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Random Arrangement of Chromosomes in *Uvularia* (*Liliaceae*)

By

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Abstract: Chromosome arrangement in interphase has been inferred from an analysis of the relative positions of the chromosomes and the chromosome arms in untreated haploid pollen grain metaphases of *Uvularia grandiflora*. The distances between centromeres forming the smallest possible circle were measured in 43 metaphases. The relative positions of the chromosomes did not differ significantly from randomness. Neither did similar-sized chromosome arms show any tendency to be next to each other. The results thus disagree both with the hypothesis of COMINGS (1968) that each chromosome occupies a definite position in the interphase nucleus and with the claim of BENNETT (1982) that similar-sized chromosome arms lie next to each other.

The problem of the position of chromosomes in an interphase nucleus has several aspects. Not only is interphase an important part of the mitotic cycle, but the question of chromosome position during it has possible implications for chromosome structure and behavior, for karyotype evolution, and for the interaction of genes.

Three main hypotheses which all assume a nonrandom arrangement of chromosomes in the mitotic interphase have been put forward. Time and again it has been claimed that homologous chromosomes are paired, or at least lie closer to each other than a random arrangement would presuppose (VOGEL & SCHROEDER 1974, AVIVI & FELDMAN 1980, COMINGS 1980). Furthermore, it has been assumed that each chromosome is attached at a specific point to the nuclear membrane (COMINGS 1968). Under another hypothesis recently proposed by BENNETT (1982; see also HESLOP-HARRISON & BENNETT 1983 a, b) nonhomologous, but similar-sized chromosome arms would have a tendency to lie next to each other.

The present observations are relevant to the last two hypotheses. The haploid pollen grain metaphases appear singularly well-suited for a study

of chromosome arrangement, since they have not been treated in any way prior to fixation nor dried-on or squashed to distort their original positions.

Material and Methods

The material consisted of haploid mitoses in pollen grains of the liliaceous species *Uvularia grandiflora*. The contents of the anthers were smeared on slides and fixed and stained with 1% orcein in 45% acetic acid. Other slides were fixed in 1 : 3 acetic acid : ethanol and stained with the Feulgen technique. The cells were not treated in any way before fixation.

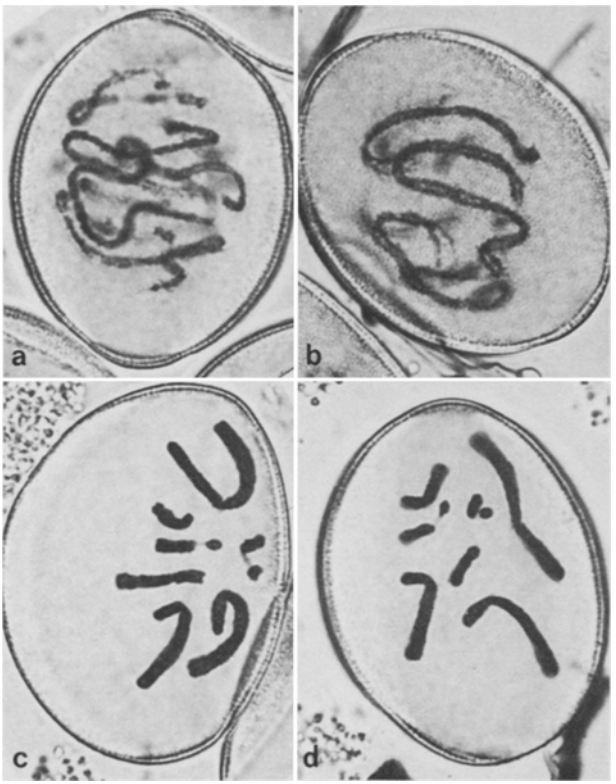
Forty-three consecutive metaphases displaying a polar view in which the centromeres were, as nearly as possible, in one plane were photographed and the distances between each centromere and all the other centromeres measured in the prints ($\times 3\,000$). From the measurements the smallest circle connecting the centromeres was determined, as described below.

