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# CHROMOSOMAL STATUS, GENE EXCHANGE AND EVOLUTION IN *DACTYLIS*

## 1. Gene exchange in diploids and tetraploids

by

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## INTRODUCTION

The genus *Dactylis* comprises a number of morphologically and geographically discrete diploid subspecies, and a widely ranging polyploid complex which can be subdivided into three taxa, *Dactylis marina*, and *Dactylis glomerata* subspecies *glomerata*, and *hispanica* (Plate 1 a, e and c). The taxonomy and phylogeny of the group have been considered previously (BORRILL, 1961a).

Up to the present our knowledge of the role of hybridisation, and our speculations concerning the introgressive consequences of gene exchange in this genus have been based on studies of naturally-occurring hybrids between the chromosomal races (MÜNTZING 1937, ZOHARY and NUR 1959).

An experimental assessment of the capacity for gene exchange between the taxa should enhance our understanding of the dynamics of the group in relation to evolution. The present series of papers will give the results of experimental hybridisation, and the detailed cytology of the hybrid and parental populations. This represents a fresh approach to the study of *Dactylis*, apart from observations by ZOHARY (unpublished) and MCCOLLUM (1958) on the inter-fertility of some diploids and colchicine-induced tetraploids.

The first paper will consider hybridisation in the diploids and tetraploids, the second (by KEITH JONES) their cytology, and the third (jointly) the cytogenetics of hybrids between the chromosome races.

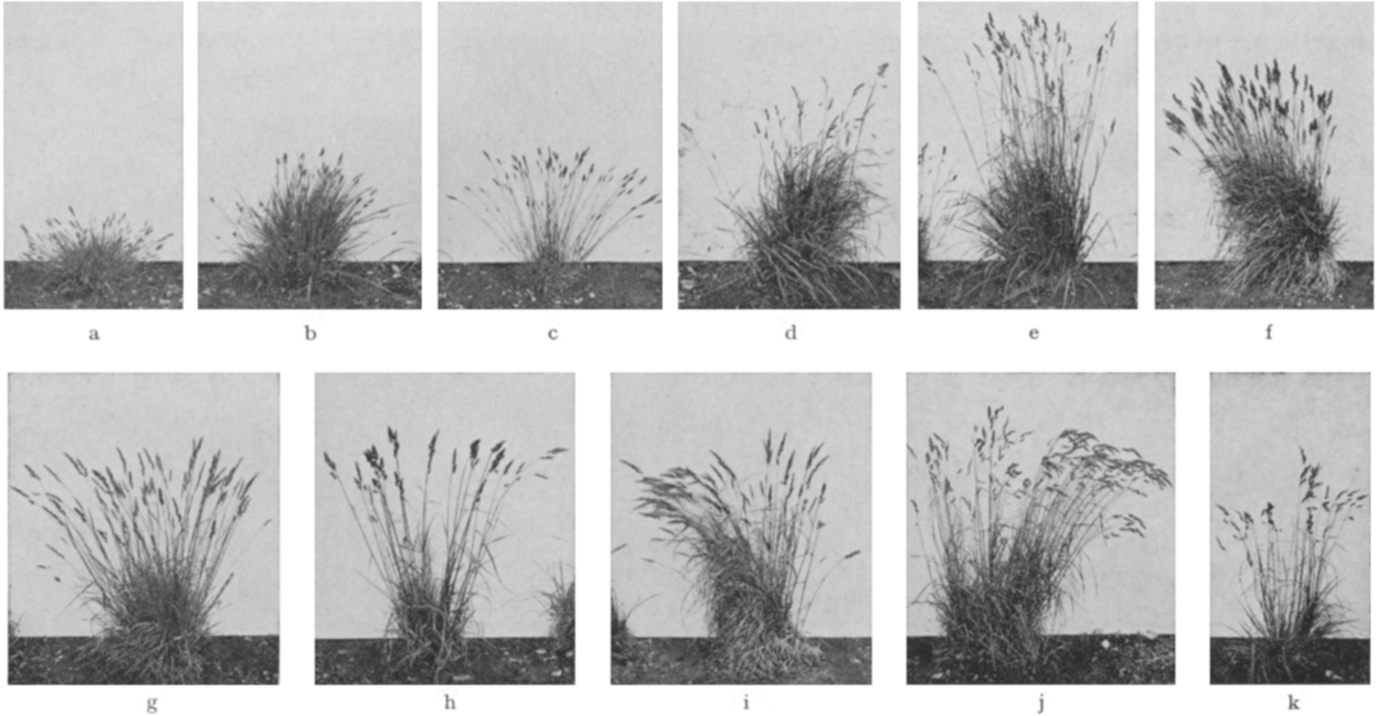

PLATE 1. *Dactylis* taxa and hybrids. a–e tetraploids and hybrids, f triploid, g–k diploids and hybrids.

a. *D. marina* Nazaré, Portugal. b. *D. marina* × *D. glomerata* subsp. *hispanica* c. *D. glomerata* subsp. *hispanica* Greece d. *D. marina* × *D. glomerata* subsp. *glomerata* e. *D. glomerata* subsp. *glomerata* Cultivar Aberystwyth S. 37 f. *D. glomerata* subsp. *glomerata* × *D. glomerata* subsp. *lusitanica* triploid g. *D. glomerata* subsp. *lusitanica* h. $F_1$ subsp. *lusitanica* × subsp. *aschersoniana* backcrossed to *lusitanica* i. $F_1$ subsp. *lusitanica* × subsp. *aschersoniana* j. $F_1$ subsp. *lusitanica* × subsp. *aschersoniana* backcrossed to *aschersoniana* k. *D. glomerata* subsp. *aschersonian*

