μm

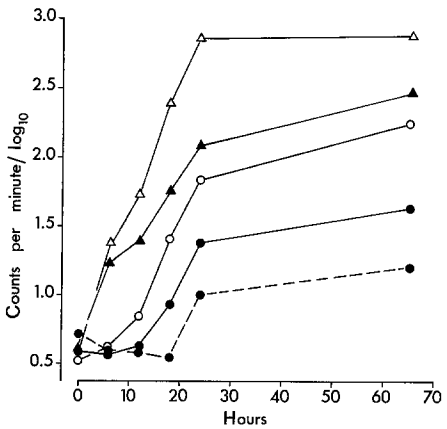

Fig. 2. Uptake of ^{3}H colchicine by *Lilium* PMCs in buds treated by the cut-shoot or needle method. PMCs from 2 buds were assayed at each of the times indicated. Measurements were also made for sepals, anther filaments, and ovaries as described in the text. The data on filaments are not plotted; their radioactivities were very close to those shown for the PMCs. – Key: △ Sepals from buds at MI-MII, ▲ sepals from buds at leptotene, ● extruded PMCs from buds at leptotene, ○ extruded PMCs from buds at MI-MII, —— cut shoot, ----- needle

Untreated meiocytes showed no evidence of incomplete pairing at pachytene, and univalents were rare (in PMCs) or absent (in EMCs) at diakinesis and first metaphase. The mean chiasma frequency was higher in EMCs (33.9) than in PMCs (26.4) as previously noted in *Lilium* by Fogwill (1958). The number of chiasmata per bivalent ranged from 1–4 in PMCs and from 1–5 in EMCs.

The karyotype of "Rosemede" is asymmetrical with two pairs of large metacentric chromosomes and ten smaller pairs of acrocentric chromosomes (Fig. 1). Careful study of diakinesis in control cells indicated that, while it was impossible to identify each bivalent, it was possible to distinguish four arbitrary groups of bivalents, on the basis of arm ratio and relative chromosome length. The length of each chromosome and each chromosome arm was measured in 10 Feulgen-stained diploid somatic anther cells at c-metaphase using a Vickers movingscale micrometer eyepiece. An idiogram of the complement (Fig. 1) made from these estimates shows the relative length of each chromosome and indicates the four groups described above.

Each of the groups was analyzed for chiasma frequency and univalency as reported under Results. Generally 40 PMCs were scored in each sampling. However, only 30 and 21 PMCs, respectively, were scored in anthers fixed 11 and 12 days after the start of pulse treatment because the increased number of univalents made it difficult to distinguish the 4 groups. Where fewer than 40 PMCs were scored, the results were adjusted by multiplying by the appropriate factor as if 40 cells had been scored.

Penetration of ^{3}H Colchicine. Colchicine (ring C, methoxy-^{3}H; specific activity 16.05 Ci/mmole) was dissolved in water with unlabelled colchicine to a final concentration of 10^{-3} M. Ten cut shoots with buds at G2-leptotene or at late meiosis were each stood in tubes containing 25 ml of colchicine solution (0.18 Ci/ml) and 20 buds at G2-leptotene were given 0.1 ml of solution (2.5 Ci/ml) by the needle method as described by Toledo et al. (1979). Groups of 2 buds were

sampled at the times shown in Figure 2. Radioactivities were determined in sepals, anther filaments, extruded microsporocytes (contaminated with tapetal nuclei), and 4 mm long segments from middle of ovaries cut into 1 mm thick slices. The tissues were suspended in "Aquasol" for counting. The two methods of application gave different results for the first 12 h, but levels of radioactivity were essentially the same in the target tissues by either method at 24 and 66 h. With exposures of 12 h or more C-metaphases were seen in buds, anthers, and ovules obtained from cut shoots. Disrupted or suppressed meiotic spindles were first seen after 24 h of exposure, the extent of the effect being greater in cut shoots than in buds treated by the needle method. Spindle suppression was total in all material fixed 66 h after the start of treatment. We estimate the threshold level of colchicine concentration to be reached about 24 h after start of treatment, assuming that spindle disruption and chiasma suppression have similar sensitivities.