Results

Even the interphase nuclei in *Uvularia* pollen grains show a polarized structure with the nucleolus situated at one end. Furthermore, most prophases display a clear RABL-orientation (Fig. 1 *a, b*) which means that the chromosomes remain through interphase in the position which they occupied in anaphase and that they will re-emerge in prophase without having changed their relative positions. Furthermore, the recent laser UV microirradiation studies have born out the long-held idea that the metaphase positions of chromosomes reflect their interphase arrangement (CREMER & al. 1982). This enables the determination of the chromosome arrangement in interphase from their relative positions in metaphase.

In the pollen grain metaphase of *Uvularia,* the 7 chromosomes can clearly be identified, the two smallest containing the nucleolar organizers (Figs. 1 *c, d,* 2). No nonrandomness is discernible in the metaphase arrangement (Figs. 1 *c, d*); the acrocentric chromosomes 6 and 7 are usually not bent at the centromere, and the short arms point to the middle of the plane.

For each of the 43 cells, the 21 centromere-centromere distances were measured and the minimum Hamiltonian circuit (the closed path through all seven centromeres of minimum length) determined. From the 43 circuits, the number of times that chromosome i was adjacent to chromosome j was determined for all i and j. For example, in cell #1 the minimum circuit is (-1-5-6-4-3-2-7-); thus the neighbors of 5 are 1 and 6, those of 3 are 2 and 4, and so on. These counts are shown in Table 1. Table 1 is not, of course, a contingency table in the standard sense because of the lack of independence (the underlying sampling distribution is not multinomial). Nevertheless, we can still test the hypothesis of no



Figs. 1 *a–d.* Mitotic stages in the pollen grains of *Uvularia grandiflora. a–b* Prophase displaying a RABL orientation of chromosomes. *c–d* Two metaphases showing random arrangement of chromosomes. × 1 500

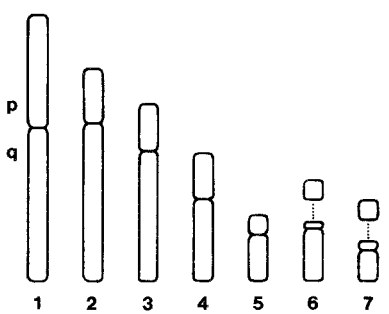


Fig. 2. Diagram of the haploid karyotype of *Uvularia grandiflora;* chromosomes 6 and 7 have satellites (p = short arm, q = long arm). × 2 000

Table 1. Neighbors: the numbers in the table are the numbers of times chromosome i was adjacent to chromosome j. Further explanation in the text

Chromosome						
2	14		$T^2 = 15.678$	P = 173/500		
3	13	21	P(MaxDev = |21–14.33|) = 249/500			
4	16	9	13			
5	10	20	14	16		
6	19	11	14	15	10	
7	14	11	11	17	16	17
	1	2	3	4	5	6
	Chromosome					

Table 2. Neighbors of 5 q (e.g. 1 p was adjacent to 5 q twice in 43 cells). Further explanation in the text

1p	1q	2q	2q	3p	3q	4p	4q	6p	6q	7p	7q	p	q
2	5	4	8	5	5	7	6	1	5	4	8	23	37
			$T^2 = 10$		P = 219/500							$[X^2 = 3.26]$	
												p > 0.06	

Table 3. Neighbors of 3 q (e.g. 1 p was adjacent to 3 q seven times in 43 cells). Further explanations in the text

1p	1q	2p	2q	4p	4q	5p	5q	6p	6q	7p	7q	p	q
7	3	8	10	2	10	1	7	3	7	1	4	22	41
			$T^2 = 22.9$			P = 3/500						$[X^2 = 5.73]$	
												p > 0.06	

association in position among the chromosomes using the test statistic $T^2 = (O_{ij}–E_{ij})^2/E_{ij}$ where O_{ij} is the observed frequency and E_{ij} the expected frequency (14.33) under the hypothesis. The value of T^2 in the observed table is 15.678. We wish to know the probability of obtaining a value of T^2 at least that large by chance (under the hypothesis of no association between chromosomes). To determine this probability, a permutation test was performed. For each of 500 sets of 43 randomly generated circuits, T^2 was calculated. In 173 cases T^2 was greater than 15.678; thus, the significance level is 0.35. There is no reason to reject the hypothesis of no association.

