

The Dynamics of Aneuploidy in an Induced Tetraploid Population of *Lolium multiflorum* × *Lolium perenne*

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Summary. Populations of induced polyploids invariably contain a substantial proportion of aneuploid individuals. A model is described which can predict the level of aneuploidy in successive generations of a closed population of *Lolium* tetraploids. The results demonstrate clearly that the proportion of aneuploid individuals increases sharply for two to three generations and then stabilizes at a level determined by the gametic output of the euploid plants. A change in the gametic output of aneuploid individuals has a relatively small effect on the final level of aneuploidy reached.

Key words: *Lolium* tetraploids – Induced polyploids – Aneuploidy

Introduction

Populations of induced tetraploids of both inter- and intra-specific origin inevitably contain a fraction of aneuploid plants (Muntzing 1943; Morrison 1956; Hagberg and Ellstrom 1959; Rommel 1961; Ahloowalia 1971; Simonsen 1973, 1975; Hossain 1978; Nagata and Okabe 1978). They arise from fertilization involving aneuploid gametes which are themselves a consequence of irregular chromosome separation at first anaphase of meiosis. This in turn is due, in part at least, to the presence of 'odd-numbered' configurations such as trivalents and univalents at first metaphase of meiosis. The situation is further aggravated in genera such as *Lolium* and *Festuca* by irregularly orientated quadrivalents.

It is a well known fact, however, that the frequencies of aneuploids in tetraploid populations 'stabilize' at between 35% and 45% after relatively few generations. Thus, there is a rapid increase in aneuploidy for two or three generations with apparently little change thereafter. The reason for this stabilization has remained a

puzzle. It is attractive to postulate that the continuous build up in the population of aneuploids from the increased aneuploid fraction is prevented by the inferior performance of aneuploids, in particular their lower fertility. However, while it has been established that aneuploids of autotetraploid rye are less vigorous and less fertile than euploids (Hagberg and Ellstrom 1959) no such evidence is available for tetraploids of *Lolium*.

The simple model which follows was developed to examine these changes in the percentage aneuploidy over successive generations of seed multiplication in greater detail. An experimental population of tetraploid *Lolium multiflorum* × *Lolium perenne* was used as the basis for the model. The model is further extended to pinpoint the factors responsible for the ultimate level of aneuploidy achieved.

All diploid *Lolium* species have a chromosome number of $2n = 2x = 14$. Euploids of both auto- and allotetraploids should therefore contain 28 chromosomes. The majority of aneuploids contain 27 or 29 chromosomes although 26 and 30 chromosome individuals can also be picked out. 25 and 31 chromosome forms are only rarely found and are therefore omitted from the present model.

The Model

To develop the working model two sets of information are used. These are:

1. The relative frequencies of 26, 27, 28, 29 and 30 chromosome plants in the base population. These can be represented as A1, A2, A3, A4 and A5 respectively so that $\sum A = 1.00$.

2. The relative frequencies of 26, 27, 28, 29 and 30 chromosome individuals in the progeny of open pollinated plants of each of the five chromosome categories listed in (1) above. These again can be represented algebraically as in Table 1.

Table 1. Frequencies of 26, 27, 28, 29 and 30 chromosome individuals in the progeny of open pollinated plants of each of the five parental chromosome classes

Chromosome number of seed parent	Fraction of parental population	Fraction of progeny with chromosome number of:				
		26	27	28	29	30
26	A ₁	b ₁	c ₁	d ₁	e ₁	f ₁
27	A ₂	b ₂	c ₂	d ₂	e ₂	f ₂
28	A ₃	b ₃	c ₃	d ₃	e ₃	f ₃
29	A ₄	b ₄	c ₄	d ₄	e ₄	f ₄
30	A ₅	b ₅	c ₅	d ₅	e ₅	f ₅

Table 2. Summary of the frequencies of 26, 27, 28, 29 and 30 chromosome progeny in each generation

Chromosome number category	Fraction of population
26	(A × b)
27	(A × c)
28	(A × d)
29	(A × e)
30	(A × f)
Total	1.00

Given this information, the relative frequencies of each chromosome category in the next generation can now be easily calculated. For example the frequency of 26 chromosome plants as a fraction of the total will be $(A_1 \times b_1) + (A_2 \times b_2) + (A_3 \times b_3) + (A_4 \times b_4) + (A_5 \times b_5)$ which can be reduced to $\Sigma(A \times b)$. Similarly the contribution of each of the other chromosome categories can be easily estimated (Table 2).

These values can now be used to calculate the composition of the subsequent generation by substituting them for the A values in Table 1 and repeating the calculations. This can be repeated for as many generations as is required.

