The allotetraploid nature of *Dactylorhiza praetermissa* (Druce) Soó (Orchidaceae) confirmed

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ABSTRACT

Examination of variation at six allozyme loci in *Dactylorhiza praetermissa* (Druce) Sóo reveals that the species is an allotetraploid that originated in parental taxa closely related to *D. incarnata* (L.) Soó s.l. and *D. fuchsii* (Druce) Soó/*D. maculata* (L.) Soó. This confirms previous hypotheses based on cytological evidence. The genus *Dactylorhiza* contains numerous allotetraploid taxa that originated from the same parental lineages that gave rise to *D. praetermissa*. The similar genomic compositions of the various allotetraploids suggests that hybridization among them is likely to result in introgression. *Dactylorhiza praetermissa* may have gained alleles by this process.

KEYWORDS: allotetraploidy, allozymes, hybridization.

INTRODUCTION

The Marsh-orchids and the Spotted-orchids, which constitute the major part of the genus Dactylorhiza Necker ex Nevski, include a large number of closely similar taxa in north-west Europe (e.g. Heslop-Harrison 1954; Hylander 1966; Soó 1980; Gathove & Tyteca 1994). Chromosome counts by Hagerup (1938) and Vermeulen (1938) demonstrated two principal ploidy levels in this group (diploids with 2n = 40 and tetraploids with 2n = 80), which indicated that the group formed a polyploid complex. Vermeulen (1938) also suggested that some taxa were allotetraploids derived from extant diploid lineages. Allotetraploids can be characterized as permanent hybrids in which the genomes of two distinct parents are combined. Because recombination only occurs between chromosomes of the same origin during meiosis, the hybrid allotetraploid genotype will be maintained through subsequent generations. Heslop-Harrison (1953) further extended our knowledge by performing cytological studies in triploid hybrids between the diploid D. fuchsii (Druce) Soó and the tetraploid D. purpurella (T. & T. A. Stephenson) Soó and D. praetermissa (Druce) Soó. He found that 20 bivalents and 20 univalents were formed in these triploids during meiosis and therefore concluded that there is a high degree of homology between the D. fuchsii chromosomes and half of the chromosomes in the tetraploid genomes. One of the parents of the tetraploids must therefore have been closely similar to D. fuchsii, and he suggested that members of D. incarnata (L.) Soó s.l. contributed the other constituent genome.

Allozymes are expressed as codominants and may be very useful to describe the structure of polyploid complexes (Roose & Gottlieb 1976; Gottlieb 1981; Crawford 1989). Enzyme electrophoresis and analysis of variation at allozyme loci (Hedrén a, in press) confirmed the general pattern described in the previous cytological studies on *Dactylorhiza*. The diploids *D. fuchsii* and *D. incarnata* s.l. were distinct at the allozyme loci examined, whereas all tetraploids had close affinities with one or both diploid lineages. *Dactylorhiza maculata* (L.) Soó was interpreted as an autotetraploid, originating in the *D. fuchsii* lineage, while *D. majalis* (Rchb. f.) P. F. Hunt & Summerh s.s., *D. traunsteineri* (Sauter) Soó, *D. sphagnicola* (Höppner) Soó, *D. lapponica* (Hartm.) Soó and *D. purpurella* had allozyme profiles conforming with allotetraploidy. Also, the allotetraploids were gradually modified by rare exchange of genes between the constituent genomes, which had resulted in a loss of some characteristic *incarnata* alleles in some populations of *D. purpurella* (Hedrén b, in press).

The studies on the allozyme variation in Dactylorhiza studies were based on material collected in

the Nordic countries, where *D. praetermissa* does not occur. However, I have now, by courtesy of British colleagues, been able to examine two populations of this species as well. Although this is a very small sample, results are sufficiently interesting to justify this brief communication.

MATERIALS AND METHODS

The nomenclature used in the present paper follows Stace (1991) for taxa occurring in the British Isles.

