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A hybrid swarm between the diploid *Dactylorhiza fuchsii* (Druce) Soó and the tetraploid *D. purpurella* (T. & T. A. Steph.) Soó in Durham

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ABSTRACT

A study was made of the pollen grain meiosis and floral characters of a random sample of a population of diploid *Dactylorhiza fuchsii* (Druce) Soó, tetraploid *D. purpurella* (T. & T. A. Steph.) Soó and apparent hybrids in a limestone quarry in Co. Durham. Eudiploids, eutetraploids and a predominance of eutriploids were found, but about a quarter of the population were aneuploid, with chromosome numbers $2n = 44$, 48, 52 and 72. A hybrid index showed that a wide range of hybrids occurred. Eutriploids, presumed to be F_1 hybrids, were very variable. They showed a wide range of univalents at meiosis, with a (rare) maximum of 20. The appearance of aneuploids was consistent with an F_2, backcross, or subsequent origin. It is suggested that population differentiation in tetraploid *Dactylorhiza* may be due to polytopic allopolyploid origin. Viability of aneuploid gametes and hybrids may be due to ancestral polyploidy. The situation is unusual because extensive F_2 and backcross hybridization seems to occur across a diploid/tetraploid barrier.

INTRODUCTION

It has long been recognized that many of the taxonomic problems associated with the genus *Dactylorhiza* in the British Isles arise from hybridization (Stephenson & Stephenson 1922). Populations containing many and varied morphological intermediates between the diploid *D. fuchsii* (Druce) Soó ($2n = 40$) and the tetraploids *D. purpurella* (T. & T. A. Steph.) Soó and *D. praetermissa* (Druce) Soó ($2n = 80$) have been studied by Heslop-Harrison (1953, 1957). His main conclusions (cited by Roberts (1975)) were that hybrids are eutriploids ($2n = 60$), although very variable, and show a high level of seed-sterility. He suggested that the few embryos which are produced arise parthenogenetically, being themselves eutriploid, and that backcrossing to parents or crossing between triploids is unlikely to occur, although the possibility is not ruled out. No aneuploids were discovered.

A more recent study (Richards 1963) of mixed populations of *D. fuchsii* and *D. purpurella* in recently abandoned magnesian limestone quarries in Durham, v.c. 66, suggested that the situation there was a more complex one. These populations contained a wide range of phenotypes, many of which showed varying degrees of intermediacy between the two species, and were morphologically consistent with extensive backcrossing of the F_1 hybrid to both parents. Furthermore, out of nine reliable chromosome counts which were made from pollen grain mitosis, two proved to be aneuploid with $n = 22$ and 37.

It also seemed that other populations occurring in long-disused magnesian limestone quarries and consisting of robust plants with broad, shallowly trilobed labella resembling T. and T. A. Stephenson's *Orchis purpurella* 'Form B' (Stephenson & Stephenson 1922), may have arisen as a result of introgression from *D. fuchsii* into *D. purpurella*.

The present work was undertaken to establish whether aneuploids of presumptive backcross origin were important in Durham populations of *Dactylorhiza*.

MATERIALS

Buds were fixed from 50 individuals in a mixed population of parent species and apparent hybrids at Quarrington, Durham, v.c. 66 (G.R. 45/330.364), on the 14th June 1974. Plants were chosen using a 1m grid and a table of random numbers. The latter generated pairs of numbers to indicate

grid intersections, and buds were fixed from the nearest flowering spike with only the bottom one or two flowers open. The labellum of the most open flower was preserved on card under transparent adhesive tape.

METHODS

Buds were fixed in 3:1 absolute ethanol/glacial acetic acid overnight and then kept in the deep-freeze. After hydrolysis in N hydrochloric acid at 60°C for 15 minutes, they were stained in Feulgen stain for 1 hour. Pollinia were excised and squashed in acetocarmine. Slides showing meiotic stages were made permanent in 'euparal'. As pointed out by Heslop-Harrison (1953), meiosis is highly synchronized; in suitable preparations large numbers of dividing cells can be observed.

