DOUBLE REDUCTION AT THE MID LOCUS IN *LYTHRUM SALICARIA*

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I. INTRODUCTION

THE gametic output from polysomic organisms of various genotypes can be expressed in terms of a single parameter, namely, the frequency of double reduction. This is the frequency with which a gamete receives two alleles derived from the same chromosome at meiosis.

The term "double reduction" was first introduced by Darlington (1929) and defined as a case "where both chromatids of one chromosome pass into the same nucleus at the second telophase". This offered an explanation for the appearance of one recessive in a progeny of 137 from a reciprocal cross between a triplex and nulliplex *Dahlia* obtained by Lawrence (1929). Similar results had been obtained by Blakeslee, Belling and Farnham (1923), who got 2 per cent. recessives from a triplex *Datura* when backcrossed to the recessive, and 1 per cent. when crossed to the simplex genotype. These early workers, however, expected to find an abnormal number of chromosomes in the recessives. Blakeslee *et al.* examined their recessives but found the numbers to be normal.

Haldane (1930) pointed out that though the passing of two identical chromatids to the same gamete in a diploid organism implied an abnormal chromosome complement in the resulting zygote, this was not the case in polyploids. He calculated the ratios to be expected if the eight chromatids of a tetraploid were distributed at random into the gametes. In the mating between a triplex and a nulliplex, one recessive in 28 could be expected on this hypothesis. Darlington (1931), however, showed that cytologically there were a number of restrictions to Haldane's theory, and concluded that intermediate conditions between random segregation of chromatids and diploid segregation were to be expected, and had in fact been observed by Lawrence.

In four crosses involving one triplex with four different nulliplex, Lawrence (1935) observed double reduction at the Y locus in tetraploid *Dahlia*. He obtained 7 recessives out of 385 plants and explained this as "probably due to chromatid segregation". At none of the three other loci studied did double reduction appear. It should be noted, however, that it was never certain that a triplex was involved in the matings.

An important contribution was made by Mather (1936) when he

pointed out that "the amount of double reduction is a function of the cross-over distance between the locus and the spindle fibre," and, therefore, will vary from locus to locus. This has since been demonstrated in *Lythrum salicaria* by Fisher (1949), who gave preliminary estimates of the frequency of double reduction at the purple/pink locus as 6.5 and at the short/non-short locus as 2.5. These estimates were obtained incidentally in an attempt to ascertain the frequencies of the eleven modes of gamete formation now known to be characteristic of linkage in tetrasomic organisms. They are based on the proportion of duplex plants obtained from simplex parents backcrossed to the recessive long-styled pink. This method involves making a second backcross to determine the genotype—whether duplex or simplex—of the dominant offspring.

An estimate based on the proportion of recessives obtained on backcrossing the triplex genotype is more direct, requiring no second backcross and can, in consequence, be based on large numbers with greater facility. This latter method has been used to estimate the frequencies of double reduction both in pollen and ovules at the mid locus in the work described here. It should be noted that for an estimate at the short locus this method cannot at present be used, since a triplex short could only be obtained by illegitimate fertilisation. All attempts to obtain such a plant have so far failed.

2. DOUBLE REDUCTION IN OVULE FORMATION

When Fisher and Mather (1943) established the existence of polysomic inheritance in *L. salicaria*, they did so by backcrossing to the long-styled form, 16 Shorts and 12 Mids obtained from a cross between a Short and a Mid, both of which had one and the same dominant mid allele. The resulting progenies showed that one Short was nulliplex for mid, 11 were simplex and 4 duplex; 8 of the Mids were simplex, 3 duplex and 1 plant, $M_{40.2}F$, gave no Longs out of 41 non-shorts. This plant was tested the next year with a progeny of 1034 (Fisher, 1944). It gave 22 Longs out of a total of 1075 in two years, and must, therefore, be a triplex and have arisen by double reduction. This first evaluation of the frequency gave an estimate of 8.19 ± 1.73 per cent.

In an attempt to obtain a Short triplex for mid, two of the Shorts that were found to be duplex for mid were crossed to a simplex Mid ($Ss_3 M_2 m_2 = s_4 M m_3$), and twenty-five Shorts obtained from this cross were tested in 1945. The twenty-five progenies are given in table 1, from which it will be seen that five plants triplex for mid were identified. Fifteen of these plants were again backcrossed, this time to pale pinks to provide triplex pales and data on the linkage of the Short and purple loci. The test progenies grown in 1946 are given in table 2, in which the Long, Mid and Short segregations alone are given. There is evidence that some stray pollen was present from the

2 Mids that appeared in the progeny of the nulliplex plant. The data from the four triplex plants will nevertheless be included in our estimate of double reduction, for, as will be shown later, a small percentage of foreign pollen does not alter the estimate appreciably. The fifth triplex plant was crossed to the triplex Mid, M_{40.2}F, in an attempt to obtain both Shorts and Mids quadruplex for mid. In 1947 eleven

TABLE 1
Test progenies from 25 Shorts (1945)

