

Statistical analysis of chromosome distribution to the poles in interspecific hybrids with variable chromosome pairing

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The behaviour of unconjugated chromosomes has been studied mainly in haploids and polyhaploids of the Triticeinae. Riley & Chapman (1957) have recently reviewed the literature on this subject; it has been found that during the first meiotic division univalents are distributed to the poles either at random or that distribution was numerically more equal. Person (1955) and Riley & Chapman (1957) interpreted the observations as consequences of the various pairwise associations which chromosomes can undergo. No study of the subject has yet been attempted in *Nicotiana*. An investigation of bivalent frequencies in various hybrids and haploids of this genus by Sficas & Gerstel (1962) provided the material for the analysis of univalent behaviour. Though the observations were made at a time when the conjugated pairs were lying across the centre of the spindles all the univalents were frequently located near the poles; this stage may be termed meta-anaphase following Tometorp (1939) and Person (1955). Only those cells out of the total sample were used in which both the number of bivalents and the univalent distribution to the poles could be clearly identified; cells with univalents near the plate were excluded from consideration.

A probability distribution was developed for testing randomness of the distribution of univalents and the observations were fitted to the theoretical expectations.

1. THE PROBABILITY DISTRIBUTION

Let n_1 be the number of chromosomes from one species and n_2 from the other, then the total number of chromosomes in the hybrid will be $N = n_1 + n_2$. Bivalents give an equal number of chromosomes to the poles and univalents will be assumed to be distributed at random to either pole. The probability that a particular univalent will move to one of the poles is $1/2$, hence, the probability that u univalents will be distributed to the two poles as x and $u - x$ without identifying poles, will be

$$\begin{aligned} D_x &= 2 \binom{u}{x} \left(\frac{1}{2}\right)^u \quad \text{for } x < \frac{u}{2} \\ &= \binom{u}{x} \left(\frac{1}{2}\right)^u \quad \text{for } x = \frac{u}{2} \end{aligned}$$

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If i bivalents have been formed, then $u = N - 2i$ and the distribution of chromosomes to the poles will be

$$\begin{aligned}
 D_{i+x} &= 2 \binom{u}{x} \left(\frac{1}{2}\right)^u \quad \text{for } x < \frac{u}{2} \\
 &= \binom{u}{x} \left(\frac{1}{2}\right)^u \quad \text{for } x = \frac{u}{2}
 \end{aligned}
 \tag{1}$$

Equation (1) can be used to calculate the distribution of chromosomes to the poles if the same number i of bivalents is observed in all PMC's. If the number of bivalents varies from cell to cell the same equation could be used as an approximation to calculate the expectations by substituting the mean number of bivalents for i . An exact distribution for this later case will be developed. Let the probability that i bivalents will be formed be B_i ($i = 0, 1, \dots, N/2$, when N is an even number, and $(N - 1)/2$, when N is an odd number). The probability that zero chromosomes will go to one pole and all N chromosomes to the other will be

$$D(0, N) = 2\left(\frac{1}{2}\right)^N B_0$$

The probability that the distribution of chromosomes to the poles will be as 1 and $N - 1$ is

$$D(1, N - 1) = 2 \binom{N}{1} \left(\frac{1}{2}\right)^N B_0 + 2 \binom{N - 2}{0} \left(\frac{1}{2}\right)^{N - 2} B_1$$

and, in general, for $y < N/2$

$$D(y, N - y) = 2 \binom{N}{y} \left(\frac{1}{2}\right)^N B_0 + 2 \binom{N - 2}{y - 2} \left(\frac{1}{2}\right)^{N - 2} B_1 + \dots + 2 \binom{N - 2y}{0} \left(\frac{1}{2}\right)^{N - 2y} B_y \tag{2}$$

and for $y = N/2$, N being an even number

$$D\left(\frac{N}{2}, \frac{N}{2}\right) = \binom{N}{y} \left(\frac{1}{2}\right)^N B_0 + \binom{N - 2}{y - 2} \left(\frac{1}{2}\right)^{N - 2} B_1 + \dots + \binom{N - 2y}{0} \left(\frac{1}{2}\right)^{N - 2y} B_y \tag{3}$$

On the basis of equations (2) and (3) numerical values for different N 's can be calculated as follows for the case of N being an even number.