Leaf material of *D. praetermissa* was obtained from two localities in southern England: Cothill, Oxfordshire (v.c. 22, Berks.; ten individuals) and a watermeadow at Winkton near Christchurch, Dorset (v.c. 11, S. Hants.; 25 individuals). *Dactylorhiza praetermissa* was compared with previously analyzed material of *Dactylorhiza* collected in northern Europe. This reference material comprised *D. incarnata* s.l. (including *D. incarnata* s.s., *D. incarnata* subsp. *ochroleuca* (Boll) P. F. Hunt & Summerh. and *D. incarnata* subsp. *cruenta* (O. F. Müll.) P. D. Sell), *D. fuchsii*, *D. maculata*, *D. purpurella*, *D. majalis*, *D. traunsteineri*, *D. sphagnicola* and *D. lapponica*. More detailed information on the origin of this material is given in Hedrén a,b (in press). Electrophoretic procedures are described in Hedrén a,b (in press). Variation was recorded at one locus in each of six enzyme systems: Phosphoglucoisomerase (Pgi, E.C. 5.3.1.9), Phosphoglucomutase (Pgm, E.C. 5.4.2.2), Triose-phosphate isomerase (Tpi, E.C. 5.3.1.1), Diaphorase (Dia, E.C. 1.6.99–), Shikimate dehydrogenase (Skd, E.C. 1.1.1.25) and Phosphogluconate dehydrogenase (Pgd, E.C. 1.1.1.44).

The material of *D. praetermissa* was compared with previously analyzed taxa by means of multivariate statistical methods (e.g. Abbott *et al.* 1985). The variation pattern in the whole group of taxa was summarized by means of a Principal Components Analysis (PCA). The alleles found at Pgi, Tpi, Pgm and Tpi were used as characters, and the number of copies of each allele as character states. Mean values were calculated for populations of each taxon, and the PCA was based on these population means. A Canonical Variates Analysis (CVA) was used to identify the best characters to discriminate among populations of the allotetraploid taxa. This analysis was based on the allozyme compositions of individual specimens, and the source population was used as the grouping variable. The CVA included information from the same four loci used in the PCA. Calculations were made using the SAS computer program (SAS Inst. 1989).

RESULTS

At the six loci analyzed, *D. praetermissa* contained no alleles other than those found in previously studied taxa (Table 1). Moreover, *D. praetermissa* was similar to the majority of the allotetraploids in that a high proportion of the analyzed individuals have at each locus two alleles in common with *D. fuchsii/D. maculata* and two alleles in common with *D. incarnata* s.l.

In the ordination plots obtained from PCA (Fig. 1), the two populations of *D. praetermissa* are grouped with populations of the allotetraploid *D. majalis*, *D. traunsteineri*, *D. sphagnicola* and *D. lapponica*. *Dactylorhiza purpurella* has also been shown to be an allotetraploid (Hedrén b, in press), but it is separated from the other taxa due to low frequencies of *incarnata* alleles at Pgm and Pgd (Table 1). All analyzed populations of *D. incarnata* s.l. were identical, whereas variation occurred in both *D. fuchsii* and *D. maculata*. The allotetraploids are intermediate between *D. incarnata* s.l. and *D. fuchsii/D. maculata* in allozyme characters, in accordance with their hybrid origin. The positions of the populations along the third principal component (Fig. 1b) add little further information, except that *D. purpurella* is placed closer to the other allotetraploids and *D. incarnata* s.l. appears more distinct.

Populations of *D. praetermissa* were also compared with other allotetraploid taxa in a CVA. In the diagram formed by the two first canonical axes (Fig. 2a), there is a tight cluster formed by populations of *D. majalis*, *D. traunsteineri*, *D. sphagnicola* and *D. lapponica* to the right. Populations of *D. purpurella* form a separate group to the left, which is in accordance with their position in the PCA ordination plots. As regards *D. praetermissa*, the Cothill population is well embedded in the main group of allotetraploid populations, whereas the Winkton population has a

