INCREASED MEIOTIC IRREGULARITY ACCOMPANYING INBREEDING IN DACTYLIS GLOMERATA L.[1]

W. M. MYERS AND HELEN D. HILL[2]
United States Department of Agriculture, State College, Pa.

Received April 14, 1943

VARIATIONS in chromosomal association and behavior during meiosis were found by MYERS and HILL (1940, 1942) among plants of *Dactylis glomerata*. Cytogenetically the plants of this species behave as autotetraploids (x=7). Among replicated clones from nine of the plants studied by MYERS and HILL (1942), MYERS (1943b) found that the differences in chiasma frequency, average number of quadrivalents per sporocyte, percentage of metaphase I sporocytes with univalents, percentage of anaphase I sporocytes with lagging and dividing chromosomes, and percentage of quartets with micronuclei, all of which had been observed in duplicate collections from the greenhouse, persisted in three replications and through two years in the field. It was presumed that the differences could be attributed to differences in genes, chromosomal differentiation, or both. For the validity of this hypothesis the progeny test provides critical evidence. In studies of first inbred generation progenies of eight of the clones, a previously unreported effect of inbreeding upon chromosomal behavior was encountered. An abstract of these results has been published (MYERS 1943a).

MATERIALS AND METHODS

Plants of the first inbred generation (from selfed seed) from the clones reported by MYERS (1943b) were used in these investigations. The inbred progenies were space-planted in the field in rows adjacent to their respective parental clones. Microsporocyte material was collected in 1940 from the plants of six progenies—namely, OG 2 (11), OG 6 (12), OG 7 (20), OG 42 (5), OG 48 (28), and OG 48 (92), and in 1941 from the plants of two progenies—namely, OG 2 (3) and OG 48 (48). All collections in each year were made on a single day to avoid introducing into the results variation due to dates of collection. The collections from the parental clones (MYERS 1943b) were also made on the same day. For the comparison of inbred progeny with parent, only data collected in the same year were used.

All material was fixed in acetic-alcohol and stored in the fixing solution at low temperature in a household refrigerator until studied. All data were obtained from fresh acetocarmine smear slides. In order to eliminate variation due

[1] Contribution No. 49, of the U. S. REGIONAL PASTURE RESEARCH LABORATORY, DIVISION OF FORAGE CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE, STATE COLLEGE, PA., in cooperation with the northeastern states.

[2] Geneticist and Agent, respectively.

to individual differences in interpretation, all meiotic data were collected by the senior author.

EXPERIMENTAL RESULTS

Chiasma frequency

The number of chiasmata per chromosome was determined from an average of 21 diakinesis sporocytes for each plant of the two inbred progenies from which material was collected in 1941 (Table 1). The class interval of 0.08, used in Table 1, is two times the standard error of the difference for a single determination calculated from the error variance obtained for this character in studies of the replicated parental clones (MYERS 1943b). Since the inbred plants were grown in rows adjacent to their parents and were handled in a similar manner, it is expected that their means were distributed with the same error variance as the means of the clones. It seems legitimate, therefore, to

TABLE 1

Average number of half chiasmata per chromosome in two parental clones of Dactylis glomerata and their respective first inbred generation (I_1) *progenies.*

FAMILY NUMBER	AVERAGE NO. OF ½ x-ta PER CHROMOSOME		NUMBER OF I_1 PLANTS WITH INDICATED NUMBER OF ½ x-ta PER CHROMOSOME						
	PARENT	I_1	1.35* to 1.43	1.44 to 1.51	1.52 to 1.59	1.60 to 1.67	1.68 to 1.75	1.76 to 1.83	1.84 to 1.91
OG 2 (3)	1.78	1.71	1	—	2	4	5	6	2
OG 48 (48)	1.77	1.82	—	—	—	—	—	5	1

* Class interval of 0.08 equals 2×S.E. of the difference for a single determination. (Based on error variance of the parental clones.)

consider any two inbred plants significantly different in chiasma frequency if they differ by more than one class interval in Table 1. Among 20 plants in the progeny of OG 2 (3) it is apparent that segregation occurred for average number of chiasmata. The extreme plants had 1.35 and 1.90 half chiasmata per chromosome, respectively. The average chiasma frequency of the inbreds was 1.71 compared to 1.78 for the parent. The difference of 0.07 is significant; $0.05 > P > 0.01$.

In the progeny of OG 48 (48), the six plants ranged from 1.80 to 1.88. Since the difference between the extreme plants is equal to exactly twice the standard error of the difference, it cannot be concluded from these data that segregation occurred in this progeny. The average chiasma frequency of the inbred plants exceeded that of the parent by 0.05, but the difference is not significant.

The two parents had nearly the same chiasma frequency but the means of their progenies differed by 0.11. In the analysis of variance (table 2), F for comparison of mean square between families with mean square within families

TABLE 2

Analysis of variance of indicated meiotic characters in first inbred generation families of Dactylis glomerata.

