Zeitschrift für Vererbungslehre, Bd. 89, S. 338—354 (1958)

INBREEDING EFFECTS IN RYE*

By

J. SYBENGA

With 3 Figures in the Text

(Eingegangen am 31. Januar 1958)

A. Introduction

* This study was started by Prof. Dr. R. PRAKKEN and handed over to the present author in 1955. Prof. PRAKKEN's valuable advice is gratefully acknowledged.

A. Introduction

For the study of the effects of inbreeding both on macromorphological and cytological characters, rye, *Secale cereale*, has been found a very useful experimental plant. Rye is naturally cross breeding, is rather easily grown and has few ($2n = 14$) and rather large chromosomes. The high self sterility is a disadvantage in early generations; later selffertility is usually attained as a result of unavoidable selection.

Mainly two methods have been used for the study of the inbreeding process in rye:

1. Using population plants as starting material (MÜNTZING et al.).
2. Using F_1 plants from crosses between homozygous lines as starting material (REES et al.).

The advantage of the first method is the naturalness of the material, as selection has not occured during previous inbreeding and thus no genes for extreme characters from the population have been lost. Furthermore homozygous lines and the crossing between these are not required.

The advantages of the latter method are: suitability for thorough statistical analysis as sufficient numbers of plants of parents, F_1, F_2, etc. can be grown simultaneously for several years; sufficient selffertility warranting sufficient offspring and continuity; acquaintance with the genetic constitution of the parents; absence of segregating recessive lethals.

The present study has been conducted on a material derived from population plants, and thus lacks the opportunity for thorough statistical analysis but is more natural than one derived from artificial crosses. More than by MÜNTZING and AKDIK (1948) have macromorphological characters been compared with cytological characters. At the same time the inbreeding could be followed over a longer period.

B. Material

The material consists of two groups of inbreeding families, each family consisting of one population plant of Petkus rye and its progeny after selfing over several generations. Observations have been made including S_6 though S_0–S_5 will be considered here mainly, as considerable elimination of inbreeding lines occured in S_6 and S_7 due to heavy frost in the winter of 1955–1956.

Group I was started in 1949 by selfing 100 population plants, 45 of which gave progeny in 1950. Group II was started in 1950 by selfing 64 population plants, 30 of which gave progeny in 1951.

Elimination of plants and even of families continued as a result of low seed set and extreme weakness of some plants. Incomplete families (of which no observations were available in one or more of the six generations) and also plants not forming a part of a continuous line of inbreeding, were excluded. Mainly families of which only few generations could be maintained were excluded. This procedure was necessary to make later generations as comparable to the preceding generations as possible. The result is doubtless a selection for self-fertility and perhaps also slightly for vigour. Thus group I was reduced to 10 families sufficiently complete for study; group II to 6 families. The different families are rather different in size and structure as splitting up into sub families has occured somewhat irregularly in different families and generations.

Table 1 gives the number of plants used for the computation of the averages for the cytological characters, pollen fertility and seedset for each generation and two examples of the same data for families. The cytological data in S_3 and S_4 for group I and S_3 for group II are lacking and thus both S_3 and S_4 are omitted for these data.

The macromorphological characters could be measured on a larger scale: sister plants to those taken for the cytological data, pollen fertility and seedset were used. For these the same numbers of plants are given in Table 1a.

All plants used for calculation of the averages originate from plants entered into the average of the previous generation. Not all plants entered into an average need have progeny, however. This is a reason later generations may contain less plants than earlier ones. Correction for differences between years might be possible only to a slight extent by the use of two groups of inbreeding families: group I started in 1949, group II in 1950. Since both groups originate from the sam de population, differences between groups may be an indication of differences between years.

Table 1. *Numbers of plants studied per generation for both groups of inbreeding families and the same for two families, one from each group. Cytological data, pollen fertility and seedset*

	S_0	S_1	S_2	S_3	S_4	S_5
Group I	10	36	53	21*	29*	49
Group II	6	24	26	12*	20	17
Total	16	60	79	33*	49*	66
Family 117–14	1	1	2	1*	2*	4
Family 277–6	1	4	4	3*	6	4

* No cytological data available.

Table 1a. *As Table 1. Macromorphological data*

	S_1	S_2	S_3	S_4	S_5
Group I	58	117	161	229	202
Group II	44	109	88	67	127
Total	102	226	249	296	329
Family 117–14	1	5	13	5	13
Family 277–6	5	14	23	22	35

C. Methods

All observations have been made on air dry or on fixed material. The cytological observations were made on paraffin sections of florets prefixed in Carnoy 1:3 and stored in KARPECHENKO's chrome acetic formalin. All staining was done in gentian or chrystal violet. Pollen fertility was scored in Belling carmine glycerin preparations.

The observations concern a number of different, more or less quantitative characters[1] with the purpose to make possible a comparison between these characters as to their reaction on inbreeding. All different observations have therefore always been made on the same plants; the macromorphological ones have been made also on an additional number of sister plants, as mentioned above.

[1] Various other characters, showing a more clear mendelian segregation and usually studied on the living plants, will be discussed in a later publication.

The following characters have been measured:

1. *Plant height*, determined as the average of the lengths of the three tallest stems, excluding the ear.

2. *Stem diameter*, the average diameter of the same three stems used for 1., measured just below the head. This gives a less variable and easier measured diameter than those measured at other places on the stem, although correlation with such other diameters need not be complete.

3. *Number of tillers* per plant. Both total number and number of tillers reaching more than $^1/_2$ of the length of the largest one.

4. *Dry weight* per plant including roots, in grams.

5. *Seed set* after selfing (bagging) on 1–4 heads per plant.

6. *Seed set* after open pollination.

For both 5. and 6. the estimation of seed set in % has been performed only in later generations. In order to obtain comparable values for all generations the plants have been assigned to four classes according to the estimated number of grains per head.

Class 1: less than 1 grain/head (sterile). Class 2: 1–10 seeds per head. Class 3: 11–50 seeds per head. Class 4: more than 50 seeds per head.