(1) For $N = n_1 + n_2 = 2$:

$$\begin{aligned}
 D(0, 2) &= B_0(0.5000) \\
 D(1, 1) &= B_0(0.5000) + B_1(1.000)
 \end{aligned}$$

(2) For $N = n_1 + n_2 = 4$:

$$\begin{aligned}
 D(0, 4) &= B_0(0.1250) \\
 D_0(1, 3) &= B_0(0.5000) + B_1(0.5000) \\
 D_0(2, 2) &= B_0(0.3750) + B_1(0.5000) + B_2(1.000)
 \end{aligned}$$

Following the above pattern a table of the coefficients of the B 's was calculated for $N = 24$ (Table 1a). This table can be used for any even number of $N \leq 24$ as follows. Let $\mathbf{D} = \text{col}[D(0, 2i) \ D(i, 2i - 1) \ \dots \ D(i, i)]$ be the column vector of the

Following the pattern Table 1*b* was calculated for $N = 19$. This table can be used for any odd number $N \leq 19$.

An approximate method can be suggested by considering as 1:1 separation of a number of chromosomes equal to the mean number of bivalents and assuming random movement of univalents. For this case formula (1) can be applied, where i is the closest to the mean bivalents integer.

Using formulae (1) and (2) (or the tables) the expected distribution is calculated for any experiment and compared with the observed one by means of a χ^2 test with degrees of freedom equal the number of classes minus one. The last low frequency classes are pooled to an expected number ≥ 5 .

If the number of chromosomes of the hybrid is large, the test of the randomness of movement of the univalents can be made by the use of the second moment about $D(N/2, N/2)$. A peaked distribution will have a smaller second moment and a flattened distribution a higher second moment than the expected. If m_2 stands for the second moment and assuming random movement of univalents to the poles, the distribution of u univalents will follow the binomial distribution with $p = 1/2$. The expected second moment about $(u/2, u/2)$ will be

$$\text{Exp. } m_2(u/2, u/2) = u(1/2)(1/2) = u/4$$

Since $u = N - 2m$, where m is the mean number of bivalents, the expected second moment about $D(N/2, N/2)$ equals to that about $D(u/2, u/2)$, hence

$$\text{Exp. } m_2(N/2, N/2) = (N - 2m)/4 \quad (5)$$

A test of significance can be performed by means of χ^2 or F

$$\chi^2_{(c-1)} = F_{(c-1, \infty)} = \text{Obs. } m_2 / \text{Exp. } m_2 \quad (6)$$

with degrees of freedom equal to $c - 1$ (where c is the number of PMC's) for χ^2 , and $c - 1$ and ∞ for F .

When N is an odd number the second moment is calculated about

$$D\left(\frac{N-1}{2}, \frac{N+1}{2}\right)$$

and has the same expected value as in (5).

The above relations are also valid when trivalents are formed by considering each trivalent as one bivalent, since the third chromosome involved will move at random to either pole. A quadrivalent should be counted as two bivalents since the distribution to the poles of its chromosomes will be $D(2, 2)$.

The problem can be reversed when it is desired to calculate the bivalent distribution given the chromosome distribution to the poles. This can be obtained by using equations (2) and (3) (or the tables) and solving backwards for the B 's. This solution is valid if it is assumed that univalents move at random to the poles.