VAR. DUE TO	D/F	AVE. X-TA PER CHROMOSOME MEAN SQUARE	F	D/F	AVE. NO. OF IV PER CELL MEAN SQUARE	F	D/F	% OF M I WITH UNIVALENTS MEAN SQUARE	F	D/F	% OF A I WITH LAGGARDS MEAN SQUARE	F	D/F	% OF QUARTETS WITH MICRONUCLEI MEAN SQUARE	F
Between families	1	0.0582	4.48*	7	0.6724	3.22†	7	1,807	4.95†	7	1,834	4.15†	7	3,596	10.41†
Within families	24	0.0130		75	0.2092		76	365		76	442		76	345	

* P is less than 0.05 (SNEDECOR 1938, Table 12).
† P is less than 0.01 (SNEDECOR 1938, Table 12).

was greater than F for P of 0.05. Therefore, the difference between families may be considered significant, indicating that the two parents differed in factors conditioning chiasma frequency.

QUADRIVALENT FREQUENCY

For determination of quadrivalent frequency, an average of 28 diakinesis sporocytes was examined from each plant. The data are summarized in table 3 in which the class interval of 0.29 equals the standard error of the difference for a single determination calculated from the error variance of the parental clones (MYERS 1943b). Therefore, differences greater than two class intervals probably may be considered statistically significant. Segregation for quadriva-

TABLE 3

Average number of quadrivalents per sporocyte at diakinesis in eight parental clones of Dactylis glomerata and their respective first inbred generation (I_1) *progenies.*

FAMILY NUMBER	AVE. NO. OF QUADRIV. OF		NUMBER OF I_1 PLANTS HAVING INDICATED AVERAGE NUMBER OF QUADRIVALENTS							
	PARENT	I_1	2.62* to 2.91	2.92 to 3.20	3.21 to 3.49	3.50 to 3.78	3.79 to 4.07	4.08 to 4.36	4.37 to 4.65	4.66 to 4.94
OG 48 (48)	3.00	3.66	—	—	1	3	2	—	—	—
OG 6 (12)	3.04	3.50	—	1	3	4	1	—	—	—
OG 2 (3)	3.50	3.50	3	2	5	3	4	2	1	—
OG 42 (5)	3.79	3.80	—	1	1	5	2	2	1	—
OG 48 (28)	3.92	3.99	—	—	—	3	3	1	2	—
OG 7 (20)	3.95	3.98	—	—	1	1	—	1	—	1
OG 48 (92)	4.06	4.04	—	—	2	1	4	2	3	1
OG 2 (11)	4.09	4.08	—	1	—	2	2	2	1	2

* Class interval of 0.29=S.E. of the difference for a single determination. (Based on error variance of the parental clones.)

lent frequency occurred in all progenies. The lowest number of quadrivalents was 2.62 found in one plant of family OG 2 (3), and the highest was 4.91 found in one plant of family OG 2 (11). These extremes are greater than any that have been found previously in *Dactylis glomerata*. There is no definite departure from a unimodal distribution in any of the inbred families and, consequently, no evidence for segregation of a single major factor conditioning quadrivalent frequency. The results seem more consistent with the hypothesis of segregation of two or more genes, chromosomal rearrangements, or both, with small cumulative effects upon quadrivalent formation. It should be borne in mind, however, that segregation of a single recessive factor usually would not be observed in autotetraploid populations of this size unless the parent was simplex (MYERS 1941b).

In two families, OG 48 (48) and OG 6 (12), the average quadrivalent frequency of the inbred plants significantly exceeded that of their respective parents, and these parental plants were the ones with the lowest numbers of quadrivalents. In one of these families, OG 48 (48), the chiasma frequency also was higher in the inbreds than in the parent, but the difference was not significant. In the remaining six families, the average quadrivalent frequency of the inbred plants was nearly identical with that of their respective parents. Included among these is family OG 2 (3) in which the inbreds were significantly lower than the parent in average chiasma frequency.

In the analysis of variance of the data on average number of quadrivalents (table 2), the mean square between families significantly exceeded that within families (P was less than 0.01), substantiating the hypothesis of heritable differences among parental clones for this character. Data for two of the families, OG 2 (3) and OG 48 (48), were taken from material collected in 1941, while data for the other families were taken from material collected in 1940. From examination of table 3, however, it is evident that seasonal influence was not responsible for the significant differences among families.

PERCENTAGE OF METAPHASE I SPOROCYTES WITH UNIVALENTS

The percentage of metaphase I sporocytes with one or more unpaired chromosomes was determined from an average of 142 cells for each inbred plant. The most striking feature of the results obtained with this character (table 4) was the consistently higher average of the inbred plants over that of their respective parents. In five of the progenies, the average of the inbreds was nearly double that of the parent, while in families OG 2 (11), OG 42 (5), and OG 6 (12), the frequency of univalents at metaphase I was three times as great in the inbreds as in the parents.

