

## Meiosis in aneuploids of tetraploid *Lathyrus odoratus* and *L. pratensis*

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Khawaja, H. I. T., Sybenga, J. and Ellis, J. R. 1998. Meiosis in aneuploids of tetraploid *Lathyrus odoratus* and *L. pratensis*. — *Hereditas* 129: 53–57. Lund, Sweden. ISSN 0018-0661. Received March 9, 1998. Accepted September 7, 1998

Meiosis was studied in one hypoploid (trisomic,  $2n = 4x - 1 = 27$ ) of an induced tetraploid ( $2n = 28$ ) of *Lathyrus odoratus* and two hyperploids (single pentasomic,  $2n = 4x + 1 = 29$  and double pentasomic,  $2n = 4x + 1 + 1 = 30$ , respectively) of an induced tetraploid of *L. pratensis*. Multivalent frequencies in the aneuploids were high, but the numbers of chiasmata were not sufficient to maintain all pairing configurations till metaphase I. Interstitial chiasmata were relatively frequent. Chiasma frequencies were considerably reduced in all three aneuploids, compared with the eutetraploids. The two pentasomes (sets of five homologous chromosomes) of the double pentasomic were both present as a quinivalent more frequently than expected when they would behave independently. The single pentasomic and the trisomic did not deviate much from expected, although, like in the eutetraploids, the multivalent frequencies were considerably higher than corresponds with the assumption that pairing initiation is restricted to the chromosome ends. The conclusion is that aneuploids of *Lathyrus* at the tetraploid level tend to behave similar to euploids, except that the chiasma frequency is considerably reduced. When maintained in the population their progeny will contain even more aneuploids than the euploids, not only because the aneuploidy will be perpetuated, but also because of lower chiasma frequencies leading to univalent formation.

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Several species of the genus *Lathyrus* are used in agriculture, some for seed production, for human consumption as well as for animal feed, and others as forage and green manure crops. Some species are natural autopolyploids, others are diploids or contain both diploid and autotetraploid forms. Some of the diploids have been doubled artificially by colchicine treatment (KHAWAJA et al. 1997). Aneuploids are regularly formed in populations of autotetraploids, including *Lathyrus* (KHAWAJA 1996) but they tend to disappear in perennial stands (KLINGA 1986, 1987). If their meiosis is sufficiently regular, they may contribute to seed production, but in general their vitality and reproductive behaviour is impaired compared to that of euploids.

The present study was set up to establish if aneuploids of *Lathyrus* at the tetraploid level follow predictions of meiotic behaviour as constructed on the basis of theoretical models, and whether or not meiosis is significantly different from that in eutetraploids.

### MATERIALS AND METHODS

Meiosis of three aneuploids is described: a. One hypoploid with  $2n = 27$ , found in the progeny after selfing of an induced autotetraploid plant of *L. odoratus*. b. Two hyperploids found in the progeny of an induced autotetraploid of *L. pratensis* (KHAWAJA

1996). Meiosis in the autotetraploids from which the aneuploids were derived, has been described by KHAWAJA et al. (1997).

Mitotic chromosome numbers were established in Feulgen stained root tip preparations, meiotic studies were made on temporary acetocarmine preparations (KHAWAJA et al. 1997).

### RESULTS

The plant of *L. odoratus* with  $2n = 27$  chromosomes was found among the few progeny of one  $C_0$  plant, and in meiosis behaves much like the tetraploids as reported in KHAWAJA et al. (1997), except for the trisome (Table 1A). Like in the eutetraploids, the distribution of multivalents over cells is random, when trivalents and quadrivalents are combined. From the trivalent frequencies it can be deduced that it is a simple trisomic. For *L. odoratus*, the chiasma frequency (here represented by the frequency  $c$  of the average chromosome arm to have at least one chiasma) is low ( $c = 0.77$ ) compared with eutetraploids ( $c = 0.962$ ), apparently a consequence of aneuploidy. In addition to the 23 cells reported, three cells had only univalents (27) and one cell had three open bivalents in addition to 21 univalents. The phenomenon that among a majority of apparently normal cells a few are totally asynaptic or nearly so,

occurs occasionally in plants with a minor abnormality, but is rarely reported. LACADENA (1965) observed a similar incidental asynapsis in nullisomic *Triticum triaristata* with  $2n = 6x - 2 = 40$ . Desynapsis after colchicine treatment of *Lathyrus odoratus* and *L. pratensis* has been described by KHAWAJA and ELLIS (1987) and in *L. odoratus* desynapsis was shown to be an inherited trait.

