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Systematics of the *Dactylorhiza euxina/incarnata/maculata* polyploid complex (Orchidaceae) in Turkey: evidence from allozyme data

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Abstract. Material of *Dactylorhiza* were sampled from 49 localities in Turkey and investigated for allozyme variation at ten loci (nine enzyme systems). Among diploids, the Anatolian *D. osmanica* and *D. umbrosa* were allozymically variable, but not distinct from each other or from *D. incarnata*. *Dactylorhiza saccifera* contained the same alleles as the European *D. fuchsii*. *Dactylorhiza iberica* and *D. euxina* were distinct from each other and the other diploids. On basis of allozyme patterns three distinct allotetraploid genotypes were distinguished, and each of them could be treated as a separate species. *Dactylorhiza nieschalkiorum* is similar to European allotetraploids, and may have arisen from hybridization between *D. incarnata* s.l. and *D. saccifera*. *Dactylorhiza urvilleana* may have arisen from parents related to present-day *D. saccifera* and *D. euxina*, but it also contains additional alleles that have not been found in any of the diploids investigated. A third allotetraploid known from four populations in the Ardahan and Kars provinces of north-eastern Turkey combines the allozyme patterns found in material of *D. incarnata* s.l. from the same area with those from *D. euxina*. It is here described for the first time as *D. armeniaca*.

Key words: Orchidaceae, *Dactylorhiza*, allozymes, evolution, phylogeny, polyploidy, systematics, Turkey.

The majority of recognized taxa in the Eurasian orchid genus *Dactylorhiza* Nevski belongs to the *D. incarnata/maculata* polyploid complex (cf. Averyanov 1983a, b, 1990; Delforge 1995; Hedrén 2001). This complex undisputedly embraces a large morphological diversity, but the variation patterns are difficult to disentangle, and there is little consensus upon the number of taxa that should be recognized. However, data from molecular studies, including allozymes (Hedrén 1996a, b, c), ITS sequences (Pridgeon et al. 1997, Bateman et al. 1997), and AFLPs (Hedrén et al. unpubl. data), combined with previous data on chromosome numbers and meiosis (Vermeulen 1938, Hagerup 1938, Heslop-Harrison 1953) can be used to describe the phylogenetic relationships and the background to the taxonomic difficulties.

In northern and western Europe the complex consists of two principal parental lineages, *D. incarnata* (L.) Soó s.l. (the diploid marsh orchids, Bateman and Denholm 1985), and *D. maculata* (L.) Soó s.l. (the spotted orchids, Bateman and Denholm 1988, that includes the diploid *D. fuchsii* (Druce) Soó and the autotetraploid *D. maculata* s.s.). These groups are morphologically and genetically distinct, but they are bridged by a large number of tetraploid forms (the tetraploid marsh orchids; Bateman and Denholm 1983) that have arisen by repeated hybridization between the two parental lineages (Heslop-Harrison 1953, 1954; Averyanov 1990). The tetraploids are functionally allotetraploids, i.e. the hybrid genotype is fixed and inherited from generation to generation (Crawford 1989). They are apparently morphologically variable. If a broad species concept is applied (Pedersen 1998) the allotetraploids could be accommodated in *D. elata* (Poir.) Soó (cf. Hedrén 2001), but most authors treat them as a series of separate species which includes, among others, *D. majalis* (Rchb.) P.F. Hunt & Summerhayes, *D. praetermissa* (Druce) Soó, *D. purpurella* (T. & T.A. Stephenson) Soó, *D. traunsteineri* (Saut.) Soó, and *D. lapponica* (Hartm.) Soó (e.g. Soó 1980).

The major reason for the complex variation pattern is that the number of origins of the allotetraploid group may be quite large – either by multiple origins from parental lineages, or perhaps less likely (cf. Pedersen 1998) by introgression from parental genotypes into existing allotetraploids. It is indicated by allozyme and AFLP data, that certain allotetraploids may be genetically coherent over quite large areas, but that there also exist many recent allotetraploids that are confined to just one or a few populations (Hedrén et al. in press). Hybridization between widespread and local forms may result in new forms that fill any morphological discontinuities, but this process is difficult to detect since hybrids between different allotetraploids are expected to be fully fertile as they have their origins in a similar set of parents (cf. Roberts 1966). Moreover, it appears that forms of similar morphological appearance have arisen independently in different parts of Europe (Hedrén et al. in press). For example, forms similar to *D. majalis* may have arisen in south-eastern Europe (i.e. *D. cordigera* (Fr.) Soó), on the British Isles (i.e. *D. occidentalis* (Pugsley) P. Delforge), and in the Alp region (i.e. *D. alpestris* (Pugsley) Aver.). Some of these taxa can not be separated from each other without knowing where they come from, and it is probable that there are similar examples where the different regional populations bear the same name.

In the present paper I will use allozymes as genetic markers to describe the relationships of Turkish members of *Dactylorhiza.* The taxonomic problems of the *Dactylorhiza* taxa occurring in Turkey are reminiscent to those in Europe, and may also be due to hybridization and polyploidy (cf. Averyanov 1983b, Renz and Taubenheim 1984, Delforge 1995). Allozymes are codominant markers and are often very useful in describing parentage of hybrid taxa in general and allopolyploids in particular (Roose and Gottlieb 1976, Crawford 1989, Weeden and Wendel 1989). I will also use the allozymes to relate the Turkish members of *Dactylorhiza* to previously examined European taxa (Hedrén 1996a, b, c, 2001).

Material and methods

Plant material. Localities and taxa sampled for the present study are presented in Table 1 and the localities shown in Fig. 1. The taxonomy has been modified according to the results of the study. However, if material from the same localities have been included in previous literature covering Turkish *Dactylorhiza* (or from the close vicinity of localities examined here), this has also been indicated in the Table with the names used by the authors. The study comprised material of the following taxa, delimited as in Renz and Taubenheim (1984).

Dactylorhiza iberica (Willd.) Soó differs in several morphological characters from the remaining taxa investigated, and it has been regarded as an isolated member of the genus (Soó 1960, Averyanov 1983b). However, ITS data indicate that it may be derived from the spotted orchids (Bateman et al. 1997). It is distributed from Greece and Macedonia in the west to Persia in the east (Renz 1978).

Dactylorhiza saccifera (Brongn.) Soó is distributed from Corsica and Italy in the west to Anatolia and Lebanon in the east (Buttler 1991). It is related to *D. maculata* s.l. and may be difficult to separate from this complex (Delforge 1995). Material from Turkey is sometimes treated as *D. bithynica* H. Baumann (Baumann 1983, Kreutz 1998).

Table 1. Localities studied for allozyme variation in Turkish *Dactylorhiza*. Code numbers refer to numbers used in Figs. 1 and 5, and Table 2; code letters refer to codes used in Fig. 3. Abbreviations: arm *D. armeniaca*, eux *D. euxina*, ibe *D. iberica*, inc *D. incarnata* s.l., nie *D. nieschalkiorum*, sac *D. saccifera*, urv *D. urvilleana*. The occurrence of dark-spotted plants has been indicated. In some cases where the localities sampled here appear in locality lists in Renz and Taubenheim (1984), Rückbrodt et al. (1992), or Kajan et al. (1992), the taxa identified by these authors are also given. Vouchers (preserved flowers) from most localities are kept with the authors

Codes	Region and site	Taxa
01	Bolu, Abant Gölü – nieschalkiorum in Renz & Taubenheim and in Rückbrodt et al.	ibe, nie
02	Bolu, 7.9 km from road Bolu–Istanbul towards Abant, Akçaalan	sac
03	Bolu, 14 km S Mengen, Eskiçağa	nie
04	Kastamonu, 9.8 km Kastamonu–Karabük	ibe
05	Kastamonu, Tosya–Kursunlu, NW Sarmasik	ibe
06	Kastamonu, Ilgaz–Kastamonu, 6.6 km N Ilgaz Geçidi	sac, urv, sac × nie
07	Kastamonu, Ilgaz–Kastamonu, 6.1 km N Ilgaz Geçidi – nieschalkiorum in Renz & Taubenheim	nie
08	Kastamonu, Tosya–Kastamonu, 500 m N Ilgaz Geçidi	nie, urv, nie × urv
09	Kastamonu, Tosya–Kastamonu, 2 km S Ilgaz Geçidi	nie, urv, nie × urv
10	Ordu, 36 km Ünye–Akkus	urv
11	Ordu, 39 km Ünye–Akkus	urv
12	Ordu, 42.1 km Ünye–Akkus, Esentepe	urv
13	Ordu, 46.7 km Ünye–Akkus, Dumantepe – plants from the area treated as nieschalkiorum and urvilleana in Renz & Taubenheim	urv
14	Ordu, Ünye–Akkus, 7 km before Akkus	urv
15	Giresun, 14.2 km S Dereli	urv
16	Giresun, 15.4 km S Dereli	urv
17	Giresun, 20.9 km S Dereli	urv
18	Giresun, Kümbet	eux, (urv)
19	Giresun, 3.6 km W Kümbet – no spotted euxina	eux, (urv)
20	Trabzon, 11 km Maçka–Gümüshane	urv
21	Trabzon, 14.7 km Maçka–Gümüshane – urvilleana in Rückbrodt et al.	urv
22	Trabzon, Maçka–Gümüshane, 3.4 km N Zigana Geçidi – a few spotted euxina	eux, urv
23	Trabzon, 21.6 km Of–Uzungöl, N Dernekpazari – plants from the area treated as urvilleana in Rückbrodt et al.	urv
24	Trabzon, Of–Uzungöl, 9.9 km S Çaykara	urv
25	Trabzon, Of–Uzungöl, 200 m N Uzungöl	urv
26	Trabzon, 9.1 km Uzungöl–Soganli Geçidi, Sekersu – about 50% spotted euxina – plants from this area treated as euxina var. markowitschii in Renz & Taubenheim, euxina and urvilleana in Rückbrodt et al.	eux, urv, eux × urv
27	Rize, 23 km Rize–Ikizdere	urv
28	Rize, Rize–Ispir, 4 km SE Ikizdere – some plants from this area treated as saccifera in Kajan et al.	urv
29	Rize, Rize–Ispir, 9.9 km SE Ikizdere	urv

Table 1 (continued)