The frequency classes of the inbred plants were arranged with the first two intervals approximately equal to the standard error of the difference for a single determination (5.23) calculated from the error variance of the parental clones, and with the remaining class intervals approximately equal to twice that amount. The standard error does not serve as well in this case as with the

chiasma and quadrivalent frequencies. Since the error variance was an average of the variances of clones ranging in mean percentage of metaphase I univalents from three to 24, the standard error probably is too high for the plants with lower frequencies of univalents and too low for those plants with the higher percentages. No better estimate of error is available for this material, however, and it seems safe to use the standard error as calculated if the limitations are borne in mind.

It is evident from the distributions shown in table 4 that segregation for percentage of metaphase I univalents occurred in all families. In families OG 2

TABLE 4

Percentage of metaphase I sporocytes with univalents in eight parental clones of Dactylis glomerata and their respective first inbred generation (I_1) *progenies.*

FAMILY NUMBER	AVERAGE % OF MI WITH UNIVALENTS		NUMBER OF I_1 PLANTS WITH INDICATED PERCENTAGE OF MI SPOROCYTES HAVING UNIVALENTS										
	PARENT	I_1	0* to 5	6 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100
OG 48 (28)	3	6	4	4	1	—	—	—	—	—	—	—	—
OG 2 (11)	4	12	3	2	4	—	1	—	—	—	—	—	—
OG 42 (5)	4	12	6	3	1	1	—	—	—	1	—	—	—
OG 48 (48)	5	10	3	—	2	1	—	—	—	—	—	—	—
OG 48 (92)	6	13	2	5	5	—	—	—	1	—	—	—	—
OG 6 (12)	9	33	1	1	1	—	2	—	2	2	—	—	—
OG 7 (20)	14	30	—	—	1	3	—	—	1	—	—	—	—
OG 2 (3)	24	42	—	—	6	2	4	1	3	1	—	2	1

* S.E. of the difference of a single determination equals 5.23. (Based on error variance of the parental clones.)

(11), OG 42 (5), OG 48 (92), OG 7 (20), and OG 2 (3), the majority of plants were distributed around the mean of the parent with one or more plants differing from the main group by at least two class intervals. Such distributions are suggestive of the segregation of a single major factor, the recessive condition of which results in a high frequency of sporocytes with univalents. The numbers of plants in each family are too small, however, to establish this hypothesis definitely.

It is interesting to note that even the plants with the highest incidence of sporocytes with univalents are not asynaptic in the usual sense. In most plants the majority of sporocytes with univalents had only one or two unpaired chromosomes. Three plants of family OG 2 (3) had over 80 percent of sporocytes with univalents. In one of these in which the total was 83 percent, 38 percent had a single univalent, 19 percent had two, 16 percent had three, and the highest number of univalents in any cell was six. In another plant with a

total of 83 percent there were seven percent with one, 18 percent with two, 13 percent with three, 14 percent with four, 14 percent with five, and the highest number of univalents observed was 11. One plant had a total of 97 percent of sporocytes with univalents, and in this plant, the most frequent types were those with six, seven, eight, and nine univalents while the maximum was sixteen.

In each family, most plants had a higher incidence of sporocytes with univalents than was found in their parents. Thus, even after the elimination of the plants in which the high univalent frequency seemed to result from the segregation of a major factor, the average of the inbred progenies is higher than that of the parents. Apparently, there has been segregation of recessive genes, of chromosomal rearrangements, or of both which are minor and cumulative in their effects on incidence of unpaired chromosomes at metaphase I.

There were in all families one or more inbred plants that had lower percentages of sporocytes with univalents than their parents. The occurrence of such plants suggests the possibility of obtaining increased meiotic regularity by selection. Two inbred plants were obtained with 0.78 and 1.26 percent of sporocytes with univalents, respectively. The incidence of asynapsis in these plants is as low as that found in normal plants of diploid *Lolium perenne* (MYERS 1941a).

That the differences among parental clones were heritable is shown by the analysis of variance summarized in table 2. Mean square for between families significantly exceeded mean square within families as judged by the F test (P was less than 0.01).

PERCENTAGE OF ANAPHASE I SPOROCYTES WITH LAGGING AND DIVIDING UNIVALENTS

The data on frequency of anaphase I sporocytes with lagging and equationally dividing univalents, obtained from an average of 90 sporocytes per plant, showed almost exactly the same relationships as were shown by the data on metaphase I univalents. It seems unnecessary, therefore, to present these data in tabular form. In the eight families, the inbred progenies had an average of two to three times as many laggards as their respective parents. Also, as in the case of unpaired chromosomes at metaphase I, a majority of plants in each inbred family had higher frequencies of lagging chromosomes than their parents.

