CHROMOSOME CONFIGURATIONS IN TRISOMIC OENOTHERAS

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In a structurally homologous diploid the number of possible primary trisomic types is strictly limited and corresponds to the haploid number of chromosomes. The classical example, of course, is in *Datura Stramonium* (BLAKESLEE, 1930), where n = 12 and the 12 distinct primary trisomic forms, besides numerous secondaries and tertiaries, have been recognised. In structurally hybrid diploids, having multiple rings of chromosomes, the conditions are considerably more complicated. In *Oenothera Lamarckiana,* for example, with a ring of 12 chromosomes and a ring pair, HÅKANSSON (1930) describes a theoretical possibility of 13 primary trisomics, since all the twelve ring-forming chromosomes are different *inter se.* All these trisomics of *O. Lamarckiana* should segregate a proportion of *Lamarckiana.* In addition, a number of other trisomics are known which do not segregate *Lamarckiana* and must therefore be of different constitution from the former class. It seems possible that these have arisen as a result of double non-disjunction on the same side, so that in the trisomic two chromosomes are duplicated and another one missing altogether (cf. GATES, 1923). They should have a peculiar type of configuration characteristic of tertiary trisomics of structurally homozygous diploids (BELLING and BLAKESLEE, 1924; BLAKESLEE, 1930) as shown in the discussion. The loss of the whole chromosome would be made up by parts of the two duplicated ones. The theoretical number of such forms is high, but in practice is probably limited by reason of the inviability of many of them. Trisomics may

also arise in the F_1 generation of crosses so that other, more complicated, configurations may be discovered in many cases, depending on the parentage of the cross and the extra chromosome involved. The chance of regeneration of the hybrid types from these trisomics would depend on individual circumstances.

The two trisomics here described have arisen in different cultures, belonging to Prof. R. R. GATES, F. R. S., which have been grown in the Royal Botanic Gardens, Regent's Park, London.

Part of the work has been carried out using a microscope for the purchase of which I have to acknowledge a grant from the Dixon Fund of the University of London.

DESCRIPTION

Oenothera nutans mutant *nana*

Introduction. This trisomic appeared in 1930, in the quadruple hybrid *O.* (*nutans* × *pycnocarpa*) × *pycnocarpa* × *nutans*). The parents of the cross had the compositions *serratans* . *dentans* and *dependens* . *nutens* respectively; the composition of the quadruple hybrid was *serratans* . *nutens*, namely *O. nutans* practically unchanged phenotypically (cf. GATES and CATCHESIDE, 1932). It had a ring of fourteen chromosomes at diakinesis (fig. 1) and metaphase I; the nucleus illustrated shows a subterminal chiasma at one point.

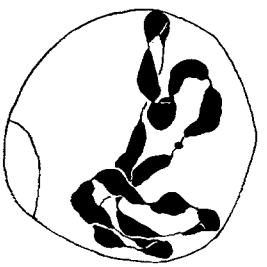


FIG. 1. *Oenothera nutans.* Pollen mother cell at diakinesis showing ring of 14 chromosomes. × 2640

The mutant was chiefly notable for its small size, shortened internodes, shorter and blunter leaves, high degree of bad pollen and infertility of the capsules under bag isolation. No selfed or crossed seeds were secured. Cytological material was fixed by KIHARA's method in Carnoy followed by ALLEN's Bouin; it was stained with HAIDENHAIN's iron alum haematoxylin. The preparations are not so good as more recent ones prepared using Flemming fixatives and gentian violet iodine as a stain; some valuable data, however, have been obtained from the material.

Meiotic Configurations. The various types of chromosome associa-

tion may best be understood from the theoretical diagram (fig. 7*a*) of the complete association possible. The extra chromosome, AB, is completely homologous with one whole chromosome in the ring and parts of it with parts of two other chromosomes, viz. BC and AP. Hence triple unions (each of two terminal chiasmata) are possible at A and B. Clearly, these two points in the ring are marked and we can identify other points relative to them, with the reservation that the following pairs are equivalent, viz.: C and P, D and O, E and N, F and M, G and L, H and K, since A and B are indistinguishable and the ring may be lettered in either direction.

In all, 80 whole nuclei have been examined and these have disclosed 37 different types of chromosome association, the frequencies

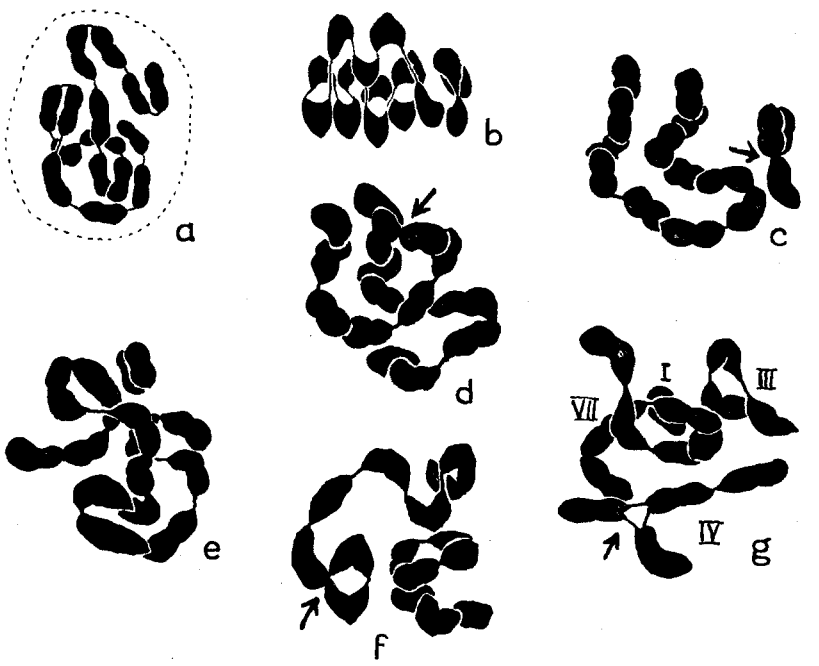


FIG. 2. *Oenothera nutans* mut. *nana*. Pollen mother cells at metaphase I. Descriptions in text: (*a*) and (*b*) × 3300, (*c*—*g*) × 4200. Note: the arrows point to triple unions (between 3 chromosomes).