The following characters (Fig. 1) were scored from the preserved labella:

1. maximum width
2. maximum length
3. vertical length of central lobe (if any) from apex to sinus
4. shape: deltoid, intermediate, trilobed
5. colour: white to pink, deep-pink, red-purple
6. markings: spots, intermediate, rings.

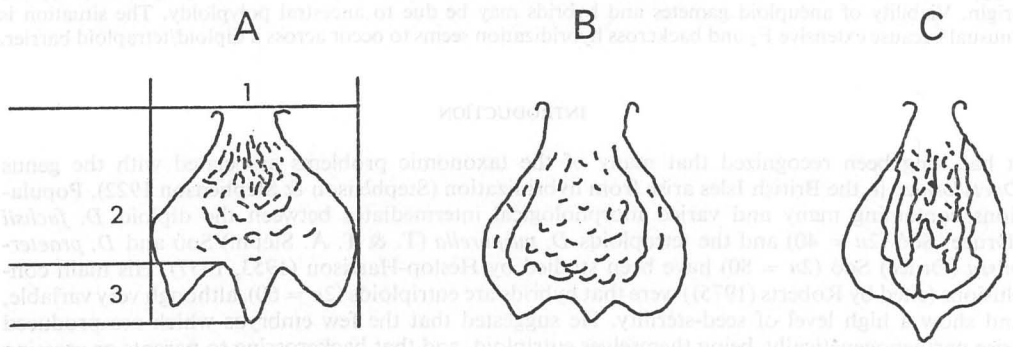

FIGURE 1. Labellum types in *Dactylorhiza*. A, *D. fuchsii*, trilobed labellum with spots; B, labellum of putative hybrid with intermediate shape and markings; C, *D. purpurella*, deltoid labellum with rings. Measurements: 1, maximum width; 2, maximum length; 3, vertical length of central lobe.

Individuals with eudiploid ($2n = 40$) and eutetraploid ($2n = 80$) chromosome counts were assumed to be pure *D. fuchsii* and *D. purpurella* respectively.

Somatic chromosome numbers were calculated from meiotic preparations by counting the number of univalents and bivalents in a number (usually about 50) cells in each individual. Some apparent variation (up to 3 chromosomes per cell) was noted within an individual plant. This is thought to be due to chromosomes being obscured, or to the occasional misinterpretation of univalents and bivalents, rather than actual variation.

RESULTS

Meiotic stages (diakinesis to metaphase I) which allowed the accurate determination of somatic chromosome number were found in 33 individuals. Of these, 5 were eudiploid ($2n = 40$), 7 were eutetraploid ($2n = 80$) and 13 were eutriploid ($2n = 60$). The remaining 8 were aneuploid, with $2n = 44$, 48, 52 and 72 (Table 1).

In 13 plants, all triploids or aneuploids, a sufficient number of cells could be analysed for the variation in the number of univalents within one plant to be counted (Table 2). Only univalents

TABLE 1. SOMATIC CHROMOSOME NUMBERS

$2n =$	No. plants	Ploidy level
40	5	Eudiploid
44	2	
48	2	
52	1	
60	13	Eutriploid
72	3	
80	7	Eutetraploid

and bivalents were seen, so counts apparently of odd numbers of univalents are probably instances of experimental error. Eudiploids and eutetraploids invariably showed regular formation of bivalents and regular disjunction.

It will be seen that a single individual may show a wide range in the number of univalents occurring in a meiotic cell. Among the triploids, individuals do not differ greatly as to mean number or range of univalents found, although the small range in plant number 2 and the high mean number in plant number 30 seem to differ from the rest. The number of univalents in an individual does not follow a normal distribution, but shows distinct peaks at 9 and 12, with perhaps a subsidiary peak at 15. 20 univalents, as constantly recorded by Heslop-Harrison (1953), rarely occurred. As might be expected, the numbers of univalents are less in subtriploid aneuploids, but here also there is a suggestion of a double peak in the distribution.