This classification is based mainly on the change from self sterility (class 1) to several degrees of self fertility (classes 2–4) during inbreeding, but nevertheless arbitrary. In compairing these characters with other characters this should be kept in mind.

7. % *Fertile pollen* as estimated from 200 grains per plant, using Bellings carmine-glycerin method.

8. *Chiasma frequency*. (Cf. p. 341.)

9. *Bivalent length*. (Cf. p. 342.)

Statistical analysis has been severely hampered by the composition of the material. No overall analysis was possible, often non-parametric methods were required. Some *t*-tests were performed, some correlations and rank correlations, and one analysis of variance calculated.

For the construction of the inbreeding curves the observed values have been combined into generation-averages per family and these into one average per generation.

Because of differences in structure between families weights had to be assigned to the family averages. These weights have been calculated as the *square root* of the number of plants in these averages. Usually variance is taken into account in the calculation of weights, but this was not possible in the present study, as variance is not only caused by sampling error and undefined errors, but also to great extent by segregation. Taking variances into the weights would mean an underweighting of the most strongly segregating families. Moreover the field plans could not be designed in a way permitting the calculation of reliable between-plant-variances.

Using the number of plants per family per generation as weight, which is the same as taking the overall averages directly from the plant values is not permitted as the starting plants are not genetically identical. It would result in an overestimating of the largest families.

No weighting would mean an overestimating of the reliability of single plant data.

The exact determination of the weights of the family means should also take into account the structure of the family in all preceding generations. Precise weighting, however, is usually not necessary as slight changes in weights have only very small effects on the average. The square root of the number of plants seemed a good compromise.

It was usually necessary to calculate the family averages in a similar way from averages of a lower rank.

Before giving the results some consideration will be given to characters 8. and 9.

Chiasma frequency is determined usually as the number of chiasmata that can be distinguished in metaphase I, as earlier stages are often less clear. The frequency can be expressed per cell or per bivalent. In rye this has been done by e.g. MATHER and LAMM (1935), LAMM (1936), REES et al. (1955 etc.). Other authors, however, used the number of bound chromosome arms per bivalent or per cell as a representative of chiasma frequency; in rye: PRAKKEN (1943), MÜNTZING and AKDIK (1948). It is then assumed that the number of bound chromosome arms is a simple function of the chiasma frequency. This is not so when chiasma localisation occurs, in which case the relation is more complicated. Nevertheless the latter method is the most usefull where, as in rye, 1. the number of chiasmata per arm is low; 2. the exact number of chiasmata is not easily determined in many plants, especially in strongly contracted bivalents and with strong terminalisation; 3. terminalisation, fusion and loss of chiasmata are common and different in different plants.

Because of 3. it is even probable that in rye the number of chiasmata counted is as well merely a function of the number of chiasmata present originally as is the number of bound chromosome arms. The latter may even be a more reliable measure of chiasma frequency as it permits the observation of a much larger number of bivalents and cells. In this study the number of bound chromosome arms was used as an estimate of chiasma frequency.

In order to get an impression of the correlation between the number of chiasmata counted and the number of bound chromosome arms, the correlation coefficient was calculated and found to be 0.987 between both values *as averages*, in 16 plants taken at random, of 100 cells per plant.

The interpretation of calculated correlation coefficients between number of chiasmata and number of bound chromosome arms *per cell* presents serious difficulties.

In the first place the regression, which is approximately linear at low and medium values, tends to approach 0 asymptotically at high values. Secondly the distribution of the cell values of both characters is very skew, again especially at high values per plant. Yet correlation coefficients have been calculated in 20 cells of each of 8 plants taken at random. All correlations are significant (Table 2), all but one highly so. The strong tendency of the correlation coefficients to become smaller at higher values of mean chiasma frequency may not be caused by the factors mentioned above only. It may be caused also by:

1. the fact that at high values the counts of chiasmata become less reliable, as mentioned earlier (p. 341); 2. non-random distribution of the chiasmata, caused by interference and certain types of localization.

A further difficulty in the estimation of the chiasma frequency and of the number of bound chromosome arms in rye is the decrease of the number of chiasmata and bound chromosome arms with progress of meiosis including metaphase. This can cause considerable variation in the estimation of chiasma frequency. The problem will be given more consideration in Sect. D III.

Table 2. *Correlation between chiasmata counted in M I and number of bound chromosome arms.* 20 cells/plant. Eight plants, taken at random

Chiasmata per cell	Bound arms per cell	Correlation coefficient
15.15	13.70	0.61
14.75	13.10	0.77
14.45	13.65	0.77
14.25	12.45	0.48
12.75	12.20	0.91
11.75	11.15	0.89
11.45	11.00	0.94
2.10	2.10	1.00

The measurement of bivalent length in M I has not been performed on all 7 bivalents per cell. Since it is usually impossible to recognize the individual bivalents in rye it would have been better to measure the whole complement. This, however, would involve a large amount of work with not very much value.

The length of the bivalents in M I depends on too many factors viz., besides on spiralization also on terminalization and localization of chiasmata and on chiasma frequency: the more chiasmata, the more, shorter, ring bivalents. As representative measure one ring was chosen, as in almost all cells ring bivalents occur but not always rod bivalents. Moreover, rings are usually stretched less than rods. The shortest but one ring was chosen as it was sufficiently easily recognized and more approaching the average ring length than e.g. the shortest ring. Some other ring could have been used as well. One value for bivalent length was obtained for each cell for which also the number of bound chromosome arms was determined. Thus the value per plant is again the average of 50 measurements.

D. Results

I. Inbreeding curves

The bulk of the data has been condensed into Fig. 1. On the abscissa the six generations of inbreeding have been set out, on the ordinate the means of all measurements for each character per generation, expressed as per cent of the mean S_0 value. Table 3 gives the absolute values.