of which are enumerated in Table I. Various conventional symbols which require explanation have been used. The arabic numerals refer to the number of chromosomes taking part in a given associa-

TABLE I. TYPES OF CHROMOSOME CONFIGURATION AT MEIOSIS IN *O. nutans* MUT. *nana*

		No. of nuclei
A. 15 chiasmata.		
(a) faillure of 1 Xa at A	(14)—(1)	3
	(13)—②	3
(b) failure of 1 Xa at C	(1)—②—(12)	1
„ E	(3)—②—(10)	1
„ F	(4)—②—(9)	1
„ H	(6)—②—(7)	1
B. 14 chiasmata.		
(a) failure of 1 Xta each at A and B	(15)	14
„	(13)+②	11
(b) failure of 1 Xa at A and 1 at C	②—(1)+(12)	1
„ D	②—(2)+(11)	1
„ G	②—(5)+(8)	1
„ H	②—(6)+(7)	2
(c) failure of 1 Xa at A and 1 at C	(13)—(1)—(1)	4
„ E	(11)—(1)—(3)	2
„ G	(9)—(1)—(5)	2
„ H	(8)—(1)—(6)	1
„ K	(7)—(1)—(7)	2
(d) failure of 1 Xa each at G and H	(7)—②—(5)+(1)	1
C. 13 chiasmata.		
(a) failure of 1 Xa each at A	②—(2)+(9)+(2)	1
and two other points at C—P	②—(6)+(4)+(3)	1
	②—(10)+(2)+(1)	1
(b) failure of 1 Xa each at A and B and D	②+(2)+(11)	1
„ E	②+(3)+(10)	2
„ F	②+(4)+(9)	1
„ G	②+(5)+(8)	3
„ H	②+(6)+(7)	2
(c) failure of 1 Xa at A and	(6)—(1)—(1)+(7)	1
at two other points (C—P)	(7)—(1)—(2)+(5)	1
	(5)—(1)—(4)+(5)	1

(d) failure of 1 Xa each at A and B and some other point (C—P)	(13)+(2)	2
	(12)+(3)	2
	(10)+(5)	2
	(9)+(6)	3
	(8)+(7)	1
D. 12 chiasmata.		
(a) failure of 1 Xa each at A and B and at E and L	②+(3)+(5)+(5)	1
(b) failure of 1 Xa each at A and B and at two points (A—P)	(8)+(6)+(1)	1
E. 11 chiasmata.		
	(2)—(1)—(1)+(7)+(3)+(1)	1
	Total	80

TABLE II. OCCURRENCE OF CHIASMA FAILURE IN *O. nutans nana*

Locations in ring.	C(P)	D(O)	E(N)	F(M)	G(L)	H(K)
Number of breaks:						
(a) definitely assigned.	7	5	6	6	8	11
(b) uncertained (divided between disputed classes).	1	2	1.5	1	3.5	3
(c) total.	8	7	7.5	7	11.5	14
Divergence (x) from mean	—1.17	—2.17	—1.67	—2.17	+2.33	+4.83
x^2/m	0.15	0.51	0.30	0.51	0.58	2.55

TABLE III. NUMBERS OF CHIASMATA AT A AND B, THE POINTS OF TRIPLE UNION, IN *O. nutans nana*

No. of chiasmata.	2/2	2/1	2/0	1/1	1/0	0/0
No. of cells.	5	14	14	45	0	0

TABLE IV. TOTAL CHIASMATA AT A AND B IN *O. nutans nana* COMPARED WITH EXPECTATION IN THE ABSENCE OF INTERFERENCE

No. of chiasmata per three sets of two segments (A and B) each.	0	1	2	3	4
Observed frequency.	0	0	59	14	5
Calculated frequency.	7.9	18.3	21.3	18.6	(12.9)

$\chi^2 = 64.3$, $n = 3$ and $P < 0.01$; variance = 0.34.

tion; a circle means a closed ring, brackets an open chain. Triple unions are recorded as follows: a ring attached to a chain by a triple chiasma is shown thus: ②—(6), e.g. fig. 2*f*; a ring attached to two chains by two triple chiasmata thus: (13)—②—(1); three chains associated by a triple chiasma thus: (7)—(1)—(7), e.g. fig. 2*d*.

The commonest configuration is a chain of 15 chromosomes (fig. 2*a*), which may be „broken" into two (fig. 2*b*) or more smaller chains. Another frequent type is a chain of 13 chromosomes and a ring pair (fig. 2*e*); in a few cases the chain of 13 has been found attached to the ring pair. The long chain may be divided into two shorter chains of which neither, one (figs. 2*c* and *f*) or both may be attached to the ring pair. Another common type is that in which three chains, one of which always consists of a single chromosome, are united in a triple chiasma (figs. 2*d* and *g*). The complete hypothetical figure (fig. 7*a*) has never been certainly identified, but all the configurations observed are clearly derivatives of it by failure of formation of one or more chiasmata. The nuclei are classified in Table I according to chiasma number and the points at which chiasma failure is believed to have occurred, equivalent points (see above) being neglected.

Chiasma Frequency. The occurrence of chiasma failures (Table II) at points other than A and B may be obtained from the data in Table I. Inability to distinguish certain pairs of points should not obscure any real discrepancies. Some 44 breaks may be assigned definitely to particular points. A further 11 breaks cannot be definitely placed (cf. Table I) but it is probably satisfactory to divide them equally between the possible locations. The figures show that there is no predisposition towards the occurrence of breaks at particular points in the ring, for (from Table II) $\chi^2 = 4.6$, $n = 5$ and P lies between 0.3 and 0.5, so that the deviations are not significant. At A and B where the conditions are rather special, total failure of chiasma formation occurred in 14 cases. Though greater than the mean (9.17) this figure is equal to the highest individual failure at pairs of points in the ring, i.e. at H and K. This is probably not significantly different from the frequencies at the other pairs of points.