The fate of the univalents is not known for certain. Limited studies of anaphase I and the second meiotic division suggest that most, if not all, migrate to one pole at anaphase I and are incorporated in one daughter nucleus. Bivalents in triploids behave normally, with regular disjunction (of 20 or more chromosomes) to each pole.

Pure populations of the parent species are rare in Co. Durham, and therefore eudiploid and eutetraploid individuals from Quarrington were used to define the morphological limits of the two species. This has the advantage of removing bias arising from phenotypic differences between populations of the same species. However, introgressive hybridization at the euploid level would not be detected.

It was found that the length, width and the length/width ratio of the labellum showed no signi-

TABLE 2. NUMBERS OF UNIVALENTS OBSERVED IN INDIVIDUAL CELLS AT METAPHASE I IN TRIPLOIDS AND ANEUPLOIDS

Plant	$2n =$	Numbers of univalents																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	60				3	6	4	2	8	6	5	3	2	2	1	3	3		2		
2	60							4	6	9	4	10	17								
3	60					2	1	3	2	3	4	6	6	4	4	2					
6	60				4	2		5	8	3	7	2	9	2		4	3		4		
12	60				3	1	2	5	3	7	2	7	7	1	1	2	2		1		
30	60										3		2	5	3	4	6	3	1	1	1
36	60				1		2	1	4	5	4	4	3	4	4	5	2	1			
39	60				1	1	1	1	4	6	1	4	3	2	1	3	2		2		1
43	60						1	1	1	3	3	4	2	2	2		3		1		
47	60			1	2		2	1	4	5	2	5	4	3	1	3	2	1		1	1
Totals ($2n = 60$)				1	14	12	13	23	39	47	35	45	55	25	17	26	23	5	11	2	3
38	48		2		2	7	5	9	4	3	2	2	1		1						
44	48		3	3	4	5	4	7	11	3											
Totals ($2n = 48$)			5	3	6	12	9	16	15	6	2	2	1		1						
50	52		1	2	6	3	2	2	2	2		1	2								

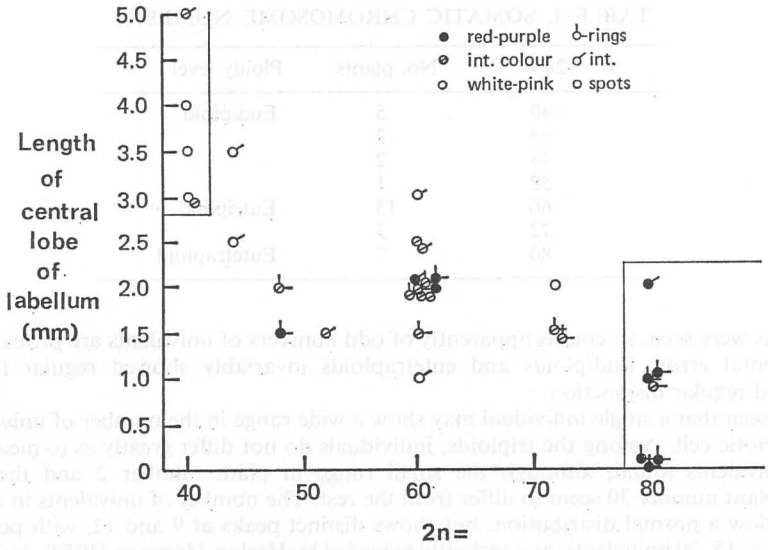

FIGURE 2. Scatter diagram of length of central lobe of labellum against chromosome number.

ficant difference between diploids and tetraploids, and these characters were not used. However, the other characters scored were considered to show such differences (Fig. 2), and it proved possible to combine these in a hybrid index (Table 3). Due to the occasional occurrence of intermediate characters in eudiploid and eutetraploid plants it was not found possible to obtain a 'pure' index score for euploid plants, but euploids could be effectively separated by this method (Fig. 3).