Looking at Table 1 more closely, we see that chromosomes 2 and 3 were neighbors 21 times (expected value, 14.33). Perhaps this deviation is significant although the overall table is not. This was also tested by a permutation test. Among 500 randomly generated tables 249 had at least one frequency as deviant as this one; i.e., a deviation this large is expected about half of the time by chance.

The arm neighbors of 5 q and 3 q are shown in Tables 2 and 3. There is

no evidence of an association in Table 2; $T^2 = 10$, Pr = 219/500. There does seem to be a tendency for 5 q to have a q neighbor rather than a p neighbor but the effect is not significant ($p > 0.06$). In Table 3, however, the effect is significant. $T^2 = 22.9$ and Pr = 3/500.. The high value of T^2 is the result of two aspects of the data: 1) the probably fortuitously higher than expected association of chromosomes 2 and 3, and 2) the apparent association of 3 q with other long arms ($p < 0.018$). This latter effect disappears if chromosomes 6 and 7 are ignored in the analysis. Both chromosome 6 and 7 are acrocentric with a tendency for their short arms to face the center of the equatorial plate. Consequently it is practically impossible for these arms to lie next to any other arm.

When a negative result is reported, the question of the statistical power of the test utilized becomes especially important. This is often a difficult matter, but we have at least estimated the power of our general test for randomness. First the 5% cutoff point under the null distribution was determined to be about $T^2_{0.05} = 23.5$. An alternate hypothesis was constructed in the following manner. A "preferred" sequence (-1-2-4-6-7-5-3-) was chosen. This is meant to represent the idea that chromosomes of similar length tend to be neighbors, but any sequence would do. Then each set of 43 sequences was randomly generated by perturbing the preferred sequence by interchanging one, two or three random pairs of centromere numbers. If only one interchange is used, 1/7 of the sequences are left unchanged and 6/7 differ from it by only one interchange. With two interchanges less than 6% are unchanged. With three interchanges the sequence is getting fairly scrambled with less than 2% the preferred sequence and many differing from it by two and three interchanges. The power calculations were as follows: one interchange: power is virtually one. With two interchanges, the power is about 99%. With three interchanges, the power drops to 46%. The test appears capable of detecting any reasonably strong tendency for the chromosomes to orient in a preferred sequence at metaphase. We find no evidence that they do.

Discussion

The RABL-orientation of the chromosomes is often directly visible in telophase-interphase which formed the basis for the classic studies of HEITZ (1933) on the localization of heterochromatin and nucleoli. In many types of nuclei it is also displayed in prophase (Figs. 1 *a, b*). Lately the constancy of the RABL-orientation through interphase and into metaphase has been confirmed by the elegant experiments of SPERLING & LÜDTKE (1981) and of CREMER & al. (1982) who used cell fusion and prematurely condensed chromosomes (PCC) for this purpose.

Some nonrandomness in chromosome arrangement may be caused by the tendency of the smaller chromosomes to lie in the middle of an untreated metaphase plate, an arrangement seen in practically every organism which has different-sized chromosomes. Also the attachment of the heterochromatic chromosome regions to the nuclear membrane and the general "stickiness" of heterochromatin may have an effect on the relative positions of the chromosomes. Furthermore, the fusion of nucleoli in interphase often brings together the nucleolar organizers in different chromosomes.

The present observations do not indicate nonrandomness of the chromosome arrangement in the haploid *Uvularia* pollen grain metaphase. This indicates that the arrangement also in interphase is random (SPERLING & LÜDTKE 1981). Our results thus do not give any support to the idea that each chromosome would occupy a definite position in the interphase nucleus (VOGEL & SCHROEDER 1974, COMINGS 1980, HAGER & al. 1982). Neither do similar-sized chromosome arms in *Uvularia* show a tendency to lie side-by-side which disagrees with the hypothesis of BENNETT (1982, see also HESLOP-HARRISON & BENNETT 1983 a, b).