TABLE 1. *Dactylis* subspecies studied

| Population or variety | Accession No. | Locality and habitat | Collector and date received |
|---|---|---|---|
| *Dactylis glomerata* subsp. *glomerata*, tetraploid | | | |
| Scilly Isles | Bc 3165<br>Bc 3171<br>Bc 3176<br>Bc 3189 | St. Mary. Amongst grass and bracken | A. R. Beddows, W.P.B.S. 15.11.47 |
| Cornwall | Bc 2862 | St. Justinian. Nr. lifeboat house | A. R. Beddows, W.P.B.S. 24. 5.46 |
| | Bc 2967 | Tregony | ,, ,, 13.11.47 |
| | Bc 3057 | Road to Sennen Cove | ,, ,, 14.11.47 |
| Scotland | Bc 4237 | South Queensferry. Open mixed deciduous wood 150 ft. alt. | J. W. Gregor, Scot. Pl. Breed. Sta. 23.1.54 |
| Aberystwyth cultivar S. 37 | Bc 2826 | Penglais Field | W.P.B.S. 1944 |
| | Bc 4373 | Gogerddan | ,, 1954 |
| Aberystwyth cultivar S. 143 | Bc 3904, 3905 | Penglais | ,, 1948 |
| | Bc 4087 | Library Field | ,, 1952 |
| Anglesey | Bc 2716 | Mixed collection from hedgerows | T. J. Jenkin, W.P.B.S. 19.1.45 |
| American cultivar | Bc 2892 | Penglais Field | W.P.B.S. 1945 |
| Lischower cultivar | Bc 4267 | Gogerddan | N.I.A.B. Cambridge 29.3.54 |
| Norway | Bc 4173 | Tjøtta. Near station farm 1 m. above sea level with *Betula*, *Salix*, *Phalaris* | Magnus Jetné, Tjøtta. 28.1.54 |
| Finland | Bc 4276 | Tikkurila. Dry sandy soil | Prof. Valle, Agric. Res. Centre. 3.4.54 |
| Denmark | Bc 4141–54 | Overbygaard, Børkop. 40 m. above sea level between fields and forest | Henrik Bögh. 28.1.54 |
| *Dactylis glomerata* subsp. *hispanica*, tetraploid | | | |
| Israel | Bc 3923 | Neveh Yaar, Drier hillsides | Amos Dovret, Agric. Expt. Sta. Neveh Yaar. 13.12.49 |
| | Bc 4037 | Matzuba, Galilee | ,, ,, ,, 15. 4.42 |
| | Bc 4155 | Rendzina soil 175 m. alt., 600–650 mm. rain | ,, ,, ,, 23. 1.54 |
| | Bc 4157 | Terra rossa 700 m. alt. 700–750 mm. rain | ,, ,, ,, 23. 1.54 |
| Cyprus | Bc 2803 | Kyperaunda, Nicosia | |
| | Bc 4081–5 | ,, ,, | Nicosia, Dept. Agric. 3.8.49 |
| | Bc 3727–8 | Roadside, central hills | |
| | Bc 4178–80 | Limestone formation | K. Jones, Dept. Agric. Nicosia. 23.1.54 |
| Sicily | Bc 3947 | San Nicola, Enna | Prof. Zanini, 1.11.50 |
| | Bc 4045 | Feudo giardinello Palermo. Collected 1950 | Prof. Zanini 24.4.52 |
| Morocco | Bc 3930 | Grand Atlas | Centre de recherches Agronomique Rabat 3.7.50 |
| | Bc 4133 | Cedar Forest, Chrea near Blidda, Algiers 1550 m. | A. Money-Kyrle. 9.7.53 |
| Portugal | Bc 3959 | | Prof. A. Camara, Estaçao Agronomica Naçional. 1.2.51 |

*Dactylis marina*, tetraploid

| | | | |
|---|---|---|---|
| Portugal | Bc 4296 | Limestone cliffs. Nazaré, Portugal. | Estaçao Agronomica naçional Sacavém |

*Dactylis glomerata* subsp. *lusitanica*, diploid

| | | | |
|---|---|---|---|
| Portugal | Bc 3948<br>Bc 3957 | No data | Prof. A. Camara, Estaçao Agronomica Naçional 1.2.51 |
| | Bc 4441 (FAO 3602) | Collected at Sao Martinho Do Porto 2–3 km. from the coast, grey-brown loam, low lying area wet in winter. Alt. 5 m., 900 mm. rain | C. A. Ncal-Smith. C.S.I.R.O. Australia. 18.10.56 |

*Dactylis glomerata* subsp. *aschersoniana*, diploid

| | | | |
|---|---|---|---|
| Sweden | Bc 2805<br>Bc 2835<br>Bc 2911 | No data | Bot. Gardens, Uppsala. 28. 1.46<br>14. 3.46<br>12. 4.47 |

*Dactylis glomerata* subsp. *woronowii*, diploid

| | | | |
|---|---|---|---|
| Iran | Bc 2912<br>Bc 4074 | No data | Bot. Gardens, Uppsala 12. 4.47<br>13.11.52 |
| | Bc 4355 | Katal Yek Chinar, Khorassan, Iran | G. L. Stebbins, Univ. Calif. U.S.A. 21.4.55 |

*Dactylis glomerata* subsp. *indeterminavit*, diploid

| | | | |
|---|---|---|---|
| Algeria | Bc 4439(FAO. 3215) | Oued Chiffa, between Edmond Daudet and Oued Mouzaia, Railway embankment 400 m. alt. 800 mm. rain, loam, a composite sample. | C. A. Neal-Smith, C.S.I.R.O. Australia. 18.10.56 |
| | Bc 4440(FAO. 3328) | 7 km. N.W. of Bouira, top of embankment. 500 m. alt. 750 mm. rain, yellow-brown clay loam and stones. Four plants. | C. A. Neal-Smith, C.S.I.R.O. Australia. 18.10.56 |

*Dactylis glomerata* subsp. *judaica*, diploid

| | | | |
|---|---|---|---|
| Israel | Bc 4302 | Kiryat Schmuel, Jerusalem. | G. L. Stebbins, Univ. Calif. U.S.A. 2.1.55. |

The authors are especially indebted to A. R. BEDDOWS, Welsh Plant Breeding Station, for providing two of the progenies on which our triploid data are in part based. He, as far as we know, was the first person to obtain experimentally triploid and other wide-cross hybrids in *Dactylis* (BEDDOWS, 1959).

## MATERIAL AND METHODS

The ability to exchange genes through hybridisation can give an insight into the relation between major taxonomic groups, and populations in the groups. Details of the *Dactylis* taxa studied from this point of view are given in Table 1.

Gene exchange is usually assessed in terms of fertility, that is to say, the ability of a female parent in an initial cross and in subsequent generations to set seed, and the viability of this seed in a germination test. Pollen fertility, as judged by staining reaction, indicates the effectiveness of the male gametes produced by such plants. The aim of the present work is to study variations in fertility which have a genetic basis.

Fitness, in the sense of ability to survive in micro-evolution, must be considered in relation to the environment of the parental plants, and this differs from fertility. Two populations may, for example, be able to exchange genes freely and the progeny be completely fertile, but the individuals may be unfit to survive in the parental environment. For fitness to remain constant the change in environment must be accompanied by proportionate genetic change, and, conversely, genetic change, as in hybrids, will achieve balance only in a hybrid environment. Fitness is, therefore, a genetical and physiological state of equilibrium in relation to a particular environment.