The close similarity of results from anaphase I with those from metaphase I was expected, since it has been shown in several species that the unpaired chromosomes at metaphase I tend to lag and divide equationally at anaphase I. Furthermore it has been shown that metaphase I univalents are the most common source of anaphase I laggards. This relationship has been demonstrated statistically for plants of *Dactylis glomerata* by MYERS and HILL (1942) and by MYERS (1943b), and it will be shown later that a high correlation co-

efficient was obtained between the two characters with the plants studied in this investigation.

That there were heritable differences among the parental clones in percentage of chromosome lagging at anaphase I was shown by the analysis of variance summarized in table 2. The variance between families was significantly greater than the variance within families ($P < 0.01$).

PERCENTAGE OF QUARTETS WITH MICRONUCLEI

An average of 129 sporocytes per plant was examined for the determination of the frequency of quartets with micronuclei. The relationship of inbred progenies to parents for this character was very similar to those reported for metaphase I univalents and anaphase I laggards. In each family there was a similar tendency for the inbred plants to have higher frequencies of micronuclei than their parents. These relationships were expected from the known correlation of incidence of micronuclei with frequency of metaphase I univalents and with presence of laggards at anaphase I. In the analysis of variance (table 2), mean square between families was larger than mean square within families ($P < 0.01$).

CORRELATIONS AMONG CHARACTERS OF MEIOTIC BEHAVIOR

Coefficients of correlation were calculated for all combinations of the meiotic characters for which data were obtained. Coefficients were calculated separately within each inbred progeny and the average correlation coefficient was obtained by the method given by FISHER (1936) in which the individual values of r were converted to z and the weighted average of z converted back to an average value of r. These data are presented in table 5. The data from family OG 7 (20) were omitted from this table because of the low number of degrees of freedom within the family.

A correlation coefficient of +0.68 between chiasma frequency and quadrivalent frequency was obtained in both inbred progenies in which data on chiasma number were available. In family OG 2 (3) that value was significant ($P < 0.01$) as was also the average value of r. In studies of the parental clones, the value of r obtained between these two characters was +0.52 (MYERS 1943b). The difference between 0.52 and 0.68 is not significant when tested by the standard error of z (FISHER 1936).

Additional evidence regarding the relation between chiasma and quadrivalent frequency is provided by the data within plants. In each sporocyte, the chiasmata were recorded separately for the chromosomes associated as bivalents and as quadrivalents. In the 26 plants of the two inbred progenies, the average chiasma frequency of the chromosomes of the quadrivalents was in each case higher than the average for the chromosomes of the bivalents. The differences varied from 0.06 to 0.28 for the different plants. The results indicate that chiasma frequency was a limiting factor in the formation of quadriva-

TABLE 5

Correlation coefficients of characteristics of meiotic chromosomal behavior compared inter se and with selfed and open-pollinated seed set within first inbred generation families of Dactylis glomerata.

CHARACTERS	OG 48 (48)	OG 6 (12)	OG 2 (3)	OG 42 (5)	OG 48 (28)	OG 48 (92)	OG 2 (11)	AVERAGE
X-ta[1] with IV[2]	+0.68(4)[8]	—	+0.68(18)§	—	—	—	—	+0.68(21)§
X-ta with MI[3]	−0.31(4)	—	−0.87(18)§	—	—	—	—	−0.83(21)§
X-ta with AI[4]	−0.38(4)	—	−0.78(18)§	—	—	—	—	−0.74(21)§
X-ta with quartets[5]	−0.19(4)	—	−0.70(18)§	—	—	—	—	−0.65(21)§
IV with MI	−0.26(4)	−0.67(7)†	−0.56(18)§	+0.09(10)	−0.32(7)	−0.14(11)	−0.48(8)	−0.37(59)§
IV with AI	−0.35(4)	−0.61(7)*	−0.46(18)†	+0.09(10)	−0.08(7)	−0.28(11)	−0.50(8)	−0.34(59)§
IV with quartets	+0.23(4)	−0.60(7)*	−0.48(18)†	+0.10(10)	−0.04(7)	−0.13(11)	−0.37(8)	−0.26(59)†
MI with AI	+0.99(4)§	+0.99(7)§	+0.98(18)§	+0.95(10)§	+0.22(7)	+0.93(11)§	+0.89(8)§	+0.95(59)§
MI with quartets	+0.68(4)	+0.97(7)§	+0.83(18)§	+0.66(10)‡	+0.29(7)	+0.94(11)§	+0.77(8)§	+0.83(59)§
AI with quartets	+0.68(4)	+0.98(7)§	+0.76(18)§	+0.81(10)§	+0.60(7)*	+0.91(11)§	+0.93(8)§	+0.86(59)§
IV with selfed seed[6]	—	—	−0.13(17)	−0.18(9)	—	−0.11(11)	−0.06(8)	−0.12(42)
MI with selfed seed	—	—	−0.39(17)*	−0.40(9)	—	−0.25(11)	−0.46(8)	−0.37(42)‡
AI with selfed seed	—	—	−0.39(17)*	−0.50(9)	—	−0.29(11)	−0.49(8)	−0.40(42)§
Quartets with selfed seed	—	—	−0.43(17)*	−0.58(9)*	—	−0.26(11)	−0.52(8)	−0.44(42)§
IV with open seed[7]	—	—	−0.56(15)‡	+0.25(7)	—	−0.32(8)	−0.19(7)	−0.31(34)*
MI with open seed	—	—	−0.22(15)	−0.28(7)	—	−0.46(8)	−0.64(7)*	−0.37(34)†
AI with open seed	—	—	−0.31(15)	−0.47(7)	—	−0.45(8)	−0.53(7)	−0.41(34)‡
Quartets with open seed	—	—	−0.26(15)	−0.52(7)	—	−0.46(8)	−0.52(7)	−0.40(34)‡