Results

1. General Aspects of Colchicine Effect

The behaviour of colchicine treated PMCs and EMCs is illustrated in Figure 3 and summarized in Figure 4. In general colchicine had the same effects in Rosemede as were noted in Black Beauty (Toledo et al., 1979), in particular reducing the chiasma frequency in diploid meiocytes, and inducing tetraploid (4x) PMCs at premeiotic mitosis which often developed until at least first metaphase. The present work is mainly concerned with the first of these effects but it seems worthwhile noting some details of chromosome behaviour in 4x PMCs although they are not the subject of further discussion. For instance, 18 days after the start of pulse treatment 4x PMCs showed a higher bivalent frequency per diploid genome (range 5–9, mean 7.3) than diploid PMCs (range 0–8, mean 3.9). The mean chiasma frequency per diploid genome was higher in 4x cells (12.7) than in diploid cells (10.3), while the mean chiasma frequency per bivalent was higher in 2x cells (2.68) than in 4x cells (1.73). Chiasma distribution differed between 2x and 4x PMCs; a given number of chiasmata tended to be distributed between a few bivalents (with high numbers of chiasmata) in 2x cells (Fig. 4e) but between more bivalents with fewer chiasmata in 4x cells (Fig. 4f). Comparing 4x cells induced by the same pulse treatment showed a significantly lower mean chiasma frequency per diploid genome in Rosemede (12.7) than in "Black Beauty" (21.1 – Toledo et al., 1979), while the mean chiasma frequency per bivalent was only slightly lower in the former (1.73) than in the latter (1.94). The reason for this difference is uncertain, but may depend on the fact that "Rosemede" is a chiasmate cultivar of a single species while "Black Beauty" is an achiasmic interspecific hybrid cultivar.

The ultimate and expected effect of the colchicine in undoubled cells is to reduce chiasma frequency and to induce univalency. From a comparison of the curves for these characters in Figure 4 with the known durations of meiotic stages (see Materials and Methods) it is to be noted that the interval of drug effectiveness does not coincide with the process of synapsis. Drug action is cumulative over an interval of about 8 days. Within that interval, the earlier treatment begins, the severer is the effect. This relationship is much more pronounced in PMCs than in EMCs, but it is unambiguous in both. Although it is not surprising that colchicine has similar effects in PMCs and