The shape of the curves appears not always to be quite regular. The leveling off seems not yet to have occured even at S_5. Those families, however, which could be studied in later generations also, did not show an observable change beyond the S_5 value. So it may be assumed that at S_5 the inbreeding minimum respectively maximum has been approached. It is clear that the different characters react differently on inbreeding as to minimum and speed of inbreeding. Most characters exhibit a clear inbreeding effect in their means, some do not. It was not found possible to estimate the significance of the deviation of the curves from any theoretical curves, due to the composition of the material.

In order to get an impression of the degree to which the inbreeding effect is consistent over the generations studied, rank correlations were calculated, and

denoted below by r, between generations (ranked according to their means) and a series of increasing numbers: 1, 2, 3, 4, 5, 6. Thus $r = +1$ and $r = -1$ indicate complete consistency, $r = 0$ indicates complete absence of consistency.

Seedset after selfing (Fig. 1: *ss*) appears to increase only after S_1, but then very rapidly percentagewise, partly an effect of selection for selffertility, which is completed in S_3 ($r = +0.8$). In absolute value (Table 3) this seedset only approaches *seedset after free pollination* (Fig. 1: *sf*), which shows a reasonably consistent ($r = -0.65$), though irregular and rather slight decrease, apparently strongly influenced by external factors.

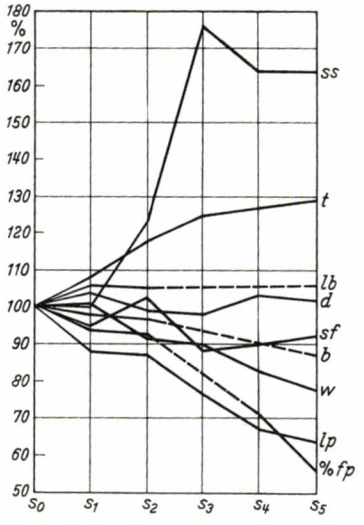

Fig. 1. Inbreeding curves. Generation averages expressed in percent of S_0. *ss* seedset after selfing; *t* number of tillers per plant; *lb* length of bivalents at M I; *d* diameter of stem; *sf* seedset after free pollination; *b* bound chromosome arms per cell; *w* plant weight; *lp* plant height; % *fp* percent fertile pollen. — — — data lacking for some generations

Percent fertile pollen (Fig. 1: *fp*) shows the strongest and also a consistent ($r = -1.0$) inbreeding effect, apparently the resultant of a number of factors, one of which is decrease in chromosome pairing in meiosis. Inbreeding seems to be more deleterious to pollen than to ovule fertility. One of the causes for this may be a certain, unvoluntary, selection for seedset during inbreeding.

Number of bound chromosome arms (Fig. 1: *b*) per cell at meiosis decreases at a similar rate as seedset (free pollination), though much more regularly ($r = -1.0$). This decrease is in accordance with the observations of MÜNTZING c. s. and REES c. s. MÜNTZING and AKDIK (1948) found a positive correlation within generations between chiasma frequency and plant vigour, measured as plant height. In the present study a similar correlation was found, particularly in the first inbred generations. Similarly a correlation between chiasma frequency and pollen fertility was found, as also mentioned by MÜNTZING and AKDIK l. c.

Bivalent length (Fig. 1: *lb*) increases with inbreeding although somewhat irregularly, not quite consistently ($r = +0.65$) and only very slightly. Plants with long bivalents do not as a rule have less bound chromosome arms as is sometimes supposed. The correlation coefficient calculated for bivalent length and number of bound chromosome arms in 20 plants taken at random from the entire material (averages of 50 cells) was $r = -0.085$. A slight negative correlation might be attributed to the contrasted change during inbreeding.

This absence of correlation is in agreement with the observations of LAMM (1935) and PRAKKEN (1943). REES (1955) found in some cells in some anthers of rye a low number of chiasmata correlated with increased length of bivalents. Some lines in the present material have also been found to contain such cells. In these cases the decreased number of chiasmata may well be caused, simultaneously with decreased spiralization, by disturbed timing relationships, as

proposed by REES. In general, however, it is apparent that bivalent length is determined independently from chiasma frequency.

The other characters concern the macromorphology.

Tillering (Fig. 1: *t*) shows a consistent ($r = +1.0$) and strong increase, partly due to the segregation of recessive factors for very large numbers of tillers (up to 70 per plant) and enhanced by wider spacing of the plants in later generations due to loss of weak plants. On the other hand some lines with reduced number of tillers appeared, indicating that at least some of the factors affecting tillering are intermediate. Since both total tillers and large tillers increase in exactly the same way percentagewise, only the first character is shown in the graph.

The fact that in a highly selected plant like Petkus rye high number of tillers is a recessive character, practically not apparent in the population, indicates that high number of tillers is not favorable for high seed production. This is conceivable since too many tillers cause strong competition within and between plants. Moreover, tillers are formed at certain intervals. The last ones will mature too late, are short and can thus not contribute to the production, yet require nutrients for their development.

Plant height (Fig. 1: *lp*) shows a strong and consistent ($r = -1.0$) decrease in a rather regular way.

Plant weight (Fig. 1: *w*), to which contribute both plant height and number of tillers, is intermediate between these, though yet decreasing considerably and consistently ($r = 0.94$). Other factors besides height and tillering apparently exert a strong influence on weight.

Diameter (Fig. 1: *d*) of the stem just below the head appears not to be influenced by inbreeding ($r = -0.029$). Both larger and smaller diameters than those of the population plants were found, suggesting that this diameter is determined mainly by intermediate factors, possibly in rather low number.

Although the calculation of variances for the material as a whole would not have been of use (see above), it was thought necessary to obtain at least an impression of some of the factors able to cause variation in the material, and of their importance. This will be dealt with in the following sections (II–IV).

II. Differences between years

Differences between years could be approached only by comparing equivalent generations of both groups of families, representing two consecutive years (Table 4). Only the three first generations are shown in Table 4 as of these a larger number of families were available than given in Table 3. The families not used for the construction of the inbreeding curves were those that did not produce progeny beyond S_2. They could be used for the present purpose, however. This was especially valuable for the characters of which the lowest number of observations were available, bound chromosome arms, pollen fertility and seedset, and these are the characters that are included in Table 4. For the remaining characters the reader is referred to Table 3.