* r exceeds value of r for P of 0.10, FISHER (1936), Table VA.
† r exceeds value of r for P of 0.05, FISHER (1936), Table VA
‡ r exceeds value of r for P of 0.02, FISHER (1936), Table VA.
§ r exceeds value of r for P of 0.01, FISHER (1936), Table VA.
[1] Average number of half-chiasmata per chromosome.
[2] Average number of quadrivalents per sporocyte.
[3] Percentage of metaphase I sporocytes with univalents.
[4] Percentage of anaphase I sporocytes with lagging and dividing univalents.
[5] Percentage of quartets with micronuclei.
[6] Number of seeds per panicle set under bag.
[7] Number of seeds per panicle set with open pollination.
[8] Numbers in parentheses are degrees of freedom.

lents in all the plants but that its effect was greater in some plants than in others. The correlation coefficient of number of half chiasmata per chromosome for the whole sporocyte with the difference in chiasma frequency between chromosomes of the bivalents and the quadrivalents was -0.59 ($P<0.01$). Apparently the differences found among plants in limitation of chiasma frequency on quadrivalent formation were partially functions of the total chiasma number. That relationship was expected. However, since total chiasma frequency accounted for only about 35 percent (r^2) of the squared variability among plants in the differences obtained, it is probable that it is not the only factor involved. If quadrivalent formation was limited differentially in the different plants by some factor other than chiasma frequency, there would be introduced a variability in the differences between chiasma frequencies of the bivalent and of the quadrivalent chromosomes which would be independent of total number of chiasmata. Thus evidence exists for factors in addition to chiasma frequency which condition differences among the inbred plants in number of quadrivalents.

Average number of chiasmata per chromosome was negatively correlated with percentage of metaphase I with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei (table 5). The correlation coefficients within family OG 2 (3) and also the average values of r were significant ($P<0.01$). In family OG 48 (48), the degrees of freedom were too low to provide an accurate evaluation of the correlation.

Quadrivalent frequency was negatively correlated with incidence of metaphase I univalents in six of the inbred families. In OG 42 (5), r was $+0.09$, a non-significant value. The values of r for families OG 6 (12), OG 2 (3), and average were negative and significant. Similar relationships were obtained for quadrivalent frequency correlated with anaphase I laggards and with micronuclei in the quartets, although the correlation coefficients tended to be lower in the latter two comparisons than for quadrivalent frequency with percentage of metaphase I univalents.

The correlations between metaphase I univalents, anaphase I univalents, and micronuclei in the quartets were in agreement with those reported previously for *Dactylis glomerata* by MYERS and HILL (1942) and MYERS (1943b). For the correlation of incidence of metaphase I univalents with anaphase I laggards, the average value of r was $+0.95$. For the other two comparisons of these three characters average r was $+0.83$ and $+0.86$. The only exceptions to this general relation were found in family OG 48 (28), in which the correlation coefficients of metaphase I univalents with anaphase I laggards and with micronuclei in the quartets were $+0.22$ and $+0.29$. Since r was based upon only seven degrees of freedom in this family, the discrepancy may be without significance.

CORRELATIONS BETWEEN CHARACTERS OF MEIOTIC BEHAVIOR AND SEED SET

For use in other experiments (MYERS 1942a, 1942b) the number of seeds per panicle set under parchment bag and with open pollination was determined

for the inbred plants involved in this study. Correlation coefficients were calculated for these two characters with the different characters of meiosis (table 5). Since seed set data were not obtained for all inbred plants, the degrees of freedom given in table 5 for these correlation coefficients are in some cases lower than for the comparisons between the meiotic characters. The coefficients have been omitted for three families because of the few degrees of freedom in each.