The trivalents derived from the trisome could not be distinguished unambiguously from those derived from the tetrasomes. Therefore, neither of the tetrasomes nor of the trisome could the pairing and chiasma parameters be estimated. There is a tendency for the trivalents to have more interstitial chiasmata, and consequently to be branched more frequently, probably a result of more pairing partner switches than quadrivalents. This phenomenon is common in polyploids (SYBENGA 1992). It is not clear to which extent this is due mainly to the trisome. Comparison with the eutetraploids is not possible because of the very different chiasma frequencies.

Two pentasomics were found among the progeny of one induced autotetraploid of *L. pratensis*. One had 29 chromosomes (Fig. 1a), the other 30. The latter was concluded to be a double pentasomic rather than a hexasomic, because hexavalents were not observed, whereas the combinations of two quinquivalents (Fig. 1b), or two trivalents, or one quinquivalent and one trivalent, were common. The results are shown in Table 1B. Again the chiasma frequency is low ( $c = 0.81$  and  $0.82$  respectively) compared to the eusomatic autotetraploid ( $c = 0.96$ ). The

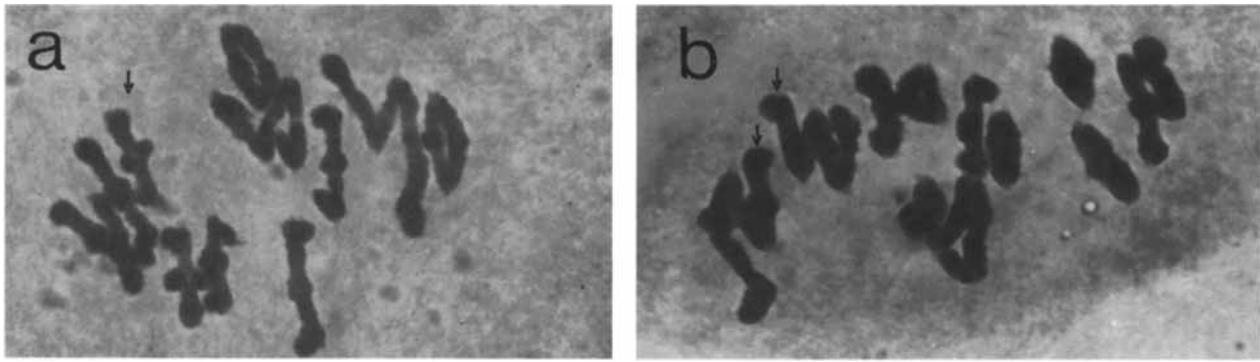
theoretical meiotic behaviour of autopolyploids has been described by JACKSON and CASEY (1982), but without working out the details. In a model assuming that meiotic pairing starts at both ends and is random between the five homologues (Fig. 2), there are twelve equally probable combinations for both arms. Numbering the chromosomes 1, 2, 3, 4 and 5, both for the left (*l*) and the right (*r*) arms (Fig. 2), pairing between *l1* and *l2* can be combined with pairing between *l3* and *l4* (5 unpaired), or with pairing between *l3* and *l5* (4 unpaired), or between *l4* and *l5* (3 unpaired). These are three combinations. Chromosome arm *l1* can also pair with *l3*, and then again there are three combinations. There appear to be four possibilities for the left arm 1 to pair, each with three other pairing combinations, or a total of twelve. The same is true for the right arm, so there are a total of  $12 \times 12 = 144$  pairing combinations. It can readily be derived that 72 (0.5) are quinquivalents, 24 (0.167) are quadrivalents with a univalent, 36 (0.25) are the combination of a trivalent with a bivalent, and 12 (0.083) two bivalents with a univalent.

