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On the ordered arrangement of the haploid complement in radial metaphases of secondary meiocytes of male grasshoppers, *Euchorthippus pulvinatus gallicus*

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Summary. Five hundred and ninety-three radial metaphase II cells from the male grasshopper, *Euchorthippus pulvinatus gallicus,* were analyzed to ascertain whether chromosomes in the haploid complement were in a fixed order. As an a posteriori hypothesis, the most probable original order of chromosomes in the metaphases was determined. The genetical significance of a suprachromosomal organization is discussed.

Key words: Suprachromosomal organization – Meiocytes – *Euchorthippus pulvinatus* – Grasshopper

Introduction

Suprachromosomal organization – that is to say, the non-random arrangement of chromosomes in the nuclei – can be analyzed by studying the meiotic and mitotic behaviour of different types of chromosomes, namely, homologous, homoeologous and genetically unrelated (neither homologous nor homoeologous) chromosomes (review Lacadena et al. 1983).

The functional genetic identity of homologous chromosomes and the functional genetic equivalence of homoeologous chromosomes have their cytological expressions both in meiotic and mitotic phenomena: autosyndetic and allosyndetic pairing, secondary association of bivalents and somatic association (Lacadena and Puertas 1969). One step more in the study of suprachromosomal organization is the analysis of the relative positions of genetically unrelated chromosomes in the nuclei.

Evidence for the non-random arrangement of chromosomes which constitute the haploid set in a diploid or a genome in a polyploid organism comes from the analysis of somatic associations in scattered metaphases (for reviews see Avivi and Feldman 1980; Avivi et al. 1982), end-to-end arrangements (Wagenaar 1969; Ashley and Wagenaar 1972, 1974; Ashley 1979; Ashley and Pocock 1981) and three-dimensional reconstruction of the co-ordinates of centromeres (Bennett 1982). Indirect evidence comes from the analysis of spontaneous and induced interchanges (review by Avivi and Feldman 1980).

An appropriate material on which to analyze the arrangement of chromosomes of the complement is haploid cells which maintain a circular metaphase arrangement when the distortion produced by cytological manipulations has not been too traumatic and where, in addition, the statistical approach for analyzing the relative positions of chromosomes of the set is the easiest.

On these bases, the possible existence of an ordered arrangement of chromosomes of the haploid complement in the radial metaphases of secondary meiocytes of grasshoppers is analyzed in the present paper.

Materials and methods

Experimental animals

Twenty-three *Euchorthippus pulvinatus gallicus* males (2n = 17,X0) were collected at two localities near Madrid.

Cytological method

Testes were fixed and stored in 1:3 acetic-ethanol. The fixed material was squashed in 45% acetic acid and stained by the Giemsa C-banding technique described by Santos and Giráldez (1978). The same technique was used to analyze the mitotic karyotype (Fig. 1, taken from Ferrer et al. 1980). Thirty cells were measured.

Five hundred and ninety-three radial metaphase II cells were analyzed, of which 283 had the X chromosome (Fig. 2) while 310 did not (Fig. 3) – consequences of the first reductional division. Chromosomes were identified by their relative

lengths and C-banding patterns. Autosomes were numbered from 1–8 according their decreasing lengths.

Squashes preparations in the material used normally produce radial metaphases. No special care in squashing was used to increase the chances of the desired radial metaphase shape.

Statistical method

The problem of the arrangement of chromosomes on the metaphase ring can be approached mathematically as the possibility of finding the original disposition of the eight or nine (when chromosome X is present) centromeres on the equatorial ring, taking into account that their relative positions can be altered by manipulations before the observations are made.