2. EXPERIMENTAL RESULTS

(i) *N. glutinosa* ($n = 12$) \times *N. sylvestris* ($n = 12$)

In 848 PMC's out of a total of 1337, examined for bivalent frequency by Sficas & Gerstel (1962), the chromosome distribution to the poles was clear and could be

recorded; the remaining cells had a number of univalents lying at the metaphase plate and could, therefore, not be scored. These 848 PMC's came from four samples, namely, from two plants and two dates of sampling for each plant. The observed data are given in Table 2 together with their statistical analysis. The expectations

Table 2. Chromosome distribution to the poles in the *N. glutinosa* × *N. sylvestris* hybrids

Source	Distribution					Lower*	Total PMC's	Mean‡	χ²§
	12-12	11-13	10-14	9-15	8-16				
Plant E ₁ , Oct. 6	59	114	70	32	19	10	304	0.97	
Exp. (adj.)†	51	94	72	46	25	16	304		15.61**
Plant E ₁ , Oct. 11	8	39	16	6	6	2	77	1.16	
Exp. (adj.)	13	24	18	12	10		77		14.99***
Plant E ₂ , Oct. 6	58	88	78	38	17	14	293	0.76	
Exp. (adj.)	49	90	69	45	24	16	293		6.40 n.s. P = 0.30-0.50
Plant E ₂ , Oct. 11	33	53	32	19	24	13	174	1.07	
Exp. (adj.)	29	54	41	27	14	9	174		13.56**

* The classes 7-17, 6-18, etc., were pooled.

† Expected distribution adjusted for the effect of bivalent frequency.

‡ Mean number of bivalent.

§ ** Significant at the 5% probability level (also in following tables). *** Significant at the 1% probability level (also in following tables). n.s. Non-significant (also in following tables).

were adjusted for the effect of the bivalents by the method described in the previous section. In three out of the four samples the more equal distributions were more frequent than expected and the unequal ones rarer however, in plant E₂ sampled on October 6 the χ² test was not significant. No definite trend was observed for the plant E₂ at the October 11 sampling.

(ii) *N. glutinosa* (n = 12) × *N. otophora* (n = 12)

In 630 out of 721 PMC's, examined for bivalent frequency by Sficas & Gerstel (1962), the distribution of chromosomes to the poles was recorded and is given in Table 3. In the remaining 91 cells some univalents were lying at the metaphase plate and these were therefore excluded. The expected numbers were adjusted for the number of bivalents.

The observed distribution deviated significantly from expectation at the 1% level in plant D9, and was not significant but close to the 5% level of significance in plant D7. A tendency was observed for equal numbers of chromosomes to go to opposite poles.

Further analysis was possible because *N. otophora* possesses five distinctly larger chromosomes. In 134 out of the 193 cells, in which these large *N. otophora* chromosomes were identified, their distribution to the poles was recorded as presented in

Table 4. Because no large chromosome was observed to pair with another large one, their distribution to the poles was not affected by the bivalent frequency, and no adjustment was needed in the expectations. The χ^2 test shows no evidence against the hypothesis that the large chromosomes of *N. otophora* move at random to either pole.

Table 3. *Chromosome distribution to the poles in the N. glutinosa* \times *N. otophora* hybrids

Source	Distribution					Lower*	Total PMC's	Mean II‡	χ^2
	12-12	11-13	10-14	9-15	8-16				
Plant D7	68	124	91	39	7	4	333	3.57	9.30 n.s. $P = 0.05-0.10$
Exp. (adj.)†	65	115	80	45	19	9	333		
Plant D9	73	108	76	25	10	5	297	3.23	17.00***
Exp. (adj.)	57	101	71	40	18	10	297		

* The classes 7-17, 6-18, etc., were pooled.

† Expected distribution adjusted for the effect of bivalent frequency.

‡ Mean number of bivalents.

Table 4. *Distribution of the large N. otophora chromosomes to the poles in the N. glutinosa* \times *N. otophora* hybrids

Source	Distribution			Total PMC's	χ^2
	2-3	1-4	0-5		
Plant D7	43	11	4	58	3.59 n.s., $P = 0.05-0.10$
Exp.*	36	22		58	
Plant D9	50	22	4	76	0.57 n.s., $P = 0.70-0.80$
Exp.	47	24	5	76	

* Expected distribution.