Tests of significance for the differences are possible only for S_0, not for other generations as there segregation and irregular splitting up into subfamilies render their variances incomparable. No significant differences were found between 1949 and 1950 in bound chromosome arms ($t = 0.37$; $P > 0.7$) and in seed set after

Table 3. *Generation means. Explanation in text*

Group I

	1949 S_0	1950 S_1	1951 S_2	1952 S_3	1953 S_4	1954 S_5
Bound chromosome arms.	13.47	13.17	12.98	—	—	11.50
Bivalent length	4.63	4.85	4.89	—	—	4.64
Percent fertile pollen . .	94.7	83.8	84.6	—	73.2	53.6
Seed set, selfed	1.6	1.6	1.9	3.1	3.0	2.9
Seed set, free	4.0	3.6	4.0	3.4	3.2	3.5
Dry weight, grams . . .	70.0	73.0	47.0	59.3	53.6	41.1
Tillers, 2 types	11.8–10.1	17.3–11.5	14.3–10.9	16.8–11.6	14.7–11.6	15.9–11.6
Height, cm	146.7	133.2	120.0	114.3	101.3	81.5
Diameter, mm	0.97	0.97	0.93	0.94	0.96	0.96

Group II

	1950 S_0	1951 S_1	1952 S_2	1953 S_3	1954 S_4	1955 S_5
Bound chromosome arms.	13.20	13.14	12.83	—	11.11	11.88
Bivalent length	4.61	4.95	4.81	—	5.02	5.44
Percent fertile pollen . .	84.2	89.5	83.9	—	49.9	44.0
Seed set, free	3.5	4.0	3.8	3.1	3.7	3.3
Seed set, selfed	1.8	1.8	2.6	2.8	2.5	2.7
Dry weight, grams . . .	53.3	51.9	72.0	53.5	50.0	62.6
Tillers, 2 types	17.5–10.0	11.8–9.1	18.7–12.8	18.2–13.4	24.5–18.0	20.9–15.8
Height, cm	140.3	127.8	130.9	102.7	85.2	107.2
Diameter, mm	0.87	0.97	0.91	0.87	0.95	0.99

Total

	S_0	S_1	S_2	S_3	S_4	S_5
Bound chromosome arms.	13.37	13.16	12.92	—	—	11.62
Bivalent length	4.62	4.90	4.87	—	—	4.90
Percent fertile pollen . .	91.1	86.1	84.3	—	64.6	50.6
Seed set, selfed	1.7	1.7	2.1	3.0	2.8	2.8
Seed set, free	3.8	3.6	3.9	3.3	3.4	3.5
Dry weight, grams . . .	63.7	64.6	58.7	57.0	52.5	49.5
Tillers, 2 types	13.9–10.1	15.0–10.5	16.4–11.8	17.4–12.3	17.7–13.6	17.9–13.2
Height, cm	144.3	127.2	125.1	109.5	96.3	91.5
Diameter, mm	0.93	0.97	0.92	0.91	0.96	0.95

Table 4. *Equivalent inbreeding generations in different years*

	S_0 1949 (group I)	S_0 1950 (group II)	S_1 1950 (group I)	S_1 1951 (group II)	S_2 1951 (group I)	S_2 1952 (group II)
Bound chromosome arms.	13.32	13.22	13.03	13.17	12.87	12.95
Percent fertile pollen . .	94.5	84.6	83.8	89.8	83.6	80.8
Seed set selfed*	1.6	1.5	1.6	1.8	2.0	2.2
Seed set free *	4.0	3.5	3.6	4.0	4.0	3.8

* Classes as in Table 3.

selfing ($t = 0.57$; $P > 0.5$); a small, just significant difference in seed set after free pollination ($t = 2.11$; $P = 0.05$). A very important difference was found in pollen fertility ($t = 3.32$; $p < 0.01$).

Apparently 1950 was a very unfavorable year for pollen fertility, as is seen again in S_1, where 1950 and 1951 can be compaired.

1954 was found to be a year in which generally the values for number of bound chromosome arms per cell were low. It is known that weather conditions have an influence on chiasma frequency. Dry conditions usually decrease the chiasma frequency, as do high temperatures. In rye a decrease in chiasma frequency due to dry weather was found by PRAKKEN (1943). LAMM (1936) found a higher chiasma frequency in the greenhouse than in the field, which may be a similar phenomenon. MÜNTZING and AKDIK (1948) did not find significant differences between years in their material. REES (1955) found no differences between days.

During the period of fixation for the estimation of chiasma frequency in 1954 and several weeks before this period no precipitation had occured while the air temperature was rather high and air moisture content low. In other years this period was appreciably moister and cooler. This may well be the explanation of the relatively low chiasma frequency found in 1954. It is in complete accordance with the observations cited in the literature.

The other characters are somewhat more irregular (Tables 3 and 4). The weight difference between 1949 and 1950 (both S_0) seems to be due to difference in length and perhaps also to difference in diameter of stems. It is rather surprising that in 1950 the S_1 surpasses the S_0 in weight. Increase in tillering in mainly the taller tillers and increase in diameter of the stems may be among the causes. Length, however, decreases as expected.

It is clear that in some instances year differences may be important and can explain some of the irregularities in the inbreeding curve, in this case somewhat reduced by the combination of two groups of families representing two years.