Genotype	Plant	L	M	S	Total
Nulliplex	S _{44.2} G ₁₅	105	...	89	194
Simplex	S _{44.2} F ₁	30	63	105	198
	S _{44.2} F ₂	39	62	97	198
	S _{44.2} F ₃	47	47	107	201
	S _{44.2} F ₄	38	60	101	199
	S _{44.2} F ₅	52	55	95	202
	S _{44.2} F ₆	39	43	114	196
	S _{44.2} G ₁	41	57	102	200
	S _{44.2} G ₅	55	62	84	201
	S _{44.2} G ₈	30	51	123	204
	S _{44.2} G ₁₆	57	51	88	196
	S _{44.2} G ₁₈	36	42	84	162
	Total	464	593	1100	2157
Duplex	S _{44.2} F ₇	12	94	95	201
	S _{44.2} G ₃	14	90	96	200
	S _{44.2} G ₄	8	91	102	201
	S _{44.2} G ₆	13	85	105	203
	S _{44.2} G ₇	16	68	114	198
	S _{44.2} G ₉	21	71	109	201
	S _{44.2} G ₁₀	18	85	98	201
	S _{44.2} G ₁₄	10	83	96	189
	Total	112	667	815	1594
Triplex	S _{44.2} G ₂	2	95	105	202
	S _{44.2} G ₁₁	1	87	111	199
	S _{44.2} G ₁₂	4	89	106	199
	S _{44.2} G ₁₃	4	93	100	197
	S _{44.2} G ₁₇	1	88	109	198
	Total	12	452	531	995

Shorts and twelve Mids obtained from this cross were backcrossed to Longs and progenies grown in 1948. It will be seen from the square-root chart in fig. 1 that five Shorts and six Mids triplex for mid were found.

The possibility that the eleven plants classified as triplex were not all that should have been so classified, *i.e.* that one or more had been classified as quadruplex, may be examined as follows. The number of Long progeny observed from nearly equal progenies with the same

frequency of double reduction, should be distributed nearly in a Poisson Series. If m is the expectation, then the frequency of cases in which none occur will be e^{-m} , and the frequency of cases with at least one

TABLE 2
Test progenies from 15 Shorts (1946)

Genotype	Plant	L	M	S	Total
Nulliplex	S44·2G15	48	2	63	113
Simplex	S44·2F3	42	44	101	187
	S44·2G1	32	41	93	166
	S44·2G5	35	51	95	181
	S44·2G8	29	55	92	176
	S44·2G16	34	40	108	182
	S44·2G18	42	44	101	187
	Total	214	275	590	1079
Duplex	S44·2G7	9	68	95	172
	S44·2G9	13	71	108	192
	S44·2G10	9	72	108	189
	S44·2G14	6	60	69	135
	Total	37	271	380	688
Triplex	S44·2G11	3	47	46	96
	S44·2G12	2	64	110	176
	S44·2G13	3	80	98	181
	S44·2G17	...	79	70	149
	Total	8	270	324	502

will be $1 - e^{-m}$. Among these latter the average number of Long plants observed must be $m/(1 - e^{-m})$. Actually the average number observed among the 11 progenies classified as from triplex seed parents

TABLE 3
Double reduction in ovule formation

Year grown	Progenies	L	M	Total
1942 and 1943	M40·2F	22	1053	1075
1945	5 Shorts	12	452	464
1946	4 Shorts	8	270	278
1948	5 Shorts	16	418	434
1946-1951	6 Mids	15	458	473
	S45·2	5	267	272
	Total	78	2918	2996

is 31/11 or 2·81. This corresponds with an estimated m of 2·61114 for which e^{-m} is 0·07345. The number of triplex progenies corresponding with 11 observed is, therefore, 11/0·07345, or 11·872. If, therefore,

we add $7/8$ of the average number of plants in a progeny, *e.g.* about 70, to the number of plants not Long observed in the progenies, *i.e.* if we take an estimate based on 31 out of 977 instead of 31 out of 907, we shall have made a reasonable allowance for the possibility that a triplex progeny has been excluded. The correction arrived at is so trifling, that more elaborate calculations are not necessary.

Another triplex, $S_{45.2}$, was identified from a repetition in 1944 of the cross, $Ss_3 M_2 m_2 = s_4 Mm_3$, made the previous year. Progenies

3. SHORTS AND MIDS QUADRUPLIX FOR MID

Amongst the plants tested in 1948, four Shorts and five Mids gave no Longs in their progenies (fig. 1). Four of these plants, two Shorts and two Mids, were tested again in 1949 with larger progenies. The combined data from both years are given in table 4.

TABLE 4
Test progenies from 2 Shorts and 2 Mids quadruplex for Mid

Plant	L	M	S	Total
S47·16 . .	...	552	575	1127
S47·23 . .	...	571	582	1153
M47·8 . .	1	546	...	547
M47·12 . .	...	567	2	569

The presence of two Shorts in the progeny of the Mid, M47·12, must be due to stray pollen at the crossing plot or to contamination in the handling of the seeds, which are very small. The Long observed in the progeny of the other Mid must also be a contaminant, since, if this Mid were a triplex 15·65 Longs would be expected from 547 plants. It was, therefore, concluded that all four plants are quadruplex for mid.