In addition to genic effects, chromosome number may introduce a considerable divergence between fertility and fitness, as for instance in the 35-chromosome polyploid race of *Holcus mollis*, where the seed-setting ability is greatly superior to the fitness of the sexual progeny. This has been discussed by JONES (1958) who cites other examples.

The assessment of fertility differences having a genetic basis in out-breeding organisms raises a number of problems for the experimenter, due to the great plant-to-plant variation in self- and cross-fertility, and the possible effect of genotype/environment interaction.

The problem is aggravated in studies which, like the present, are linked to a plant introduction programme in which the material has to be utilised as it becomes available, and hybridisation carried out in successive seasons.

The effects of genotype/environment interaction have been minimised as far as possible in handling the material. The techniques used are briefly described:

*The assessment of self- and cross-fertility.* The methods of making crosses were based on those described by JENKIN (1924, 1931). The florets selected as female units were enclosed with inflorescences of the pollen parent in glassine paper bags. Where self-fertility was being studied, groups of heads from the same plant were enclosed in pollen-proof bags. Cross-fertilisation depended on the almost complete self-incompatibility of the majority of *Dactylis* plants, and this necessitates the rejection of genotypes with high self-fertility for use as female parents. In the very few instances where self-fertile plants had been used as female parents, some of the progeny was obviously inbred, as evidenced by their lack of vigour, consequent winter killing and the presence of chlorophyll-deficient seedlings. The great majority of selfing of the female parent was carried out at the same time as hybridisation, so that self- and cross-fertility were assessed under comparable environmental conditions.

*Environmental effects on the assessment of cross-fertility.* The availability of pollen, and the ripening of seed are susceptible to unfavourable conditions. In controlled crosses in the glasshouse care was taken to bring together inflorescences which were correctly phased relative to pollen production and stigma exsertion, and to place the pollen donors higher in the bags so that on shaking the pollen would fall onto the stigmas. Where possible units were put up for crossing on warm dry days. Cool, humid weather may lead to reduced seed-ripening in enclosed heads. Such conditions were met with on one occasion in 1956, and countered to some extent by removing the bags and heating the glasshouse with electric radiators. The possible effects of cool, wet weather on pollination amongst plants in the field could not be mitigated and remained a potential source of genotype/environment interaction. With regard to seed ripening, fertility was assessed in a manner which largely compensates for the results of adverse conditions. Ripe inflorescences were threshed by hand and caryopses detectable by touch were removed, this is termed the 'threshable' seed. A sample of this seed was tested for germination in a warm glasshouse. The chaff which remained after threshing was also sown, as in many instances where the yield of threshable seed was depressed, many small viable caryopses were present in the chaff and germinated to give vigorous seedlings. The data were, therefore, pooled to give the number of seedlings obtained for every 100 spikelets used on the female parent. This assesses the potential contribution to the next generation and can be termed the 'fertility index' = F.I. By this means, whenever pollination was effective the viable caryopses were detected.

The data suggest that when sub-optimal environmental conditions occurred they affected seed maturation, i.e., the formation of the endosperm and caryopsis size, and not embryo development. It is reasonable to suppose that in these *Dactylis* crosses, pollination is not a limiting factor. Embryo formation appears to be less sensitive to the effects of genotype/environment interaction, than is endosperm development.

Pollen was taken from anthers just prior to exsertion and stained in aceto-carmine. In the hybrids there was a gradation between normal and aborted grains, and more variation in grain size than in the parents. Only cells full of cytoplasm and well-stained nuclei were counted as fertile. Two preparations of at least 200 grains were made from each plant.

The object of the experiment was to compare the cross-fertility of populations and taxa and so to ascertain any differences in their ability to exchange genes. In practice the plant-to-plant variation in F.I. is great. In a sample of 50 plants of *Dactylis glomerata* subsp. *lusitanica* inter-pollinating under favourable conditions the F.I. ranged from 10 to 900. It is clear that to find average compatibility levels between populations large numbers of hybridisations, or of individual hybrid plants, must be studied because of the wide variation between genotypes occurring in outbreeding plants (COOPER 1959). Comparisons within and between the major taxa were made in each successive season, and the data pooled because there were no worthwhile differences in fertility between the sub-groups, for instance, as between the various pairs of diploid subspecies. The high plant-to-plant variation in fertility indicates that substantial reductions in cross-fertility must occur before they can be reliably detected. The data were therefore grouped into crosses between taxa and crosses within taxa, i.e., between populations of the same taxonomic group. Comparisons were made with data from plants within a single interbreeding population growing in what were judged to be optimal conditions, and which therefore acted as a standard for fertility index. The frequency of the components of fertility in the various groups is shown