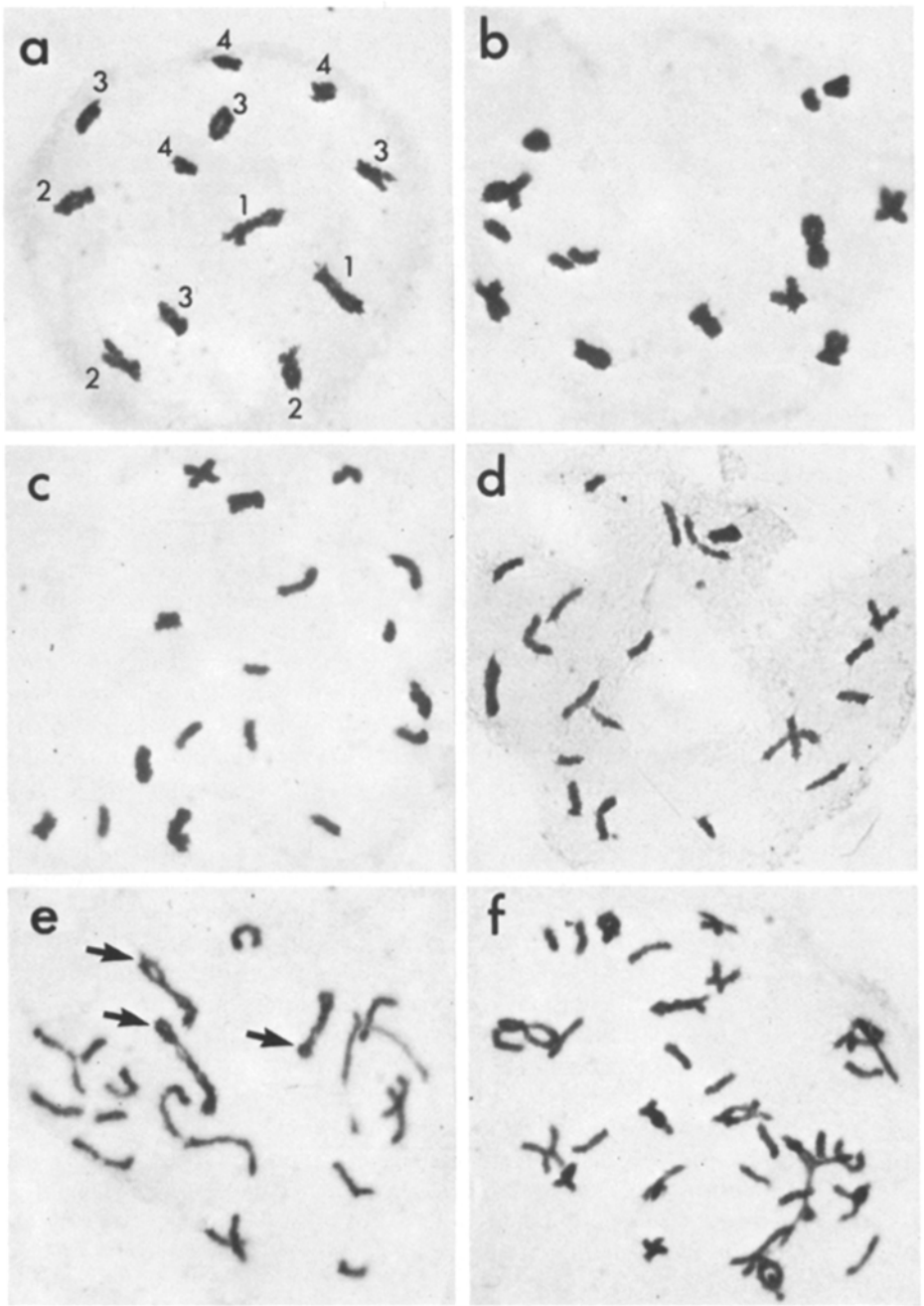

Fig. 3a–f. Diploid PMCs (**a–e**) and colchicine induced tetraploid (**f**) PMC at diakinesis from control (**a**) or 3 day pulse, colchicine treated shoots fixed 10 (**b** and **d**) or 18 (**c–f**) days after the start of treatment showing: **a** 12^{II} assigned to the 4 arbitrary groups, **b** $10^{II}+4^{I}$; **c** $7^{II}+10^{I}$; **d** $2^{II}+20^{I}$; **e** 3^{II} (arrowed) with typically high numbers of chiasmata; and **f** IIs and Is