Codes		Region and site	Taxa
30		Rize, Rize–Ispir, 17 km SE Ikizdere – urvilleana in Renz & Taubenheim and in Rückbrodt et al. – maculata and euxina also reported from this area in Kajan et al.	urv
31		Rize, Rize–Ispir, 1 km N Sivrikaya – many spotted euxina	eux, urv
32		Rize, Rize–Ispir, 0.7–1.1 km S Sivrikaya – many spotted euxina – euxina var. euxina in Renz & Taubenheim	eux, urv
33		Rize, Rize–Ispir, Ovitdagi Geçidi – no plants spotted – euxina var. markowitschii in Renz & Taubenheim	eux
34		Artvin, 5 km E Savsat	arm
35		Artvin, 8 km E Savsat – armeniaca treated as euxina var. euxina in Renz & Taubenheim	arm, urv, arm × urv
36	A	Kars, 5.3 km Ardahan–Savsat	inc
37	B	Kars, 76 km Kars–Ardahan	inc
38		Kars, 28.9 km Kars–Ardahan – plants from the area treated as euxina var. euxina in Renz & Taubenheim	arm
39	C	Kars, 62.4 km Horasan–Kars – plants from the area treated as umbrosa in Rückbrodt et al.	arm, inc
40	D	Erzincan, Sivas–Refahiye, 2.2 km E Kizildağ Geçidi – 1 plant spotted – plants from the area treated as D. osmanica in Rückbrodt et al.	inc
41	E	Erzincan, 7 km E Refahiye – osmanica and umbrosa in Rückbrodt et al., umbrosa in Kajan et al.	inc
42	F	Erzincan, Refahiye–Erzincan, 6 km W Sakaltutan Geçidi – plants from this area treated as umbrosa in Rückbrodt et al.	inc
43	G	Tunceli, a few km towards Pülümür from road Erzincan–Erzurum – osmanica var. anatolica, sp. A in Renz & Taubenheim, osmanica in Rückbrodt et al. and in Kajan et al.	inc
44	H	Erzurum, Ascale–Trabzon, 3 km N Kop Geçidi – a few plants spotted – osmanica var. osmanica and cruenta in Renz & Taubenheim, umbrosa in Rückbrodt et al.	inc
45	I	Erzurum, 6.7 km Ascale–Trabzon – a few plants spotted – osmanica, umbrosa in Rückbrodt et al.	inc
46	J	Erzurum, Erzincan–Erzurum, 6 km E Ascale – umbrosa in Rückbrodt et al.	inc
47	K	Erzurum, Erzurum–Horasan, 8–10 km E Erzurum	inc
48	L	Erzurum, Horasan–Ağri, 1 km W Sac Geçidi	inc
49	M	Ağri, 7 km Ağri–Tutak – a few plants spotted – umbrosa in Renz & Taubenheim	inc

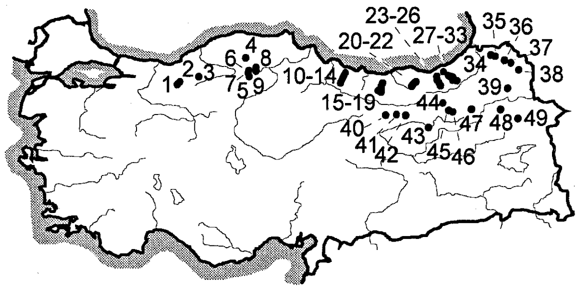

Fig. 1. Position of sampled localities. The localities are numbered as in Table 1

Dactylorhiza urvilleana (Steud.) H. Baumann Künkele is restricted to north-eastern Turkey and Caucasus, but it may be conspecific with *D. lancibracteata* (C. Koch) Renz which occurs in the Caspian area. It is similar to *D. saccifera* in the flat leaves that are spotted above and the pale flowers with a large spur.

Dactylorhiza nieschalkiorum H. Baumann & Künkele is endemic for north-western Turkey. The plants are robust with usually non-spotted leaves and have large flowers.

Dactylorhiza euxina (Nevski) S.K. Cherepanov is a mountain plant known from Caucasus and north-eastern Turkey. It is characterized by the lip of the flower that is broader than long, wrinkled and provided with an irregular margin. Low-grown and typically unspotted plants from high altitudes have been segregated as var. *markowitschii* (Soó) Renz & Taub., whereas medium-sized, often dark-coloured plants from somewhat lower altitudes are treated as var. *euxina*.

Dactylorhiza incarnata (L.) Soó has a wide distribution area in Europe and is also known from north-western Turkey. No population from this area was sampled for the present study, but some more eastern populations contained plants similar to this species, for instance plants from sites 37, 39, 41, 45, and 48 (Table 1).

Dactylorhiza osmanica (Klinge) Soó is endemic to central Anatolia. It is a sturdy plant characterized by having broadly lanceolate leaves and flowers with relatively short spurs. Tall plants have been separated as var. *anatolica* (E. Nelson) Renz & Taub.

Dactylorhiza umbrosa (Kar. & Kir.) Nevski has a wide distribution that extends from eastern Anatolia, northern Iraq and Persia to the mountain regions of central Asia. It is variable in size and may be quite large. The flowers have a long and narrow, slightly decurved spur, but there are also forms that approach *D. osmanica* (Renz and Taubenheim 1984).

Renz and Taubenheim (1984) also separated some dark-coloured plants as *D. cruenta* (O.F. Müll.) Soó. No pure population of dark-coloured plants was included in the present study, but such plants were sampled from several localities containing *D. osmanica* or *D. umbrosa*, as indicated in Table 1.

Enzyme electrophoresis. Parts of leaves were detached from living specimens in the field and kept as cool as possible until they could be refrigerated at 4 °C in the laboratory. For each individual, about 1 cm^2 of leaf area was ground with a small amount of washed sea sand in 80 µl of a Tris-HCl grinding buffer (Soltis et al. 1983) modified by replacing β-mercaptoethanol by dithiothreitol (Lönn and Prentice 1990). Enzymes were absorbed onto chromatography paper wicks and were either stored at −80 °C until electrophoresis, or run immediately after extraction.

Proteins were separated on 9–11% horizontal starch gels at ca. 10 Vcm^{-1}. A Lithium-borate/Tris-citrate buffer system (Ashton and Braden 1964), modified according to Lönn and Prentice (1990) was used to separate allozymes of Glucose-6-phosphate isomerase (*GPI* (*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), Menadione reductase (*MNR*, E.C. 1.6.99.-), and Diaphorase (*DIA*, E.C. 1.6.99-). A Tris-citrate/Histidine-EDTA buffer system at pH 7.0, modified from King and Dancik (1983), was used to separate allozymes of Isocitrate dehydrogenase (*IDH*, E.C. 1.1.1.42), Malate dehydrogenase (*MDH*, E.C. 1.1.1.37), and Shikimate dehydrogenase (*SKD*, E.C. 1.1.1.25). This system comprised an electrode buffer of 0.125 M Tris adjusted to pH 7.0 with 1 M citric acid and a gel buffer containing 10 mM L-histidine, 0.28 mM EDTA (tetrasodium salt), and 20 mM Tris adjusted to pH 7.0 with 1 M hydrochloric acid. The gel buffer was prepared as a stock solution of 5× concentration. A Histidine-citrate buffer system at pH 5.7 (Wendel and Weeden 1989) was used to separate allozymes of Phosphogluconate dehydrogenase (*PGD*, E.C. 1.1.1.44). Staining recipes followed Wendel and Weeden (1989) with only minor modifications.

In systems where more than one locus was interpreted (i.e. *TPI*) the loci were numbered sequentially beginning with the locus containing the most rapidly migrating allozyme. The allele annotation is based on Hedrén (1996a) in which alleles were denoted by alphabetic letters in order of migration distance of the corresponding allozymes. Additional alleles encountered in the present study have been inserted according to the same principle. Relative migration distances (R_m values) were calculated relative to the common allele in European *D. incarnata* at each locus, except for *Tpi-1* where the common allele at *Tpi-2* was used. The R_m values are based on material used in Hedrén (1996a, b, c), and on material examined for the present study.

Data analysis. The allozyme composition of the Turkish material investigated in the present paper was compared to a dataset of European *Dactylorhiza* compiled from Hedrén (1996a, b, c, and from unpublished data). The European material was reinterpreted in connection with the present study whereby some modifications were made. Thus, the allele denoted as Pgm^c in Hedrén 1996a, b) was subdivided into two alleles denoted as Pgm^j and Pgm^k in the present study. Also, the alleles Skd^c and Skd^d reported in those studies are here treated collectively as Skd^d, and similarly the alleles Pgd^e and Pgd^f were amalgamated to Pgd^e. The remaining changes regarded interpretation of single plants.

Non-metric multidimensional scaling analyses (MDS) (Kruskal 1964a, b; Sneath and Sokal 1973) were adopted to describe genetic differentiation patterns between populations. The differentiation between each pair of populations was described by means of Rogers' genetic distance (Rogers 1972). The MDS analyses were then performed on the resulting distance matrix, and were repeated ten times to avoid problems with local minima. The run with the lowest stress value (lowest amount of ranking order distortion between genetic distances in the original matrix of Rogers' genetic distances and distances in the final MDS solution) was chosen for interpretation. The MDS solution was rotated in a principal components analysis (PCA, Sneath and Sokal 1973) in order to maximize the variance along the horizontal axis. Population samples containing less than 10 individuals were excluded in these analyzes.

Material of *D. incarnata* s.l. was analyzed separately in a PCA in which alleles were used as characters and allele frequencies as character states. At each locus the allele with the lowest frequency was excluded in order to avoid redundant information (as the sum of allele frequencies at a locus is 1).

The multivariate analyses were performed in NTSYS-pc 1.80 (Rohlf 1994) or in SAS (SAS Inst. 1990).

Results

Allozyme patterns. Ten loci were found to be variable and interpretable at the nine enzyme systems investigated. Two loci were interpreted at *TPI*, whereas one locus was interpreted at each of the remaining systems.

All loci contained additional alleles to those found in previous studies on European taxa. A total of 14 alleles were recognized at *Pgm*. Some of these, e.g. Pgm^l and Pgm^d, produced bands that were close to each other on the electrophoresis gels, but that were apparently confined to different taxa. Running these taxa adjacent to each other on the gels it could be ascertained that the allozymes had different mobilities, but it was not possible to test all populations in this way. The same difficulty also applied to other loci with small differences in allozyme mobility. In some other cases, e.g. in Pgm^j, it was suspected that allozymes had slightly different mobilities in the European and Turkish material. However, it was not possible to re-run these materials on the same gel and using a conservative approach these allozymes had to be related to a single allele.

Two of the *Tpi-1* alleles, $Tpi\text{-}1^g$ and $Tpi\text{-}1^d$, invariably gave rise to three-banded patterns. These patterns may be due to enzyme modifications, or to the presence of a "superallele" containing two different genes. Either explanation is compatible with the band patterns found in heterozygotes containing one of these alleles (Fig. 2). Heterodimers are formed with the proteins forming the slow and the fast band, but not with the middle band, and it is indicated that the fast and the slow bands are themselves homodimers, whereas the middle band is a heterodimer.