The distribution of hybrid index scores in the sample is rather uniform (Fig. 4), but with nearly half the individuals morphologically resembling *D. purpurella* (hybrid index 6–8). However, it is clear that a wide and continuous range of morphs occurs. The distribution of hybrid index scores

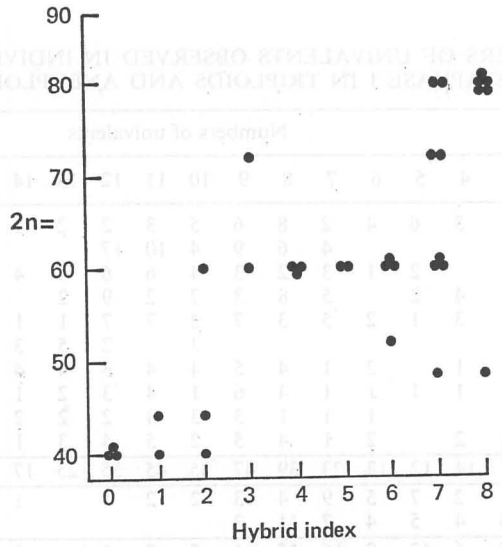

FIGURE 3. Scatter diagram of hybrid index against somatic chromosome number. Note separation of diploids and tetraploids, and wide morphological range of triploids.

TABLE 3. THE SCORING OF LABELLUM CHARACTERS USED IN THE CONSTRUCTION OF A HYBRID INDEX

	D. fuchsii	Intermediates	*D. purpurella*
Score	0	1	2
Length central lobe	3 mm +	2·1–2·9 mm	0–2·0 mm
Shape	Trilobed	Intermediate	Deltoid
Colour	White to pink	Intermediate	Red-purple
Markings	Spots	Intermediate	Rings

against chromosome number (Fig. 3) is of interest. Eudiploids ($2n = 40$) score 0–2, and eutetraploids ($2n = 80$) 7–8, two-thirds of euploids showing maximum differentiation (0 or 8). Eutriploids ($2n = 60$) have a wide range of hybrid index scores (2–7), although with very little overlap with diploids and tetraploids. These results confirm those of Heslop-Harrison (1957), who suggested that the wide range of intermediates occurring in hybrid populations of *Dactylorhiza* can be due to triploids, presumably of F_1 origin. However, in this population, about one-quarter of the plants are aneuploids, presumably of F_2, backcross or later generations. These plants show variable and often anomalous phenotypes. Although $2n = 44$ plants resemble *D. fuchsii*, $2n = 48$ and $2n = 52$ plants more closely resemble *D. purpurella*. $2n = 72$ plants are variable, with scores of 3 and 7.

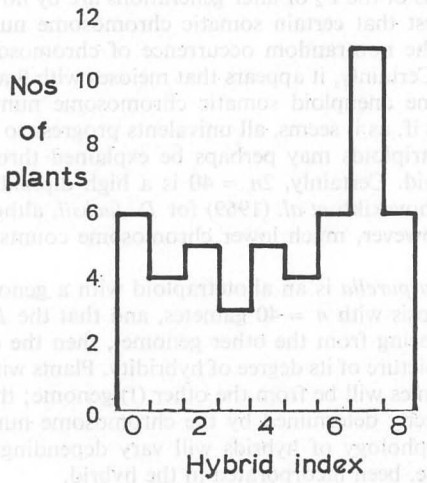

FIGURE 4. Distribution of hybrid index scores for all individuals.

DISCUSSION

It is usually considered that tetraploids are effectively reproductively isolated from related diploids, hybrid triploids showing a very high level of sterility. This can generally be shown to be due to the irregularity of meiosis in the triploid, with the formation of univalents and often trivalents resulting in meiotic products which are aneuploid and inviable. A few cases have been reported in which introgression may have occurred from a diploid to a tetraploid or vice-versa. However, in high polyploids, fertile hybrids between different ploidy levels are more common, presumably because the difference between gene dosages found in aneuploids is buffered by polyploidy.