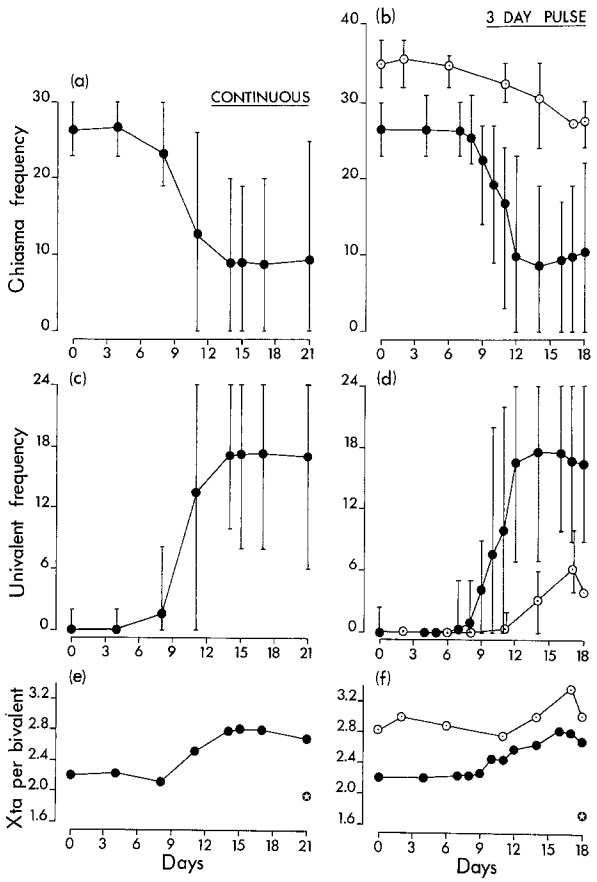

Fig. 4a–f. Mean chiasma frequency per cell (**a** and **b**), mean univalent frequency per cell (**c** and **d**), and mean chiasma frequency per bivalent (**e** and **f**) in diploid PMCs (●) and EMCs (⊙) of controls and of buds fixed up to 21 days after the start of continuous (**a**, **c** and **e**) or 3 day pulse (**b**, **d**, and **f**) colchicine treatment

EMCs, it should be noted that colchicine induced univalency and reduced chiasma frequency (Fig. 4b, d) have not been reported previously in female meiocytes. Hereafter our comments are addressed to the data on PMCs because they are more readily analysed.

No effect of colchicine was observed in buds treated 5 days or less before the meiocytes reached diakinesis; the first significant increase in univalency was seen in PMCs treated 7 days before reaching diakinesis. If one day is allowed for colchicine to reach a threshold concentration, the 6-day minimum implies that the drug can suppress chiasma formation if present during the first half or more of the synaptic process, but that it can no longer be effective after cells have reached mid to late zygotene. The latter results are in agreement with previous studies of *Lilium* (Shepard et al., 1974). The novel evidence is that colchicine begins to have its cumulative effect at about the midpoint of premeiotic interphase, approximately 14 days before the cells reach diakinesis.

The boundaries of the effective interval are difficult to define precisely both because of the spread of effects, as indicated by the vertical lines in the figures (e.g. Fig. 4a–d), and because of the long persistence of colchicine activity once absorbed by the cell. The persistence makes pulse exposures impossible because they are the equivalent of continuous ones. Barber (1942) demonstrated long ago that colchicine was active in *Fritillaria* buds 2 months after pulse application. In the present work, and in that of Toledo et al. (1979), persistence was of the order of 22 days. However, despite the imprecision in defining the interval of colchicine action on chiasma formation, the data clearly demonstrate that colchicine absorbed 4–5 days prior to pairing has a stronger effect on subsequent chiasma formation than if absorbed at a later time. This effect is unlikely to be due to differences in colchicine intracellular concentration; pulse and continuous treatments give the same result. The mechanism by which colchicine produces its effects on chiasma formation is beyond the reach of these experiments. Nevertheless, it is apparent that colchicine can interfere with the process both well before and during chromosome pairing, but not after pairing has occurred.

2. Chromosome Length and Univalency

The classification of *Lilium* chromosomes into 4 identifiable sizegroups (Fig. 1) made it possible to examine colchicine action in relation to chromosome size. If the cumulative effect of colchicine reflects a progressive modification of chromosomes or chromosome-associated components, longer chromosomes might be less susceptible to colchicine action than shorter ones. It is evident from Figure 5 that the two groups of shorter chromosomes become univalents more rapidly than the chromosome complement as a whole, and that the two groups of longer chromosomes do so more slowly. Chromosome size is thus a significant factor in the time required for colchicine to suppress chiasma formation.

The data in Figure 5 may, however, be explained by a random action of colchicine on factors involved in chiasma formation. On sustained exposure to colchicine, bivalents of longer chromosomes, which have higher chiasma frequencies, would be more likely to retain at least one chiasma, and thus bivalency. The possibility of random chiasma loss was examined by comparing the decline in chiasma frequencies among the various bivalents with a set of theoretical curves derived from an analysis of 40 untreated PMCs. Bivalents in such cells may be divided into 4 categories, each category having either 1, 2, 3, or 4 chiasmata. Curves were constructed to trace the expected numbers of bivalents in each of the categories as the mean chiasma frequency per meiocyte dropped from 26.4 (the mean for untreated cells) to 0. For one set of curves, the probability of chiasma loss was considered to be independent of the number of chiasmata in the bivalent. For the other set, the probability was considered to be proportional to chiasma number. The curves so obtained are drawn as dashed and dotted lines for each of the 4 categories of bivalents in Figure 6. These should be compared with the solid lines which trace the actual changes in bivalent number as mean chiasma frequency per cell declined.