The two sets of three homologous segments at A and B mutually interfere in pairing and hence there may be 2, 1 or 0 chiasmata at these points. NEWTON and DARLINGTON (1929) have shown that in trivalents only two chromosomes pair at any one point, hence the

possible length in which chiasmata can be formed is the same as in a normal bivalent. For this reason, too, chiasma numbers are very little higher in triploids than in diploids (CATCHESIDE, 1931*a*). We may therefore expect a similar frequency distribution of chiasmata to that in bivalents. It is possible at metaphase I to count up to four chiasmata at A and B together; the figures in Table III show the respective numbers of chiasmata in the two pairing regions of the chromosomes. The mean number of chiasmata per „trivalent" group is 2.31 a value which is very close to that of 2.27 found at late diplotene in the homozygous diploid *Oenothera blandina*. All the cases except two (D(*b*) and E in Table I) can be placed definitely in one class or another. Of the unplaced examples, D(*b*) could be either 2/1 or 2/0 while E could be either 1/1 or 1/0. It is probably better to omit them from the computations; this 2.5% omission is not likely to introduce an error even equal to, let alone greater than that due to sampling. In Table IV the figures have been grouped as occurrence of 0—4 chiasmata in the two regions combined. The calculated figures have been obtained on the assumption that the proportions in the different classes should conform to a Poisson series (HALDANE, 1932). They seem to show interference similar to that in the case of the bivalents of an homologous diploid, for the divergences are highly significant and the variance (0.34) is considerably less than the mean (2.31). But the two cases areprobably somewhat different, since in the trisomic a triple chiasma signifies the presence of two non-recurrent chiasmata in the same arm of one of the chromosomes. We have no data on the relative frequency of recurrent and non-recurrent chiasmata.

Tetraploidy. One loculus contained 14 tetraploid (4n + 2) cells at metaphase II; they must be the result of somatic doubling at least five divisions prior to meiosis. Similar tetraploid tissue has been found by other authors, e.g. VERBRUGGE (1933). The frequencies of different distributions of chromosome numbers were found to be as follows:

Distribution	No. of cells
13+17	1
14+16	4
15+15	9

Two cells have been figured, a 15+15 distribution at fig. 3*a* and a 14+16 distribution at fig. 3*b*. Such tetraploid cells would yield functional gametes no more frequently than a corresponding entirely tetraploid sporophyte. The growth of diploid pollen tubes is reduced in diploid styles and therefore they are relatively unsuccessful in competition with haploid ones (WATKINS, 1932). Hence, sporadic somatic doubling just prior to meiosis, with reduction of a tetraploid nucleus can hardly be the usual source of triploids and tetraploids in *Oenothera*.

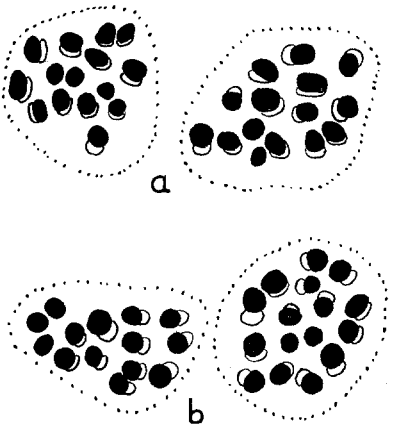


FIG. 3. *Oenothera nutans* mut. *nana*. Pollen mother cells at metaphase II, taken from tetraploid tissue. (*a*) 15+15, (*b*) 14+16 distributions × 3300.

Oenothera h-blandina . paenevelans mutant *lata*

Origin and Genetics. Selfed seeds of the trisomic *O. Lamarckiana pallescens* grown in 1930, yielded two mature plants. One was a presumed half-mutant agreeing in all particulars with *O. rubrinervis*, and having a ring of six chromosomes and four ring pairs (CATCHESIDE, 1931*b*); the other was a dwarfish plant which matured late and from which no cytological material was obtainable. The former plant was selfed and also pollinated with the homozygote *O. blandina*. The *lata* trisomic arose in the F_1 hybrid family.

Of 244 selfed seeds of *O. rubrinervis*, 163 had large healthy-looking embryos, while 81 were quite empty; there was thus 67% good seeds. In the case of the F_1 hybrid of *rubrinervis* with *blandina*, on the contrary, 273 out of 287 seeds had good embryos, while 14 were quite empty; here there were 96% good seeds.

One selfed family of the supposed half-mutant was germinated, well over 100 seedlings being obtained from the seeds of one capsule. About 20 plants were put out, but owing to their excessive brittleness several were damaged and only 16 could be classified finally. There was considerable uniformity with respect to most characters,

and it was only at the flowering stage that division into two types was possible. Ten plants (*rubrinervis*) had relatively broad bracts more or less strongly crinkled like the foliage leaves; six plants (*deserens*) had relatively narrower bracts, and both the bracts and the leaves were smoother than in *rubrinervis*.

The *rubrinervis* type had a ring of 6 chromosomes and 4 ring pairs like the parent plant, while *deserens* had 7 ring pairs at meiosis. The latter is therefore cytologically a homozygote and must be a segregate arising from mating of two a-lethal gametes of the same type. It is known that trisomic *pallescens* gives, besides *pallescens* and *Lamarckiana* and other forms, a small proportion of *rubrinervis* (DE VRIES, 1911); while *rubrinervis* regularly segregates homozygous *deserens* (DE VRIES, 1919). The constitution of *rubrinervis* is *subvelans* . *paenevelans* according to HOEPPENER and RENNER (1928), while *deserens* is *subvelans* . *subvelans*; *paenevelans* . *paenevelans* is non-viable and represented by empty seeds.

The hybrid population of *O. rubrinervis* × *blandina* F_1 germinated well, over 100 seedlings being obtained from the seeds of one capsule. It was early found possible to distinguish two well marked types among the young rosettes; one resembling *rubrinervis*, was commoner then the other, which more nearly resembled *blandina*. The proportions were about 3 : 2, but absolutely accurate analysis was not possible at so early a stage. Not all the plants were grown to maturity, 45 only being put out; these included one rosette, strikingly different from the rest, which was classified as mutant *lata*. All the plants put out were brought to maturity with ease, the stems of every one being tough (in contrast to those of the seed parent). Of the 44 diploid plants, 28 belonged to the *rubrinervis* type while the other 16 belonged to the *blandina* type.