The model is no doubt an oversimplification. No attempt is made to allocate 'relative fitness' values to the

various aneuploid classes in comparison with the euploids. It is also assumed that each seed parent category produces the same fraction of aneuploid an euploid progeny irrespective of variation in genotype and in the pollen composition in successive generations. However, results which we have obtained from controlled crosses indicate that aneuploid pollen is largely ineffective. The results derived from the model can therefore be taken as a reasonably accurate picture of the dynamics of aneuploid production in polyploids.

Application of the Model to a Tetraploid Population of *Lolium multiflorum* × *Lolium perenne*

F₂ seed of an experimental population of tetraploid *L. multiflorum* × *L. perenne* labelled as Bx.164 was obtained from the Welsh Plant Breeding Station. This was originally derived from a cross between autotetraploid forms of the parental species.

An isolated polycross block of 182 spaced plants was established in the field and the chromosome number of each plant determined by cytological analysis of pollen mother cells at anaphase 1 of meiosis. As expected the chromosome number ranged from 26 to 30. At harvest, seed was collected from individuals in the central area of the block only. Five plants containing 26, 27, 28 and 29 chromosomes were available but only 3 individuals containing 30 chromosomes could be sampled from this region.

At least 10 progeny from each parent plant were subsequently grown in small pots in the greenhouse and their chromosome content determined at anaphase 1 of meiosis in the pollen mother cells. In all a total of 337 progeny from the five parental classes were 'counted'. The average frequencies of 26, 27, 28, 29 and 30 chromosome progeny produced by each of the five parental ploidy classes are given in Table 3.

These data were then used to examine the changes in the frequency of euploid/aneuploid individuals in a population of this type using the model given earlier and starting from a situation where all the individuals

Table 3. The average frequencies of euploid and the four classes of aneuploid progeny produced by each of the five parental chromosome classes

Seed parent class	Fraction of the progeny with chromosome number of				
	26	27	28	29	30
26	0.1728	0.6926	0.1346	0	0
27	0.0334	0.4821	0.4845	0	0
28	0.0420	0.1262	0.7759	0.0559	0
29	0.0110	0.0230	0.5650	0.3468	0.0540
30	0	0	0.3220	0.6100	0.0673

Table 4. Predicted composition of successive generations of Bx. 164

Generation	Fraction of population with chromosome number of				
	26	27	28	29	30
P	0	0	1.0000	0	0
F ₁	0.0420	0.1262	0.7759	0.0559	0
F ₂	0.0447	0.1891	0.7004	0.0628	0.0030
F ₃	0.0441	0.2120	0.6775	0.0627	0.0036
F ₄	0.0439	0.2197	0.6710	0.0618	0.0036
F ₅	0.0438	0.2224	0.6690	0.0612	0.0036
F ₆	0.0438	0.2234	0.6685	0.0608	0.0035
F ₇	0.0438	0.2238	0.6683	0.0606	0.0035
F ₈	0.0438	0.2239	0.6683	0.0605	0.0035

are assumed to be euploids, i.e. A_3 was given a value of 1.00 and A_1 , A_2 , A_4 and A_5 given values of zero.

The relative proportion of euploids and the four aneuploid classes up to F_8 are given in Table 4. The data generated by the model confirms in a striking manner the largely undocumented observations of many plant breeders over the last three decades of a very sharp drop in the euploid fraction in the first two generations followed by a period of much less drastic chang-

es. It can be seen that equilibrium is achieved as early as the F_4 to F_5 generation. Presumably by this stage the frequency of aneuploids produced by euploid plants is equal to the frequency of euploids produced by aneuploid plants.

It will be recalled that in these calculations the euploid and the various aneuploid fractions are assumed to produce a specific ratio of euploid to aneuploid progeny at all times. For example the euploid fraction is

Table 5. The relative frequencies of 26, 27, 28, 29 and 30 chromosome plants in the progeny of a range of parental plants from each of the five ploidy classes

Parental chromosome number	Plant number	Fraction of progeny with chromosome number of				
		26	27	28	29	30
26	1	0.4000	0.4000	0.2000	0	0
	2	0	1.0000	0	0	0
	3	0.1820	0.7270	0.0910	0	0
	4	0.1820	0.6360	0.1820	0	0
	5	0.1000	0.7000	0.2000	0	0
27	1	0	0.6154	0.3846	0	0
	2	0.1000	0.5000	0.4000	0	0
	3	0	0.4620	0.5380	0	0
	4	0	0.5000	0.5000	0	0
	5	0.0667	0.3330	0.6000	0	0
28	1	0	0.2000	0.7333	0.0667	0
	2	0	0.1540	0.7691	0.0769	0
	3	0.1330	0.2000	0.6670	0	0
	4	0	0	0.9412	0.0588	0
	5	0.0769	0.0769	0.7690	0.0769	0
29	1	0	0	0.5000	0.5000	0
	2	0.0660	0	0.4667	0.4000	0.0660
	3	0	0	0.5263	0.4210	0.0530
	4	0	0	0.5830	0.4170	0
	5	0	0.0714	0.7140	0.1430	0.0714
	6	0	0.0666	0.6000	0.2000	0.1333
30	1	0	0	0.4440	0.4440	0.1110
	2	0	0	0.2720	0.6360	0.0910
	3	0	0	0.2500	0.7500	0