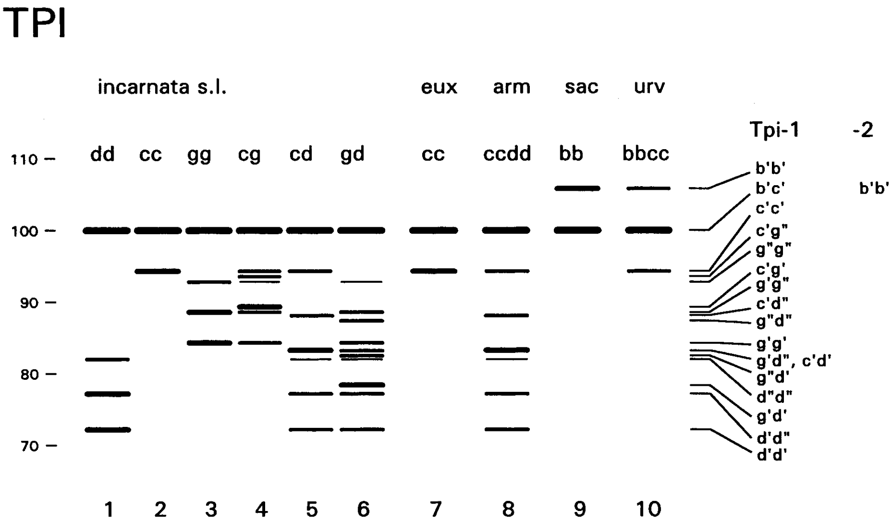

Fig. 2. Interpretation of enzyme patterns found at *TPI*, which is a dimeric enzyme. Lanes 1–6 show some of the patterns seen in Turkish *D. incarnata* s.l., whereas lanes 7–10 show the common patterns seen in *D. euxina*, *D. armeniaca*, *D. saccifera*, and *D. urvilleana*, respectively. The pattern given as lane 1 is also the common pattern in European *D. incarnata.* The enzymes have migrated from the bottom towards the top of the figure. The scale to the left show their mobilities relative to the homodimer produced by the common allele at *Tpi-2*, i.e. *Tpi-2*^{b}. The proposed subunit composition of each allozyme is shown to the right, and the genetic interpretation of the samples are shown above each lane. All samples shown are homozygous for *Tpi-2*^{b}, and the b′b′ homodimer appears as a strong band that masks the b′c′ heterodimer at *Tpi-1* in *D. urvilleana.* Two of the alleles in *D. incarnata* s.l., *Tpi-1*^{g} and *Tpi-1*^{d} each give rise to two different subunits, denoted g′ and g″, and d′ and d″, respectively. See text for possible explanations

The allozyme bands at *Gpi*, *Tpi-1*, *Tpi-2*, *Pgm* and *Pgd* often differed clearly in staining intensity in the tetraploid plants and it was generally possible to estimate the numbers of allele copies from the band intensities. The correlation between staining intensity and allele copy number was corroborated in plants where more than two alleles occurred at a locus, in which either one strong and two weak bands, or, less frequently four weak bands were seen. In the remaining loci it was more difficult to estimate numbers of allele copies, and a conservative approach was often necessary, assuming that alleles occurred in equal numbers if two different ones were present.

Under the running conditions used here allozymes at *Mdh* formed ladders. The interpretation of this locus was nevertheless straightforward when plants were homozygous at the locus, but more difficult when plants were heterozygous with overlapping bands, especially as regards estimates of allele copy numbers in polyploids.

Based on the overall variation patterns at all loci and all individuals within the populations, the ploidy levels of the taxa were estimated, and previous reports on chromosome numbers were either accepted or rejected. For instance, tetraploidy was indicated by the occurrence of three or four alleles at a locus, or the presence of unbalanced heterozygotes, allotetraploidy by the presence of fixed heterozygosity or the dominance of certain heterozygous genotypes at some loci. Diploidy was indicated by simple variation patterns with homozygotes and balanced heterozygotes, and by heterozygote frequencies according to expectations (Hardy–Weinberg equilibria) within populations.

Dactylorhiza iberica was found to contain completely different alleles compared to the remaining taxa and it was early concluded that this species was not closely related to the other taxa investigated. Accordingly, the species was excluded from further comparisons, and alleles occurring in *D. iberica* have not been recognized.

The alleles recorded in the remaining taxa and populations are presented in Table 2. Each column gives the percentage of samples that contains a particular allele. Mean percentages (over populations) have been calculated for each taxon. Some localities contained two allozymically distinct taxa, but the separation of these was mostly straightforward. However, a few localities contained some putative hybrids that are not included the Table.

The Turkish taxa are compared with material from Europe in Table 3. For each taxon mean percentages of individuals containing the different alleles have been calculated as in Table 2. For the total material of European allotetraploids it has only been indicated whether alleles are present or absent.

Taxonomic subdivision indicated by genetic data. Three allozymically distinct diploid groups could be distinguished in the Turkish material (Table 2). The delimitation of two of these coincide with the circumscription of *D. saccifera* and *D. euxina*. *Dactylorhiza saccifera* is closely similar to the European *D. fuchsii* in allozyme composition (Table 3). *Dactylorhiza euxina* is not closely similar to any European taxon previously investigated. A third group includes material of *D. osmanica* and *D. umbrosa*, and will subsequently be treated as *D. incarnata* s.l. This group was genetically variable, but there was no correlation between allozyme differentiation and the proposed delimitation into taxa (cf. Table 1). Although the Turkish material contained more alleles per locus than did the European material (Table 3), some populations also contained the typical European *D. incarnata* alleles in quite high frequencies and were morphologically similar to *D. incarnata* from Europe. In addition, the Turkish material contained some alleles that have not been found in European *D. incarnata*, but that should belong to the *D. incarnata* genome according to variation patterns seen in the European allotetraploids (e.g. *Pgm*^{a}, cf. Hedrén 1996a). The variation patterns in Turkish *D. incarnata* has been summarized by a PCA given as Fig. 3.

There are also three allozymically distinct allotetraploids in Turkey. Two of the allotetraploids coincide with the taxonomic circumscriptions of *D. nieschalkiorum* and *D. urvilleana*, respectively. The third allotetraploid is here described as *D. armeniaca* (Appendix 1). The allotetraploids combine alleles from the diploid groups in all three possible ways. Thus, all populations, and most individuals of *D. nieschalkiorum* contain alleles from *D. saccifera* and *D. incarnata* s.l. at each locus. *Dactylorhiza nieschalkiorum* also reflects much of the allelic variation seen the two diploids, but at some loci, e.g. *Pgm*, it contains high frequencies of alleles that are rare or absent from Turkish populations of these taxa.

Dactylorhiza urvilleana combines subsets of alleles present in *D. saccifera* and *D. euxina*, but in addition it also contains alleles at, i.e. *Tpi-2*, *Idh*, and *Mdh* that have not been encountered in any of the two diploids. *Dactylorhiza armeniaca* combines equal proportions of alleles present in Turkish *D. incarnata* s.l. and *D. euxina* at each locus, and there are no additional alleles in *D. armeniaca* that has not been found in any of these diploids. *Dactylorhiza armeniaca* contains high proportions of the allele *Idh*^{a} that has also been found in high proportions in populations of *D. incarnata* s.l. from the same area, e.g. from localities 37 and 39.

The allozyme differentiation found in the investigated material was generally in agreement with determinations made in the field, or with previous literature reports (Table 1). However, some populations had to be reinterpreted. *Dactylorhiza nieschalkiorum* and *D. urvilleana* overlap in the Kastamonu region and at least two localities (localities 8 and 9) contained a mixture of the two different allotetraploid genotypes (Table 1, Fig. 4). Some populations hitherto included in

D. euxina were reinterpreted as the new allotetraploid *D. armeniaca*.

All Turkish populations comprising more than 10 individuals were compared in a MDS given as Fig. 5. The three diploid groups are distinct, but *D. euxina* is apparently more similar to *D. incarnata* than to *D. saccifera*. The allotetraploids occupy positions intermediate between different pairs of diploids.

A few hybrids were found in the investigated material. Two F_1 plants between *D. armeniaca* and *D. urvilleana* were recorded at locality 35, which was the only locality where both taxa were found together. A single F_1 plant between *D. euxina* and *D. urvilleana* was found at locality 26. No hybrids were recorded from the remaining five localities where both species were found together. Four plants from locality 6 were interpreted as F_1 hybrids between *D. nieschalkiorum* and *D. saccifera*. F_1 hybrids between *D. nieschalkiorum* and *D. urvilleana* were found at localities 8 and 9. Two plants combining alleles from *Dactylorhiza nieschalkiorum* and *D. urvilleana* were also found at locality 7. However, these plants could not be interpreted as F_1-plants, but must represent F_n hybrids or backcrosses.

Discussion

Correlation between allozymes and other data. The Turkish populations of *D. incarnata* s.l. constitute a variable group, which includes *D. osmanica*, *D. umbrosa* and material closely similar to European populations of *D. incarnata* s.s. This result contrasts to treatments of previous authors who recognized *D. osmanica* and *D. umbrosa* as separate species (e.g. Baumann and Künkele 1981, Renz and Taubenheim 1984). However, there is no indication from genetic data that the group could be subdivided into discrete taxa (Fig. 3), and the genetic differentiation between populations described in the present study is not correlated with variation patterns in floral characters, including spur size, lip shape and various other floral characters (data not shown). It is also apparent from available taxonomic literature that it is difficult to delimit *D. osmanica* and *D. umbrosa* from each other (Renz and Taubenheim 1984, Rückbrodt et al. 1992, Delforge 1995, Kreutz 1998). Intermediate specimens have been interpreted as hybrids and variable populations as hybrid swarms (Baumann and Künkele 1981, Renz and Taubenheim 1984). This interpretation may have contributed to the view that hybridization would be frequent in Turkish *Dactylorhiza*. However, although a broad circumscription of *D. incarnata* s.l. in Turkey seems necessary, it should not be disregarded that this is a morphologically variable taxon, and for instance it should be highly interesting to correlate variation in spur morphology with pollinator fauna. To enable a more precise taxonomy reflecting these variation patterns, extended morphological studies should be performed, and it should also be relevant to compare Turkish populations of *D. incarnata* s.l. with European material.

Plants with leaves spotted on both sides appear in both what has been considered as *D. osmanica* and *D. umbrosa*. The extent of spotting varies, in some plants the spots are confluent and the leaves almost completely dark-coloured. This situation is similar to that of European *D. incarnata* s.l. in which plants with spotted leaves are often recognized as *D. cruenta*, and this name has also been applied to Turkish plants (Renz and Taubenheim 1984).