The most important differences between the two types lay in the rosette leaf characters. Those of the *rubrinervis* type were relatively short and broad (av. 15×4 cms.), deep green in colour, strongly crinkled, acuminate, glabrous and shiny and with a white midrib. The leaves of the *blandina* type were longer and relatively narrower (av. 20×4.5 cms.), light green (the young leaves yellow green) smoother acute hairy and with a dull surface midrib white. The *rubrinervis* type was less branched, the branches being mostly suprabasal and the inflorescence was relatively lax. The *blandina* type had

a ring of basal branches like *blandina* and the inflorescence was relatively dense. No other striking differences could be noted, but these were more than sufficient to separate the plants into the two classes. The *rubrinervis* type undoubtedly has the constitution *subvelans* . h-*blandina*, while the paler plants, more closely similar to *blandina*, are *paenevelans* . h-*blandina*.

These two types had different linkages at meiosis. *Subvelans* . h-*blandina* had a ring of 6 chromosomes and 4 ring pairs (fig. 4*a*); *paenevelans* . h-*blandina* a ring of 4 chromosomes and 5 ring pairs (fig. 4*b*). As in *rubrinervis*, the meiotic conditions clearly demonstrate that the attachments of the chromosomes to one another are true chiasmata. For, in the case of strictly terminal attachments, the connection was seen to be double in every case suitable for examination, and it therefore represented a terminal chiasma. When the association was more or less interstitial, clear exchanges of partners between four chromatics could often be seen, showing that these points are

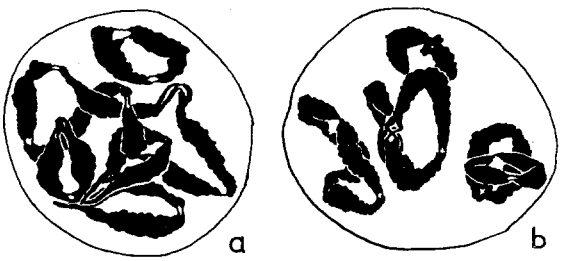


FIG. 4. Pollen mother cells at diakinesis. (*a*) *subvelans* . h-*blandina*, showing ring of 6 chromosomes and 4 pairs; (*b*) *paenevelans* . h-*blandina*, showing ring of 4 chromosomes and 5 pairs. × 3360.

true interstitial chiasmata. Examples of both conditions are shown in the two figures. The linkage found for *subvelans* . h-*blandina* agrees with that determined for the F_1 of *deserens* × *blandina* (GATES and CATCHESIDE 1931, 1932) and may be regarded as confirmatory evidence of the correctness of the identifications.

The *lata* mutant was strikingly different from the remainder of the culture at all stages. The leaves were a medium clear green colour, different from the deeper green of *subvelans* . h-*blandina* and the yellow green of *paenevelans* . h-*blandina*. The leaves were rather

short, 11 × 4.5 cms., strongly crinkled, mucronate, hairy and with a red ege; the midribs were white above and below. The bracts were similar and very broad. The green stem was stout and fleshy, but rather weak and brittle, finally reaching about 0.5 m. in height; it had numerous red papillae and very short patent hairs. Branches were relatively few, distinctly short, and exclusively suprabasal. The buds were very stout, short and conical, yellow-green in colour and shortly hairy; the sepal tips were green and erect or practically appressed. Flowering commenced July 20, the petal size being 34 × 32 mms; the petals were markedly crumpled. The stout green capsules had four broad red stripes. Few or no seeds were set on selfing, but plenty when the plant was pollinated with *blandina*; these seeds, however, have failed to germinate.

At meiosis, the maximum configuration found was a ring of four chromosomes attached to a ring pair by a seventh chromosome (fig. 5*a* and 7*d*), with a triple chiasma at each end. The remaining eight chromosomes usually formed four ring bivalents, but there was an occasional rod bivalent, due to failure of chiasma formation between one pair of arms. The nature of the maximum catenation shows that the trisomic is *paenevelans* . h-*blandina* plus one of the *subvelans* ring-forming chromosomes. Recognition of this gives a clue to the type of non-disjunctional configuration giving rise to the 8 chromosome gamete which mated to a h-*blandina* gamete, gave the present trisomic. This is fully described in the discussion.

The different types of configuration observed, together with their frequencies, are listed in Tabel V. It is obvious that every configuration found may be derived from the maximum by failure of one or more chiasmata (and hence terminal unions) at different points. The types selected for illustration are marked in Table V with the number of the figure.

As in the diploid forms, the association of the chromosomes at metaphase I is usually by terminal chiasmata. Terminal connections between two chromosomes in all favourable cases may be seen to be double; terminal connections between three chromosomes may often be observed to be triple, e.g. figs. 5*a*, *g* and *m*. These observations confirm the situation found in diploids and triploids, and demonstrate that the connections are true (terminal) chiasmata. Occasionally, as in figs. 5*c* and *k*, a chiasma may be slightly interstitial; this occurs