In the total distribution of chromosomes to the poles there was evidence against the hypothesis of random movement at least in plant D9, but one cannot reach the conclusion that non-random movement is restricted only to the small chromosomes because the data in Table 4 were obtained from a smaller sample (about six times smaller) than was used in the test of the total distribution. However, here is a point for further investigation.

(iii) *N. tabacum* ($n = 24$) \times *N. glutinosa* ($n = 12$)

A large number of univalents was lying on or near the metaphase plate in most of the cells. For this reason only in 399 out of 874 cells analysed for bivalent frequency by Sficas & Gerstel (1962), could the chromosome distribution to the poles be scored. From the plant C6 at June 18 only nine cells were recorded out of 194 and will not be used for statistical analysis. Hence, the remainder of 390 cells represents the plant C2 at two dates and the plant C6 at one date of sampling. The

approximate method of testing the randomness of the distribution of the univalents to the poles is used, since the number of chromosomes is large and pairing frequency is relatively small. In Table 5 the experimental data are given together with the mean number of bivalents (m), and the expected distribution considering that a number of chromosomes, equal to 36 minus twice the mean number of bivalents, move at random.

Table 5. *Chromosome distribution to the poles in the N. tabacum × N. glutinosa hybrid*

Source	Distribution							Total PMC's	Mean* II	χ^2
	18-18	17-19	16-20	15-21	14-22	13-23	Lower*			
C ₂ , May 28	9	21	13	9	11	1	2	66	2.54	
Exp. ($u \ddagger = 30$)	8	18	15	11	7	7		66		5.83 n.s.
C ₂ , July 11	30	86	56	31	23	7	5	238	2.94	
Exp. ($u = 30$)	33	64	55	40	26	14	6	238		13.56**
C ₆ , Apr. 29	18	26	20	6	7	5	4	86	3.87	
Exp. ($u = 28$)	14	23	19	14	9	7		86		3.06 n.s.

* Lower classes were pooled for presentation but they were used separately in calculating the second moments.

† Mean number of bivalents.

‡ u = univalents.

The analysis was performed separately for the various dates and plants because the mean number of bivalents was different. In all cases a tendency was towards an equal distribution of chromosomes to the poles. Only one of the three cases was statistically significant.

(iv) *Haploid N. tabacum* ($n = 24$)

Two tobacco haploids were studied for bivalent frequency by Sficas & Gerstel (1962). In one of the haploids most of the chromosomes were scattered throughout the cytoplasm, hence very few cells could be analysed for chromosome distribution to the poles. The other haploid was of the 402 variety but it possessed a substituted chromosome from *N. plumbaginifolia* carrying resistance to the black shank disease. This haploid had, therefore, 23 tobacco and one *N. plumbaginifolia* chromosomes. In this last haploid 450 out of the total of 500 PMC's were recorded for chromosome distribution to the poles and are given in Table 6 together with the expected one

Table 6. *Chromosome distribution to the poles in haploid N. tabacum (23t + 1pb)*

Source	Distribution							Total PMC's	Mean II	χ^2
	12-12	11-13	10-14	9-15	8-16	7-17	6-18			
Plant 23t + 1pb	56	122	82	70	51	40	19	450	0.35	
Exp. (adj.)*	73	136	106	70	38	18	9	450		83.98***

* Expected distribution adjusted for the effect of bivalent frequency.

assuming random movement of univalents, the adjustment being made by the exact method presented in this paper. It is observed that a significant deviation from randomness occurred in the distribution, with a tendency towards a more unequal distribution.