Depending on the formation of chiasmata, these or lower frequencies are expected at diakinesis-metaphase I (Fig. 2D–G). In the single pentasomic *L. pratensis*, these configuration frequencies are expected for one chromosome, in the double pentasomic for two. For the first,  $c$  equals 0.82, for the second, 0.81. This implies that several pairing multivalents will be present at metaphase I as smaller configurations as a result of insufficiency of chiasmata. In the single pentasomic (Table 1B), 11 quin-

Table 1. Meiotic configurations of one hypoploid of *L. odoratus* ( $2n = 27$ ) (A) and two hyperploids of *Lathyrus pratensis* ( $2n = 29$  and  $2n = 30$ , resp.) (B). a: total observed. b: average per cell. c,d: same after pentasome removed, and frying pans etc. taken together with chains, and trivalent including univalent. e: probability of any chromosome to be involved in configuration specified

A																
2n	cells	V	ring IV	chain IV	fr.pan IV	OK IV	X IV	Y IV	chain III	fr.pan III	Y III	ring II	open II	I	c	
27	23	a	47	29	10	0	3	1	21	10	4	23	34	42		
		b	2.04	1.26	0.43	0	0.13	0.04	0.91	0.43	0.17	1	1.48	1.83	0.77	
B																
29	35	a	11	51	47	10	7	9	3	11	6	0	111	83	13	
		b	0.31	1.46	1.34	0.29	0.2	0.26	0.09	0.31	0.17	0	3.17	2.37	0.37	0.82
		c	–	49	72	–	–	–	–	1	–	–	98	76	2	
		d	–	1.4	2.06	–	–	–	–	0.03	–	–	2.8	2.17	0.06	
		e	–	0.23	0.34	–	–	–	–	0.01	–	–	0.23	0.18	0.01	
30	38	a	22	58	39	5	2	1	5	33	3	0	103	116	44	
		b	0.58	1.53	1.03	0.13	0.05	0.03	0.13	0.87	0.08	0	2.71	3.05	1.16	0.81

Note: c: average frequency per arm of having at least one chiasma. V: quinquivalent; IV: quadrivalent; III: trivalent; II: bivalent; I: univalent. fr. pan: frying pan; OK: configuration with two chromosomes forming a ring, to which two others are attached, each with one arm only. X: configuration with four chromosomes attached at one point. Y: configuration of three chromosomes in a chain and the fourth attached at the side