Population/taxon		Pgi									Pgm					Трі			Dia			Pgd						Skd			
	n	а	b	с	d	е	а	b	с	d	е	f	g	h	а	b	с	d	а	b	с	а	b	с	d	e	f	a	b	с	d
D. praetermissa									-		5		9																		
Ćothill	10	15	38	0	0	48	0	50	0	35	15	0	0	0	0	50	0	50	50	50	0	48	25	25	0	2	0	50	50	0	0
Winkton	25	0	50	0	0	50	6	43	0	18	15	18	0	0	0	50	0	50	50	50	0	50	50	0	0	0	0	50	50	0	0
Other taxa																															
D. incarnata																															
subsp. incarnata	86	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0	0	0	100	0	0	0
subsp. ochroleuca	32	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0	0	0	100	0	0	0
subsp. cruenta	64	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0	0	100	100	0	0	100	0	0	0	0	0	100	0	0	0
D. fuchsii	80	1	99	0	0	0	0	0	3	24	61	11	1	0	1	99	1	0	0	99	1	0	47	52	1	0	0	0	92	2	6
D. maculata	100	13	85	2	0	0	0	0	6	51	38	5	0	>0	11	89	0	0	0	95	5	0	27	70	3	0	0	0	95	3	1
D. purpurella	116	0	50	0	0	50	0	6	44	3	47	0	0	0	0	50	0	50	50	50	0	14	76	10	0	0	0	50	47	1	2
D. majalis s.s.	113	2	48	0	0	51	4	46	0	40	10	0	0	0	0	44	6	50	50	50	0	51	38	9	0	2	0	50	50	0	0
D. traunsteineri	307	2	48	0	>0	50	0	50	3	36	10	0	0	0	0	50	0	50	51	49	0	51	2	36	4	0	7	49	50	1	0
D. sphagnicola	121	6	56	0	>0	38	1	50	0	43	7	0	0	0	0	50	0	50	50	50	0	49	1	26	23	0	1	50	34	16	0
D. lapponica	30	12	38	0	0	50	0	50	9	41	0	0	0	0	0	50	0	-50	50	50	0	50	0	23	0	0	27	50	50	0	0

TABLE 1. ALLELE FREQUENCIES (PER CENT) IN TWO POPULATIONS OF DACTYLORHIZA PRAETERMISSA, COMPARED WITH SEVERAL OTHER DACTYLORCHID TAXA OCCURRING IN NORTHERN EUROPE (DATA FROM HEDRÉN A, B, IN PRESS)

n: number of individuals.

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FIGURE 1. Resulting scatter plots from a Principal Components Analysis on allozyme markers in populations of the orchid genus *Dactylorhiza*. The two populations of *D. praetermissa* are indicated by arrows. The analysis was based on mean allele numbers in 83 populations of various diploid and tetraploid taxa, representing altogether 1084 individuals. Twenty-three variable characters (alleles) from four allozyme loci were used in the data matrix. The first three principal components (PC1, PC2, PC3) described 30.1%, 12.0% and 7.8%, respectively, of the total variance. (a): Plot of the first and second principal components. (b): Plot of the first and third principal components.

strongly negative value along axis 2. The reason why this population deviates from the main group, is the frequent occurrence of the allele Pgm^{f} in many individuals (Table 1). The third canonical axis (Fig. 2b) separates populations of *D. sphagnicola* from the other allotetraploids but reveals no further information on *D. praetermissa*.

DISCUSSION

Most of the alleles found in *D. praetermissa* are also present in *D. incarnata* s.l. or *D. fuchsii/D. maculata*. The two exceptions are rare occurrences of Pgm^a and Pgd^e (Table 1). However, these rare alleles have been found in other tetraploid taxa, indicating that they may be present in non-analyzed populations of *D. incarnata* s.l. or *D. fuchsii/D. maculata*. Alternatively, they have been lost from the ancestral lineages after the origin of the allotetraploids. Thus, the allozyme composition of *D. praetermissa* indicates that the taxon is an allotetraploid with close origin in the *D. incarnata* s.l. and *D. fuchsii/D. maculata* lineages, as predicted by Heslop-Harrison (1953), although these ancestral lineages may have been slightly modified since *D. praetermissa* evolved.