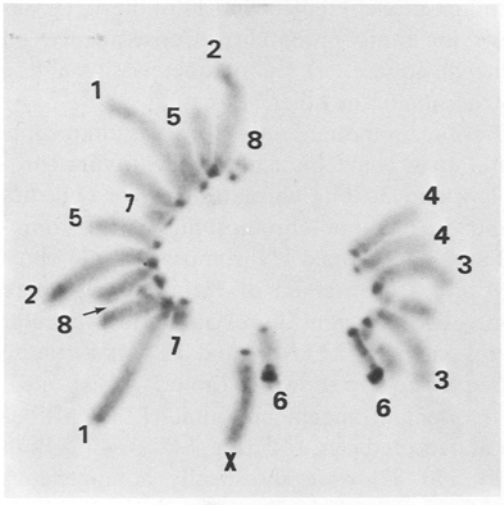


Fig. 1. C-banded radial mitotic metaphase from an embryo of the male grasshopper, *Euchorthippus pulvinatus gallicus* (2n = 17, XO) (magnification 1,875×)

The frequencies of the observed dispositions will depend on two factors, namely 1) the original dispositions of the chromosomes in the living cell, and 2) which were the causes and how they act on such arrangements. Any statistical analysis to be used can only aim at testing the validity of one of several hypotheses formulated a priori about the above mentioned two factors. Therefore, if the hypotheses do not discriminate between them, the statistical analysis will only refer to the evaluation of both factors as a whole but not to any one of them in particular.

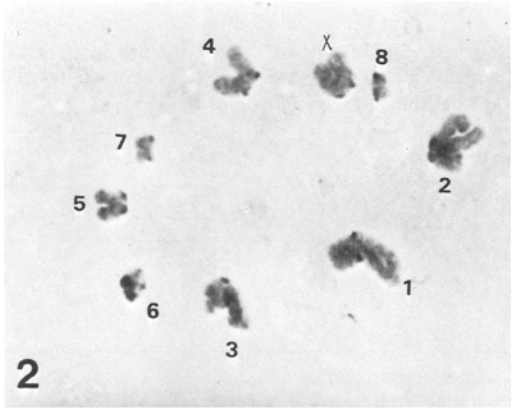
In our case there are no cytological data which might favour a unique prediction of the non-random original chromosome arrangement on the equatorial ring or of the mechanism of distortion. In the present circumstances, the only a priori possibility would be to evaluate the hypothesis "existence of an original chromosome arrangement plus random distortion mechanism" versus the infinite possibilities that it does not happen.

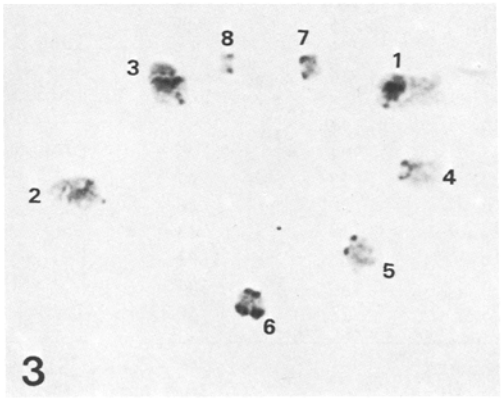
To begin with, chromosome X was discarded a priori from the statistical analysis because it did not reduce drastically the sample size.

In order to analyze the hypothesis by a chi-square test, a minimum of 12,500 metaphase II cells ($1/2{,}500 \times N \geqq 5$) should be observed since there are 2,500 ($7! \times ½$) different non-symmetrical circular autosomal arrangements. Obviously, such a high number of required cells is a handicap to this statistical approach.

However, in the material we have analyzed, chromosomes 7 and 8 are very small and show a strong tendency to lie inside the radial metaphase – thus they can also be discarded from the statistical analysis. On this assumption, the number of different dispositions would be 60, surely not too many classes for the size of the sample available. However, it is desirable to reduce the number of classes still further. So, chromosome 6 can also be discarded from the analysis although it will be taken into consideration later. Thus, the number of different non-symmetrical circular arrangements of chromosomes 1, 2, 3, 4 and 5 is 12 ($4! \times ½$).

Methodologically one can assume that if an original disposition existed in the living cells and the distortion produced by cytological manipulations does not disturb it completely, one can except that one out of the twelve possible circular arrangements should appear with a frequency higher than that of the remaining 11 dispositions (which should show very similar frequencies).