The correlation coefficients of selfed seed set with quadrivalent frequency, percentage of metaphase I sporocytes with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei were negative in all families and in the average. Average r was not significant for selfed seed set with quadrivalent frequency, but average r was significant for selfed seed set with incidence of metaphase I univalents ($r=-0.37$, $P<0.02$), lagging chromosomes at anaphase I ($r=-0.40$, $P<0.01$), and micronuclei in the quartets ($r=-0.44$, $P<0.01$). Open-pollinated seed set likewise was negatively correlated with the four meiotic characters, the average values of r being -0.31 ($P<0.10$), -0.37 ($P<0.05$), -0.51 ($P<0.02$), and -0.40 ($P<0.02$), respectively.

DISCUSSION

The most important feature of the data obtained in these investigations was the large increase in incidence of unpaired chromosomes at metaphase I which accompanied inbreeding in all families. The metaphase I univalents tend to lag and divide equationally at anaphase I and therefore tend to be left in the cytoplasm as micronuclei in the quartets. It was suggested by MYERS (1943b) that the unequal distribution and tendency for loss of the metaphase I univalents and their division products probably were more important factors in conditioning production of aneuploids and decreased fertility in plants of *Dactylis glomerata* than was the presence of quadrivalents *per se*. Thus inbreeding has resulted in an increase in all families of the features of meiotic irregularity which are considered of greatest importance in this species, while quadrivalent frequency on the average has been unaffected in six progenies and increased in two.

There is a striking similarity between the effects of inbreeding on meiotic irregularity found in these studies and the effects of inbreeding upon self- and open-pollinated seed set reported for *Dactylis glomerata* by MYERS (1942a). Plants of the first inbred generation set on the average only 37 percent as many seeds per panicle under bag and 62 percent as many seeds with open pollination as their parental clones. In comparison, in the present investigations the average frequency of univalents at metaphase I in the inbred progenies was increased two to threefold as compared with their parents. If there is a causal relationship between these two results, there should be a negative correlation of the frequency of asynapsis, chromosome lagging, and chromosome loss with seed set within inbred progenies. Significant negative correlation coefficients

were obtained. On the average, about 16 percent (r^2) of the squared variability in self- and open-pollinated seed set among inbred plants within families may be attributed to variation in incidence of metaphase I univalents and the resulting laggards at anaphase I and micronuclei in the quartets.

This relationship is surprisingly high when it is considered that number of seeds per panicle set under bag and with open pollination show a great amount of non-heritable variation (MYERS 1942b). Since the data for selfed seed set were obtained from three determinations per plant while those for open-pollinated seed set were obtained from a single determination, it is apparent that the values for individual inbred plants were subject to considerable inaccuracy. In studies of seed set using first inbred generation progenies including those studied in the present investigations, MYERS (1942a) found that about 38 percent and 23 percent, respectively, of the squared variability of seed set under bag and with open pollination could be attributed to heritable factors. Thus, it seems probable that a considerable proportion of the heritable variation in seed set was attributable to meiotic irregularities.

It has been pointed out (MYERS 1942a, 1942b), that number of seeds per panicle set with open pollination is conditioned by the number of florets per panicle and by the percentage fertility of the florets. Number of seeds per panicle set under bag is further influenced by a third factor, self-compatibility. With the data available to date, it has been impossible to estimate how much of the heritable variation in number of seeds per panicle may be attributed to variations in fertility and how much to the other factors. Nevertheless, it is known that great differences occur among plants of *Dactylis glomerata* in number of florets per panicle and in self-compatibility (MYERS 1942a). Variations in meiotic irregularity would condition differences in seed set by effects upon female fertility but would not be expected to influence number of florets. Neither would meiotic irregularity be expected to affect self-compatibility except in cases where pollen viability became a critical factor.

The correlation coefficient obtained between meiotic irregularities and seed set was probably lowered also by the fact that in family OG 2 (3), the one with the most degrees of freedom, seed set data were obtained in 1940, while the microsporocyte material was collected in 1941. In studies of the parental clones, MYERS (1943b) found significant year and clone×year effects upon the meiotic characters studied. Also, MYERS (1942b) found significant clone×year interactions for number of seeds per panicle set under bag. In spite of these limitations, significant correlation coefficients were obtained between incidence of metaphase I univalents, anaphase I laggards, and micronuclei in the quartets on the one hand and number of seeds per panicle on the other. It seems probable that in *Dactylis glomerata* these meiotic irregularities have a major role in conditioning variations in fertility among plants within families and the reduction in fertility which accompanies inbreeding. Experiments have been started which should provide a more critical measure of the importance of irregularities of meiosis in that regard. The significant negative correlation

coefficients of metaphase I univalents, anaphase I laggards, and micronuclei with seed set lend support to the hypothesis that these features of meiotic chromosomal behavior are more important criteria of regularity than average number of quadrivalents *per se*.