4. DOUBLE REDUCTION IN POLLEN FORMATION

To obtain an estimate of α in pollen formation it was necessary to use the triplex Mid as pollen parent and the recessive Long as seed parent. As demonstrated by Fisher and Mather (1943), the advantages of the technique of open pollination, which we have used for all tests in this investigation, far outweigh the disadvantage of the risk of contamination by foreign pollen, and they found this to be less than 1 per cent. in their progenies. It should be pointed out, however, that the risk of error in the estimate of double reduction due to the same percentage of foreign pollen is about fifty times greater when the seed parent is a Long than when it is a Mid or Short. This becomes apparent when it is realised that all the ovules from a Long will, if fertilised by pollen carrying neither mid nor short, increase the proportion of long-styled individuals in the progeny. Since calculation shows that 42 per cent. of such pollen exists in a theoretical population (Fisher, 1941), even 5 per cent. stray pollen at the crossing plot would almost double the percentage of Longs observed in the progeny. On the other hand, when the triplex Mid is the seed parent, only those ovules not carrying mid, that is about 2 per cent., can if fertilised by pollen carrying mid, result in a deficiency of Longs in the progeny. If 5 per cent. foreign pollen were present, the deficiency would be less than 0·05 per cent., a difference that could make no serious difference to the estimate of α .

With this very real source of error in mind, great care was taken in 1948 to find a plot far from any known *Lythrum* populations for the pollination of a long-styled plant by the triplex Mid, M40.2F. To make plenty of the right pollen available, the Mid was divided and planted in a ring round the Long. Seed taken from the Long was grown first in 1949 and again in 1951. The data are given in table 5.

TABLE 5
Double reduction in pollen formation

Year	L	M	S	Total
1949 . .	10	374	...	384
1951 . .	21	943	...	964
Total . .	31	1317	...	1348

The percentage of Longs—2.300 per cent.—is slightly less than that observed in ovule formation. This, and the fact that no Shorts were observed in either year, is convincing evidence that foreign pollen was absent at the crossing plot. The estimate of α is 9.199 per cent. The data for both years are homogeneous— $\chi^2_{(1)} = 0.2225$.

The data from all years, 1943-1951, from both ovules and pollen combined are homogeneous, $\chi^2 = 5.110$ for 6 degrees of freedom, indicating that seasonal variations do not affect the frequency of double reduction. Taking the two estimates together we get $2.509 \pm .237$ per cent. Longs and $\alpha = 10.037 \pm .949$ per cent., and $\chi^2 = 0.351$ for 1 degree of freedom, on the crude data. If we include a twelfth progeny from the 1948 families, the percentage of Longs is $2.469 \pm .234$ per cent., giving $\alpha = 9.878 \pm .936$ per cent., and $\chi^2 = 0.232$ for 1 degree of freedom. Our estimate is only slightly higher than that forecast by Fisher and Mather in 1943.

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5. SUMMARY

About the time when it was realised that the gametic output from polysomic organisms of various genotypes could be expressed in terms of a single parameter, viz. the frequency of double reduction, α , an attempt was first made to estimate this frequency at the mid locus in *Lythrum salicaria*. When Fisher and Mather (1943) established polysomic inheritance in *L. salicaria*, they obtained a triplex mid that could only have arisen by double reduction. This plant was backcrossed to the recessive long-styled form and in two seasons gave 22 Longs out of 1075. The proportion of Longs represents a quarter of α in ovule formation, which was then estimated at 9.18 per cent. Similar

data has been obtained in subsequent years. In 1948 triplex and quadruplex plants were distinguished only by their performance in producing or not producing Longs. Allowance has been made for the possibility that one or more triplex had been classified as a quadruplex by adding $7/8$ of the average number of plants in a progeny, 70, to the Mids observed. The corrected total of 78 out of 3066 plants were obtained in five years. The proportion of Longs is 2.544 per cent. giving $a = 10.176$ per cent.

To obtain an estimate of a in pollen formation the reciprocal cross was made in 1948. In such a cross the risk of error from contamination by foreign pollen is about 50 times greater than when the triplex plant is the seed parent. Since the open pollination technique was used throughout, special care was taken in selecting a site for the pollination plot for this cross. In two years 31 Longs were obtained out of 1348 plants; no Shorts were observed, thus providing evidence that foreign pollen was absent. The percentage of Longs is 2.300 per cent., giving an estimate of $a = 9.199$ per cent. in pollen formation, very close to the value for ovules. Combining the data from ovules and pollen the proportion of Longs is $2.469 \pm .234$ per cent., and $a = 9.878 \pm .936$ per cent. The data obtained from 1942 to 1951 are homogeneous, $\chi^2_{(6)} = 5.110$, indicating that a is not affected by seasonal differences.

Of the Shorts and Mids likely to be quadruplex for mid, two of each type have been tested with adequate numbers and their genotype verified. All four were morphologically normal showing the completeness of dominance at the mid locus.

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