Figs. 2 and 3. Secondary meiocytes of the male grasshopper, *Euchorthippus pulvinatus gallicus.* **2** Radial metaphase II cell with nine chromosomes (8 autosomes plus the X chromosome). **3** Radial metaphase II cell with eight autosomes

Results

Measurements of the mitotic karyotype are shown in Table 1.

The results obtained on chromosome arrangement are shown in Table 2. Since, as indicated in "Methods", there was not a unique prediction, the 593 radial metaphase II cells observed were considered at first as two samples, one corresponding to the 310 secondary meiocytes lacking the X chromosome (sample 1, S1) and the other to the 283 cells in which the chromosome X was present (sample 2, S2). Both samples were statistically homogeneous ($\chi^2 = 4.78$; d.f. = 11; $0.90 < P < 0.95$).

From the analysis of the distribution of sample 1, it was deduced, as an a posteriori hypothesis, that the most probable original chromosome arrangement was 1–2–3–4–5. Taking this arrangement as the unique prediction of an a priori hypothesis for sample 2, a good agreement was found.

Since the homogeneity test of the two samples was not significant, both distributions were summed up. On the other hand, it is worth mentioning the fact that the three distributions (sample 1, sample 2 and their sum) not differing significantly from random (i.e. the classes having the same probability), have no significance since the non-random model predicts that 11 of the 12 classes should have the same probability. Consequently, on applying the chi-square test, the unique class would be masked by the sum of the other 11 classes.

It is possible, although not probable, that some experimental error could be made in identifying chromosomes 1, 2 and 3 (long chromosomes) or chromosomes 4 and 5 (medium chromosomes). If chromosomes 1, 2 and 3 are named L chromosomes and chromosomes 4 and 5 are named M chromosomes, there will be only two different non-symmetrical circular arrangements, namely, LLLMM and LLMLM, whose observed frequencies are shown in Table 3. As expected, the most frequent arrangement found (LLLMM) includes the above predicted ordering 1–2–3–4–5. Both samples (S1 and S2) were statistically homogeneous ($\chi^2 = 5.14$; d.f. = 5; $0.3 < P < 0.5$).

It is also possibe to arrange chromosome 6 with respect to L and M chromosomes. According to the results shown in Table 4, the most probable arrangement is 6–L–L–M–M–L, also in agreement with the previously inferred arrangements.

Table 1. Measurements of the mitotic karyotype of the grasshopper *Euchorthippus pulvinatus gallicus*

Chromosome	Sample size	Relative length	Arm index[a] (long:short)
1	30	0.1130 ±0.0058	1.5924 ±0.1079
2	30	0.1045 ±0.0056	1.7739 ±0.1300
3	30	0.0908 ±0.0042	1.4852 ±0.0800
4	30	0.0413 ±0.0023	
5	30	0.0338 ±0.0019	
6	30	0.0332 ±0.0027	
7	30	0.0181 ±0.0025	
8	30	0.0151 ±0.0017	
X	30	0.0489 ±0.0029	

[a] Arm indices of acrocentric chromosomes were not calculated

Table 2. Observed frequencies of the 12 radial dispositions of chromosomes 1, 2, 3, 4 and 5 at metaphase II

Arrangement	Cells		
	S1[a]	S2[b]	Total
1–2–3–4–5	37	35	72
1–2–3–5–4	25	25	50
1–2–4–3–5	21	25	46
1–2–4–5–3	26	25	51
1–2–5–3–4	25	19	44
1–2–5–4–3	31	25	56
1–3–2–4–5	30	21	51
1–3–2–5–4	25	21	46
1–3–4–2–5	22	25	47
1–3–5–2–4	26	20	46
1–4–2–3–5	24	19	43
1–4–3–2–5	18	23	41
Totals	310	283	593
Means	25.83	23.58	49.42

[a] S1 = cells with 8 chromosomes
[b] S2 = cells with 8 + X chromosomes
Comparison with a binomial distribution (p = ½):
$72 = 49.42 + 3.36\ \sigma$; $P = 0.0005$

Table 3. Observed frequencies of the two possible radial dispositions in which L (long) substitutes for chromosomes 1, 2 and 3, and M (medium) for chromosomes 4 and 5