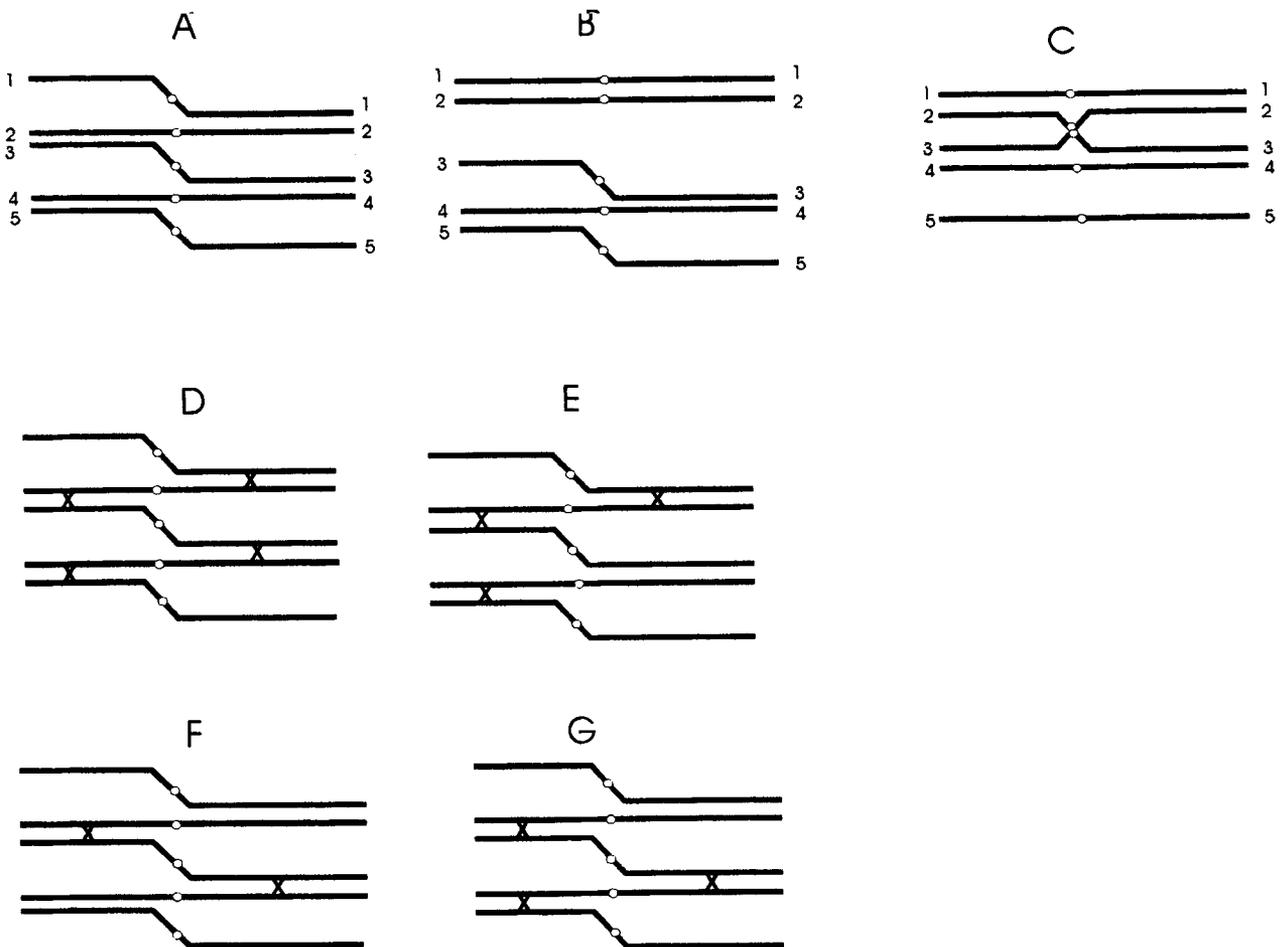
**Fig. 1a and b.** Photomicrographs of metaphase I cells of two hyperploids (pentasomics) of autotetraploid *Lathyrus pratensis*. **a** Cell of a single pentasomic with one quinquivalent (arrow), four quadrivalents and four bivalents. **b** Cell of a double pentasomic with two quinquivalents (arrows), three quadrivalents and four bivalents.

quivalents, 17 trivalents without univalent (specific for the pentasome) and 7 univalents without a trivalent were observed. Because the majority of the chromosomes in the cell were tetrasomic and formed quadrivalents as well as bivalents in large numbers, a quadrivalent with a univalent formed by the pentasome could not be distinguished from a bivalent pair with a univalent. A univalent without trivalent must have been derived from a pentasome. The trivalents without (single) univalent must be accompanied by an open bivalent or a pair of univalents. The expected numbers of quinquivalents, trivalents without univalent and univalents without trivalent, calculated as shown above, are: 17.5, 8.75 and 8.75, respectively, a considerable difference with the 11, 17 and 7 observed. The small number of quinquivalents is mainly due to lack of chiasmata, undoing the effect of multivalent pairing, as also found in the eu-tetraploids. The excess of trivalents is mainly a result of this phenomenon.