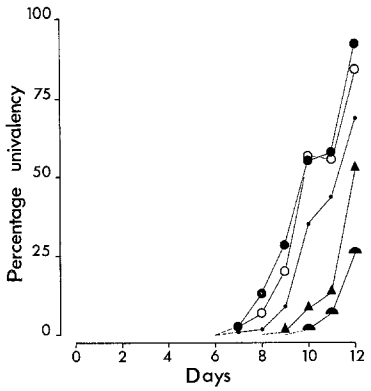

Fig. 5. The mean percentage univalency per PMC (·) and in chromosomes of group 1 (◓); 2 (▲); 3 (○); and 4 (●) in PMCs fixed up to 12 days after the start of 3 day pulse colchicine treatment. (N.B. Symbols are not displayed for values of zero, and the first occurrence of univalents is joined by a dotted line to the preceding sample time when no univalents were seen)

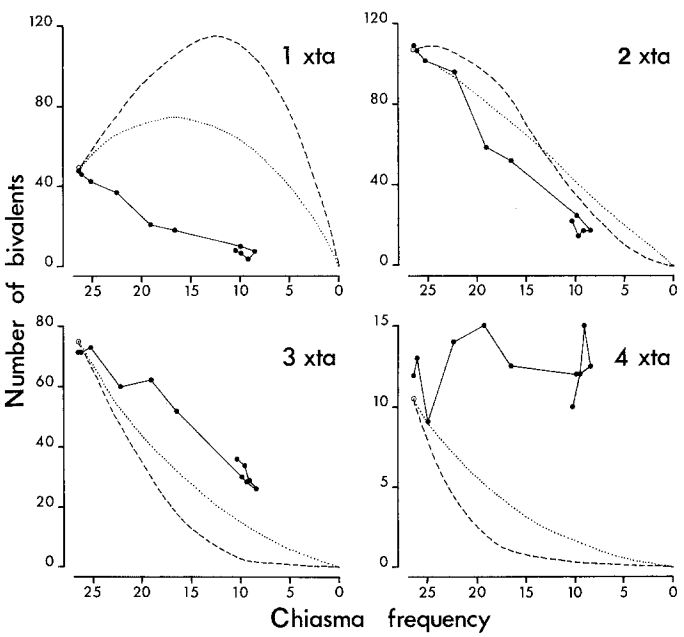

Fig. 6. The total numbers of bivalents with 1, 2, 3, or 4 chiasmata observed in samples of 20 PMCs plotted against their estimated mean chiasma frequency per cell (●——●), and curves for the expected numbers in 20 PMCs as the mean chiasma frequency per cell tends to zero assuming either that (.....) all bivalents have equal probabilities of losing a chiasma, or (-----) that the probability of losing a chiasma is directly proportional to the number of chiasmata per bivalent

It is at once apparent that none of the 4 categories of bivalents loses chiasmata randomly. As the mean frequency of chiasmata per cell falls, the number of bivalents with one chiasma declines much more rapidly than predicted, and the number with two chiasmata slightly more rapidly than expected. By contrast, bivalents with 3 or 4 chiasmata have higher numbers than those predicted by random chiasma loss. Indeed, in the case of bivalents with 4 chiasmata, the number remains unchanged over a broad range of mean cellular chiasma fre-

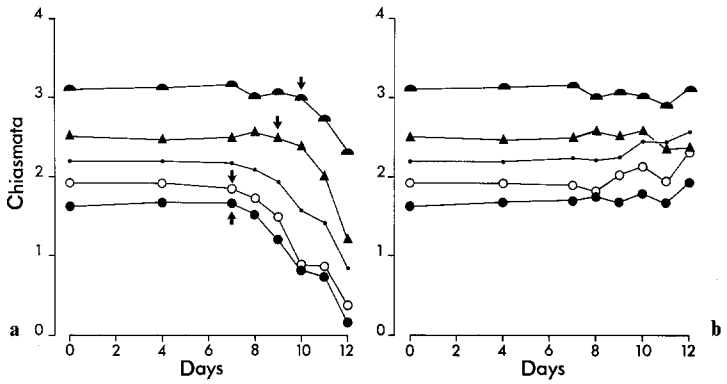

Fig. 7a and b. The mean chiasma frequency (**a**) per two chromosomes and (**b**) per bivalent in PMCs fixed up to 12 days after the start of 3 day pulse colchicine treatment for chomosomes of group 1 (▲); 2 (▲); 3 (○); 4 (●); and, per cell (·). (N.B. The time of first appearance of univalents in chromosomes of each class is indicated in **a** by arrows)