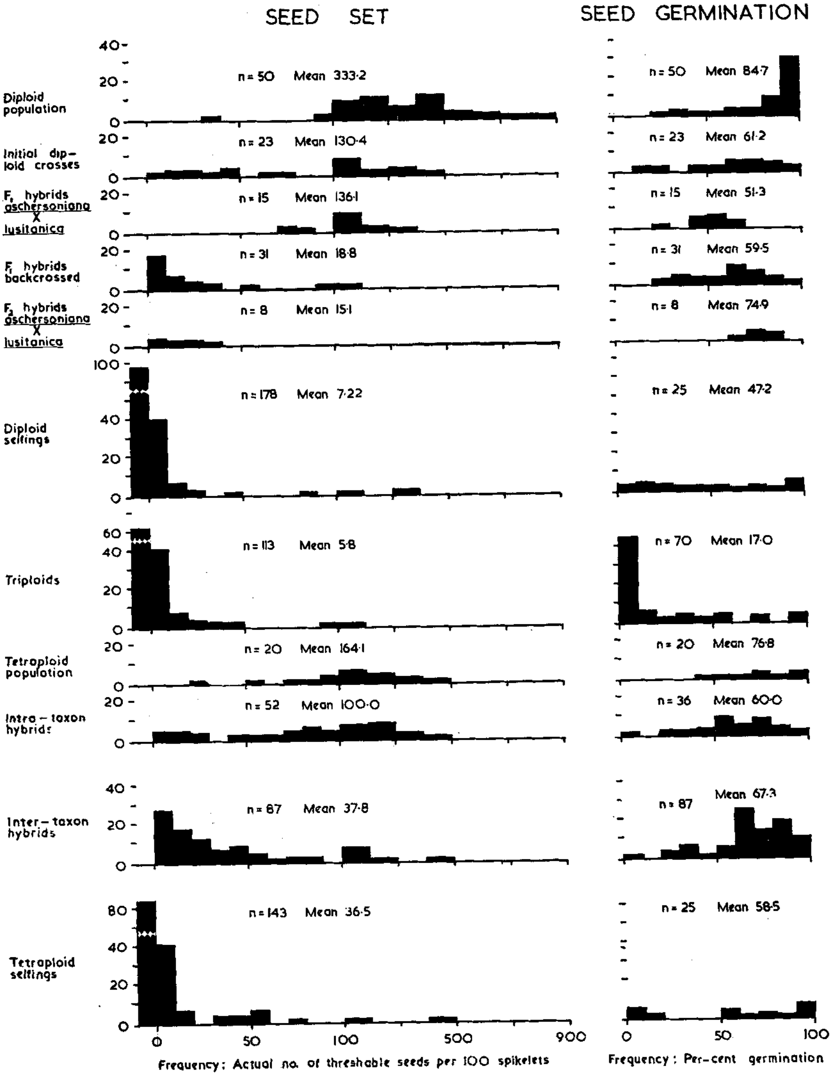

Fig. 1. Frequency distribution of components of fertility in *Dactylis*, populations and hybrids. Threshable seed per 100 spikelets, and percent germination.

in Figs. 1 and 2. The wide range of seed-setting from cross to cross, and plant to plant, means that in some instances most of the seed is produced by a few very compatible individuals, whereas in actual fact most plants set few seeds or none at all. To demonstrate more clearly what occurs in these plants a scaling for seed-set and F.I. has been used: from 0 to 100, the frequency classes are 10 units; above 100, they are 100 units each.

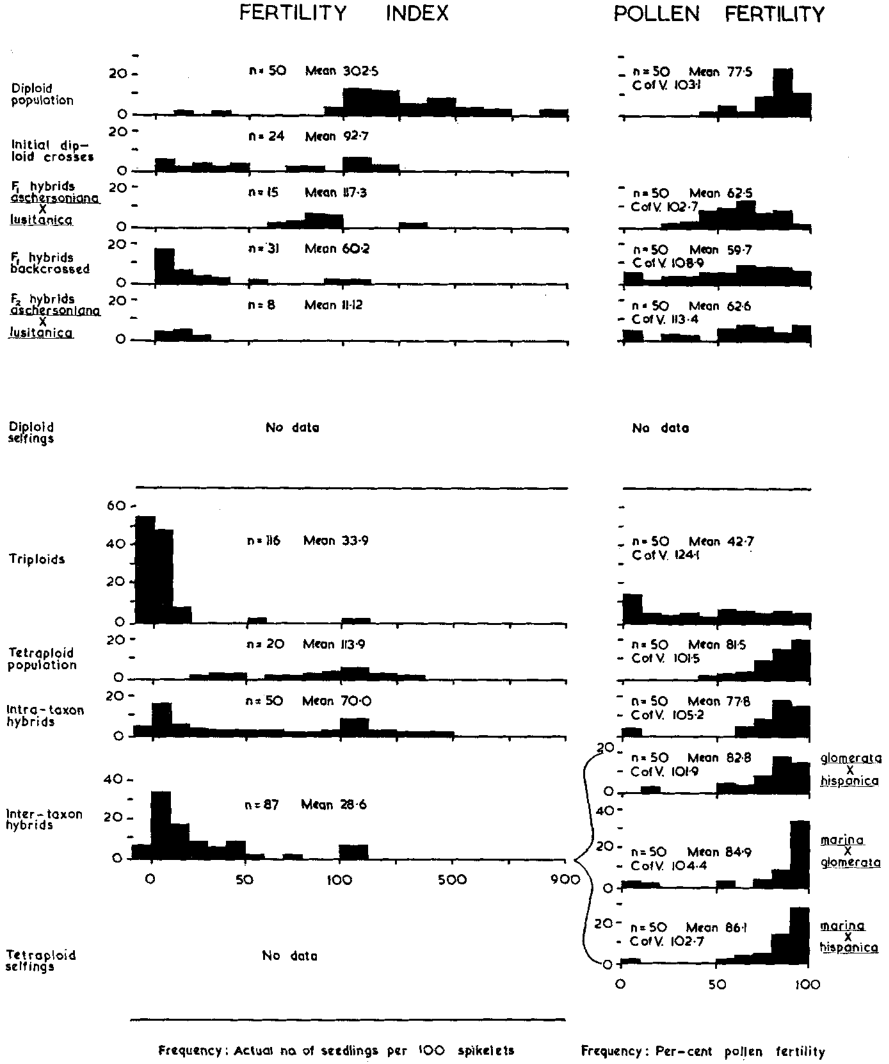

Fig. 2. Frequency distribution of components of fertility in *Dactylis* populations and hybrids. Fertility index and pollen fertility.

## EXPERIMENTAL RESULTS

The results for the components of fertility, namely, seed-set per 100 spikelets, percentage germination, seedlings established per 100 spikelets (F.I.) and percentage pollen fertility, will be discussed under four headings, diploids, tetraploids, self-fertility and triploids.

### DIPLOIDS

An aggregate sample of *Dactylis glomerata* subsp. *lusitanica* (Plate 1 g) was used as a standard of fertility. This was chosen because it grows luxuriantly and ripens seed very well at Aberystwyth. Experience has shown that for some Mediterranean diploids the climate in West Wales is sub-optimal for growth and seed setting. Fertility was high in this population, which had an F.I. of 302.5. The seed-setting and F.I. data show an approximately normal frequency distribution, but the germination and pollen stainability are skew, with the mode in the direction of the higher fertilities. Hybrids were made between subspecies *aschersoniana, lusitanica,* and *woronowii,* as follows, a different genotype being used in each cross:

| Subspecies | No. of crosses | Mean seed-set per 100 spikelets |
|---|---|---|
| *aschersoniana* × *woronowii* | 8 | 120.3 |
| *aschersoniana* × *lusitanica* | 8 | 158.9 |
| *woronowii* × *lusitanica* | 8 | 160.7 |
| | 24 | 146.6 |

Nearly all the crosses and selfings were made at the same time and no reciprocal differences were observed. Further study was concentrated on hybrids between *aschersoniana* and *lusitanica* (Plate 1, k and g), selected because they are well adapted to the climate at Aberystwyth, and on morphological grounds are a pair of closely-related subspecies (Borrill, 1961a). This would suggest that any reduced capacity for gene exchange in this instance might be exaggerated in other pairs of subspecies originating from more dissimilar environments and showing greater morphological differences. Fifteen $F_1$ hybrid plants of *aschersoniana* × *lusitanica* grown in the field were examined for fertility (Plate 1, i).