Increased meiotic irregularity accompanying inbreeding in rye has been reported by LAMM (1936). Inbred plants on the average, had lower chiasma frequencies than plants of the normal population. Chiasma frequency was inversely correlated with frequency of metaphase I univalents that lagged and divided equationally at anaphase I. Certain other results are interesting in relation to the decreased fertility which accompanied inbreeding in Dactylis. LINDSTROM and HUMPHREY (1933) found in *Lycopersicum esculentum* that the homozygous autotetraploid obtained by chromosome doubling from the haploid was extremely infertile, while tetraploids from diploid plants and from F_1 varietal crosses were fairly fertile. The diploid obtained from the haploid, on the other hand, was markedly fertile. SPARROW, RUTTLE, and NEBEL (1942), working with Antirrhinum, found that autotetraploids of varieties were lower in fertility than the hybrids between the autotetraploid varieties, although the differences could not be correlated with quadrivalent frequency. The authors concluded that sterility was a concomitant of, but was probably not conditioned by, a greater amount of pollen abortion, a significant difference in laggards at anaphase I, and a significant difference in 18:14 distributions at anaphase I. In maize, autotetraploids produced from inbreds were less fertile than those from hybrids or open-pollinated stocks (RANDOLPH 1941), but meiotic irregularities were no more common in sterile than in fertile lines (FISCHER 1941). Such results indicate that increased homozygosity tends to be accompanied by decreased fertility, at least in autopolyploids, but the behavior in Antirrhinum and maize differs from the results in *Dactylis glomerata* in that in the former species the increased homozygosity was not accompanied by increased meiotic irregularity *at least* to such a striking degree. Decreased fertility accompanying inbreeding has been encountered commonly in the naturally cross-pollinated forage species. In fact, this reduction in fertility has been one of the principal limiting factors in a successful inbreeding program with some of these species. The results with *Dactylis glomerata* raise the question of whether or not the decreased fertility has been accompanied by increased meiotic irregularity in some of these cases.

In addition to the lowered fertility caused by the greater meiotic irregularity of the inbred plants, there is also expected an increased incidence of aneuploid plants among their progenies. Thus while inbreeding might increase uniformity as a result of greater homozygosity in some progenies, in others it might increase phenotypic variability due to the occurrence of aneuploids.

An explanation of the increased incidence of unpaired chromosomes at metaphase I which accompanies inbreeding is of considerable importance. Percentage of metaphase I sporocytes with univalents was negatively correlated with chiasma frequency. Therefore, a decrease in number of chiasmata would

be accompanied by an increase in univalents at metaphase I. Data on chiasma frequency were obtained in only two progenies. In OG 2 (3), the average chiasma frequency was 0.07 per chromosome lower in the inbreds than in their parent, while the average percentage of metaphase I univalents was 18 percent higher. In the parental clones (MYERS 1943b), the regression coefficient (b) of metaphase I univalent frequency on chiasma frequency was -79.63. Using this value of b, the calculated percentage of metaphase I with univalents for the inbreds was 36 compared with the 42 percent obtained. Thus the reduced chiasma frequency did not account for all of the increase in metaphase I univalents. In OG 48 (48) the percentage of metaphase I with univalents increased from five percent for the parent to 10 percent for the average of the inbreds. The chiasma frequency of the inbreds also was higher than that of the parent, but the difference was not significant. In this case the increase of metaphase I univalents cannot be attributed to fewer chiasmata. Here the relationship between these two characters is complicated by an average increase in quadrivalent frequency for the inbreds. From the data available it seems probable that in some inbred progenies the greater incidence of metaphase I univalents may result in part but not entirely from a lower chiasma frequency, while in other progenies decreased chiasma frequency is not a factor in that regard. Data from more progenies are required to determine how generally these relationships apply. The results in these two inbred families are consistent with those obtained from the parental clones (MYERS 1943b) where it was shown by the analysis of covariance that frequency of metaphase I univalents was affected significantly by some factor or factors in addition to chiasma frequency.

In studies of plants from open-pollinated populations, MYERS and HILL (1942) and MYERS (1943b) obtained small non-significant correlation coefficients between quadrivalent frequency and percentage of metaphase I univalents. The inbred progenies in the present study, on the other hand, yielded significant negative coefficients for two progenies and for the average. These results are consistent with the hypothesis proposed by MYERS (1943b) to account for the absence of correlation between these two characters in the data obtained from the parental clones. Differences among plants in amount of chromosomal differentiation or other factors conditioning preferential prophase pairing should be less on the average within inbred families than among unrelated plants. Consequently, chiasma frequency would have a relatively greater role in conditioning differences among plants in quadrivalent frequency and incidence of metaphase I univalents within inbred families than among the parental clones. The relationship of quadrivalent frequency and incidence of metaphase I univalents to chiasma frequency is such that the two characters will tend to be negatively correlated in cases where chiasma frequency plays a dominant role (MYERS 1943b). Hence, the significant negative correlation coefficients obtained in this investigation.