After having separated some allotetraploid populations as *D. armeniaca*, the remaining populations of *D. euxina* constitute a genetically well characterized diploid species. It is more similar to *D. incarnata* than to *D. saccifera* in allozymes, and it is morphologically distinct from other diploid members of *Dactylorhiza* in having flowers with broad, wrinkled lips with very irregular margins of the side-lobes. The leaves are soft, flat and obtuse at the apex. Like in *D. incarnata*, populations may be polymorphic containing green and dark-coloured individuals. However, the dark-coloured individuals differ from those of *D. incarnata* s.l. in having streaks rather than

Table 2. Allozyme composition of Turkish members of the *Dactylorhiza incarnata*/*maculata* polyploid complex. For each population and allele, the percentage of samples containing that particular allele has been given. Mean frequencies (over populations) have been indicated for each taxon. For population numbers, see Table 1. Abbreviations: inc = *D. incarnata* s.l. (including *D. osmanica* and *D. umbrosa*), sac = *D. saccifera*, eux = *D. euxina*, nie = *D. nieschalkiorum*, arm = *D. armeniaca*, urv = *D. urvilleana*

Taxon	Pop	n	Gpi										Pgm														Tpi-1								Tpi-2					
			f	g	a	b	c	h	d	i	e	j	a	b	i	j	k	l	d	m	e	n	f	o	g	h	a	b	c	e	f	g	h	d	a	b	c	d	e	f
inc	36	19	0	0	0	0	11	63	68	11	16	0	5	100	0	0	0	0	0	5	0	0	0	0	0	0	0	0	84	0	0	84	0	0	0	100	11	0	0	0
inc	37	8	13	63	0	0	0	0	50	0	25	0	0	13	13	0	0	0	100	0	0	0	0	0	0	0	0	0	88	0	0	25	0	0	0	100	0	0	0	0
inc	39	3	0	33	0	0	0	33	67	33	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	33	0	100	67	0	0	0
inc	40	25	0	12	0	0	44	32	60	8	0	0	0	24	8	0	56	0	56	24	0	0	0	0	0	0	0	0	92	0	0	44	0	16	8	100	8	0	0	0
inc	41	19	0	26	0	5	32	47	53	0	21	0	0	0	11	0	79	0	42	5	5	0	0	0	0	0	0	0	74	0	0	53	0	16	0	100	5	0	0	0
inc	42	20	0	0	0	0	5	60	45	5	40	0	5	40	0	0	80	0	25	15	0	0	0	0	0	0	0	0	80	0	0	65	0	10	5	95	0	0	0	0
inc	43	8	0	0	0	0	25	75	25	13	0	0	0	0	0	0	88	0	0	63	0	0	0	0	0	0	0	0	50	0	0	63	0	0	0	100	0	0	0	0
inc	44	23	0	4	0	9	17	43	70	4	35	0	4	52	13	0	17	0	9	65	9	0	0	0	0	0	0	22	43	0	0	74	0	9	0	100	0	0	0	0
inc	45	21	0	0	0	5	24	57	48	14	29	0	0	100	0	0	5	0	0	14	0	0	0	0	0	0	0	0	81	0	0	81	0	5	0	100	5	0	0	0
inc	46	21	0	0	0	0	5	48	76	0	24	0	0	100	5	0	10	0	0	10	0	0	0	0	0	0	0	5	76	0	0	81	0	5	0	100	10	0	0	0
inc	47	21	0	0	0	0	14	62	29	0	38	0	0	100	0	0	14	0	0	14	0	0	0	0	0	0	0	0	55	0	0	85	0	15	0	100	0	0	0	0
inc	48	22	0	0	0	0	27	55	45	5	32	0	0	82	27	0	14	0	5	5	9	0	0	0	0	0	0	0	71	0	0	86	0	10	0	100	0	0	0	0
inc	49	30	0	0	0	3	3	27	83	10	40	3	0	100	0	0	3	0	0	27	0	0	0	0	0	0	0	3	67	0	0	73	0	10	0	100	7	0	0	0
mean			1	11	0	2	16	46	55	8	23	0	1	62	6	0	28	0	18	19	2	0	0	0	0	0	0	2	66	0	0	70	0	10	1	100	9	0	0	0
sac	2	27	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	7	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0
sac	6	29	0	0	24	90	7	0	0	0	0	0	0	0	0	0	0	0	10	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0
mean			0	0	12	95	4	0	0	0	0	0	0	0	0	0	0	0	9	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0
eux	18	1	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	19	17	18	94	0	0	0	0	0	0	0	0	0	0	0	0	6	88	12	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	22	31	10	100	0	0	0	16	0	0	0	0	0	0	0	10	0	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	26	47	9	96	0	2	0	21	0	0	0	0	0	0	0	32	0	100	0	0	0	0	0	0	0	0	0	0	100	0	9	0	0	0	0	100	15	0	0	0
eux	31	34	18	100	0	21	0	0	0	0	0	0	0	0	0	50	0	94	0	0	0	0	0	0	0	0	0	0	100	0	6	0	0	0	0	100	0	0	0	0
eux	32	66	11	98	0	20	0	2	0	0	0	0	0	0	0	27	0	98	0	0	2	0	0	0	0	0	0	2	100	0	2	0	0	0	0	100	0	0	0	0
eux	33	16	0	94	0	25	0	0	0	0	0	0	0	0	0	25	0	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
mean			9	97	0	10	0	6	0	0	0	0	0	0	0	21	1	97	2	0	0	0	0	0	0	0	0	0	100	0	2	0	0	0	0	100	2	0	0	0
nie	1	25	0	0	0	96	4	4	8	28	60	4	100	10	0	0	0	0	10	0	38	5	86	5	0	0	0	8	92	0	0	0	0	100	0	100	0	0	0	0
nie	3	8	0	0	0	100	0	0	75	75	0	0	88	0	0	0	0	0	0	0	88	0	63	0	0	0	0	100	20	0	0	0	0	100						
nie	7	24	0	0	0	96	0	4	8	50	29	17	85	35	0	0	0	0	19	0	96	0	8	0	0	0	0	100	0	0	0	0	0	100	0	100	0	0	0	0

nie	8	3	0	0	0	100	0	0	67	100	0	0	67	67	0	0	0	0	33	0	100	0	67	0	0	0	0	100	0	0	0	67	0	100	0	100	0	0	0	0
nie	9	5	0	0	0	80	0	40	0	60	0	40	100	0	0	0	0	0	0	0	80	0	100	0	0	0	0	100	0	0	0	0	0	100	0	100	0	0	0	0
mean			0	0	0	94	1	10	32	63	18	12	88	22	0	0	0	0	12	0	80	1	65	1	0	0	0	82	22	0	0	13	0	100	0	100	0	0	0	0
arm	34	30	47	93	0	0	0	0	3	0	100	0	0	97	0	17	0	87	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0
arm	35	15	53	87	0	0	0	0	0	0	100	0	0	100	0	13	0	87	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0
arm	38	26	12	100	0	4	0	4	23	0	100	0	0	100	0	23	0	85	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	100	4	0	0	0
arm	39	18	17	89	0	0	0	22	50	0	100	0	0	94	0	50	0	56	0	6	0	0	0	0	0	0	0	0	100	0	0	0	0	100	6	100	0	0	0	0
mean			32	92	0	1	0	7	19	0	100	0	0	98	0	26	0	79	0	2	0	0	0	0	0	0	0	0	100	0	0	0	0	100	2	100	1	0	0	0
urv	6	2	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	0	100	0	0
urv	8	15	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	47	7	0
urv	9	1	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	100	0	0
urv	10	3	0	100	0	100	0	0	0	0	0	0	0	0	0	33	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	0	0	0
urv	11	5	20	80	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	60	0	0
urv	12	27	4	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	55	0	0
urv	13	31	0	97	0	100	0	0	0	0	0	0	0	0	0	7	0	97	7	0	97	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	35	0	0
urv	14	1	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	100	0	0
urv	15	2	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	50	0	0	0	0	0	0	100	0	100	0	0
urv	16	24	4	96	0	96	0	0	0	0	0	0	0	0	0	13	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	46	0	0
urv	17	37	14	100	0	100	0	5	0	0	0	0	0	0	0	22	0	97	0	0	100	0	3	0	0	0	0	100	100	0	0	0	0	0	0	100	8	53	0	0
urv	18	14	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	93	0	0	0	0	0	0	100	0	7	0	0
urv	20	21	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	46	0	0
urv	21	14	0	100	0	100	0	0	0	0	0	0	0	0	0	14	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	50	0	29
urv	22	30	0	100	0	100	0	0	0	0	0	0	0	0	0	27	0	100	0	0	93	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	3	31	7	0
urv	23	5	0	100	0	100	0	0	0	0	0	0	0	0	0	20	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	0	0	60
urv	24	7	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	71	0	14
urv	25	20	0	100	0	100	0	0	0	0	0	0	0	0	0	10	0	95	0	0	95	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	10	0	0
urv	26	13	8	92	0	85	8	8	0	0	0	0	0	0	0	15	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	8	0	0	100	0	31	0	0
urv	27	21	0	100	0	100	0	0	0	0	0	0	0	0	0	5	0	100	0	0	100	0	5	0	0	0	0	100	100	0	0	0	0	0	0	100	0	71	0	14
urv	28	6	0	100	0	100	0	0	0	0	0	0	0	0	0	17	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	33	0	0
urv	29	17	0	100	0	100	0	0	0	0	0	0	0	0	0	29	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	50	0	0
urv	30	20	0	100	0	100	0	0	0	0	0	0	0	0	0	0	0	95	5	0	95	0	5	0	0	0	0	100	100	0	0	0	0	0	0	100	5	70	0	10
urv	31	25	0	100	0	100	0	0	0	0	0	0	0	0	0	18	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	0	0	0	0	100	0	36	4	12
urv	32	23	0	100	0	100	0	0	0	0	0	0	0	0	0	13	0	100	0	0	100	0	4	0	0	0	0	100	100	0	0	0	0	0	0	100	0	24	0	5
urv	35	13	0	100	0	92	0	0	0	0	15	0	0	8	0	0	0	100	0	0	100	0	0	0	0	0	0	100	100	0	0	23	0	15	0	100	15	8	38	0
mean			2	99	0	99	0	1	0	0	1	0	0	0	0	9	0	99	0	0	99	0	1	0	0	0	0	100	94	0	0	1	0	1	0	100	1	47	2	6

Table 2 (continued)