Backcrosses were made to the diploid parents (Plate 1, h and j).

Four diploid hybrid progenies were used, 557bC(1), 558bC(1), 560bC(1) and 562bC(1), each resulting from the crossing of a different pair of parental genotypes. Individual hybrid genotypes were backcrossed to a number of different genotypes of both parents. No reciprocal differences were observed. The backcrosses to *lusitanica* were apparently more successful than those to *aschersoniana* but this is not a consistent trend:

| Hybrid parent No. | Diploid parent | No. of crosses | Mean seed-set per 100 spikelets |
|---|---|---|---|
| 557 bC(1) | *lusitanica* | 6 | 53.0 |
| | *aschersoniana* | 7 | 7.9 |
| 558 bC(1) | *lusitanica* | 4 | 5.0 |
| | *aschersoniana* | 5 | 14.8 |
| 560 bC(1) | *lusitanica* | 3 | 12.3 |
| | *aschersoniana* | 2 | 3.5 |
| 562 bC(1) | *lusitanica* | 2 | 6.0 |
| | *aschersoniana* | 2 | 29.0 |
| Backcrosses to *lusitanica* | | 15 | 25.8 |
| ,, *aschersoniana* | | 16 | 12.2 |

In addition, individual genotypes within $F_1$ hybrid progenies 557bC(1), 558bC(1), and 562bC(1), were crossed together to give an $F_2$ generation. Figs. 1 and 2 indicate that hybridisation between the diploids results in reduced fertility. There was no significant difference in the fertility of the different subspecies combinations. In the initial crosses there is a drop in mean seed-set, and seed germination is substantially reduced, consequently the F.I. is depressed. Comparisons can legitimately be made between the pooled data for the initial crosses and $F_1$ hybrids, and the backcrosses and $F_2$ plants involving only *aschersoniana* × *lusitanica*, since the fertility data for the initial hybrids between all three diploid taxa are essentially similar. In the backcrosses and $F_2$ plants a successive reduction in fertility occurs. The fertility index means go down progressively as follows:

| *lusitanica* population | | All initial crosses | | *aschersoniana* × *lusitanica* Backcrosses | | $F_2$ |
|---|---|---|---|---|---|---|
| 303 | → | 92.7 | → | 60.2 | → | 11.1 |

The average fertility of individual $F_1$ plants of *aschersoniana* × *lusitanica* in the field was a little higher than the corresponding initial crosses in the glasshouse. Accompanying the reduction in average fertility is a change in the shape of the frequency curve. In *lusitanica* the F.I. is distributed normally, germination and pollen fertility have a skew distribution with a mode well towards the high fertility. In the $F_1$ the distribution curve becomes broad and shallow, and in the backcrosses and $F_2$ plants the curve is skew but in the opposite direction, the majority of crosses having an F.I. between 0 and 10.

Mean pollen fertilities of the groups were compared using the *t* test. The backcrosses and $F_2$ hybrids showed a reduction significant at 5% when compared to *lusitanica*. The coefficients of variation showed that variability in *lusitanica* and the $F_1$ was similar at 103, whereas in the backcrosses and $F_2$ there was a marked increase significant at 5%, up to 109 and 113 (Fig. 2). All the data indicate that hybridisation between these diploid taxa leads to a reduction in hybrid fertility and consequent decline in ability to exchange genes.

## TETRAPLOIDS

Seed-setting was studied in a sample of plants of wild Danish and the Aberystwyth cultivar S. 37 (Bc 4141–4154, Bc 4373) (Fig. 1e). These belong to subsp. *glomerata* and were chosen because they were well adapted to the climatic conditions at Aberystwyth. The seed ripened well and heads were harvested singly under favourable conditions. The seed-setting and F.I. data are distributed normally, pollen fertility showed a skew distribution with a mode between 90–100%. The germination figure (available for wild Danish only) was 77%. The fertility index of these tetraploids, 114, was not as high as in *lusitanica*, 333, but the data show a similar distribution. STAPLEDON (1931) found that in the tetraploids fertility varied from 78 to 364 seedlings per panicle, which is equivalent to F.I. of 62 to 288 (mean 129), using the figure of 126 spikelets per panicle as a conversion factor (BORRILL 1961a). Our tetraploid sample is above average for fertility in *Dactylis;* the *lusitanica* is highly fertile. The data indicate that the percentage of viable seeds found in *Dactylis* ranges from 15.4 to 72.2 with an average of 32.3. On the average, only between 1 and 3 florets set viable seeds out of 5 or 6 in each spikelet.

The pattern of phenotypic variation and distribution is more complex in tetraploid *Dactylis* than in diploid *Dactylis* (BORRILL, 1961a).

The boundaries of the major groups are less easily defined, apart from *Dactylis marina* (BORRILL 1961b). Using the tetraploid populations detailed in Table 1, hybrids were made between the taxonomic groups, and between populations within these groups, to ascertain any broad differences in their ability to exchange genes, in accordance with their degree of morphological dissimilarity. The within-taxon populations are more phenotypically similar than those from different taxa. No account is taken of geographical distance which varied greatly, and in the tetraploids is of less significance than microclimate; populations separated by quite short distances often show considerable morphological variation.

*Hybrids within taxa.* Hybrids were made between populations within each of the main taxonomic groups, using a different pair of genotypes in each cross.

| | Populations | Hybridisations |
|---|---|---|
| Within *marina* | 5 | 27 |
| ,, *glomerata* | 6 | 13 |
| ,, *hispanica* | 4 | 12 |
| | 15 | 52 |

The results were of a similar nature and the data for the 52 crosses were pooled, Figs. 1 and 2. There is a definite reduction in average fertility compared to Danish and Aberystwyth cultivar S. 37, and a tendency for some of the crosses to give a low seed-set; this is expressed in a wide and shallow frequency curve. Seed germination is also reduced (mean 60%). The F.I. frequency shows a suggestion of skew distribution, with about half the crosses giving an F.I. of less than 40.