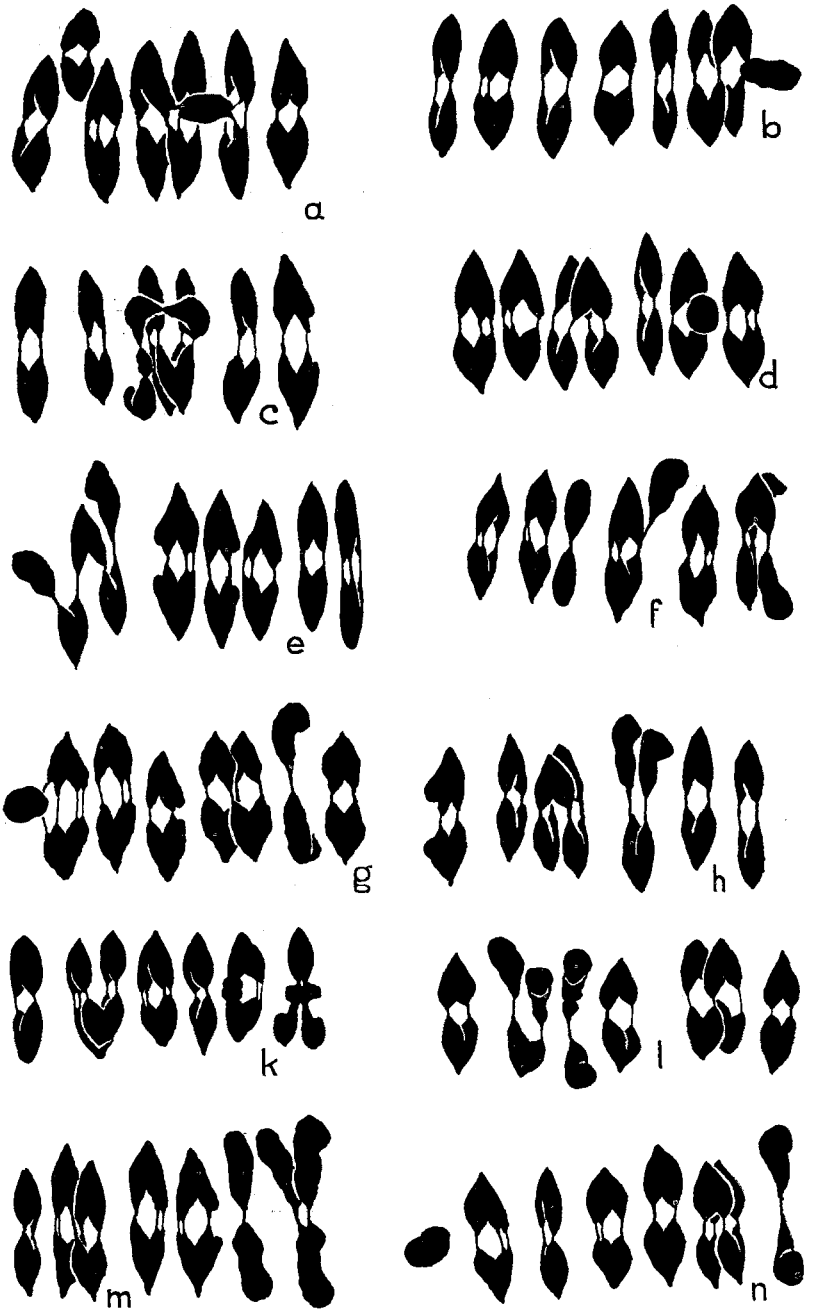


FIG. 5. *Paenevelans* . h-*blandina* mut. *lata*. Pollen mother cells at metaphase I in profile view. Descriptions in text. × 4200.

TABLE V. TYPES OF CHROMOSOME CONFIGURATION AT MEIOSIS IN *O. paenevelans* . h-*blandina* MUT. *lata*

Configuration.	Fig. no.	No. of p.m.cs.	No. of Xta at H	No. of Xta at A
A. 16 chiasmata. (4 cells).				
④—(1)—②+4②	5a	4	2	2
B. 15 chiasmata. (28 cells).				
④—①—②+3②+(2)	—	1	2	2
④—(1)+5②	5b	6	2	1
④—(3)+4②	5c	5	2	1
④+②—(1)+4②	5d	16	1	2
C. 14 chiasmata. (92 cells).				
(7)+4②	—	1	1	1
(5)+5②	5e	9	1	1
(4)+②—(1)+4②	5f	5	0 or 1	2
④+②—(1)+3②+(2)	5g	1	1	2
④+5②+(1)	—	26	1	1
④+(3)+4②	5h	35	1	1
④+Y-trivalent+4②	5k	15	1	2
D. 13 chiasmata. (26 cells).				
(4)+(3)+4②	—	4	0 or 1	1
④+(3)+3②+(2)	5l	3	1	1
(4)+Y-trivalent+4②	—	1	0 or 1	2
④+Y-trivalent+3②+(2)	5m	1	1	2
(4)+5②+(1)	—	1	0 or 1	1
④+4②+(2)+(1)	5n	15	1	0 or 1
5②+(2)+Y-trivalent	—	1	2	1
E. 12 chiasmata. (4 cells).				
④+(3)+2②+2(2)	—	2	1	1
(4)+4②+(2)+(1)	—	2	0 or 1	0 or 1
F. 10 chiasmata. (1 cell).				
(4)+Y-trivalent+2②+(2)+2(1)	—	1	0 or 1	2
Total		155		

more frequently in trivalents than in bivalents. In the case of a Y or ring-and-rod trivalent, two of the chromosomes are often associated interstitially and the third terminally. The structure of the trivalent in fig. 5*k* is exceptional and probably anomalous. Apparently, two chiasmata have been formed, one between paired terminal segments of two chromosomes (A and B) and the other between an interstitial segment in A and an homologous terminal segment of C. The structure is similar to that of the unequal bivalent occurring in haploid *Oenothera blandina* (CATCHESIDE, 1932).

Disjunction is subject to the usual anomalies characteristic of trisomic and ring-forming plants. Non-disjunction of the ring or chain of four is, however, quite exceptional. Lagging and division of a univalent is frequent and chromosomes associated by interstitial chiasmata also lag on the spindle in a characteristic manner, pending the time when the chiasma will be fully terminalised. Clear examples are shown in figs. 6*a* and *b*. The drawings show stages in the resolution of interstitial chiasmata which leave no doubt of the real nature of the associations. The figures of GATES and THOMAS (1914) may be compared, since they show identical and conditions in other trisomics.

Breakdown of the double terminal attachments does not always occur simultaneously in both of the connections. In a number of cases (figs. 5*c*, *e*, *g*, *h* and *m*), one of the connections is broken, while the other is still intact. The two chromatids whose connection has been separated into its components, have contracted backwards away from each other and their ends have become rounded. It is significant that the first breakdown occurs between the „outside" pair of chromatids. Since they occur on the outside, it is easily conceivable that the greater length they have to occupy results in a greater longitudinal tension within them. Hence, the forces tending to separate them will exceed those holding them together, before the same limiting condition is reached in the case of the inside pair. It is also quite possible that the outside connection could break down before metaphase if, by any mechanical chance, the tension between the two chromatids, usually delicately adjusted to a value below the attractive force, were to increase much above the normal. The terminal chiasma mechanism thus appears to be exceedingly finely adjusted to answer two purposes: (1) to maintain the attachment of the chromosomes until they are properly orientated on the spindle and (2) to

TABLE VI. CHIASMA FREQUENCIES IN THE TRIPLICATED SEGMENTS (A AND H) OF *O. paenevelans* . h-*blandina* MUT. *lata*

	Segment A			Segment H		
No. Xta per set of 3 segments.	2	1	1 or 0	2	1	1 or 0
No. cases.	45	93	17	17	124	14
Xa frequency per set of three segments:						
Maximum.		1.29			1.11	
Minimum.		1.18			1.02	

TABLE VII. TOTAL CHIASMATA AT A AND H IN *O. paenevelans* . h-*blandina* MUT. *lata*, COMPARED WITH EXPECTATION IN THE ABSENCE OF INTERFERENCE

No. of chiasmata per three sets of two segments (A and H) each.	0	1	2	3	4
Observed frequency.	2	20	83	45	5
Calculated frequency.	17.2	37.8	41.5	30.4	(28.1)

$\chi^2 = 98.6$, n = 3 and $P < 0.01$; variance = 0.64.

secure rapid disjunction by a relatively slight increase in the force of repulsion between the paired chromosomes. Any considerable lagging, as in the case of bivalents with interstitial chiasmata, might result in a high proportion of restitution nuclei and hence diploid gametes, with a consequent disturbance in the process of sexual reproduction and in the stability of the species.