assumed to produce progeny comprising 77.59% euploids and 22.44% aneuploids, the 27 chromosome fraction to produce 48.45% euploids and 51.55% aneuploids and the 29 chromosome fraction to produce 56.50% euploids and 43.50% aneuploids (Table 3). These of course are average figures obtained from a specific set of plants under a specific set of conditions. Different populations of tetraploids would almost certainly give different values. Indeed the evidence from the Bx.164 population surveyed here indicates that there can be substantial variation in the composition of the progeny from plants of identical chromosome number from within the same population. Table 5 gives the proportion of euploids to aneuploids in the progeny of a range of 27, 28 and 29 chromosome plants from this Bx.164 population.

Whilst there is no clear evidence from this type of analysis as to the heritable component of this variation it would not be unreasonable to suppose that some of it at least is genetic in origin. It is therefore of considerable interest to determine what effects a change in the nature of the progeny of both euploid and aneuploid fractions would have on the ratio of euploids to aneuploids in the population at equilibrium. This will give some idea of which fraction of the population is mainly responsible for the final level of euploidy/aneuploidy in the whole population. It might also give some idea as to the practicality of 'improving' the population by selection.

Consequences of a Change in the Composition of the Progeny of Euploid and Aneuploid Plants

Simple predictions can be made of these consequences by using the general model given earlier and changing the level of euploid to aneuploid production in an arbitrary manner. This was done in the first place by changing the euploid content of the progeny of euploid plants by steps of 5% from 30 to 100. It was considered that no euploid plants would produce less than 30% euploid progeny. The various aneuploid fractions in the progeny of these 28 chromosome plants had, of course, to be varied proportionally to account for the changes made. The composition of the progeny of the 26, 27, 29 and 30 chromosome plants were left unaltered. The results of this type of exercise taken through eight generations of the model are illustrated graphically in Fig. 1. It is patently obvious that the relationship between the degree of euploidy in the population at equilibrium and the euploid output of the euploid fraction is not linear. Nevertheless, it is quite clear, as indeed might be expected, that the output of the euploid fraction has a profound effect on the nature of the aneuploid/euploid balance of the population at equilibrium. It is also quite

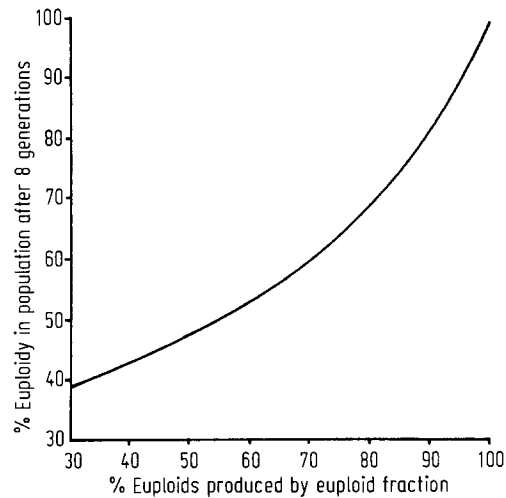


Fig. 1. The pattern of variation in the euploid content of the population at equilibrium in response to changes in the euploid output of the euploid fraction

clear that for most of the range the level of euploidy in the population at equilibrium is lower than the level of output from the euploid plants. However, when the output of euploids by the euploid fraction is below 45%, the equilibrium point is actually higher.