*Hybrids between taxa.* A range of genotypes from populations of different taxa was hybridised. Those between *glomerata* and *hispanica* were more fertile than the other combinations. The overall level of fertility was much lower in those crosses than in the hybrids within taxa, and the data were pooled for comparison, Figs. 1 and 2. Hybrids between *D. marina*, subsp. *glomerata* and *hispanica* are shown in Plate 1, d and b.

| Subspecies and No. of populations | Hybridisations | Mean seed-set per 100 spikelets |
|---|---|---|
| *glomerata* (10) × *hispanica* (6) | 26 | 48.7 |
| *marina* (1) × *glomerata* (5) | 23 | 20.9 |
| *marina* (1) × *hispanica* (8) | 38 | 16.2 |
| Total of populations 31 | | |
| ,, ,, hybridisations 87 | | |
| Overall mean seed-set per 100 spikelets 28.6 | | |

In *marina* a range of genotypes from Nazaré, Portugal was used, the other populations of this subspecies not then being available. The result of hybridisation between the main taxa is a definite reduction in fertility. The frequency for seed-set and F.I. is skew with a low mode. By far the majority of plants have a low F.I. (Fig. 2). Seed germination is higher than in the intra-taxon hybrids, but probably not significantly so. The F.I. declines progressively as we move from a single population, (114), to crosses between populations in the same taxon, (70), and finally to crosses between different taxa, (28).

The pollen stainability results taken from the female parents of these initial crosses are all similar in average value and frequency distribution (Fig. 2). The coefficient of variation is also similar. Male gamete production is therefore effective in the female parents of these crosses. The data are included here because they show the occurrence of a few individual plants with poorly-staining pollen. The cytological data suggest these may be interchange heterozygotes, which have been found in population plants and hybrids (JONES 1961).

The initial effect of crossing individual plants from different tetraploid populations is a reduction in fertility. This is greater when populations from separate taxa are involved, but is less than that observed in diploid hybrids.

## SELF-FERTILITY

178 selfings were performed in the tetraploids and 143 in the diploids. The large majority of genotypes are self-incompatible (Figs. 1 and 2). Germination is very variable, the frequency histogram showing about equal numbers of plants in all the classes. The conse-

quence of poor germination is a very low F.I. both in the diploids (0.83) and in the tetraploids (0.23). There is an apparent slightly greater overall fertility in the diploids. No pollen data are available for the selfings. These results are in close agreement with those of STAPLEDON (1931) and BEDDOWS (1959).

### TRIPLOIDS

The fertility and chromosomal behaviour of the triploids (Plate 1f) will be considered in detail in a further paper. The fertility data for the initial crosses are, however, summarised here (Figs. 1 and 2) for comparison with the situation in diploids and tetraploids. Seed-setting and germination are low with a skew distribution, the mode being at a very low level of fertility. Consequently the F.I. for the 116 crosses is only 3.39. Pollen stainability fluctuates widely. The mean of 42.7% is significantly lower ($P < 0.05$) than the diploid or tetraploid populations, and the coefficient of variation (124) is significantly the highest obtained. Compatibility and frequency distribution in the triploids are very similar to those obtained on selfing. It is apparent that there is a considerable barrier to gene exchange between the ploidy levels.

### CORRELATION BETWEEN THE COMPONENTS OF FERTILITY

In this study it has been observed that as a result of hybridisation there is an average reduction both in seed-setting and in the stainability of the pollen and also a lowering of the capacity of the seed to germinate. It is pertinent, therefore, to inquire whether there is any plant-to-plant correlation between these factors.

*Seed-setting and germinability.* In a genetically-balanced population of the diploid subsp. *lusitanica* (Fig. 3) there is no plant to-plant correlation. Germination is high, out of 50 plants only 3 were below 50%, and only 9 below 80%. Seed-setting fluctuates to a greater extent. It is worth noting that although many plants with low seed-setting had good germination it is very rare to find a plant which sets a lot of seed and has poor germination.

In the $F_1$ hybrids the diploids and tetraploids behaved similarly; the diploid data are shown in Fig. 4. As in the diploid population there is no significant correlation between the variables. There are,

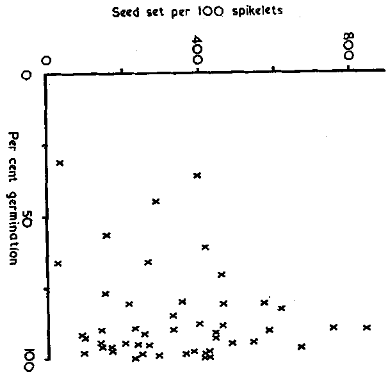

Fig. 3. Relation between seed set and germination, *Dactylis glomerata*, subsp. *lusitanica*.

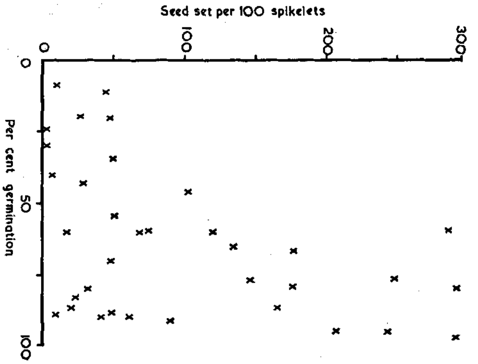

Fig. 4. Relation between seed set and germination, diploid hybrids.

however, more genotypes with lowered germination, and these *tend* to be the low seed-setting plants. In the diploid hybrids a lower average fertility suggests a degree of genic unbalance compared to the diploid population, and in a proportion of the genotypes this appears to affect seed-set and germinability in the same direction.

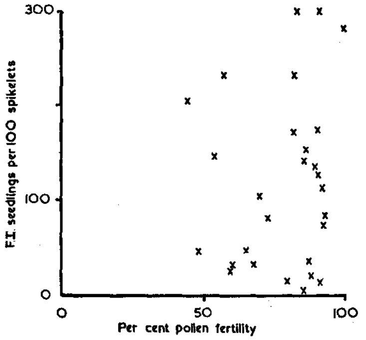

Fig. 5. Relation between F.I. and pollen fertility, diploid and tetraploid population plants.

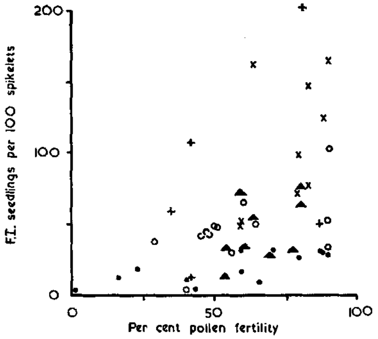

Fig. 6. Relation between F.I. and pollen fertility, diploid hybrids.