Taxon	Pop	Dia					Mnr							Pgd								Skd						Idh					Mdh							
		d	a	b	c	e	a	b	c	f	d	e	g	a	h	i	b	j	c	d	e	a	b	c	e	f	g	a	b	c	d	e	a	b	c	d	e	f	g	h
inc	36	0	100	16	0	0	0	0	0	0	100	0	5	63	21	68	0	0	0	0	0	0	21	0	95	0	0	0	32	100	0	0	0	0	0	89	0	11	0	0
inc	37	25	100	0	0	0	0	0	0	0	100	0	14	29	43	86	0	0	0	0	0	0	67	17	33	0	0	88	0	13	0	0	0	0	0	100	0	0	0	0
inc	39	0	100	0	0	0	0	0	0	0	100	0	0	67	33	100	0	0	0	0	0	33	67	0	100	0	0	33	100	0	0	0	0	0	0	100	0	0	0	0
inc	40	0	100	0	0	0	0	0	0	0	100	0	16	60	28	36	4	0	0	0	0	24	24	68	44	4	0	0	100	16	0	0	0	0	0	79	0	83	0	0
inc	41	0	100	0	0	0	0	0	0	0	100	0	5	21	84	26	32	0	0	0	0	11	26	26	63	11	0	0	100	0	0	0	0	0	0	82	0	71	0	0
inc	42	5	100	5	0	0	0	0	15	0	95	0	0	15	50	60	35	0	0	0	0	10	5	10	65	55	5	5	100	30	0	0	0	0	0	100	0	32	0	0
inc	43	0	100	0	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
inc	44	0	100	0	0	0	0	0	4	0	100	9	4	43	48	43	4	0	0	0	0	0	13	17	61	48	9	0	87	43	0	0	0	0	0	90	0	10	0	0
inc	45	5	100	5	0	0	0	0	0	0	100	0	19	14	62	76	0	0	0	0	0	5	5	0	100	14	0	0	43	93	0	0	0	0	0	95	0	5	0	0
inc	46	10	100	0	0	0	0	0	0	0	100	0	0	57	33	67	5	0	0	0	0	10	0	0	100	5	0	0	44	100	0	0	0	0	0	95	0	14	0	0
inc	47	5	100	18	0	0	0	0	0	5	100	5	0	15	60	55	35	0	0	0	0	5	10	0	100	5	0	0	38	90	0	0	0	0	0	95	0	5	0	0
inc	48	0	100	0	0	0	0	0	5	0	100	0	0	27	23	86	0	0	0	0	0	5	0	0	100	5	0	0	64	82	0	0	0	0	0	100	0	5	0	0
inc	49	0	100	0	0	0	0	0	3	0	100	0	0	57	53	20	17	0	3	0	0	0	23	10	87	7	0	0	97	17	0	0	0	0	0	83	0	0	0	0
mean		4	100	3	0	0	0	0	2	0	100	1	5	44	41	56	10	0	0	0	0	8	20	11	81	12	1	10	70	45	0	0	0	0	0	93	0	18	0	0
sac	2	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	100	0	30	0	0	–	–	–	–	–	–	–	–	–	–	–	0	30	0	0	93	0	41	0
sac	6	–	–	–	–	–	0	0	100	0	0	0	0	0	0	0	24	0	90	0	0	0	100	0	0	0	0	–	–	–	–	–	0	90	0	14	57	0	5	0
mean		0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	62	0	60	0	0	0	100	0	0	0	0	–	–	–	–	–	0	60	0	7	75	0	23	0
eux	18	–	–	–	–	–	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	0	100	0	0	0	0	–	–	–	–	–	0	0	0	100	0	0	0	0
eux	19	0	100	0	0	0	0	0	0	0	100	0	0	100	12	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	22	10	94	0	0	0	0	0	0	0	100	0	0	97	0	0	32	0	3	0	0	0	7	100	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	26	0	100	0	0	0	0	0	0	0	100	0	0	98	0	19	4	0	0	11	0	0	0	91	28	17	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	31	0	100	0	0	0	0	0	3	0	100	0	0	100	6	9	6	0	9	0	0	0	23	97	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0
eux	32	0	100	10	0	0	0	0	2	3	98	2	2	98	0	4	9	0	6	2	0	0	2	100	2	0	0	0	100	0	0	0	0	2	0	100	0	0	0	0
eux	33	0	100	0	0	0	0	0	0	0	100	0	0	75	19	0	25	13	25	0	0	0	0	25	0	0	0	–	–	–	–	–	0	0	0	100	0	0	0	0
mean		2	99	2	0	0	0	0	15	0	85	0	0	95	5	5	11	2	6	2	0	0	19	73	4	2	0	0	100	0	0	0	0	0	0	100	0	0	0	0
nie	1	–	–	–	–	–	0	0	96	0	100	12	0	100	0	0	76	0	56	0	0	–	–	–	–	–	–	–	–	–	–	–	0	25	0	100	50	25	0	0
nie	3	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	29	0	0	–	–	–	–	–	–	–	–	–	–	–	0	0	0	100	100	0	0	0
nie	7	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	83	0	50	0	0	–	–	–	–	–	–	–	–	–	–	–	0	0	0	67	100	0	0	0
nie	8	–	–	–	–	–	0	0	100	0	100	33	0	100	0	0	67	0	100	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
nie	9	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	60	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
mean		–	–	–	–	–	0	0	99	0	100	9	0	100	0	0	85	0	59	0	0	–	–	–	–	–	–	–	–	–	–	–	0	8	0	89	83	8	0	0

arm	34	0	100	7	0	0	0	0	0	0	100	0	0	100	0	93	0	0	0	0	0	100	4	100	0	0	0	100	100	0	0	0	0	0	0	100	0	0	0	0
arm	35	0	100	0	0	0	0	0	0	0	100	0	0	100	0	93	0	0	0	0	0	100	0	100	0	0	0	100	100	0	0	0	0	0	0	100	0	0	0	0
arm	38	0	100	12	0	0	0	0	0	0	100	0	0	100	0	81	0	0	0	0	0	100	0	88	28	0	0	100	100	0	0	0	0	0	0	100	0	0	0	0
arm	39	0	100	0	0	0	0	0	0	0	100	0	6	100	0	83	0	0	0	0	0	100	25	63	56	0	0	72	100	0	0	0	0	0	0	100	0	0	0	0
mean		0	100	5	0	0	0	0	0	0	100	0	2	100	0	88	0	0	0	0	0	100	7	88	21	0	0	93	100	0	0	0	0	0	0	100	0	0	0	0
urv	6	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	0	0	100	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	8	–	–	–	–	–	0	0	100	0	100	0	0	80	0	0	87	0	20	7	7	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	9	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	100	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	10	0	100	100	0	0	–	–	–	–	–	–	0	100	0	0	100	0	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	11	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	100	100	0	0	0	–	–	–	–	–	20	0	0	100	0	0	0	0
urv	12	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	–	–	–	–	–	–	–	–	–	–	–	0	85	0	100	15	0	0	0
urv	13	0	100	100	0	0	0	0	100	0	100	0	0	100	0	0	100	4	0	0	0	–	–	–	–	–	–	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	14	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	100	100	0	0	0	–	–	–	–	–	100	0	0	100	0	0	0	0
urv	15	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	16	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	4	4	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	17	–	–	–	–	–	0	0	100	0	97	0	0	97	0	0	100	0	0	0	0	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	18	–	–	–	–	–	0	0	100	0	100	0	0	100	0	0	100	0	7	0	0	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	20	0	100	100	0	0	0	0	100	0	100	0	5	86	0	0	100	0	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	21	8	100	100	0	0	0	0	85	0	100	0	0	86	0	0	100	0	7	0	0	0	92	92	0	0	0	0	100	0	0	29	0	75	0	100	0	0	0	0
urv	22	3	100	93	23	0	0	0	100	0	100	0	0	97	0	0	97	0	7	3	0	0	97	93	7	0	0	0	100	0	3	7	0	97	0	97	3	0	0	0
urv	23	20	100	80	60	0	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	100	100	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0
urv	24	0	100	57	43	0	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	100	100	0	0	0	0	100	0	0	0	0	86	0	100	0	0	0	0
urv	25	5	100	79	26	0	0	0	100	0	100	0	0	89	0	0	100	0	0	0	0	0	100	100	0	0	0	0	100	0	5	0	0	100	0	100	0	0	0	0
urv	26	8	100	92	0	0	0	0	100	0	100	0	0	100	0	0	100	0	0	0	0	0	91	91	18	0	0	0	100	0	0	0	0	45	0	55	0	0	0	0
urv	27	–	–	–	–	–	0	0	100	0	100	0	0	95	0	0	100	0	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urv	28	–	–	–	–	–	0	0	100	0	100	0	67	33	0	0	100	0	0	0	0	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	29	–	–	–	–	–	0	0	100	0	100	0	41	53	0	0	100	0	6	0	0	0	100	100	0	0	0	–	–	–	–	–	0	92	0	100	0	0	0	0
urv	30	0	100	92	42	8	0	0	100	0	88	0	20	85	0	0	90	0	15	0	0	–	–	–	–	–	–	–	–	–	–	–	0	95	0	100	0	0	0	0
urv	31	0	100	100	0	0	0	0	100	0	100	0	0	91	4	0	100	9	4	4	0	0	100	100	4	0	0	0	100	0	24	0	0	64	0	100	0	0	0	0
urv	32	0	100	88	35	0	0	0	100	0	100	0	14	90	0	0	95	5	14	0	0	0	100	100	0	0	0	–	–	–	–	–	0	100	0	100	0	0	0	0
urv	35	0	100	85	23	0	0	0	100	0	100	0	31	100	0	8	92	0	8	0	0	10	100	100	0	0	0	8	100	8	8	0	0	92	0	100	0	0	0	0
mean		3	100	90	19	1	0	0	99	0	99	0	7	92	0	0	95	1	11	1	0	1	99	99	2	0	0	1	100	1	5	5	6	83	0	98	1	0	0	0

Table 3. Comparison of allozyme composition of Turkish and European members of the *Dactylorhiza incarnata*/*maculata* polyploid complex. For each taxon and allele, the mean over population of samples containing that particular allele has been given as a percentage. + denote frequencies below one percent. Relative migration distances (R_m) has been calculated relative to the common *D. incarnata* allele at each locus, except for *Tpi–1* where migration distances are calculated relative to *Tpi-2*^{b}. For *Tpi-1*^{g} and *Tpi-1*^{d}, which produced triplet bands (see text and Fig. 2.), the mobility of the most slowly–migrating band has been given. Data for European material are based on Hedrén (1996a, b, c). Additional alleles present in material from northern Europe (N), western Europe (W), and central Europe (C) has been indicated (Hedrén, unpublished data). Only presence/absence of alleles has been given in the row summarising European allotetraploid FFII taxa. The genome composition of each taxon has been indicated

Taxon	Locus	Gpi										Pgm														Tpi–1								Tpi–2					
	alleles	f	g	a	b	c	h	d	i	e	j	a	b	i	j	k	l	d	m	e	n	f	o	g	h	a	b	c	e	f	g	h	d	a	b	c	d	e	f
	R_m	126	123	122	119	116	113	108	104	100	95	101	100	98.5	98	97	96	95	93	91	90	88	86	84	81	113	106	94	89	87	84	78	72	109	100	90	82	79	76
incarnata s.l. Turkey II		1	11	0	2	16	46	55	8	23	+	1	62	6	0	28	0	18	19	2	0	0	0	0	0	0	2	66	0	0	70	0	10	1	100	9	0	0	0
incarnata s.l. Europe II		0	0	0	0	0	0	0	0	100	0	W	100	0	W	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	100	0	99	+	0	0	0
saccifera FF		0	0	12	95	4	0	0	0	0	0	0	0	0	0	0	0	9	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	0	0	0	0
fuchsii FF		0	0	1	100	0	0	0	0	0	0	0	0	1	0	4	0	37	0	84	0	21	0	4	0	1	100	1	0	0	0	0	0	0	100	0	0	0	0
maculata FFFF		0	0	18	100	4	0	0	0	0	0	0	N	0	0	16	0	80	0	77	0	23	0	0	+	21	100	0	0	0	0	0	0	0	100	5	0	0	0
euxina EE		9	97	0	10	0	6	0	0	0	0	0	0	0	21	1	97	2	0	+	0	0	0	0	0	0	+	100	0	2	0	0	0	0	100	2	0	0	0
nieschalkiorum FFII		0	0	0	94	1	10	32	63	18	12	88	22	0	0	0	0	12	0	80	1	65	1	0	0	0	82	22	0	0	13	0	100	0	100	0	0	0	0
majalis FFII		0	0	6	98	0	0	0	0	100	0	14	97	0	0	0	0	94	0	33	0	0	0	0	0	0	96	16	0	0	0	0	100	0	100	0	0	0	0
traunsteineri FFII		0	0	10	97	1	0	1	0	100	0	0	100	0	0	11	0	84	0	31	0	0	0	0	0	0	100	x	0	0	1	0	99	0	100	1	0	0	0
sphagnicola FFII		0	0	34	89	0	0	0	0	88	0	6	99	0	0	0	0	97	0	8	0	0	0	0	0	+	98	x	0	0	0	0	100	0	100	0	0	0	0
lapponica FFII		0	0	62	58	0	0	0	0	100	0	0	100	0	0	10	0	92	0	2	0	0	0	0	0	0	100	0	0	0	0	0	100	0	100	0	0	0	0
purpurella FFII		0	0	0	100	0	0	0	0	100	0	0	16	0	78	22	0	7	0	97	0	0	0	0	0	0	100	0	0	0	0	0	100	0	100	0	0	0	0
praetermissa FFII		0	0	10	89	0	0	0	0	100	0	5	98	0	0	0	0	38	0	60	0	18	0	0	0	0	90	0	0	0	0	0	100		0	100	0	0	0
Summary European FFII		–	–	x	x	x	–	x	–	x	N	x	x	–	x	x	–	x	N	x	–	x	–	–	–	x	x	x	–	–	x	–	x	–	x	x	–	–	N
armeniaca EEII		32	92	0	1	0	7	19	0	100	0	0	98	0	26	0	79	0	2	0	0	0	0	0	0	0	0	100	0	0	0	0	100	2	100	1	0	0	0
urvilleana EEFF		2	99	0	99	+	1	0	0	1	0	0	+	0	9	0	99	+	0	99	0	1	0	0	0	0	100	94	0	0	1	+	1	0	100	1	47	2	6