Heslop-Harrison (1953, 1957) reported that diploid × tetraploid hybrids in *Dactylorhiza* were invariably triploid and highly sterile, and that 20 univalents were regularly formed (some of his figures show fewer univalents, perhaps due to some being masked by others). He considered that this result was consistent with an allopolyploid origin of the tetraploids *D. purpurella* and *D. praetermissa* (FFII) with one parent *D. fuchsii* (FF) and the other probably *D. incarnata* (L.)

Soó (II). This conclusion has not been doubted and is agreed with here. Indeed, it is possible that the allopolyploids *D. purpurella*, *D. praetermissa*, *D. majalis* (Reichb.) Hunt & Summerh. and *D. traunsteineri* (Sauter) Soó are the product of crosses between the various subspecies of *D. incarnata* and *D. fuchsii*, allopolyploidy having arisen polytopically.

In the present work, it was found that 20 univalents rarely occur in the triploid F_1 hybrid between *D. fuchsii* and *D. purpurella* (FFI), it being common to find between 4 and 18 univalents (and 21 and 28 bivalents). It is therefore likely that some homology exists between the F and I genomes. Unfortunately meiosis of the diploid hybrid *D. fuchsii* × *D. incarnata*, which is occasionally found (Roberts 1975), has not been described.

Where 20 univalents occur in the triploid F_1, fertility of the resulting gametes might depend on the segregation of the univalents. If they stayed together and migrated to one pole at anaphase I, euploid spores of $n = 20$ and $n = 40$ would be formed, which would probably produce viable gametes and result in diploid ($2n = 40$), triploid ($2n = 60$) or tetraploid ($2n = 80$) offspring. It is not inconceivable that the few triploid embryos reported by Heslop-Harrison (1953) originated in this way, rather than parthenogenetically as suggested.

The occurrence of a substantial number (24%) of aneuploids suggests that the triploids are by no means totally sterile, but may frequently cross among themselves, or backcross to the parents. Earlier meiotic studies of both parents (Richards 1963, and unpublished) confirm the results of Heslop-Harrison (1953) and the present work in that eudiploids and eutetraploids show a regular meiosis, and thus aneuploids are unlikely to have arisen except from triploids. The chromosome numbers of aneuploid hybrids of the F_2 or later generations are by no means randomly distributed (Table 1). This might suggest that certain somatic chromosome numbers are more viable but, equally, it may be due to the non-random occurrence of chromosome numbers in the meiotic products of the triploid F_1. Certainly, it appears that meioses with 9 and 12 univalents occur most frequently, and at least some aneuploid somatic chromosome numbers (48, 52, 72) might be explicable on this hypothesis if, as it seems, all univalents progress to one pole at anaphase I.

The apparent fertility of triploids may perhaps be explained through the suggestion that *D. fuchsii* is ancestrally polyploid. Certainly, $2n = 40$ is a high diploid number, and one count of $2n = 20$ is recorded in Bolkhovskikh *et al.* (1969) for *D. fuchsii*, although this might have been a parthenogenetic haploid. However, much lower chromosome counts are recorded in the related genus *Orchis*.

If it is assumed that *D. purpurella* is an allotetraploid with a genome from *D. fuchsii*, that *D. purpurella* has a regular meiosis with $n = 40$ gametes, and that the *D. fuchsii* genomes in the F_1 hybrid pair (the univalents being from the other genome), then the chromosome number of the hybrid can give an accurate picture of its degree of hybridity. Plants with $2n = 40$ will be *D. fuchsii*, and all additional chromosomes will be from the other (I) genome; thus the hybrid 'dose' in each individual can be rather strictly determined by the chromosome number, between $2n = 40$ and $2n = 80$. However, the morphology of hybrids will vary depending on which chromosomes of the I genome have, by chance, been incorporated in the hybrid.

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(*Accepted July* 1976)