As mentioned above, a quinquivalent pairing configuration falls apart into a quadrivalent and a univalent (Fig. 2G) when either the left-most or the right-most chromosome fails to be associated with the remaining four by lack of chiasmata. A trivalent and bivalent result when either a left or a right interstitial arm lacks chiasmata, which also represent two separate cases (Fig. 2E). A trivalent with two univalents (Fig. 2F), is formed when the three chromosomes in the middle are associated by chiasmata and the other two are univalent (one case). A trivalent can thus be derived from a pairing quinquivalent in three cases. With a chiasmate association frequency of 0.82 (see above) the frequency of trivalents derived from quinquivalent pairing thus equals  $2 \times 0.82^3 \times (1 - 0.82)$  (trivalent + bivalent) +  $3 \times 0.82^2 \times (1 - 0.82)^2$  (trivalent + two univalents), or 0.282. However, a number of pairing trivalents is also broken down into smaller configurations, but less fre-

quently, because the trivalent pairing configuration is less frequent than the quinquivalent. In addition, chiasma failure in the accompanying bivalents does not affect the trivalent frequency. For the 17.5 expected quinquivalents, this leads to 4.94 broken down into a trivalent and other configurations, and to be added to less than the 8.75 trivalents expected with complete chiasma formation. Together these processes could result in about 13 trivalent, not quite as many as the 17 observed. A quinquivalent is expected only when all arms have a chiasma, which has a probability of  $0.82^4 \times 17.5 = 7.91$ , also less than the 11 observed. When the two arms have different probabilities of forming a chiasma, the expected quinquivalent frequency would be even lower. It must be concluded that multivalent pairing does not fit the assumption that pairing initiates at the ends exclusively, with only one point of pairing partner exchange. This had also been concluded for eutetraploids in *Lathyrus* (KHAWAJA et al. 1997).

Except in a few cells where some uncertainty existed, it was possible to determine which configurations (other than a quinquivalent) were derived from the pentasome. Thus it was also possible to determine the frequencies of configurations derived from the six tetrasomes ( $2n = 24$ ) together (Table 1B, rows c and d). From these pairing and chiasma parameters can be estimated (SYBENGA 1975) and compared with those of the corresponding tetraploid reported by KHAWAJA et al. (1997). It appears that the overall chiasmate association frequency per chromosome arm *c* is 0.817, apparently equal to that of the cell average including the pentasome, but much lower than for the eutetraploid. The average frequency for the longer arm equals 0.983, that for the shorter arm, 0.650. After quadrivalent pairing the corresponding values are 0.993 and 0.579, respectively, and after bivalent pairing, 0.959 and 0.822 respectively. The frequency *f* of quadrivalent pairing equals 0.706,



**Fig. 2A–G.** Diagrams of pairing configurations and effects of chiasma frequency and localization on final configurations of a pentasome. Centromeres: white circles. Chiasmata: crosses. **A** One possibility of forming a pairing quinquivalent: left arm 1 is unpaired, 2 and 3 are paired, as are 4 and 5. In the right arm 1 and 2, as well as 3 and 4 are paired and 5 is unpaired. **B** Pairing resulting in a bivalent and a trivalent. **C** pairing resulting in a quadrivalent and a univalent. **D–G** Effects of chiasmata. **D** Pairing quinquivalent is maintained till metaphase I because all arm pairs have a chiasma. **E** Pairing quinquivalent breaks down into trivalent and bivalent because of lack of chiasmata in one arm. **F** Pairing quinquivalent breaks down into a trivalent and two univalents because two arm pairs lack chiasmata. **G** Pairing quinquivalent breaks down into a quadrivalent and a univalent.

whereas the natural autotetraploid *L. pratensis* studied by KHAWAJA et al. (1997) had an  $f$ -value of 0.750, an artificial autotetraploid had 0.613, and a hybrid between the two had 0.660. The aneuploid is well within the range of the eutetraploids, with a quadrivalent pairing frequency considerably higher than expected when only chromosome ends are capable of initiating pairing.