Table 3 (continued)

Taxon	Locus	Dia					Mnr						Pgd									Skd						Idh					Mdh							
	alleles	d	a	b	c	e	a	b	c	f	d	e	g	a	h	i	b	j	c	d	e	a	b	c	e	f	g	a	b	c	d	e	a	b	c	d	e	f	g	h
	R_m	107	100	92	83	77	180	150	129	105	100	70	119	100	97	89	85	73	69	53	34	100	88	78	65	56	50	113	100	92	90	83	117	109	107	100	96	90	82	70
incarnata Turkey II		4	100	3	0	0	0	0	2	+	100	1	5	44	41	56	10	0	+	0	0	8	20	11	81	12	1	10	70	45	0	0	0	0	0	93	0	20	0	0
incarnata Europe II		1	99	N	0	0	0	0	0	0	100	0	0	99	0	0	W	0	1	0	0	99	0	0	0	1	0	0	100	0	0	0	0	0	0	100	0	0	0	0
saccifera FF		0	0	100	0	0	0	0	100	0	0	0	0	0	0	0	62	0	60	0	0	0	100	0	0	0	0	–	–	–	–	–	0	60	0	7	75	0	23	0
fuchsii FF		0	0	100	1	0	3	20	81	0	5	0	0	0	0	0	74	0	74	3	0	0	99	14	0	0	0	–	–	–	–	–	–	–	–	–	N	–	–	N
maculata FFFF		N	1	100	7	N	6	18	99	0	2	2	0	1	0	N	65	0	91	17	6	0	100	3	0	0	0	–	N	–	–	–	0	N	0	0	100	0	0	N
euxina EE		2	99	2	0	0	0	0	15	+	85	0	0	95	5	5	11	2	6	2	0	0	19	73	4	2	0	0	100	0	0	0	0	0	0	100	0	0	0	0
nieschalkiorum FFII		–	–	–	–	–	0	0	99	0	100	9	0	100	0	0	85	0	59	0	0	–	–	–	–	–	–	–	–	–	–	–	0	8	0	89	83	8	0	0
majalis FFII		0	100	100	0	0	0	10	94	0	100	0	0	100	0	0	90	0	34	0	8	100	100	0	0	0	0	0	96	12	0	0	0	0	0	100	92	26	0	0
traunsteineri FFII		0	100	100	0	0	0	33	86	0	100	0	0	100	0	0	9	0	82	11	24	99	100	2	0	0	0	–	–	–	–	–	0	0	0	100	60	70	0	0
sphagnicola FFII		0	100	100	0	0	0	13	96	0	100	0	0	100	0	0	3	0	61	61	1	99	69	57	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–
lapponica FFII		0	100	100	0	0	0	0	100	0	100	0	0	100	0	0	2	0	71	0	53	100	100	0	0	0	0	–	–	–	–	–	0	0	0	100	0	100	0	0
purpurella FFII		0	100	100	0	0	0	85	100	0	14	0	0	24	0	0	99	0	33	0	0	100	86	15	0	0	0													
praetermissa FFII		0	100	90	0	0	–	–	–	–	–	–	0	100	0	0	48	0	58	0	2	100	100	0	0	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–
Summary European FFII		N	x	x	–	–	–	x	x	–	x	–	W	x	–	–	x	–	x	x	x	x	x	x	–	–	–	–	x	x	–	–	–	N	–	x	x	x	–	–
armeniaca EEII		0	100	5	0	0	0	0	0	0	100	0	2	100	0	88	0	0	0	0	0	100	7	88	21	0	0	93	100	0	0	0	0	0	0	100	0	0	0	0
urvilleana EEFF		3	100	90	19	1	0	0	99	0	99	0	7	92	+	+	95	1	11	1	+	1	99	99	2	0	0	1	100	1	5	5	6	83	0	98	1	0	0	0

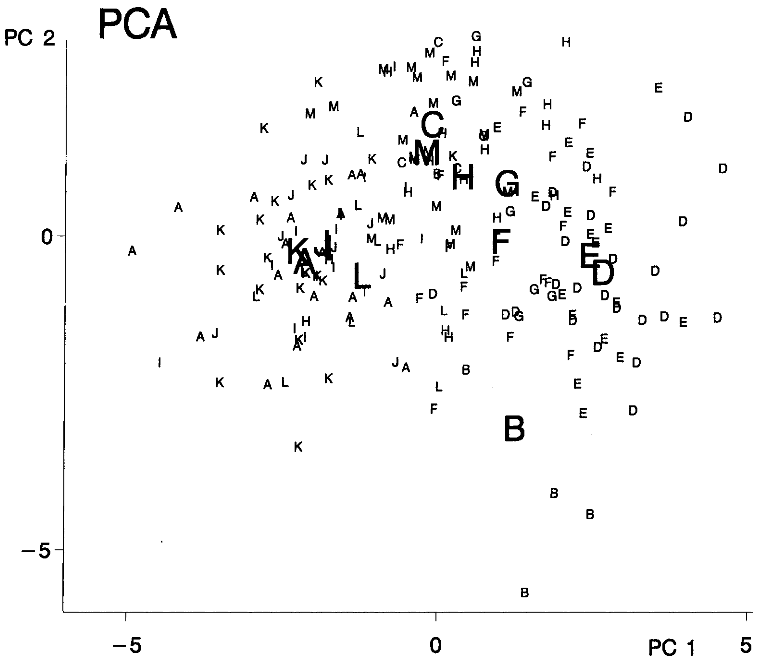

Fig. 3. Principal components analysis of Turkish material of *Dactylorhiza incarnata* s.l. Population centroids are given by large code letters. For population codes, see Table 1. The first and the second principal component accounted for 11.6% and 7.0%, respectively, of the total variance in the data set

spots. The streaks are concentrated along the veins and are distributed on both sides of the leaves as well as on the stem. In some specimens the streaks may be dense and confluent. In non-coloured individuals the leaves are pure green on both sides, as in *D. incarnata.*

Dactylorhiza euxina has often been associated with *D. majalis* (Soó 1960, Renz and Taubenheim 1984), which, however, is an allotetraploid taxon with no affinity to *D. euxina.* The diploid chromosome number 2n = 40 reported by Averyanov (1983a, 1983b) is in agreement with allozyme data.

Dactylorhiza armeniaca is intermediate between *D. euxina* and *D. incarnata* s.l. in morphology (Appendix 1). It has the lip characters of *D. euxina*, but is often much larger with large erect, somewhat acute leaves, a swollen stem, and many-flowered inflorescences. No dark-coloured plants have been seen.

Dactylorhiza armeniaca may have evolved in north-eastern Turkey in the area where it occurs today, and it may be of relatively recent origin. No mixed populations of the putative parental taxa *D. incarnata* s.l. and *D. euxina* were found in the present study. However, they occur in the vicinity of each other in north-easternmost Turkey (cf. Renz and Taubenheim 1984, Buttler 1991, Rückbrodt et al. 1992, Fig. 4), and both may be found at high altitudes. Still, populations of *D. armeniaca* were separated by as much as 90 km, and unless the species has multiple origins it should have taken some time to cover such a distance. No other alleles than those present in the putative parents were found in *D. armeniaca.* A significant feature of the species is high frequencies of the otherwise rare allele *Idh*^{a}. This allele was also found in high frequencies in populations of *D. incarnata* s.l. from the same area, again indicating an origin in situ.

Dactylorhiza saccifera is similar to European populations of *D. fuchsii* in having flat leaves that are bluish green on the lower side and often spotted on the upper side. The flowers are pale with a deeply three-lobed lip, but they differ from those of *D. fuchsii* in being larger and in having a longer and thicker spur. Soó (1960) and Averyanov (1983b) indicated that the species should be a tetraploid, but the examined Turkish populations were both diploid which is in agreement with information given in Buttler (1991) and Delforge (1995).

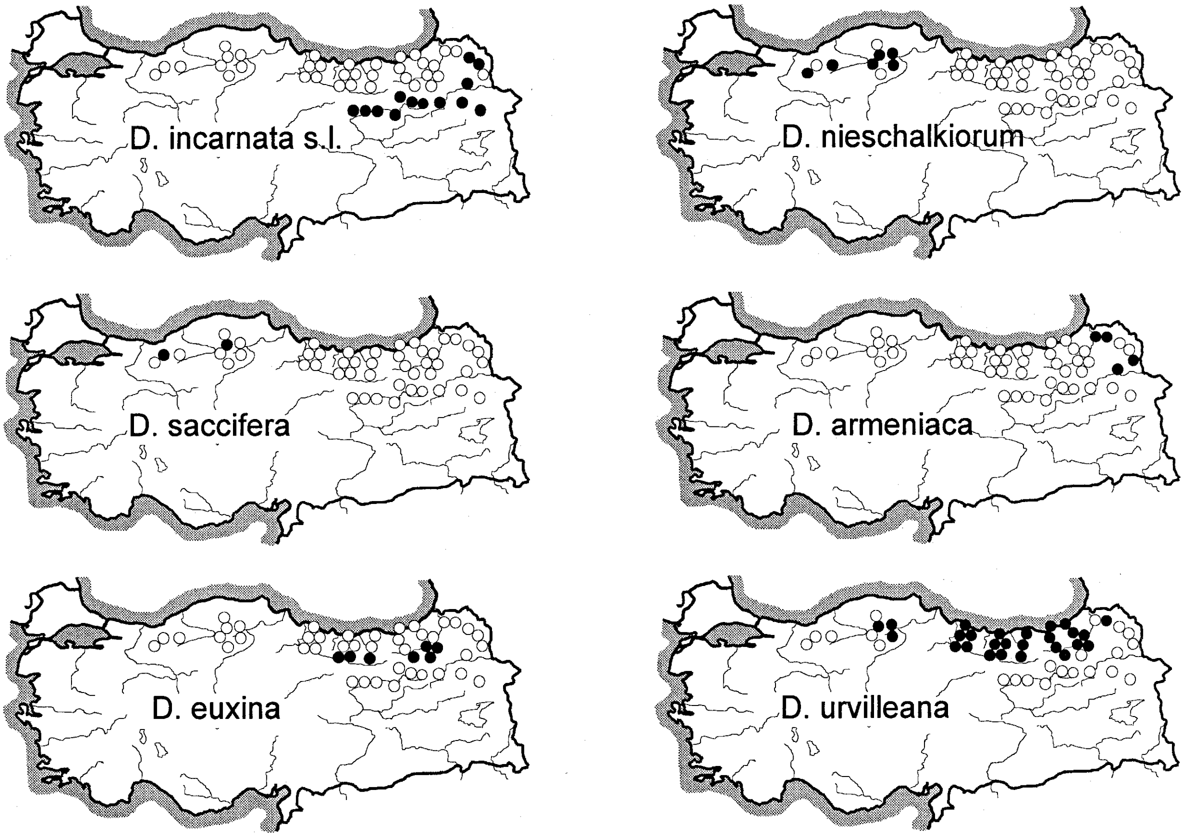

Fig. 4. Maps showing the distribution of Turkish members of the *Dactylorhiza incarnata/maculata* polyploid complex at the examined localities. Some symbols have been slightly displaced