Various mechanisms have been evoked to explain the multitude of closely similar forms in *Dactylorhiza*, including hybridization followed by polyploidization, hybridization and introgression between taxa at different ploidy levels, and hybridization among taxa at the tetraploid level (e.g. Vermeulen 1938; Heslop-Harrison 1953, 1954; Lord & Richards 1977; Bateman & Denholm 1983; Jenkinson 1991). The analyses of the allozyme variation in northern European *Dactylorhiza* indicated that polyploidization was the most important mechanism, and that allotetraploids have



FIGURE 2. Resulting scatter plots from a Canonical Variates Analysis performed on allozyme data from allotetraploid populations of *Dactylorhiza*. Symbols as in Fig. 1. The two populations of *D. praetermissa* are indicated by arrows. The analysis included 691 individuals from 42 allotetraploid populations. Population membership was used as the grouping variable. The data matrix analyzed by the CVA included 19 variable characters (alleles) from the same four loci analyzed by the PCA. The three first canonical axes (CAN1, CAN2, CAN3) described 56.2%, 13.0% and 9.4%, respectively, of the total variance. (a): Plot of the first and second canonical axes.

originated repeatedly from the *D. incarnata* s.l. and *D. fuchsii/D. maculata* lineages (Hedrén a, in press). The allozyme composition of *D. praetermissa* is consistent with this view. At any given allotetraploidization event, at most two different alleles from *D. fuchsii* or *D. maculata* could be transferred to the allotetraploid derivative. *Dactylorhiza praetermissa* is the only allotetraploid in which the *fuchsii* allele Pgm^f has been found (Table 1). With the inclusion of *D. praetermissa* in the group of allotetraploids, the number of Pgm alleles shared by *D. fuchsii/D. maculata* and the allotetraploid group is at least four. This strengthens the view that the allotetraploid group has multiple origins; *D. praetermissa* may have originated as an independent polyploidization event.

It should also be observed that the two analyzed populations of D. praetermissa have three Pgm alleles in common with D. fuchsii/D. maculata. It is possible that D. praetermissa itself has multiple origins, but it may also be the case that alleles have been transferred to D. praetermissa by hybridization with other allotetraploid taxa. From a theoretical standpoint, hybridization among the allotetraploids may well result in back-crossing and introgression (Hedrén a, in press). It is sometimes also proposed that hybridization with D. maculata or with the diploids may contribute to increased variation in the allotetraploid taxa. However, F_1 hybrids formed by these combinations are expected to have comparatively low fertility (Hedrén a, in press), and in mixed populations of D. maculata and the various allotetraploid taxa studied in Sweden the proportion of F_2 hybrids plus back-crossed individuals to F_1 hybrids is small (Hedrén a, in press). Still, hybrids between D. *maculata* and the allotetraploids may sometimes have higher fertility than expected (Roberts 1975; Malmgren 1992), and aneuploid back-crosses have been identified in a mixed population of the diploid D. fuchsii and the tetraploid D, purpurella (Lord & Richards 1977). Therefore, the possibility of gene transfers from D. maculata or D. fuchsii to an allotetraploid cannot be excluded. Other allotetraploid dactylorchids have also been found to share three or more alleles with D. fuchsii/D. maculata at some loci, which must also be explained by either multiple origins or introgression from other taxa.

The better-known allotetraploid dactylorchid taxa appear to be fairly homogeneous over large parts of their distribution, both in external morphology and in genetic characters. Furthermore, different taxa may have evolved independently from the same ancestral lineages. The allotetraploids should have poorly developed internal barriers to hybridization, as is indicated by hybridization data (compiled by Roberts 1975). They must accordingly be isolated by external

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barriers reflecting differences in flowering period, habitat preference, or distribution. However, many allotetraploid *Dactylorhiza* are partly sympatric. In areas of overlap they are often extremely difficult to separate from each other (Bateman & Denholm 1983; Jenkinson 1991), and at some localities intermediate forms connect the different taxa (e.g. *D. traunsteineri* and *D. sphagnicola* in Sweden (pers. obs.), *D. majalis* and *D. lapponica* in the Alps (S. Hansson, pers. comm.), and various allotetraploid taxa in the British Isles (Jenkinson 1991)). The poor genetic isolation and the morphological intergradation in areas of sympatry indicate that treatment of the allotetraploid marsh-orchids as taxonomic species may not be justified. They might be better treated as subspecies of a single variable species, *D. majalis*, as suggested by some recent studies (e.g. Bateman & Denholm 1983; Jenkinson 1991; see also Sundermann 1975; Soó 1980). This subspecific status still recognizes that these taxa may have evolved independently, and that they have at least partly different distributions and contrasting habitat requirements.

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