quencies. In effect, the progressive decline in mean chiasma frequency and bivalent number per cell is accompanied by a net increase in chiasma frequency per bivalent. This relationship was further analysed because the curves drawn in Figure 6 do not distinguish between a selective retention of bivalents with higher chiasma frequencies and an increased chiasma-forming capacity among residual bivalents.

To distinguish between the 2 possibilities, the changes in mean chiasma frequency of each of the 4 chromosome size-groups were measured against time of exposure to colchicine. The results are shown in Figure 7. A selective retention of the larger bivalents, which also have the higher mean chiasma frequency, is evident. The greater the mean chromosome length of the group, the longer the time of exposure to colchicine before a decrease in mean chisma frequency per group is observed, and the smaller the decrease in that frequency over a fixed period of time (Fig. 7a). If the chiasma frequencies per remaining bivalent in each group are plotted instead of the frequencies per group, the results obtained are shown in Figure 7b. The approximate constancy in mean chiasma frequency per remaining bivalent is striking. Except for the slight but significant increase in mean chiasma frequency for the 2 groups of smaller bivalents, it would appear that residual bivalents have the mean number of chiasmata chracteristic of their group. The conclusion may tentatively be drawn that the non-random loss in chiasmata is the combined result of selective retention of larger bivalents and an increased chiasma-forming capacity among residual bivalents.

Compensatory formation of chiasmata under conditions in which certain chromosomes or chromosomal segments have been excluded from chiasma formation has been noted by a number of investigators (Mather, 1936; Parker, 1975). Thus, the present above-mentioned increase noted among the smaller bivalents is in line with the published observations. This relationship, however, also clearly indicates that colchicine does not affect the chiasma forming mecha-

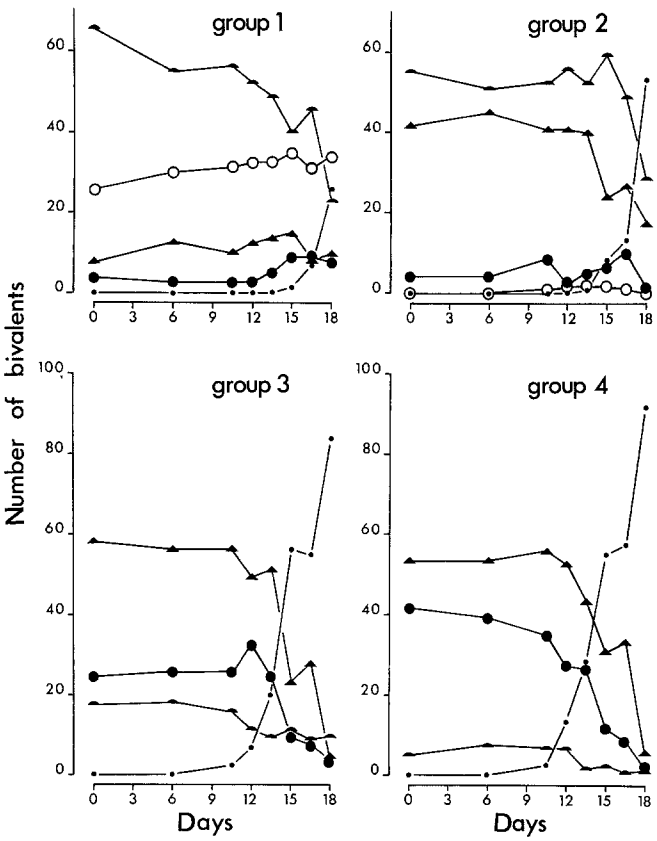

Fig. 8. The percentage of bivalents with: 0[a] (·); 1 (●); 2 (▲); 3 (◓); or 4 (○) chiasmata in chromosomes of group 1, 2, 3 or 4 of samples of 40 PMCs fixed up to 18 days after the start of 3 day pulse colchicine treatment.