The closest extant representatives of the lineages from which the allotetraploid *D. urvilleana* arose appear to be *D. saccifera* and *D. euxina*. *Dactylorhiza urvilleana* is similar to *D. saccifera* in the flat, obtuse leaves that are spotted above and slightly bluish green beneath, and the flowers that are fairly pale and provided with a long and wide spur. The flower lip is wider than long, and have side-lobes with irregular margins, like in *D. euxina*. Some specimens also approach *D. euxina* in having streaks on the stem. The examined material of *D. saccifera* lacks such streaks.

The distribution area of *D. urvilleana* coincides with that of *D. euxina* in Turkey, but it extends somewhat further to the west (Rückbrodt et al. 1992, Fig. 4). *Dactylorhiza saccifera* has a more scattered distribution. It is a low to medium altitude plant and there are no reliable records that it has been found together with *D. euxina*. Furthermore, *D. saccifera* is often confused with *D. urvilleana*, and it appears that reports of *D. saccifera* from NE Turkey may be erroneous. The wide distribution area of *D. urvilleana*, the occurrence of rare alleles that have not been encountered in any other taxon, and the absence of localities where *D. euxina* has been found in sympatry with *D. saccifera* indicate that *D. urvilleana* may not have a recent origin, and/or that the parental populations were somewhat different from those investigated here.

Allozyme data clearly indicate that *D. nieschalkiorum* has originated from hybridization between the *D. incarnata* s.l. and *D. maculata* s.l. lineages, but it contains high frequencies of alleles that are rare in the *D. incarnata* s.l. samples studied here, e.g. at *Gpi, Pgm*, and *Tpi-1* (Tables 2 and 3). It is still possible that these alleles are more common in *D. incarnata*

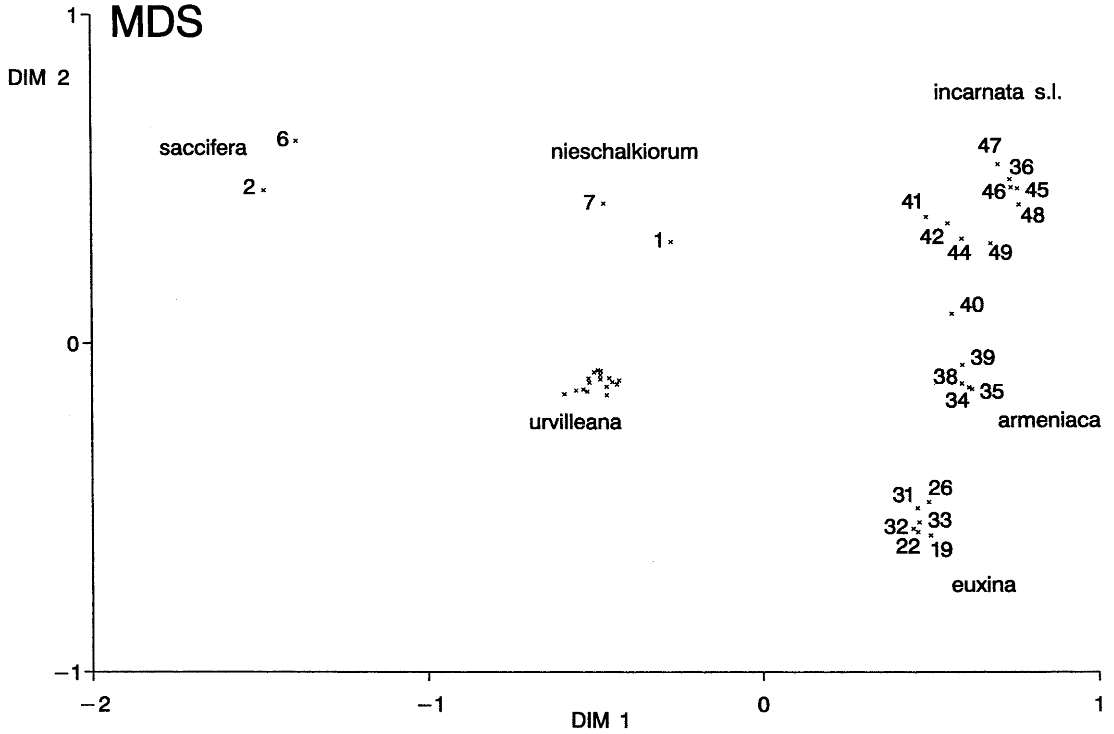

Fig. 5. Non-metric multidimensional scaling analysis on Rogers' genetic distances illustrating the genetic differentiation in Turkish members of the *Dactylorhiza incarnata/maculata* polyploid complex. Stress = 0.10239

from western Turkey and it should be of interest to perform extended sampling from this area. Similarly, the allele *Pgm*^{f} that is present in *D. nieschalkiorum* was not found in *D. saccifera*, although it is quite common in the European representatives of *D. maculata* s.l. However, only two populations of *D. saccifera* were examined.

Dactylorhiza nieschalkiorum is characterized by its large size, the usually unspotted leaves, and the large flowers, but the origin from a hybridization between the *D. incarnata* s.l. and *D. maculata* s.l. lineages is not directly evident from its morphology. However, the large group of European allotetraploids with origin in these lineages apparently contains a large morphological diversity as well, and some of these forms approach *D. nieschalkiorum* in external morphology. Thus, if a wider species concept is applied (Pedersen 1998), *D. nieschalkiorum* may be included in *D. elata* s.l. together with the remaining European allotetraploids.

Dactylorhiza nieschalkiorum extends to the Kastamonu region in the east where it co-occurs with *D. urvilleana*, sometimes in mixed populations (Fig. 4). Allotetraploids from this area have sometimes been treated as *D. ilgazica* C. A. J. Kreutz (Kreutz 1997, 1998).

Hybridization. A low number of hybrids were found in the allozymically defined species. The majority of these were apparently F_1 hybrids. However, a few plants from the Kastamonu region must represent F_n hybrids or back-crosses between *D. nieschalkiorum* and *D. urvilleana*, and it is possible that some gene flow may occur across the species borders. Still, most plants from the area clearly belonged to either of the two species. It is concluded that gene flow between the species recognized in the present study should be negligible, which is contradictory to the assertion given in Renz and Taubenheim (1984) that most species in the genus hybridize freely.

Although few hybrids were confirmed by allozyme data, it should be observed that hybrids involving the allotetraploid taxa, which are of hybrid origin as such, only differ quantitatively from the pure allotetraploids. This would also apply to the external morphology. Furthermore, judging from the situation in northern European populations (Hedrén 1996a) where allotetraploids often grow together with one or both of their diploid progenitors, primary hybrids may look quite different from each other from time to time they arise. It is possible that mixed populations including many hybrid plants may be encountered in Turkey as well, but a variable hybrid population is thus not necessarily indicative of a hybrid swarm.

Concluding remarks. In Europe the *D. incarnata/maculata* polyploid complex could be subdivided into three broadly defined species, which are morphologically variable, but genetically coherent and distinct from each other. *D. incarnata* s.l. is a diploid that could be described by the genome II, whereas *D. maculata* s.l. contains diploid and tetraploid populations that could be described by the genomes FF and FFFF, respectively. *Dactylorhiza elata* s.l. comprises allotetraploid taxa that have arisen from hybridization between these species (or related ancestral taxa) and it could be described by an FFII genome (Hedrén 1996a).

The species relationships of Turkish *Dactylorhiza* are more complex (Fig. 6). In addition to the diploid groups that occur in Europe, there is a third genetically divergent species, *D. euxina*, which could be described by the genome EE. This species has produced allotetraploid derivatives by hybridization with both *D. maculata* s.l. resulting in *D. urvilleana* (genome EEFF) and with *D. incarnata* resulting in *D. armeniaca* (genome EEII). In total, the *Dactylorhiza incarnata/maculata* polyploid complex in Turkey contains three extant genetically distinct diploid lineages, and three allotetraploid derivatives that combine genomes from these lineages. Judging from allozyme data, *D. nieschalkiorum* may be included in *D. elata* s.l. and *D. saccifera* in *D. maculata* s.l.

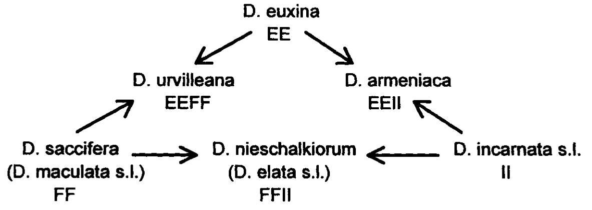

Fig. 6. Suggested species relationships in the *Dactylorhiza incarnata/maculata* polyploid complex in Turkey. Capital letters denote the genome composition of each species

Knowledge on the phylogeny of the allotetraploids may provide a better understanding of the taxonomic problems in Turkish *Dactylorhiza*. The allotetraploids bridge the gaps in morphological discontinuity between the diploids, and they may be difficult to separate from each other as they partially share the same ancestors.

Another contributing factor for species delimitation problems in Turkey could be the recognition of too many taxa at species level within *D. incarnata* s.l. Allozyme data demonstrate that the species is variable, but that there are no discontinuities in the variation pattern, and this situation appears to be reflected also in external morphology.

Despite of the problems described above, the systematic relationships revealed by allozymes generally have support from morphological data. The six groups in the *D. incarnata/maculata* polyploid complex recognised here apparently constitute good biological species, and delimiting the taxonomic species in the same way should result in relatively clear taxonomy with a minimum of poorly defined taxa and problematic samples that need to be treated as hybrids.

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Appendix 1

Dactylorhiza armeniaca Hedrén spec. nov.