III

a) Chiasma frequency, *loss of chiasmata.* Since in some instances rather large differences have been found in chiasma frequency determined in M I between

Table 5. *Early and late metaphase I compared. Number of bound chromosome arms and of chiasmata per cell. Averages of 50 cells. Variance and t-values for number of bound chromosome arms.*
For terminalization see text

Plant number	Early metaphase I				Late metaphase I				
	bound arms	variance	chias-mata	termina-lization	bound arms	variance	chias-mata	termina-lization	t
395–3	12.46	1.08	12.90	0.55	12.32	1.06	12.80	0.60	0.68
948–1	12.98	1.10	13.44	0.80	12.34	1.42	12.78	0.84	2.83
365–2	13.64	0.27	14.24	0.82	13.34	0.42	13.90	0.84	2.52
157–2[a]	13.90	0.09	14.22	0.85	13.64	0.31	13.90	0.80	2.89
598–2	12.98	0.86	13.16	0.88	12.42	0.96	12.52	0.91	2.90
970–1	12.42	1.52	12.66	0.76	11.54	1.69	11.76	0.74	3.44
626–1	13.30	0.61	13.96	0.63	12.94	0.54	13.78	0.74	2.42
1496–7	12.08	1.47	12.34	0.80	11.68	1.58	11.88	0.86	1.54
1499–12	11.82	1.83	12.04	0.68	11.06	2.62	11.36	0.73	2.52
1258–7	11.98	2.06	12.74	0.64	11.74	2.12	12.28	0.60	0.82
1506–14	12.68	0.98	12.90	0.72	12.50	0.69	12.70	0.69	0.97
1506–8	12.40	0.80	13.02	0.67	11.96	1.72	12.48	0.70	1.94
959–1	10.46	2.21	10.86	0.70	10.08	2.03	10.26	0.66	1.29
1504–9	10.44	2.29	10.66	0.57	9.62	4.16	9.76	0.56	2.26
1504–16	11.86	1.36	12.22	0.59	12.00	1.32	12.70	0.60	0.60

groups of cells genetically identical and physiologically apparently not in a different state, a more thorough study of first metaphase of meiosis was made.

It was then found, that, when in a group of genetically identical cells distinction could be made between early and late metaphase I, the cells in early metaphase I practically always showed a higher number of chiasmata and of bound chromosome arms than those in late metaphase I. In several cases this difference was statistically significant, especially when very early and very late metaphase I were compared. In Table 5 in all cases, except one, more chiasmata are found in early than in late metaphase I; this one case, however, is not of much value, as here the classification of metaphase was doubtfull.

This difference between early and late metaphase means that not all chiasmata are maintained till anaphase and that "terminalization" does not stop at the chromosome ends; in other words it means that "anaphase" starts already at early metaphase, increases during metaphase and reaches its peak at anaphase proper. In several cases the shape of the bivalents suggests that ring bivalents change to rod bivalents and rod bivalents to univalent pairs (Figs. 2, 3). Apparently the movement of the unbound arm is very rapid to a new equilibrium position, once the last chiasma is released.

This latter can be concluded from the fact that the number of times this unbinding of arms is seen is much smaller than the number of bound chromosome arms lost.

Many oriented univalents may have arisen from rod bivalents in this way. A similar phenomenon has been observed by LAMM (1936) and again by PRAKKEN (1943) in asynaptic rye. It has also been observed by other authors in other organisms [vide GAUL (1954)] but has usually not been considered of much importance for the determination of chiasma frequency. There is no reason to assume that the chiasmata lost would be of another kind than those maintained, or even not be real chiasmata at all. It is interesting to note (Table 5) that the loss of chiasmata is in general accompanied by an increase in variance. This increase is the normal increase expected when the mean is lowered in a skew distribution (see below). This again is an indication that the loss of chiasmata is at random. Table 5 shows that chiasmata behave in the same way as bound chromosome arms, as expected.

The terminalization coefficient has been determined here as the ratio between the number of terminal chiasmata and the total number of chiasmata that are in a position to be terminalized, i.e. the outermost chiasma in each arm.

This coefficient permits a better comparison between bivalents with many and bivalents with few interstitial chiasmata than does the terminalization coefficient that takes into account all chiasmata present.

From Table 5 appears that the terminalization coefficient is on the whole not influenced by the decrease in number of chiasmata and bound chromosome arms. If terminalization would stop at the end of the chromosome arms and the unbinding would be independent of terminalization, the coefficient could be expected to be higher at late than at early metaphase. On the other hand, the terminalization coefficient would be lower at late than at early metaphase if terminal chiasmata would be lost after the arrest of terminalization only. It would also be lower, though in a much lower degree, in case two or more chiasmata occur frequently in one chromosome arm. Loss of the terminal chiasma would then probably not be accompanied by the immediate terminalization of the next

interstitial chiasma and thus tend to decrease the terminalization coefficient. This latter is unusual in the present material, however, as infrequently more than one chiasma per arm occurs.