× *lusitanica* × *aschersoniana*
○ *lusitanica* × *woronowii* (Uppsala)
● *aschersoniana* × *woronowii* (Iran)
significant at $P < 0.05$
+ *lusitanica* × *judaica*
△ *aschersoniana* × *woronowii* (Uppsala)

*Fertility index and pollen fertility.* In the genetically-balanced diploid and tetraploid populations (Fig. 5) there is no correlation on the same plant between F.I. and pollen fertility. It is notable that stainability exceeds 50% in all except 2 out of 28 plants. In the diploid hybrids (Fig. 6) each inter-group combination is shown separately, and in many plants pollen fertility is greatly reduced as well as the F.I. There is, therefore, a trend in these hybrids, although this reaches statistical significance in only one subspecies combination. Again it appears that where genic unbalance is great enough to lead to overall reduction in fertility, as in these hybrids, there is a suggested correlation on the same plant between seeding and male gamete effectiveness.

Observations on pollen diameter were made during the course of these studies (Fig. 7). The results indicate that the means for diploid populations are generally smaller than those for the tetraploids. The difference between all diploids and all tetraploids is significant at 5%. Some individual pairs of diploid and tetraploid do not differ significantly. Some, such as diploid subsp. *lusitanica* and tetraploids from Cyprus, are very similar. Pollen diameter cannot, therefore, be used as a reliable character to separate material of *Dactylis* into chromosome races. The hexaploid obtained from twin seedlings ex tetraploid seed has smaller grains than the tetraploids.

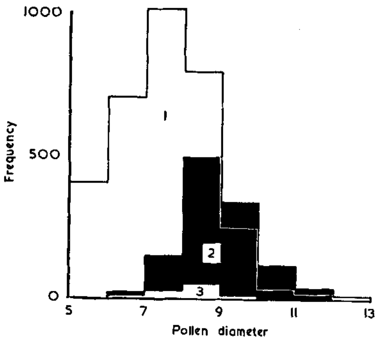

Fig. 7. Pollen diameter in *Dactylis* (arbitrary units)
1 Diploid 2 Tetraploid 3 Hexaploid

*Pollen diameter (arbitrary units)*

| Diploids | | | Tetraploids | | | Hexaploid | |
|---|---|---|---|---|---|---|---|
| Subspecies | No. of plants | Average diam. | Sub-species | No. of plants | Average diam. | | Average diam. |
| *aschersoniana*<br>Bc2911, Bc2835 | 10 | 7.85 | *glomerata*<br>Lischower<br>Bc 4267 | 8 | 8.67 | One genotype<br>Bc 4379 | 8.55 |
| *woronowii*<br>Bc4074, Bc2912<br>(Bot. Gard.) | 10 | 7.77 | Finland<br>Bc 4276 | 6 | 9.60 | | |
| *woronowii*<br>Bc 4355 (Iran) | 8 | 8.30 | Scotland<br>Bc 4237 | 8 | 9.17 | | |
| *judaica*<br>Bc 4302 | 10 | 7.96 | *hispanica*<br>Portugal<br>Bc 3959 | 9 | 9.17 | | |
| Algeria F.A.O.<br>Bc 4439 | 4 | 7.13 | Cyprus<br>Bc 4179 | 8 | 8.76 | | |
| *lusitanica* ex<br>F.A.O.<br>Bc 4441 | 4 | 7.73 | *D. marina* ex<br>Portugal<br>Bc 4296 | 16 | 9.20 | | |
| *lusitanica*<br>ex Portugal<br>Bc 3957 | 8 | 8.72 | | | | | |
| *lusitanica*<br>ex Portugal<br>Bc 3948 | 4 | 7.67 | | | | | |

## DISCUSSION

In *Dactylis* little experimental hybridisation has previously been attempted. In the diploids, ZOHARY (1956, unpub.) made hybrids between subspecies *aschersoniana, hookerii, woronowii, judaica* and *lusitanica,* and reported that all the $F_1$ hybrids showed a considerable reduction in pollen fertility, chiefly of the order of 20–50% aborted pollen. Big differences were found to exist between individual plants of the same hybrid combination. There was a slight reduction in seed-setting when compared to the parents.

MC. COLLUM (1958) by contrast found a slight but not significant increased seed-set in the $F_1$ hybrids as compared to the parents. This is probably a consequence of the small number of hybrids studied, and also that in some cases the diploid parents grown in a winter glasshouse had an abnormally low seed-set. Pollen fertility was studied and no biologically significant differences or trends were found.

According to HERTZSCH (1959) WEIBULL found that *aschersoniana* and *woronowii*, and the diploids from Iran, Israel and Grigna, (N. Italy) were fully interfertile. As far as the tetraploids are concerned Weibull also found that *glomerata* and *hispanica* produce fully fertile hybrids .The view is expressed by STEBBINS and ZOHARY (1959) that the tetraploids give fully fertile $F_1$ and $F_2$ generations.

The extensive results of the present study are in agreement with those of ZOHARY (1956) regarding reduction in fertility on hybridisation of the diploids, and the great plant-to-plant variation in fertility within a progeny. They go further, however, and demonstrate that when sufficient hybrid combinations are attempted to show the general trend the magnitude of the drop in fertility in the $F_1$, and particularly $F_2$, is substantial and is of real significance.

In the tetraploids there is a drop in fertility on initial hybridisation. The cytological evidence, to be given in a subsequent paper, shows that regular bivalent pairing is the rule in the diploid populations and hybrids. In the tetraploids, however, preferential pairing amongst the four similar chromosomes is especially sensitive to small differences between the parental chromosomes. We are able to detect a highly significant increase in bivalent pairing without change in chiasma frequency. The consequences of differentiation between the parental chromosome sets have been noted cytologically and in hybridisation.

The situation in *Dactylis* regarding its ability to exchange genes is similar to that reported for a number of other plants. GRANT (1958) considering genetic recombination in relation to sterility barriers, cites 72 instances of varying degrees of inter-sterility between taxa, some of which, (notably *Streptanthus glandulosus* KRUCKEBERG (1957) and *Aster* AVERS (1953)) are relevant to the situation in *Dactylis*. In the apparently cross-fertilising *Streptanthus glandulosus* complex a large number of hybridisations were made between populations from the same and different recognised taxa. Hybrid fertility, judged by pollen staining, was shown to be related to seed-setting. Varying degrees of hybrid sterility were observed. In *Streptanthus*, as in diploid *Dactylis*, regular 14 bivalent pairing was observed at meiosis in all the hybrids. This hybrid sterility consequently arises from genic and/ or fine structural disharmonies in the paired chromosomes. As we cannot distinguish between these KRUCKEBERG (1957) suggests the name "cryptic hybridity".