3. DISCUSSION

Pairing of chromosomes assures their distribution to the poles in equal numbers which is necessary for viable gamete production and fertility. In species hybrids, haploids and asynaptic organisms where a variable number of chromosomes remain unpaired at the metaphase stage, their distribution to the poles is variably unequal. The chromosomes that remain univalents may move independently and at random to either pole. Such a random distribution was indicated in an investigation conducted by Riley & Chapman (1957) in some haploids and polyploids of *Aegilops* and *Triticum*. In *T. timopheevii*, on the other hand, the distribution was not random. Person (1955) concluded that non-random distribution was due to 1:1 disjunction of associated chromosomes not joined by chiasmata.

Table 7. Summary tabulation and comparison of criteria for testing randomness of distribution of chromosomes to the poles

Plant*	Distribution compared with expected	χ^2 for distribution	Moment about $D\left(\frac{N}{2}, \frac{N}{2}\right)$		F test for moments
			Observed	Expected	
E ₁ Oct. 6	Peaked	15.61**	4.53	5.51	1.22**
E ₁ Oct. 11	Peaked	14.99***	4.45	5.42	1.21 n.s.
E ₂ Oct. 6	Peaked	6.40 n.s.	4.95	5.62	1.13 n.s.
E ₂ Oct. 11	No pattern	13.56**	6.40	5.46	1.17 n.s.
D7	Peaked	9.30 n.s.	3.36	4.21	1.25***
D9	Peaked	17.00***	3.15	4.38	1.39***
C ₂ May 28	Peaked	5.83 n.s.	6.77	7.75	1.15 n.s.
C ₂ Jul. 11	Peaked	13.56**	5.59	7.50	1.34***
C ₆ Apr. 29	Peaked	3.06 n.s.	6.52	7.05	1.08 n.s.
23t + 1pb	Flattened	83.98***	9.10	5.82	1.56***

* E = *N. glutinosa* × *N. sylvestris*; D = *N. glutinosa* × *N. otophora*; C = *N. tabacum* × *N. glutinosa*; 23t + 1pb = haploid *N. tabacum* (with a foreign substitution chromosome).

Two cases of non-random distribution were observed in the present work: (1) deviation from randomness towards a more nearly equal distribution (peaked curve) (2) deviation giving a flattened curve. A summary of the results is given in Table 7 where the second moments about $D(N/2, N/2)$ were also calculated and compared with the expected ones as described in the present paper. Both criteria (distribution fitness and second moments) are in agreement but they do not coincide in the significance level in all cases.

The first kind of non-randomness was noticed in all three interspecific hybrids which were tested. Of the nine distributions, eight were peaked (four of which significant) and one without a definite pattern. (This last case might be attributed to a

chance effect.) An explanation, similar to that given by Person (1955), may be offered: it is possible that some chromosomes pair and without forming a chiasma start moving to opposite poles. This type of pairing may be similar to that observed in certain insects by Wilson (1925) and Schrader (1940), who used the term of 'touch and go pairing'.

The second type was found in the haploid of allopolyploid tobacco with the substituted *N. plumbaginifolia* chromosome. It constitutes a tendency of the whole complement to go to one of the poles leaving a varying smaller portion to move to the other pole. This might be related to Goodspeed's (1952) observation that haploid tobacco gives a higher than expected number of restitution nuclei, which can result either from failure of cell wall formation or movement of all the chromosome to one of the poles.

4. SUMMARY

A probability distribution of chromosome separation to the poles was developed to test the randomness of movement of univalents in asynaptic material where a variable amount of meiotic pairing occurs. Two tables were calculated, one for 24 chromosomes which can be used for any even number equal or less than 24, and the other for 19 chromosomes which can be used for any odd number equal or less than 19.

Three *Nicotiana* hybrids, namely *N. glutinosa* × *N. otophora*, *N. glutinosa*, *N. sylvestris*, and *N. tabacum* × *N. glutinosa*, and one polyhaploid were investigated. All hybrids had a tendency towards an equal distribution of unpaired chromosomes to the poles. The polyhaploid *N. tabacum* (with a substituted *N. plumbaginifolia* chromosome) had an opposite tendency, i.e. towards a flatter distribution than expected from random distribution of univalents. A short discussion of the problem is given.

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