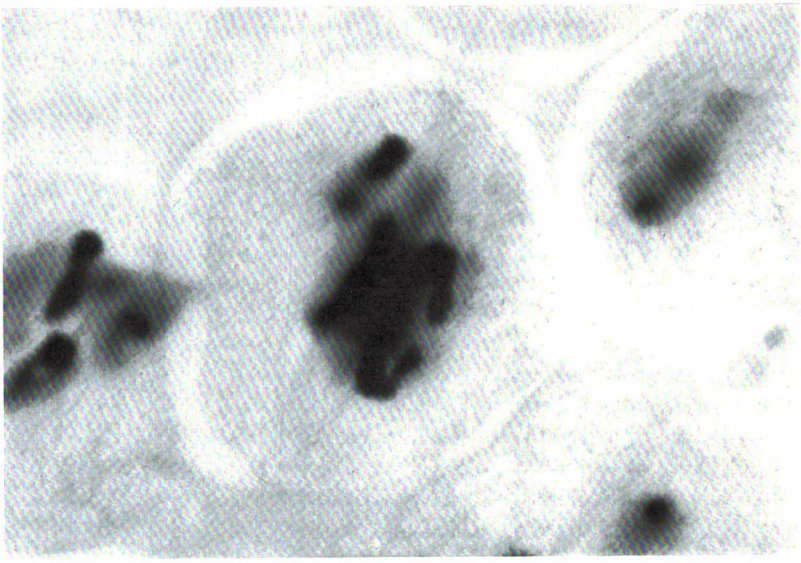

Fig. 2. Ring bivalent changing into rod bivalent

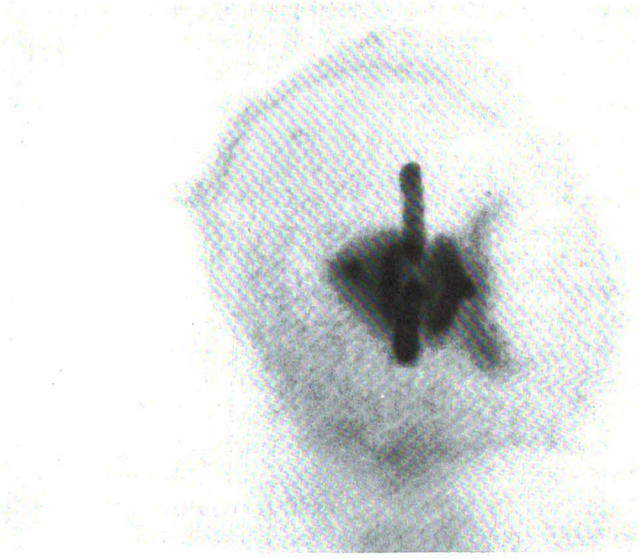

Fig. 3. Rod bivalent changing into univalent pair

Since the terminalization coefficient does not change with loss of chiasmata and bound chromosome arms it is probable, in view of the above mentioned points, that this loss is a continuation of the normal process of terminalization of chiasmata past the end of the chromosome arm.

The phenomenon of asynapsis in rye (PRAKKEN 1943), or better desynapsis, is to some extent similar to the phenomenon mentioned here, though of much greater intensity.

Classification of the plants in Table 5 according to their chiasma frequency shows that, when plants are considered and not stages in metaphase, the terminalization coefficient tends to increase when chiasma frequency increases. This may be explained in this way that, when more chiasmata than one occur in one chromosome arm, the outermost chiasma is more inclined to terminalize than when only one chiasma occurs in one arm. Then the centromere would have a weaker influence on the final stages of terminalization than a second chiasma. The phenomenon may also be explained by a stronger loss of terminal chiasmata *prior to* metaphase in those plants that are found to have a lower chiasma frequency *in* metaphase. Part of the difference in chiasma frequency between plants is then to be explained by a difference in loss of chiasmata prior to metaphase. The loss of terminal chiasmata not immediately followed by terminalization of interstitial chiasmata would decrease the terminalization coefficient.

Quantitative evaluation of the loss of chiasmata and bound chromosome arms is severely hampered by the difficulty of estimating the stage of metaphase I. It is usually necessary to compare both extremes in a gradient from early to late metaphase. For this reason it was impossible in this preliminary study to establish differences between inbred lines, although there are some indications that such differences may exist. Usually the variation within lines is too large.

It is clear that a loss of chiasmata during metaphase can be and in several cases is found to be a cause of differences as observed between anthers, florets and fixations within plants, physiological differences then being of less importance. It is a serious source of error in the determination of chiasma frequencies, which can be avoided to some extent by taking as much as possible the same stage of metaphase in one experiment.

b) Chiasma frequency, *variance within anthers.* In many cases variances have been computed for the comparison of different means of number of bound chromosome arms. This was usually the variance between cells in 50 cells in one anther. The distribution of the cell values (number of bound chromosome arms) within anthers is probably binomial; very skew and similar to a negative Poisson distribution at high average number of bound chromosome arms, symmetrical at medium averages and again skew and similar to a positive Poisson distribution at low averages. The same is true, though less pronounced at the higher values, for chiasma frequency. Already at medium values and stronger at low values, the numbers of chiasmata and of bound chromosome arms tend to become identical, as is also pointed out by PRAKKEN (1943). As a result of these types of distribution of the cell values, the variances are strongly correlated with the means and therefore not very interesting in themselves, especially for bound chromosome arms.

This relation between mean and variance implies that, in case heterosis is found to result in higher chiasma frequencies in the region of the negative Poisson distribution (relatively high numbers of chiasmata and bound chromosome arms) the variance is bound to decrease. Thus such decrease of the variance cannot entirely be attributed to "homeostasis" (cf. REES, 1955).

MATHER and LAMM (1935) found for number of chiasmata per bivalent in population plants of rye a smaller variance between cells than within cells. LAMM

(1936) found this difference in inbred plants in some of the plants, not in others. PRAKKEN (1943) working with asynaptic rye, found the distribution of chiasmata per bivalent within cells identical with that between cells. At the higher numbers of chiasmata per bivalent, as in population plants, the counting of chiasmata becomes less reliable and the distribution skewer. Analysis of variance thus becomes less trustworthy in this region. Yet in the present study again an analysis of variance was worked out for 5 plants of Table 5, 50 cells per plant, for early and late metaphase; Table 6.

Table 6. *Analysis of variance, chiasmata per bivalent. Inbred lines, different stages of inbreeding*

Plant	Early metaphase I			Late metaphase I		
	chiasmata/cell	M.S. between cells	M.S. within cells	Chiasmata/cell	M.S. between cells	M.S. within cells
395–3 (S_2)	12.90	0.194	0.250	12.80	0.220	0.360
157–2[a] (S_1)	14.22	0.612	0.059	13.90	0.082	0.090
970–1 (S_4)	12.66	0.302	0.225	11.76	0.273	0.317
365–2 (S_2)	14.24	0.716	0.028	13.90	0.175	0.161
948–1 (S_4)	13.44	0.218	0.217	12.78	0.282	0.262

There are no indications that the variance within cells is greater than the variance between cells. On the contrary, the plants with the highest number of chiasmata, so most resembling the population plants studied by MATHER and LAMM (1935), have a much greater between-cell than within-cell-variance. Yet by dividing the anther into groups of early and late metaphase a reduction in between-cells variance in comparison to the whole anther, could be expected.