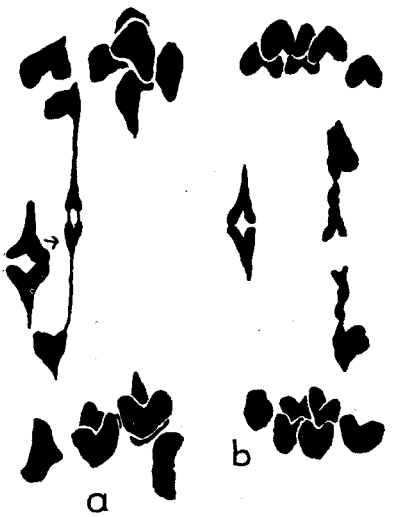


FIG. 6. *Oenothera paenevelans* . h-*blandina* mut. *lata*. Pollen mother cells at anaphase I, showing lagging bivalents and dividing univalents. × 3360.

In the *lata* trisomic, the extra chromosome, AH (for nomenclature, see discussion), can nearly always be identified precisely. „A" is the segment pairing with segments in the bivalent, „H" that pairing with segments in the ring of four. As shown in the right-hand columns of Table V, the numbers of chiasmata formed between the triplicated segments can be counted separately. The frequencies of 2, 1 and 1 or 0 chiasmata in A and H are summarised in Table VI, which shows also the maximum and minimum metaphase chiasma frequencies in the two segmental regions. In certain cases it is not possible to decide whether a particular segment (A or H) has 0 or 1 chiasmata; the maximum frequency is calculated on the assumption that all these cases mean 1 chiasma, the minimum on the counter assumption that they mean 0 chiasmata. It is clear that the minimum frequency for A is higher than the maximum frequency for H. Further, two chiasmata occur with a considerably higher frequency between the three A segments than between the three H segments. Hence, the chiasma frequency between three homologous segments is higher when two of them belong to bivalent-forming chromosomes than when the two belong to ring-forming chromosomes. This conclusion is in agreement with the finding that there is a higher frequency of chiasmata, per pair of pairing segments, in bivalents than in larger rings in various

hybrid combinations in *Oenothera* (CATCHESIDE, 1933). Both facts probably mean that pairing end-segments are longer in bivalents than in rings and so are capable of forming more chiasmata.

The figures may also be assembled to show interference between chiasmata, similar to that in *O. nutans* mut. *nana*. In Table VII, the frequencies in A and H are taken together, and in each of the disputed (0 or 1 chiasmata) cases, the lower figure has been adopted. This has the effect of increasing the dispersion of the figures, and so reducing any appearance of interference. The mean number of chiasmata on this basis is 2.2 (note that the maximum possible mean is 2.4), and the variance is 0.64; interference is therefore marked. Further analysis, by counting half-chiasmata per chromosome, does not add materially to our knowledge owing to the wide range of possibilities.

DISCUSSION

Chromosomal Interrelationships of some of the Complexes

In the following account, each letter signifies one arm (or pairing segment) of a chromosome, and each arm has a different homology. Each pair of letters represents one chromosome, made up of two arms. Identical arms, described by the same letter are capable of pairing and therefore of leading to an association of the chromosomes possessing them.

We know (GATES and CATCHESIDE, 1932) that if h-*blandina* is

A . B C . D E . F G . H K . L M . N O . P

then h-*deserens* (= *subvelans*) is

B . C D . G A . H E . F K . L M . N O . P

Paenevelans gives with h-*blandina* a ring of 4 and 5 pairs, and with *subvelans* a ring of 6 and 4 pairs. *Paenevelans* therefore differs from *subvelans* by two exchanges involving the three chromosomes B . C, D . G and A . H; further, *paenevelans* must possess one of A . B, C . D and G . H. The possible formulae for *paenevelans* are therefore

1. A . B C . G D . H
2. C . D A . G B . H } E . F K . L M . N O . P
3. G . H A . C B . D

Subvelans and *paenevelans* may be compared with the two com-

plexes of *rubricalyx*, the possible formulae of which have been obtained by GATES and CATCHESIDE (1932), as follows

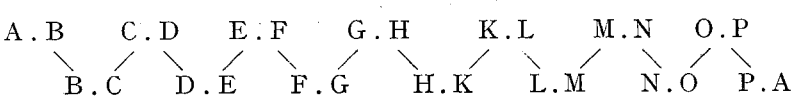
rubricalyx α	1.	A . B D . G C . H	E. F K . L M . N O . P
	2.	C . D A . H B . G	
rubricalyx β	1.	D . E F . G A . H	K . L M . N O . P B . C
	2.	D . F E . G A . H	
	3.	D . G A . F E . H	
	4.	D . G A . E F . H	

Rubricalyx α corresponds to *paenevelans*, *rubricalyx* β to *subvelans*. Although the chromosomes cannot be definitely identified, it is clear that *rubricalyx* β differs from *subvelans* by one exchange involving two chromosomes, while *rubricalyx* α probably differs from *paenevelans* also by one exchange. Therefore *O. rubricalyx* has undergone sundry segmental interchanges in the course of its history, perhaps since the dominant red bud and stem colour arose as a point mutation. Possibly, the American strain with a ring of 8 and 3 pairs is an offshoot at some point. A thorough knowledge of the chromosomes should enable us to reconstruct the cytological history of *O. rubricalyx*.