On the other hand, our results correspond to those of SPERLING & LÜDTKE (1981) who followed the arrangement of muntjac chromosomes through interphase and into metaphase using the cell fusion and PCC technique. They found no tendency to mitotic pairing or any other nonrandomness in the relative positions of the chromosomes. Further evidence for a random chromosome arrangement has been provided by DRETS & STOLL (1974) who did not find in *Gryllus argentinus* nonrandomness in the attachments of the heterochromatic telomeric regions. Similarly the telomeric attachments of human chromosomes in the THIBERGE-WEISSENBACH syndrome involved the different chromosomes at random (DUTRILLAUX & al. 1978).

A different conclusion has been reached by VOGEL & SCHROEDER (1974) and by HAGER & al. (1982) from studies of chromosome exchanges in variously treated human cells and in lymphocytes of BLOOM's syndrome and FANCONI's anemia patients. They reported that specific chromosome combinations were more frequent than would be expected, if the translocations occurred at random. They concluded that exchanges preferentially took place between chromosomes lying next to each other and that therefore the relative positions of interphase chromosomes could be inferred from the frequencies of the different exchanges.

Obviously two broken chromosome ends must come in contact with each other to form a translocation. However, the determination of chromosome positions in interphase nuclei from translocations presupposes that the chromosomes in most cells are in the same relative

positions and that the interphase domains which they occupy are regular and definite like slices in an orange. However, since the human chromosomes vary considerably in size and are enormously extended in interphase, their domains can hardly be definite or regular. Furthermore, although the closeness of two chromosomes may be one of the factors that determine their involvement in translocations (DUTRILLAUX & al. 1981), the exchange frequencies could equally well be explained by assuming a random arrangement combined with nonrandom breakage and rejoining. Moreover, in addition to the chromosomes carrying the ribosomal RNA genes, other nonhomologous chromosomes might have homologous segments which could be the cause of preferential exchanges; this may be the reason, for instance, of the frequent translocations between human chromosome arms 11 q and 22 q (PIHKO & al. 1981).

It seems even less possible to draw conclusions about chromosome positions from the frequencies of ROBERTSONIAN translocations between the different human acrocentric chromosomes (HAGER & al. 1982). Although the satellite associations seem to some extent to be nonrandom, they certainly do not agree with the ratios of the frequencies of (13 q 14 q and 14 q 21 q) : (any other combination except 14 q 14 q) : (14 q 14 q) which are in the range of 100 : 10 : 1 (THERMAN unpublished). Some type of crossing-over seems to provide the best explanation for the origin of ROBERTSONIAN exchanges, especially since they differ from other translocations also in that X-rays do not increase their frequency whereas radiation induces other translocations (THERMAN 1980).

Although the present observations are not relevant to the question of mitotic chromosome pairing, a few remarks may be in order, since many of the papers quoted above, also deal with this aspect of chromosome arrangement. At first sight the evidence for mitotic pairing in the articles advocating this phenomenon appears impressive (VOGEL & SCHROEDER 1979, AVIVI & FELDMAN 1980, COMINGS 1980). However a closer look gives a different picture. Certainly studies published before 1920, quoted in these reviews, have mainly historical interest, and in many of the later papers, not only is the statistical treatment often insufficient, but the cytological claims are not adequately documented. Furthermore, observations on real or assumed chromosome pairing are much more apt to be published than observations showing a random chromosome arrangement which most cytologists have anyway taken for granted.

It is naturally possible that, even apart from diptera, in some types of cells and/or under certain conditions (cold treatment?) a certain degree of statistically nonrandom closeness of homologous chromosomes may be present. However a great number of recent studies provide convincing evidence against mitotic pairing (WALTERS 1970, COHEN & al. 1972, ZORN

& al. 1979, KORF & DIACUMAKOS 1977, SPERLING & LÜDTKE 1981, THERMAN & KUHN 1981, CREMER & al. 1982). Our experience (THERMAN & KUHN 1981) is in complete agreement with the summing up of SPERLING & LÜDTKE (1981, p. 552): "In conclusion our data on prematurely condensed muntjac chromosomes reveal no evidence for homologous chromosome pairing or any other suprachromosomal organization."

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