The extent of hybrid incompatibility in *Streptanthus* is roughly proportional to the distance between the parent populations. There is a similar situation in *Gilia capitata* subspecies (GRANT 1952). In these species, distance, or topographical barriers, permit the accumulation of genetic differences resulting in loss of fertility when hybridised. In such flowering plants, which are largely insect pollinated, effective isolation depends a good deal on the behaviour of the vectors, whilst in wind-pollinated grasses the situation is simpler, in that isolation of 200 yards effectively reduces introgression, permitting genetical and morphological differences to accumulate to the extent of near, or complete, inter-sterility, as in *Elymus* (SNYDER 1950, 1951) or in *Glyceria* (BORRILL, 1958). A full discussion of this point is given in BORRILL (1958).

In *Dactylis* our assessment of the occurrence of sterility barriers must be related to the chromosomal status and geographical distribution of the taxa. The endemic diploids, and certain others, are morphologically and geographically distinct entities. This applies to the subspecies on which our hybrid data are based. The living subspecies probably represent relics of a widespread diploid complex depleted by the last glaciation. Their geographical isolation has, therefore, lasted a long time and conditions have been favourable for the accumulation of genetic differences which are thus a consequence of isolation. The extent of the reduction in diploid hybrid fertility can be judged by the percentage F.I. (using 6 as the average number of florets per spikelet, BORRILL (1961a). Comparing the average fertility with that of the most fertile individual in each generation, it may be supposed that stringent selection for fertility would be less effective in successive generations. A further decline might therefore be expected in the $F_3$.

| F.I. | Diploid populations | Initial crosses | Subspecies *aschersoniana* and *lusitanica* | | |
|---|---|---|---|---|---|
| | | | $F_1$ | Back-crosses | $F_2$ |
| Per cent average | 50 | 16 | 20 | 10 | 1.9 |
| , ,, highest individual | 133 | 83 | 50 | 16 | 3.0 |

In the tetraploids, *glomerata* and *hispanica* are complexes based on several existing diploids or their ancestors. *D. marina* (BORRILL 1961b) is a natural group the relatives of whose diploid ancestors, *D. smithii* and *D. ibizensis* occur today. It is not surprising, therefore, to find that *D. marina* shows its distinctness in respect of the lowest inter-fertility with the other groups:

| Subspecies | Mean seed set per 100 spikelets |
|---|---|
| *glomerata* × *hispanica* | 48.7 |
| *marina* × *glomerata* | 20.9 |
| *marina* × *hispanica* | 16.2 |

The inter-fertility of populations in these groups does not show the kind of relationship to distance observed in *Streptanthus*. The complex origin, particularly of *glomerata* and *hispanica*, and the wide ecological adaptability of *Dactylis* in general, may indicate the absence of such a relationship. When crosses between populations in the same tetraploid taxon are considered it seems that a re-patterning of the chromosomes has occurred to about the same extent throughout. The consequent lower inter-fertility is probably a transient initial result of hybridisation, since recombination between parental chromosomes in quadrivalents may lead to restored fertility.

The results are of significance in relation to evolution in *Dactylis*. They show that the diploids are partly inter-sterile, as a consequence of long periods of geographical isolation. One might plausibly argue that tetraploids originated on many occasions from hybrids between living diploids and some of these might have been successful because more fertile than the diploid hybrids. It is a matter for speculation, however, whether the diploids as we know them today were the ancestors of the tetraploids. In the past, diploids were probably more or less continuously distributed in temperate and sub-tropical regions, and consequently were inter-fertile. (The relict subsp. *smithii* is endemic in the sub-tropical zone of the Canaries). The reason for the initial success of the tetraploids is a matter for conjecture.

The degree and duration of isolation between tetraploids are less than between diploids, and inter-fertility, therefore, is much higher and opportunities for introgression occur widely. STEBBINS and ZOHARY (1959) describe 11 diploids which they regard as subspecies in preference to species, largely on the grounds that the diploids are potentially inter-fertile, a conclusion at variance with the present results, and indeed, with those of ZOHARY (1956) for the $F_1$ hybrids. Such considerations as ploidy, and potentiality for gene exchange, are misplaced as criteria for a taxonomic classification, which must recognise morphologically identifiable taxa. It may be noted that although *D. marina* can meet and hybridise with *hispanica* little introgression occurs, and *marina* is a perfectly good species. A taxonomic revision of the group will be presented elsewhere.

The practical consequences of loss in fertility lie at the diploid level, where the plant breeder seeking to utilise these 'pure' sources of genes by hybridisation may find difficulty. A more promising approach would appear to be the production of tetraploid hybrids by means of colchicine. Initially these would be expected to show more preferential pairing, and hence infertility, than natural tetraploid hybrids, but this could be rapidly overcome by selection. This will be discussed in a later paper.

## SUMMARY

Two main ploidy levels occur in *Dactylis*, namely, diploids and tetraploids, and the object of the study was to compare the cross-fertility of local populations and of taxa and so ascertain any differences in their ability to exchange genes.

Methods of measuring fertility, seed setting, germination and pollen staining were compared. The use of a 'fertility index' (F.I.) namely, the number of seedlings obtained per 100 female spikelets, was shown to have the advantage of minimising the effects of genotype/environment interaction when comparing cross-fertilities.

The fertility index of population plants varied greatly and showed no plant-to-plant correlation with pollen staining which was always over 50%. In hybrids, there was some plant-to-plant correlation between fertility index and pollen staining. Pollen staining may, therefore, not reliably indicate the seed setting capacity of genotypes in balanced populations.

A considerable reduction in ability to exchange genes was observed when diploid taxa were hybridised. The loss in fertility when crossing tetraploids was less marked. In the tetraploid populations, ease of hybridisation was in accordance with their taxonomic grouping.

The loss of fertility in the hybrids is interpreted in terms of genic repatterning of chromosomes in the spatially-isolated parental populations. In the diploid hybrids where bivalent pairing is the rule, the consequences of genic unbalance are considerable, since fertility is 30 times less in the $F_2$ than in a balanced population. This would have practical implications for the plant breeder wishing to combine qualities from two or more diploids.

In the tetraploids the differentiation between parental chromosomes leads to preferential pairing as bivalents and a reduced quadrivalent frequency. The harmful effect of hybridisation on fertility is expected to be transient at the tetraploid level, because of the extensive opportunities for the repatterning of parental chromosomes in the quadrivalents at meiosis.

These results are considered in relation to the pattern in some other outbreeding plants, and to evolution in *Dactylis*.

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