In the double pentasomic, the numbers of trivalents and univalents per cell were small, and it could be concluded that almost all were derived from the pentasomic, few from breakdown of one of the other multivalents, as a result of insufficient chiasma formation. However, the ring and open bivalents were frequent, and there was no way of telling whether they were derived from tetrasomes or pentasomes. In the 38 cells of the double pentasomic, the two penta-

somes could be concluded to have formed two quinquivalents in nine cells; four cells had one quinquivalent together with one trivalent with bivalent (ring or open); ten had two trivalents with a bivalent; 11 had one trivalent with a bivalent (ring or open), and must also have included either a quadrivalent with a univalent or a bivalent pair with a univalent; four cells had only bivalents, quadrivalents and univalents. The combination of one quinquivalent and one univalent (combined with a quadrivalent or a bivalent pair not distinguished from those formed by the tetrasomes) was not observed. The average quinquivalent frequency  $q$ , was 0.289, so  $(1 - q) = 0.711$ . Assuming independence of the two pentasomes in respect to quinquivalent pairing, in the 38 cells studied,  $q^2 \times 38 = 3.2$  are expected to have two (9 found),  $2q(1 - q) \times 38 = 15.6$  are expected to have one (4

found) and  $(1 - q)^2 \times 38 = 19.2$  are expected to have no quinquivalent (25 found). The difference is considerable. If the two pentasomes would have different quinquivalent frequencies, the combination of two quinquivalents would be less frequent than when they would have equal frequencies, whereas the opposite is found. A possible reason for the deviation is variation between cells in chiasma frequency, which produces an irrelevant correlation. This results in more cells with two quinquivalents as a result of infrequent breakdown of the quinquivalents in cells with high chiasma frequencies. At the same time, cells with relatively few chiasmata tend to have no quinquivalent at all. There is indeed a correlation between numbers of bound arms per cell and numbers of quinquivalents, but it is not quite significant and can partly be explained by the fact that the quinquivalents necessarily have larger numbers of arms bound. There may well be an additional reason for the coincidence of two quinquivalents.

In the double pentasomic it was not possible to determine unambiguously which configurations had been derived from the five tetrasomes, and it was not attempted to estimate pairing and chiasma parameters.

## DISCUSSION

Multivalent frequencies are high, both in natural and induced tetraploids of the genus *Lathyrus* (KHAWAJA et al. 1997). The aneuploids described in this report are no exception. It is clear that meiosis in the aneuploids studied here at the tetraploid level does not fundamentally deviate from that in the eutetraploid, except for considerably lower chiasma frequencies. As a consequence, there are fewer multivalents, but correction for low chiasma frequency shows that the initial multivalent pairing frequency is the same in aneuploids and euploids, and the same in trisomes and pentasomes, as in tetrasomes. The trivalents have more interstitial chiasmata in all cases.

Low chiasma frequencies may somewhat decrease fertility by increasing the probability of univalent formation, which in turn results in more dysfunctional gametes, in addition to low fertility as a result of reduced vigour of aneuploids in general. More seriously, increased univalent formation leads to new aneuploidy, and thus contributes to lower vigour and reduced fertility of the population. Like in any tetraploid in the population, new aneuploidy results

also from asymmetric segregation of the multivalents formed by all tetrasomes in the aneuploids. However, the main source of aneuploidy in the progeny is the aneuploidy already present in the parent.

In the hypoploid of tetraploid *L. odoratus*, a number of cells formed univalents (almost) exclusively. In the embryosac mother cells, fertility would potentially be reduced, but this would be masked by the almost complete female fertility of the autotetraploid types, for which the reason is not known. Exceptionally, completely asynaptic cells may form unreduced gametes, but no evidence for this was obtained in the present study.

There is no explanation for the coincidence of two quinquivalents in the double pentasomic.

## ACKNOWLEDGEMENTS

The material and facilities provided by the Department of Botany and Microbiology, University College, London, and the National Agricultural Research Centre, Islamabad, are gratefully acknowledged. Also, logistic assistance provided by the Pakistan Genetical Society is acknowledged. Dr. J. H. de Jong of the Wageningen Agricultural University gave valuable comments on the manuscript.

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