[a] (N.B. A pair of univalents is regarded as a bivalent with no chiasma in this comparison)

nism, but rather a condition permitting chiasma formation, probably effective pairing. A very simple interpretation of the results contained in Figures 5 and 7 is that colchicine affects that condition in an all-or-none manner. The relationship would be analagous to that observed when isochromosomes are exposed to colchicine and are unaffected in their chiasma frequency even though chiasma formation may be greatly reduced in the remainder of the chromosome complement (Driscoll and Darvey, 1970). The analogy would provide a valid explanation of the results thus far obtained if it could be shown that individual pairs of homologs behave in all-or-none fashion.

Additional analysis of the data indicate, however, that the all-or-none effect is an oversimplification. The constancy in mean chiasma frequency recorded for each of the chromosome groups does not extend to individual chromosomes within the groups. The percentage of bivalents with 1–4 chiasmata in each of the 4 size groups of chromosomes was measured during a 12-day exposure to colchicine. The numbers of bivalents within a particular frequency category

did not remain constant in any of the groups (Fig. 8). Among the largest bivalents (Fig. 8; Group 1), there was a marked loss of bivalents with 3 chiasmata during days 7–10 which was compensated by an increase in the numbers of bivalents with 2 and 4 chiasmata. The increase in number of bivalents with one chiasma must reflect a loss of chiasmata from other bivalents as there was negligible formation of univalents during that interval. Among the second largest group of bivalents (Fig. 8; Group 2), the number of bivalents with 3 chiasmata increased during days 7–10 whereas those with 2 chiasmata showed a considerable decrease. The contrasting behaviour of bivalents with 3 chiasmata in Groups 1 and 2 may be attributed to the independent responses of chromosome arms in a metacentric. Such metacentrics of group 1 usually have a single chiasma in one of their arms whereas the acrocentrics of group 2 often have all three chiasmata in the one arm. Such behaviour is consistent with previous evidence that bivalents with higher chiasma frequencies lose chiasmata at a lower rate than those with low chiasma frequencies (Fig. 6). Nevertheless, arm length rather than chiasma number may be the critical factor. Bivalents with one chiasma in Group 1 do not become univalents between days 7–10 (no univalents are formed in this group), but those in Group 4 fall markedly during that same interval.

The results shown in Figure 8 point to a complex of factors governing the ultimate number and distribution of chiasmata in meiocytes perturbed by colchicine treatment. The constancy in chiasma frequency of any particular group of bivalents that remain after colchicine exposure is not the product of a constancy in its individual members. The chromosomes behave as though there were a redistribution of chiasmata among remaining bivalents. There is, of course no process of redistribution that can be directly traced. Redistribution is inferred from the data provided in Figures 7 and 8. The data represent an outcome of the interactions between the cumulative effect of colchicine on what we presume to be effective pairing, the lengths of chromosome arms as they influence the magnitude of that effect, the undefined threshold condition required for bivalency to persist until chiasmata can form, and the chiasma forming machinery which appears to be capable of compensatory action. The extent to which these different considerations contribute to our understanding of the relationship between pairing and chiasma formation is best deferred to the discussion.

Discussion

The two major issues which arise from these studies are the mechanism of colchicine action, and the manner in which it affects the sequence of events leading to chiasma formation. Neither issue is novel, and both have been subject to inconclusive debate (Levan, 1939; Driscoll and Darvey, 1970; Dover and Riley, 1973; Hotta and Shepard, 1973; Shepard et al., 1974). The distinctive and well-established effect of colchicine on microtubular organization has led understandably to the conclusion that this same effect must carry over, though indirectly, to chromosome synapsis (Driscoll and Darvey, 1970). The extensive

and cytologically detailed studies of colchicine effects on wheat meiosis have led to the conclusion that such effects are products of colchicine action on premeiotic processes (Driscoll et al., 1967; Dover and Riley, 1973). The outcome of the results reported in this paper is to cast strong doubts on the model of spindle fibres as primary sites of colchicine action in relation to synapsis. It also expands the interval of known colchicine effectiveness in disrupting chiasma formation in *Lilium* so as to encompass both premeiotic and prophase stages. It should be emphasized in passing that while there are important differences between *Triticum* and *Lilim* with respect to the end of colchicine sensitivity [i.e., at zygotene in *Lilium*, but during premeiotic interphase in *Triticum* (Dover, 1973), they are now shown to be similar insofar as the onset of sensitivity is well before the start of meiosis in both.]