The occurrence of significant differences between inbred progenies indicates

that there were heritable differences among parents in meiotic chromosomal behavior. Since the irregularities of meiosis were correlated with fertility, it should be possible to effect an appreciable increase in fertility in *Dactylis glomerata* by selection, just as it was possible to increase fertility in autotetraploid maize in that manner (RANDOLPH 1941).

SUMMARY

Chromosomal association and behavior during meiosis was studied in plants of the first inbred generation progenies of eight parental clones of *Dactylis glomerata*.

Frequency of half-chiasmata per chromosome was determined from plants of two progenies, and in one of these the average chiasma frequency of the inbred plants was significantly lower than that of the parent. In the other family the difference between progeny and parent was not significant. In six families the average number of quadrivalents per sporocyte was sensibly the same for the inbreds as for their respective parents, while in the other two families there were significant increases in quadrivalent frequency with inbreeding.

The average percentages of metaphase I sporocytes with univalents of the inbred progenies were from two to three times as great as for their respective parents in all families. Increases of similar magnitude from parent to inbred progeny were obtained for percentage of anaphase I sporocytes with lagging and dividing chromosomes and for percentage of quartets with micronuclei.

Chiasma frequency was positively correlated with quadrivalent frequency and negatively correlated with percentage of metaphase I sporocytes with univalents among plants within families. Significant negative correlation coefficients were obtained for quadrivalent frequency with incidence of metaphase I univalents, anaphase I laggards, and micronuclei within families, whereas the correlation coefficients for these characters were not significant among plants from open-pollinated populations. An explanation of this difference has been proposed. Percentages of metaphase I sporocytes with univalents, anaphase I with laggards, and quartets with micronuclei were positively and significantly correlated, *inter se*.

Number of seeds per panicle set under bag and with open pollination were both negatively correlated respectively with incidence of metaphase I univalents, anaphase I laggards, and micronuclei.

It was shown that the increased incidence of metaphase I univalents and, consequently of anaphase I laggards and micronuclei, which accompanied inbreeding could be attributed in part in one family to decreased chiasma frequency. In the other family in which data were obtained chiasma frequency was not reduced with inbreeding.

Statistically significant differences among families compared with variation within families indicated that there were heritable differences among parental clones in chiasma frequency, quadrivalent frequency, percentage of metaphase I with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei.

LITERATURE CITED

FISCHER, H. E., 1941 Causes of sterility in autotetraploid maize. Genetics **26**: 151.

FISHER, R. A., 1936 Statistical methods for research workers. Ed. 6. London: Oliver & Boyd.

LAMM, R., 1936 Cytological studies on inbred rye. Hereditas **22**: 217–240.

LINDSTROM, E. W., and L. M. HUMPHREY, 1933 Comparative cyto-genetic studies of tetraploid tomatoes from different origins. Genetics **18**: 193–209.

MYERS, W. M., 1941a Variations in chromosomal behavior during meiosis among plants of *Lolium perenne* L. Cytologia **11**: 388–406.

1941b Genetical consequences of chromosomal behavior in orchard grass. J. Amer. Soc. Agron. **33**: 893–900.

1942a Heritable variations in seed set under bag among plants of orchard grass, *Dactylis glomerata* L. J. Amer. Soc. Agron. **34**: 1042–1051.

1942b Analysis of non-heritable variations in seed set under bag among plants of orchard grass, *Dactylis glomerata* L. J. Amer. Soc. Agron. **34**: 1114–1124.

1943a The effects of inbreeding upon meiotic irregularity in plants of *Dactylis glomerata*. (Abstract.) Genetics **28**: 83–84.

1943b Analysis of variance and covariance of chromosomal association and behavior during meiosis in clones of *Dactylis glomerata* L. Bot. Gaz. **104**: 541–552.

MYERS, W. M., and HELEN D. HILL, 1940 Studies of chromosomal association and behavior and the occurrence of aneuploidy in the autotetraploid grass species, orchard grass, tall oat grass, and crested wheatgrass. Bot. Gaz. **102**: 236–255.

1942 Variations in chromosomal association and behavior during meiosis among plants from open-pollinated populations of *Dactylis glomerata*. Bot. Gaz. **104**: 171–177.

RANDOLPH, L. F., 1941 An evaluation of induced polyploidy as a method of breeding crop plants. Amer. Nat. **75**: 347–363.

SNEDECOR, G. W., 1938 Statistical methods. Ames, Iowa: Collegiate Press.

SPARROW, A. H., MABEL L. RUTTLE, and B. R. NEBEL, 1942 Comparative cytology of sterile intra- and fertile intervarietal tetraploids of *Antirrhinum majus* L. Amer. J. Bot. **29**: 711–715.