The difference is completely lost, however, when metaphase proceeds. Possibly this can be explained by the fact that in the group "early metaphase" a considerable number of late metaphases have been included without being recognized. This would have caused an increase in variance but mainly in the between-cell-variance. In "late metaphase" such later cells would have changed to full anaphase and as such not included in the average. The within-cell-variance increases consistently with decrease of the mean.

About these problems of decrease of chiasma frequency, of difference between inter-cell and intra-cell-variance and of their interrelation, much can be speculated. For solution of the problems more very thorough study is required, however.

c) Chiasma frequency, *zones within anthers.* For all cytological observations the anther was thought divided into five zones, zone 1 at the tip, zone 5 at the base. Where possible 10 cells were taken in each zone and where this was not possible, the zone was noted from which the cells were taken. In some cases it was found that a significant difference existed between zones; this was attributed mainly to: 1. chance and 2. difference in stage of metaphase I as treated in detail in the preceeding sections.

In several anthers a gradient of stage of metaphase I was observed that caused a gradient in chiasma frequency. At first it was thought that the tip of the anther was usually in a more advanced stage of meiosis than the base, but on the whole this could not be maintained.

In 20 plants taken at random from those in which all 5 zones were in metaphase I simultaneously in one anther, the non-parametric method of "m rankings" was applied to discover a possible gradient in the anther for number of bound chromosome arms per cell. The zones were ranked 1 to 5 according to their average number of bound chromosome arms per cell (10 cells per zone); 1: lowest average. The following result was obtained:

zone 1	zone 2	zone 3	zone 4	zone 5	zones
55.5	60.0	62.0	58.5	64.0	totals
1	3	4	2	5	order

Although zone 1 is lowest and zone 5 highest, this is apparently due to chance. The "coefficient of concordance" M is 4.25, while it has an expectation value of 20 (random distribution of averages over zones) and a maximum of 400. Thus there is a strong indication that, on a average, all zones are equivalent with respect to number of bound chromosome arms per cell. The low value of M may have been caused by opposed gradients, as have indeed been found and which were caused by gradients in the stage of metaphase.

d) Chiasma frequency, *differences between florets etc.* Another source of variability may be a difference between florets in one fixation and between fixations in one plant.

Table 7. *t-values for the differences between 2 florets per fixation and between 2 fixation per plant*

2 florets		2 fixations		
Plant	t	Plant	t	
927–3	1.30	157–1	0.63	
365–14	0.10	413–1	1.50	for 98 d.f.
909–16	1.12	265–1	1.38	$P=0.05$ $t=1.960$–2.042
970–1	5.19**	968–4	1.45	$P=0.01$ $t=2.576$–2.750
968–4	1.79	598–2	0.69	
608–3	0.30	117–16	1.33	
1256–8	1.28	1627–12	1.89	
1256–2	0.81	209–2	0.77	
628–1	0.36			
1262–3	1.12			
609–2	1.37			

Table 7 gives the t-values for the cases where sufficient material was available for a comparison. As the distribution within means is far from normal, t-values are more appropriate than an analysis of variance.

One individual case shows a significant difference, although on the whole the differences are not significant. The differences found can again be attributed to: 1. chance error and 2. difference in stage of metaphase I as treated in detail in Sect. III a, as well as to specific differences between florets and between fixations.

It is not denied, that such specific differences, especially between fixations, may exist. The differences found are no proof, however, that this is the case here.

IV. Between plant variances

In the complete material the inbreeding effect could be studied only with the aid of averages, not of variances. For the study of the change in variability in

Table 8. *Variance (s^2), mean ($\bar{x}$) and coefficient of variability (c.v.) for 4 characters. One plant per family per generation.* Explanation in text

Group I (10 plants in each generation)

	1949 S_0	1950 S_1	1951 S_2	1952 S_3	1953 S_4	1954 S_5
			Bound chromosome arms			
s^2	0.050	0.432	0.285	—	—	0.583
s	0.2236	0.657	0.534	—	—	0.764
$\bar{x}$	13.47	13.09	12.96	—	—	11.58
c.v.	1.66	5.16	4.12	—	—	6.59
			Plant height			
s^2	136.3	364.9	212.7	587.2	117.2	91.6
s	11.66	19.10	14.59	24.23	10.82	9.57
$\bar{x}$	147	124	118	118	105	88
c.v.	7.93	15.40	12.36	20.53	10.30	10.88
			Diameter			
s^2	0.029	0.010	0.089	0.020	0.046	0.043
s	0.170	0.100	0.298	0.141	0.214	0.207
$\bar{x}$	0.97	0.99	0.97	0.88	0.82	0.91
c.v.	17.53	10.10	30.62	16.02	26.10	22.75
			Fertile pollen			
s^2	24.03	117.36	105.66	—	161.50	729.60
s	4.899	10.817	10.295	—	12.689	27.019
$\bar{x}$	94.7	80.4	86.8	—	69.0	52.3
c.v.	5.17	13.45	11.10	—	18.39	51.66

Group II (6 plants in each generation)

	1950 S_0	1951 S_1	1952 S_2	1953 S_3	1954 S_4	1955 S_5
			Bound chromosome arms			
s^2	0.063	0.618	0.480	—	1.439	0.413
s	0.251	0.786	0.693	—	1.20	0.643
$\bar{x}$	13.20	12.77	12.52	—	11.36	11.94
c.v.	1.90	6.16	5.61	—	10.56	5.39
			Plant height			
s^2	151.20	235.02	334.00	364.80	151.20	66.60
s	12.29	15.33	18.28	19.10	12.29	8.16
$\bar{x}$	140	129	129	102	91	110
c.v.	8.78	11.88	14.19	18.71	13.51	7.42
			Diameter			
s^2	0.003	0.071	0.030	0.017	0.070	0.086
s	0.055	0.266	0.173	0.130	0.264	0.293
$\bar{x}$	0.87	0.97	0.95	0.75	0.98	0.98
c.v.	6.29	27.43	18.23	17.28	26.92	29.89
			Fertile pollen			
s^2	84.36	68.34	51.36	—	152.70	443.46
s	9.187	8.264	7.169	—	12.369	21.048
$\bar{x}$	84.2	87.8	84.6	—	62.4	46.7
c.v.	10.90	9.41	8.47	—	19.82	45.07

some of the characters mentioned above a part of the material that would permit such a study was taken out. Of each family one line of inbreeding was taken at random, having one plant in each generation in direct descendance. Mean and coefficient of variability of all families were calculated for each generation and for both groups of inbreeding separately (Table 8).