Arrangements	Cells		
	S1[a]	S2[b]	Total
L–L–L–M–M	174	152	326
L–L–M–L–M	136	131	267
Total	310	283	593

[a] S1 = cells with 8 chromosomes
[b] S2 = cells with 8 + X chromosomes
Comparison with a binomial distribution (p = ½):
$326 = 296.50 + 2.42\ \sigma$; $P = 0.008$

Table 4. Observed frequencies of the six possible radial dispositions involving chromosome 6 and the L(1, 2 and 3) and M (4 and 5) chromosomes

Arrangement	Cells		
	S1[a]	S2[b]	Total
6–L–L–L–M–M	68	60	128
6–L–L–M–L–M	55	61	116
6–L–M–L–L–M	46	48	94
6–L–L–M–M–L	81	63	144
6–L–M–L–M–L	35	22	57
6–M–L–L–L–M	25	29	54
Total	310	283	593

[a] S1 = cells with 8 chromosomes
[b] S2 = cells with 8 + X chromosomes
Comparison with a binomial distribution (p = ½):
144 = 118.6 + 2.60 σ; $P = 0.005$

Table 5. Frequencies with which the chromosomes 1, 2, 3, 4, 5 and 6 appeared adjacent to one another in radial metaphase II cells

Chromosome	Chromosome						
	1	2	3	4	5	6	Total
1	–	249	227	219	252	239	1,186 (2×593)
2		–	242	219	223	253	
3			–	245	224	248	
4				–	272	231	
5					–	215	

A further attempt was to locate chromosome 6 with respect to the ordering 1–2–3–4–5. As can be deduced from Table 5, the most probable arrangement is 1–2–6–3–4–5, which agrees both with the 1–2–3–4–5 and the 6–L–L–M–M–L arrangements previously established.

Finally, it is worth mentioning that the hypotheses in this work are a posteriori and, consequently, we did not even try to evaluate the significance of the results since any statistical analysis would be of doubtful value and would conflict with the cytological approach to the problem used in this work.

Only for its possible indicative value do we present in Tables 2–4 the probabilities of equal or larger deviations, testing the ordering 1–2–3–4–5 as an a posteriori hypothesis. Obviously, multiplying them by 12 to obtain the a posteriori probabilities would not be correct.

Discussion

Radial metaphases of secondary meiocytes constitute an optimum material for studying the relative positions of chromosomes of the haploid set. This type of cell has been previously used by other authors to analyze the chromosome arrangement (Nur 1973, in the grasshopper *Melanoplus femur-rubrum*; Juricek 1974, in Chinese hamster).

Our experimental data seem to suggest the existence of a determined chromosomal arrangement in the haploid set of the grasshopper *Euchorthippus pulvinatus gallicus*. This fact is in agreement with previous investigations by other authors in which a specific ordered arrangement of non-homologous chromosomes with respect to one another has been found. This is the case of the phenomenon of end-to-end arrangement described in several higher plants (Wagenaar 1969; Ashley 1979; see references in Avivi and Feldman 1980). Even Ashley and Wagenaar (1974) pointed out that the chromosomal sequence is maintained throughout the life cycle of the plant.

Bennett (1982) described a model for predicting the position of each chromosome in a haploid genome as well as the analysis that was used to test for non-random chromosome dispositions in general, and for the unique prediction of the model, in particular. This unique prediction is based on the ordering of the set of chromosomes bringing together the most similarly sized pairs of chromosome arms throughout the haploid complement. Bennett made the analysis by entering the three-dimensional co-ordinates of each centromere in a haploid genome into a Kontron Videoplan microcomputer. Bennett's model successfully predicted orders with significantly closer arrangements than those randomly found in the three materials tested (*Secale cereale, S. africanum* and *Hordeum vulgare*).

Obviously, from the existence of an ordered arrangement of chromosomes at metaphase one can infer a similar situation at interphase. So, a question immediately arises, namely, what is the genetical significance of an ordered arrangement of chromosomes at interphase? Or, in other words, is there a certain order in the genetic organization of the organisms or, on the contrary, is the genetic information randomly distributed in their genomes and in their nuclei a genetical chaos? Recently, Lacadena et al. (1983) pointed out a cytogenetical rationale and experimental evidence supporting the suprachromosomal organization.