Similar calculations were repeated separately for both the 27 and 29 chromosome fractions. The results are illustrated graphically in Fig. 2. Since the 26 and 30 chromosome plants form an insignificant part of the population their influence was not considered. Again

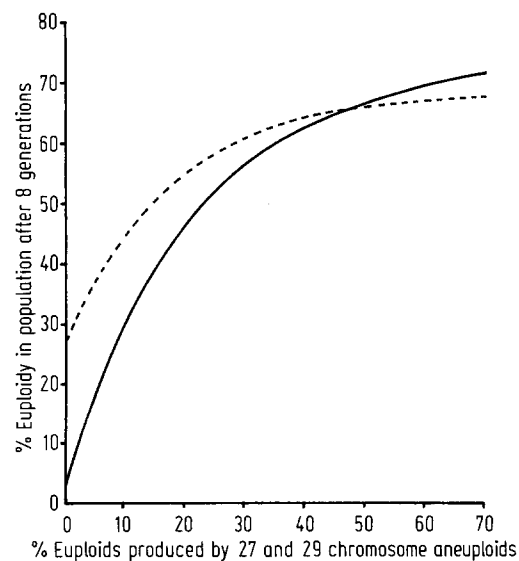


Fig. 2. The pattern of variation in the euploid content of the population at equilibrium in response to changes in the euploid output of the 27 and 29 chromosome fractions. Effects of 27 chromosome fraction —; effect of 29 chromosome fraction -----

the relationship between euploid output and equilibrium point is not linear but the pattern is quite different from that discussed above. The effect of a change in the euploid output of both 27 and 29 chromosome plants is most marked when the values fall below about 30–35%. Above this there is very little effect on the equilibrium point. For example a change in output of euploids from 0% to 35% from the 27 chromosome plants alters the euploid content of the population at equilibrium from 6% to 60.5% but a subsequent increase of from 35% to 70% in euploid output only raises the equilibrium point by a further 11.5% to 72%. Corresponding figures for the 29 chromosome fraction are 32.5% to 63% to 68%.

It is relevant at this point to consider these results in conjunction with the range of values of euploid progeny obtained from different plants of the Bx.164 population. The data are given in Table 5. Although the number of plants sampled within each class is admittedly low, it nevertheless gives some idea of the range in this particular tetraploid population. It will be noted that in the euploid (28 chromosomes) class the euploid output of individual plants ranges from 66.7% to 94.1%. If these figures represented the euploid output of the foundation plants of different populations and it is assumed that the same pattern is repeated in successive generations then according to the projections illustrated in Fig. 1 this would result in a range of euploid values at equilibrium of from 57.5% to 88.5%. Similarly the range in euploid output of the aneuploid fractions of Bx.164 were 38.5% to 60% from 27 chromosome plants and 46.7% to 71.4% from the 29 chromosome plants. Although these changes are not quite as large as for the 28 chromosome plants they are nonetheless quite substantial. However, a similar projection using the graphs in Fig. 2 gives a range in population euploid content at equilibrium of only 62% to 70% for the 27 chromosome changes and 66% to 68% for changes in the 29 chromosome class.

The inevitable conclusion must therefore be that the ratio of euploids to aneuploids in the population at equilibrium is mainly determined by the ratio of euploids to aneuploids produced by the euploid fraction. The aneuploid fractions only become important if their output of euploids falls below 35–30%.

Discussion

Although the data used in this report relate to a specific population grown under a specific set of conditions the conclusions reached are nevertheless applicable to any polyploid population multiplied through several seed generations. That an essentially static level of aneuploidy is reached in very few generations is inevi-

table. This stems from the fact that while euploid plants produce a substantial proportion of aneuploid progeny the aneuploid plants produce an equivalent amount of euploid progeny.

The plant breeder, however, is also concerned with the identification of the factors which determine the ultimate level of aneuploidy reached at equilibrium. The present model serves to clarify this problem to some extent at least. It was shown in the preceding section that aneuploidy at the population level is not dependent upon the existence of a proportion of aneuploids in the previous generation. The importance of the euploid genotypes bears significantly upon the relative rate of production of aneuploidy, not only in the initial generation but throughout successive generations of seed multiplication. The segregation of functional aneuploid gametes from the euploid genotypes determines not only the rate of aneuploid establishment, but also the final level reached and thereafter maintained over successive generations of seed multiplication. The euploid output of the aneuploid fraction has to be very low (less than 30%) to have any profound effect on the dynamics of the population.

In the present model we have not considered the implications of differences in 'fitness' of euploids and aneuploids. In tetraploid rye at least it was established by Hagberg and Ellstrom (1959) that aneuploids were less vigorous and produced less grain than their euploid counterparts. There is no such detail available for *Lolium*s but even if an extreme situation were to occur where each euploid plant produced twice as many seed as each aneuploid the increase in the euploid percentage at equilibrium would still be marginal although the time (generations) taken to reach this level would be increased slightly.

It would appear therefore that improvement of the chromosomal stability of tetraploid populations rests almost entirely on the feasibility of selecting euploids which consistently produce a high proportion of euploid progeny. This would almost certainly entail establishing euploids with a more regular disjunction of chromosomes at anaphase one of meiosis and this would best be achieved by a more regular pairing of chromosomes in the early stages of meiosis. The exact relationship between these three parameters will be examined in more detail in a later paper.

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