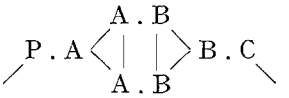
Non-disjunction and the Genesis of Trisomics

It is obvious, from this and other recent studies of *Oenothera* trisomics, that all the chromosomes of both complexes are represented once and one of them twice. No trisomics, with a few possible exceptions (cf. HÅKANSSON, 1930) so far found, have been deficient in one chromosome and duplicated in respect of two others. The configurational types, as will be seen below, establish this point.

The simpler type of configuration, characteristic of *O. nutans nana*, the primary trisomics of *O. Lamarckiana* (viz. *lata*, *pallescens lata*, *dependens*, *stricta*, *longepetiolata*, *pallescens*, *liquida* and *pulla* (HÅKANSSON, 1930) all of which segregate *Lamarckiana*) and the trisomics studied by GOODWIN (1933) and VERBRUGGE (1933), is illustrated diagrammatically at fig. 7*a*. We may conveniently label the ringforming chromosomes of diploid *O. nutans* as follows:



Supposing that the extra chromosome in *O. nutans nana* is A . B, then the configuration in the trisomic is represented by



Clearly, then, three chromosomes (P . A, A . B and B . C) adjacent in the ring have passed into the same gamete; this means that they

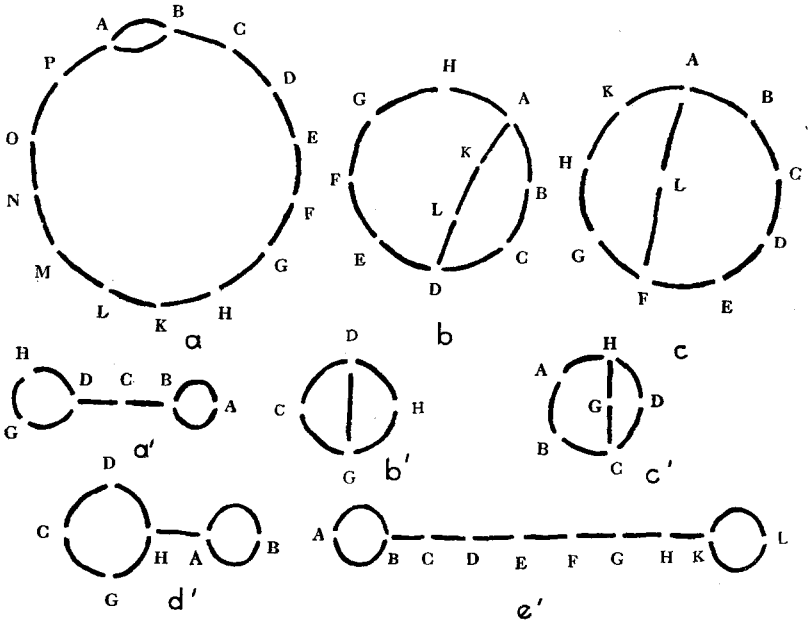
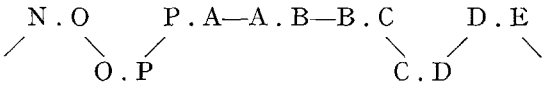


FIG. 7. Diagrams of configurations in trisomic Oenotheras: (*a*) type II (*O. nutans nana*); (*b*, *b'*, *c* and *c'*) type III; (*d*) type IV (*O. paenevelans* . h-*blandina lata*); (*d'*) type IV; (*e*) type IV (*O. Lamarckiana curta*). Full descriptions in text.

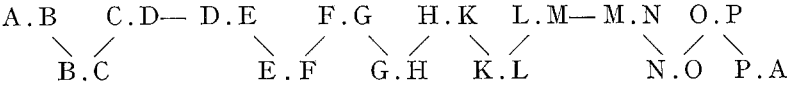
have disjoined together at anaphase I. The necessary disjunctional arrangement is



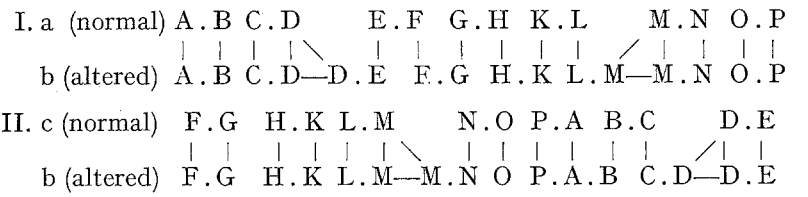
and is probably met in practice by the familiar non-disjunctional type in which the chromosomes P . A and B. C are suspended between

A . B and O . P and C . D respectively (SHEFFIELD, 1927), or else by non-disjunctional chains of chromosomes. The requirements are fully realised only if A . B passes to the same pole as P . A, B . C, D . E, etc.

The other frequent non-disjunctional type, leading to an 8+6 distribution of the chromosome, is that styled double non-disjunction on the same side. In it the chromosomal arrangements would be of the form



The 8 chromosomes gamete consists of a mixture of the two complexes and, with normal gametes, would give rather complicated configurations as follows:



The essential structure here consists of two bivalents joined by an odd-numbered chain of chromosomes (cf. fig. 7*e*), the length of which depends on the relative positions of the non-disjunctions. The only case I can find is *O. Lamarckiana curta* HERIBERT-NILSSON (HÅKANSSON, 1930), in which the maximum configuration appears to be ②—(7)—②+2②. This trisomic is constant, not segregating *Lamarckiana*. The reason is clear if one considers the probable chromosomal construction to be that given above as I. The functional gametic types are essentially two only, the unaltered (a) and the altered (*b*); of the zygotic types, aa is inviable since a is lethal in the homozygous condition, ab repeats the trisomic, while bb also may be inviable. In any case bb does not represent the parental form, which cannot reappear.

O. Lamarckiana nitens DE VRIES, studied by HÅKANSSON (1930), appears to be different, since it splits off a diploid form, *distans*, which has a ring of 8 and 3 pairs. It is probably one of the normal type, consisting of a chain of seven chromosomes closed by a ring pair. In its origin there seems to have been a combination of seg-

mental interchange, like that leading to half-mutant formation (cf. DARLINGTON, 1931), with non-disjunction. It is only able to segregate the new interchange form, *distans*, in F_2 and the parent, *Lamarckiana*, cannot reappear.