Genetic interactions between genes located on homologous, homoeologous and non-homologous chromosomes have been reported. For instance, Avivi et al. (1982) mentioned the activation of one allele by its homologous partner in salivary gland chromosomes of *Drosophila melanogaster* (Ashburner 1967), and the case in which the degree of somatic association between

homoeologous chromosomes in common wheat intensified the interaction between homoalleles, thereby affecting the phenotype of the alcohol dehydrogenase zymogram (Avivi et al. 1972). Finally, there is the transvection effect observed in *Drosophila melanogaster* by lack of proximity between interacting genes of non-homologous chromosomes (Lewis 1954).

Summing up, the suprachromosomal organization is an expression of the structure-function binomial which is a constant in biology (Lacadena et al. 1983).

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References

Ashburner M (1967) Gene activity dependent on chromosome synapsis in the polytene chromosomes of *Drosophila melanogaster.* Nature 214:1159–1160

Ashley T (1979) Specific end-to-end attachment of chromosomes in *Ornithogalum virens.* J Cell Sci 38:357–367

Ashley T, Pocock N (1981) A proposed model of chromosomal organization in nuclei at fertilization. Genetica 55:161–169

Ashley T, Wagenaar EB (1972) End-to-end attachment of haploid chromosomes of *Ornithogalum virens.* Can J Genet Cytol 14:716–717

Ashley T, Wagenaar EB (1974) Telomeric association of gametic and somatic chromosomes in diploid and autotetraploid *Ornithogalum virens.* Can J Genet Cytol 16: 61–67

Avivi L, Efron Y, Feldman M (1972) Effect of somatic chromosomal association on the zymogram of alcohol dehydrogenase (ADH) in common wheat. Genetics 71 (Suppl):2–3

Avivi L, Feldman M (1980) Arrangement of chromosomes in the interphase nucleus of plants. Hum Genet 55:281–295

Avivi L, Feldman M, Brown M (1982) An ordered arrangement of chromosomes in the somatic nucleus of common wheat, *Triticum aestivum* L. 1. Spatial relationships between chromosomes of the same genome. Chromosoma 86:1–16

Bennett MD (1982) Nucleotypic basis of the spatial ordering of chromosomes in eukaryotes and the implications of the order for genome evolution and phenotypic variation. In: Dover GA, Flavell RB (eds) Genome evolution. Academic Press, London, pp 239–261

Ferrer E, Lacadena JR, Jódar B (1980) Análisis de la asociación somática de cromosomas homólogos en *Euchorthippus pulvinatus gallicus* Maran (Acrididae, Orthoptera). Rev R Acad Cienc Exactas Fis Nat Madrid 74:639–651

Juricek DK (1975) Non-random chromosome distribution in radial metaphases from the Chinese hamster. 1. Uncultured cells. Chromosoma 50:313–326

Lacadena JR, Jòdar B, Ferrer E (1983) Suprachromosomal organisation: Cytogenetical rationale and experimental evidence. In: Brandham PE, Bennett MD (eds) Kew Chromosome Conf II. Allen and Unwin, London, pp 81–90

Lacadena JR, Puertas MJ (1969) Secondary association of bivalents in an allohexaploid, *Aegilops triaristata* Willd 6×. Genet Iber 21:95–116

Lewis EG (1954) The theory and application of a new method of detecting chromosomal rearrangement in *Drosophila melanogaster.* Am Nat 88:225–239

Nur U (1973) Random arrangement of chromosomes in a radial metaphase configuration. Chromosoma 40:263–267

Santos JL, Giráldez R (1978) The effect of C-heterochromatin in chiasma terminalisation in *Chorthippus biguttulus* L. (Acrididae, Orthoptera). Chromosoma 70:59–66

Wagenaar EB (1969) End-to-end attachments in mitotic interphase and their possible significance to meiotic chromosome pairing. Chromosoma 26:410–426