In most cases the increase in variability already in S_1 is striking. This is doubtlessly due to segregation. Large variability in later inbreeding generations may partly be due to decrease in phenotypic stability with homozygosis. The year influence on pollen fertility (1950) appears also in the variance.

Diameter of the stem behaves abnormally in group I, the reason for which is not clear.

For the study of the change in variability during inbreeding the availability of not more than one plant per family is a great disadvantage. It causes, besides a large sampling error, a large non-genetic error, especially when growing conditions are not under complete control. The failure of rank correlations even between later generations of inbreeding to approach unity, is an indication of the importance of this error. They do increase, however, indicating that segregation decreases. Such rank correlations

have been calculated, but are not given here, being incomplete. Table 9 shows rank correlations for earlier generations.

The same problem is encountered when attempts are made to draw conclusions about segregations from such data. Characters that are not severely

Table 9. *Rank correlation coefficients between generations (mother-daughter correlation); 10 pairs in group I; 6 pairs in group II*

S_0–S_1	Group I	Group II	S_1–S_2	Group I	Group II
Bound arms	0.095	0.869	Bound arms	0.879	0.086
Plant height	0.115	0.557	Plant height	—0.003	0.800
Diameter	0.188	0.857	Diameter	—0.294	0.950
Fertile pollen	0.161	0.657	Fertile pollen	0.423	0.486

affected by the environment, as often chiasma frequency, are somewhat more favorable.

E. Selection

Finally a short note on the effect of unavoidable selection during inbreeding in a small material. During the winter 1955–1956 a large number of lines were lost due to frost damage. This was the reason the analysis of the inbreeding effects could not be carried on beyond this season. The results of this loss of lines may be a strong selection for winterhardiness. On the other hand it was observed that a shift had taken place in the averages of other characters also, apparently at random, as both groups of inbred lines can show shifts to opposed directions. This can be seen in Table 10, which is based on Table 8.

Table 10. *Number of bound chromosome arms per cell. Shift in means resulting from selection for frost resistance*

	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7
				Group I				
Surviving 1956	13.44	12.71	12.66	—	—	11.26	—	11.48
Dead 1956	13.50	13.47	13.27	—	—	11.91	—	†
Average	13.47	13.09	12.96	—	—	11.58	—	—
				Group II				
Surviving 1956	13.09	13.01	13.00	—	11.81	12.38	12.58	—
Dead 1956	13.43	12.69	12.31	—	10.91	11.49	†	—
Average	13.28	12.83	12.75	—	11.30	11.94	—	—

For bound chromosome arms in group I the means in all *preceeding generations* of the plants that survived are lower than of those that died. Thus the group average has shifted to a lower value as a result of elimination of lines with high values. In group II a shift into the other direction can be observed.

Other characters, for which the figures are not given here, react much in the same way, although in some cases no shift is found, or the data are too irregular to base a conclusion upon.

During earlier generations self fertility has been selected upon very strongly. This, and any narrowing down of the material, as has appeared inevitable at

several occasions in this material, may have had effects that have not been recognized. Selection, besides year differences and sampling errors, including systematic sampling errors as encountered in chiasma frequency (early and late metaphase), may be considered the major causes of irregularities in the inbreeding curves.

Summary

1. The effects of continued inbreeding by forced selffertilization in Petkus rye was studied over 6 generations (S_0–S_5).

2. There were two groups of inbreeding families; one started in 1949, the other in 1950. Measurements were taken of nine more or less quantitative characters: plant height; stem diameter; number of tillers; dry plant weight; seed set after selfing; seed set after free pollination; % fertile pollen; chiasma frequency; bivalent length. The number of plants measured is given in Table 1.

3. In general strong inbreeding effects were observed. The main results are condensed into Fig. 1 (p. 343) and Table 3 (p. 345).

4. An increase in variance due to segregation was observed in the inbred generations in comparison to S_0.

5. Some factors affecting the results were studied. These were for all characters: year effects and selection; for chiasma frequency: loss of chiasmata during metaphase, variability within anthers, differences between florets and between fixations.

Literature

GAUL, H.: Asynapsis und ihre Bedeutung für die Genomanalyse. Z. indukt. Abstamm.- u. Vererb.-Lehre **86**, 69—100 (1954). — LAMM, R.: Cytological studies on inbred rye. Hereditas (Lund) **22**, 217—240 (1936). — MATHER, K., and R. LAMM: The negative correlation of chiasma frequencies. Hereditas (Lund) **20**, 65—70 (1935). — MÜNTZING, A., and S. AKDIK: Cytological disturbances in the first inbred generations of rye. Hereditas (Lund) **34**, 485—509 (1948). — PRAKKEN, R.: Studies of asynapsis in rye. Hereditas (Lund) **29**, 475—495 (1943). — REES, H.: Genotypic control of chromosome behaviour in rye. I. Inbred lines. Heredity **9**, 93—116 (1955). — Heterosis in chromosome behaviour. Proc. roy. Soc. B **144**, 150—159 (1955). — REES, H., and J. B. THOMPSON: Genotypic control of chromosome behaviour in rye. III. Chiasma frequency in homozygotes and heterozygotes. Heredity **10**, 409—424 (1956).

Dr. Ir. J. SYBENGA, Laboratorium voor Erfelijkheidsleer, Landbouw Hogeschool, Wageningen, Nederland

Druck der Universitätsdruckerei H. Stürtz AG., Würzburg