The origin of *O. paenevelans*. h-*blandina* mut. *lata* may be explained satisfactorily by supposing that three adjacent chromosomes in the ring of six of *rubrinervis* had disjoined to the same pole. If we select the first of the three possible formulae for *paenevelans* (the differences are irrelevant to our present purpose) then *paenevelans*. h-*blandina* is:

paenevelans A . B C . G H . D E . F K . L M . N O . P
h-*blandina* A . B G . H D . C E . F K . L M . N O . P

The extra chromosome, from *subvelans*, must therefore be either B . C or A . H (assumed to be the latter in fig. 7*d*). The presence of D . G would give a different and quite novel configuration of the type shown in fig. 7*b'*; the actual configuration would consist of a ring of four chromosomes linked from opposite points (D and G) by a fifth chromosome. Supposing that B . C is the extra chromosome, then the disjunctional type would be the well-known configuration:

D . H A . B—B . C—C . G
H . A G . D

It is figured at text fig. 4*c* in an earlier paper (CATCHESIDE, 1931). Double non-disjunction on the same side in the rin gof *rubrinervis* would not yield the configuration observed, but either a ring of three attached to a ring of two by a chain of two chromosomes (fig. 7*d'*), or a ring of five chromosomes with a chain of two joining the ring at two points two chromosomes apart (fig. 7*c'*). No trisomics of these types have been discovered so far, but odd-numbered closed rings certainly seem possible in trisomics of immediately hybrid parentage.

Types of Configurations in Oenothera Trisomics

In view of the complications of structural hybridity, it seems preferable to classify *Oenothera* trisomics broadly according to the maximum type of configuration which they show at meiosis. This has been attempted in the following scheme, in which the important unit is the chromosome segment rather than the whole chromosome.

Type I. The extra chromosome is a bivalent-forming chromosome in the diploid; the three chromosomes therefore form a trivalent of which the „triple arc" is the maximum. A secondary trisomic from *O. Lamarckiana* mut. *pulla* DE VRIES, described by HÅKANSSON (1930), appears to belong to this category, though the critical „triple arc" was never observed. Could occur only in selfed populations. [1])

Type II. The extra chromosome is a ring-forming chromosome in the diploid. The configuration in the trisomic (fig. 7*a*) shows the extra chromosome connecting two adjacent points in the ring; it could occur only in selfed populations. Examples: *O. Lamarckiana* muts. *lata, cana, pallescens,* (HÅKANSSON, 1926, 1930); *O. Lamarckiana* mut. *cana* (GOODWIN, 1933); *O. rubricalyx* β. h-*blandina* mutant (VERBRUGGE, 1933) and *O. nutans* mut. *nana.*

Type III. Two points in an even (or odd) numbered ring are joined by an odd (or even) numbered chain of chromosomes (figs. 7*b*, *b'*, *c* and *c'*). No examples are known yet and they could appear only in trisomics of immediately hybrid parentage. The extra chromosome would be a ring-forming chromosome in the parent contributing the 8-chromosome gamete.

Type IV. The triplicated segments occur in different rings of chromosomes (containing two or more chromosomes). The configuration therefore consists of two rings of chromosomes, either bivalents or larger rings (not necessarily of the same size) connected by one chromosome (fig. 7*d*) or an even (fig. 7*d'*) or odd numbered chain (fig. 7*e*) depending on whether the extra segments are in the same or different chromosomes. *O. paenevelans* . h-*blandina* mut. *lata,* described above, belongs here; its configuration is represented diagrammatically at fig. 7*d*. *O. Lamarckiana* mut. *curta* HERIBERT NILSSON (cf. HÅKANSSON, 1930) possibly has two ring pairs connected by a chain of seven chromosomes, together with two free pairs (cf. fig. 7*e*) and may have arisen through double non-disjunction on the same side. It does not segregate *O. Lamarckiana.* This type of trisomic may appear in selfed lines following double non-disjunction on the same side, or in hybrid lines following any non-disjunctional method giving viable 8-chromosome gametes.

[1]) This type has been found by Mr. E. D. SWEET in a trisomic appearing in the F_2 of *deserens. nutens* from *O. deserens* × *nutans*; it has (10) + (2) + triple arc as maximum configuration.

SUMMARY

The chromosome associations at meiosis in two *Oenothera* trisomic plants have been studied. Both exhibited characteristic variations based on a maximum configuration and derived from it by failure of one or more of the possible unions. In mutant *nana* from *O. nutans* (ring of 14 chromosomes at meiosis) the maximum was probably a chain of 13 chromosomes closed by a ring pair. In a *lata* mutant arising in the F_1 of *rubrinervis* (ring of 6 and 4 pairs) $\times$ *blandina* (7 pairs) the maximum was a ring of 4 joined to a ring of 2 by a seventh chromosome, and accompanied by 4 ring pairs. Triple unions (chiasmata) occur where three chromosomes meet. *Rubrinervis* $\times$ *blandina* gave twin types in the F_1, namely *subvelans* . h-*blandina* (ring of 6 and 4 pairs) and *paenevelans* . h-*blandina* (ring of 4 and 5 pairs); the *lata* mutant was therefore *paenevelans* . h-*blandina* plus a *subvelans* chromosome from the ring in *rubrinervis*.

The non-disjunctional arrangements, giving rise to the 8-chromosome gametes which are the source of these trisomics, must satisfy the condition that the 7 chromosomes constituting a particular complex pass, with one chromosome of the other complex, to the same pole. These trisomics are capable of segregating the parental form (or corresponding hybrid in the cases of trisomics of immediately hybrid parentage). The majority of known trisomics come into this category.

Eight chromosome gametes, formed through double non-disjunction on the same side at anaphase, should theoretically be capable of yielding trisomics. These would have a special type of configuration, and be incapable of segregating the parental type. The evidence for their occurrence is uncertain.

A number of points concerning metaphase chiasma frequencies have been deduced from the data, particularly of the two points at which triple chiasmata can be formed. The figures can be assembled

to show cytological interference, the significance of which is here doubtful owing to our lack of knowledge of zygotene pairing in relation to chiasma frequency in trivalents, when combined with terminalisation. Chiasma failure appears to be at random in *O. nutans* mut. *nana*. In *O. paenevelans*. h-*blandina* mut. *lata* the metaphase chiasma frequency is higher between three homologous segments when two of them take part in bivalent-forming chromosomes, than when two of them take part in ring-forming chromosomes.

I am indebted to Prof. R. R. GATES F.R.S. for several useful criticisms during the course of the work.

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