The cumulative pattern of colchicine action is difficult to account for in terms of a mechanism similar to that of spindle inactivation. The basis for such a mechanism might have been strong if the severity of colchicine effect could have been tied to a progressive increase in intracellular concentration. Although the data on ^{3}H colchicine uptake point to a small accumulation after 24 h, the significance of that accumulation is denied by the fact that continuous and pulse exposure to colchicine have virtually identical effects if the exposures are begun at the same developmental interval. It seems probable that the cumulative effect results from either a time-dependent progressive interaction of colchicine with a component ultimately essential to chiasma formation or to interference with accumulation of such a component. The evidence that colchicine can interfere with nucleoside transport into cells (Mizel and Wilson, 1972) adds plausibility to these alternatives. Intercellular transport between tapetum and microsporocytes and intracellular transport between cytoplasm and nucleus are both critical to meiotic development. The highly dampened effect of colchicine on megasporocyte chromosomes is best explained by the buffering action of the enveloping nucellular tissue and the much larger megasporocyte cytoplasm. In the case of wheat, evidence has been accumulated demonstrating an interference by colchicine with transport between tapetum and microsporocyte (Bennett, unpublished).

If the primary action of colchicine is on a general cellular process such as transport, the action is compatible with the relatively broad developmental interval over which it is effective. To be sure, the generalized effect of colchicine reduces its usefulness as a specific probe of meiotic mechanisms, but it nevertheless lends emphasis to the observations on the critical importance of presynaptic events to the synaptic process. Since we are lacking a molecular account of these events, it is impossible to identify specific mechanisms that link the presynaptic and synaptic intervals. However, certain inferences, that have a significant bearing on the relationship between synapsis and chiasma formation can be drawn from the responses of meiocytes to colchicine.

It is most unlikely that colchicine acts directly on chiasma formation. The small but unequivocal increase in chiasma frequency among short and large chromosomes that remain bivalent under colchicine exposure, the decrease in univalents on exposures to colchicine for 15–17 days (Fig. 4c, d), and the formation of chiasmata in colchicine-induced amphidiploids of cv. "Black Beauty"

(Toledo et al., 1979), all point to the conclusion that colchicine is indifferent to the immediate processes of chiasma formation. On the other hand, it seems most likely that colchicine acts on a precondition for chiasma formation. It is attractive to suppose that this precondition is effective chromosome pairing. The problem is to reconcile the effectiveness of colchicine during zygotene when synapsis is occurring with its effectiveness during premeiotic interphase when synapsis is not occurring. Since colchicine has no effect on the paired structure, it must act on some process essential to effect a stabilized pairing. There may be 2 stages leading to effective synapsis and it is conceivable that colchicine can disturb different processes essential to both.

Several models could be drawn to account for the fact that larger chromosomes are more resistant to the meiotic action of colchicine. Some of these have already been considered under Results. There is no point in repeating the nature of the models except to emphasize that univalent formation, a very critical event in meiosis, cannot be accounted for by a random process of chiasma loss. A distinction must be made between the capacity of the chiasma-forming mechanism, and the condition for chiasma formation. Colchicine affects the latter but not the former. One observed consequence of this selective action by colchicine is an inter- and intrachromosomal effect on chiasma frequency under conditions of increasing univalency. To the extent that univalents represent chromosomes that lacked synapsed, or effectively synapsed, regions at the time of chiasma formation, their increase may be viewed as representing a decrease in available sites for an undiminished chiasma-forming mechanism and thus a source of higher chiasma frequencies per residual bivalent. The idea of limited chiasma-forming capacity has been discussed by a number of investigators (Darlington, 1940; Tease and Jones, 1976).

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