Type: Turkey, Ardahan adm. region, ca. 5 km east Savsat. Hedrén & Hansson s.n. 9 June 2000, LD holotypus.

Diagnosis: Species nova inter D. euxinam (Nevski) Czerep. et D. incarnatam (L.) Soó, differt ab prima planta robustiore caule inflato plurifloro foliis acutis, ab secunda labiis rugosis lobis lateralibus labii erosis.

Description: Plant up to ca 70 cm, robust with a hollow stem. Leaves up to 12.5 × 2.5 cm or more, rather soft, lanceolate, widest near the base, green on both sides, unspotted, apex acute. Inflorescences rather dense, many-flowered with up to ca 30 flowers. Bracts lanceolate with prominent veins, lower ones 30–52 × 6–9.5 mm, often somewhat dark green, purplish on the edges and towards the base but with no other markings. Flowers intensely

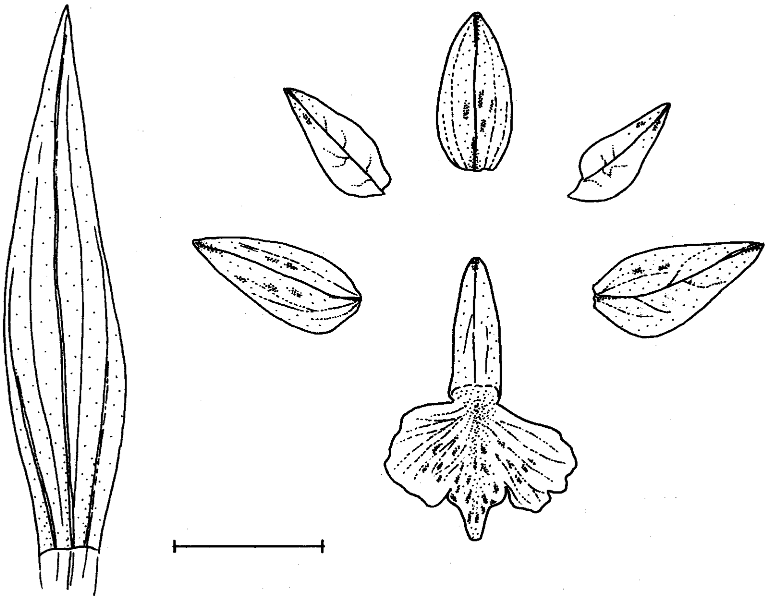

Fig. 7. *Dactylorhiza armeniaca.* Flower with bract from the lower part of the inflorescence. Scale bar = 10 mm. Hedrén and Hansson s.n., LD

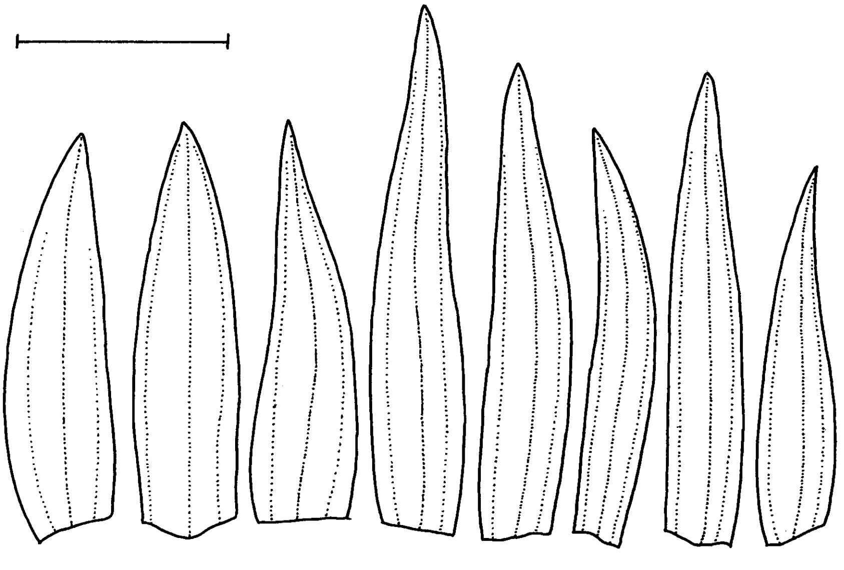

Fig. 8. *Dactylorhiza armeniaca.* Variation in leaf shape at the type locality. Scale bar = 50 mm. Hedrén and Hansson s.n., LD

purple-coloured, whitish at spur entrance; lateral sepals at an angle of ca 45° relative to the median sepal, ovate with a pattern of solid markings and rarely bows or annular markings, ca 12 × 5 mm; median sepal ovate, ca 11 × 5 mm; lateral petal ovate-lanceolate, thin, ca 10 × 3.5 mm; labellum three-lobed, rugose with an irregular margin of sidelobes, length from spur entrance to apex of mid-lobe 8–11.5 mm, to base of sinus separating lobes 6.5–8.5 mm, and to apex of right lateral lobe 6–9 mm, width 8–15 mm, widest at about the middle, lateral lobes ± deflexed, with markings of streaks and bows distributed over the major part of the labellum or concentrated to the mid-part, markings intensely purplish-lilac contrasting against a pale background; spur straight to somewhat decurved, tapering, 8.5–11.5 mm long, 2.5–3.5 mm wide at spur entrance, 2–3.5 mm wide at the middle. Ovary up to ca 16 mm – Figs. 7 & 8.

References

Ashton G. C., Braden A. W. H. (1964) Serum ß-globulin polymorphism in mice. Austral. J. Biol. Sci. 14: 248–254.

Averyanov L. V. (1983a) The genus *Dactylorhiza* (Orchidaceae) in the USSR. I. Bot. Zhurn. 68: 889–895.

Averyanov L. V. (1983b) The genus *Dactylorhiza* (Orchidaceae) in the USSR [cont.]. Bot. Zhurn. 68: 1160–1167.

Averyanov L. V. (1990) A review of the genus *Dactylorhiza*. In: Arditti J. (ed.) Orchid Biology: Reviews and Perspectives, V. Timber Press, Portland, Oregon, pp. 159–206.

Bateman R. M., Denholm I. (1983) A reappraisal of the British and Irish dactylorchids, 1. The tetraploid marsh-orchids. Watsonia 14: 347–376.

Bateman R. M., Denholm I. (1985) A reappraisal of the British and Irish dactylorchids, 2. The diploid marsh-orchids. Watsonia 15: 321–355.

Bateman R. M., Denholm I. (1988) A reappraisal of the British and Irish dactylorchids, 3. The spotted orchids. Watsonia 17: 319–349.

Bateman R. M., Pridgeon A. M., Chase M. W. (1997) Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 2. Infrageneric relationships and reclassification to achieve monophyly of Orchis sensu lato. Lindleyana 12: 113–141.

Baumann H., Künkele S. (1981) Beiträge zur Taxonomie orientalischer *Dactylorhiza*-Arten. Mitt. Bl. Arbeitsk. Heim. Orch. Baden-Württ. 13: 220–266.

Buttler K. P. (1991) Field guide to the orchids of Britain and Europe, revised English edition. Crowood.

Crawford D. J. (1989) Enzyme electrophoresis and plant systematics. In: Soltis D. E., Soltis P. S. (eds.) Isozymes in Plant Biology. Dioscorides Press, Portland, pp. 146–164.

Delforge P. (1995) Orchids of Britain and Europe. HarperCollins, London.

Hagerup O. (1938) Studies on the significance of polyploidy. II. *Orchis*. Hereditas 24: 258–264.

Hedrén M. (1996a) Genetic differentiation, polyploidization and hybridization in northern European *Dactylorhiza* (Orchidaceae): evidence from allozyme markers. Plant Syst. Evol. 201: 31–55.

Hedrén M. (1996b) Electrophoretic evidence for allotetraploid origin of *Dactylorhiza purpurella* (Orchidaceae). Nord. J. Bot. 16: 127–134.

Hedrén M. (1996c) The allotetraploid nature of *Dactylorhiza praetermissa* (Orchidaceae) confirmed. Watsonia 21: 113–118.

Hedrén M. (2001) Conservation priorities in the taxonomically complex genus *Dactylorhiza*. Lindleyana 16: 17–25.

Hedrén M., Fay M. F., Chase M. W. (2001) Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). Amer. J. Bot. (in press).

Heslop-Harrison J. (1953) Microsporogenesis in some triploid dactylorchid hybrids. Ann. Bot. n.s. 17: 539–549.

Heslop-Harrison J. (1954) A synopsis of the dactylorchids of the British Isles. Bericht über das geobotanische Forschungsinstitut Rübel in Zürich 1953: 53–82.

Kajan E., Kreutz K., Jansen H. (1992) Mit dem Reisemobil durch die Türkei. Tagebuchaufzeichnungen einer Orchideen-Exkursion im Mai 1988. Ber. Arbeitskr. Heim. Orchid. 9: 161–176.

King J. N., Dancik B. P. (1983) Inheritance and linkage isozymes in white spruce (*Picea glauca*). Canad. J. Genet. Cytol. 25: 430–436.

Kreutz C. A. J. (1997) Zwei neue Orchideenarten aus der Türkei, *Dactylorhiza ilgazica* und *Epipactis turcica*. Eurorchis 7: 46–66.

Kreutz C. A. J. (1998) Die Orchideen der Türkei. Beschreibung, Ökologie, Verbreitung, Gefährdung, Schutz. Selbstverlag, Landgraaf.

Kruskal J. B. (1964a) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29: 1–27.

Kruskal J. B. (1964b) Nonmetric multidimensional scaling: A numerical method. Psychometrika 29: 28–42.

Lönn M., Prentice H. C. (1990) Mosaic variation in Swedish *Petrorhagia prolifera* (Caryophyllaceae): the partitioning of morphometric and electrophoretic diversity. Biol. J. Linn. Soc. 41: 353–373.

Pedersen H. Æ (1998) Species concept and guidelines for infraspecific taxonomic ranking in Dactylorhiza (Orchidaceae). Nord. J. Bot. 18: 289–310.

Pridgeon A. M., Bateman R. M., Cox A. C., Hapeman J. H., Chase M. W. (1997) Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis* sensu lato. Lindleyana 12: 89–109.

Renz J. (1978) *Dactylorhiza* Necker ex Nevski. In: Rechinger K. H. (ed.) Flora Iranica, vol 126. Akademische Druck- u. Verlagsanstalt Graz, Graz, pp. 118–139.

Renz J., Taubenheim G. (1984) *Dactylorhiza* Necker ex Nevski. In: Davis P. H. (ed.) Flora of Turkey and the East Aegean Islands, vol. 8. Edinburgh University Press, Edinburgh, pp. 535–551.

Roberts R. H. (1966) Studies on Welsh Orchids, III. The coexistence of some of the tetraploid species of Marsh Orchids. Watsonia 6: 260–267.

Rogers J. S. (1972) Measures of genetic similarity and genetic distance. Studies in Genetics 7: 145–153.

Rohlf F. J. (1994) NTSYS-pc, Numerical taxonomy and multivariate analysis system. Exeter software, Setauket.

Roose M. L., Gottlieb L. D. (1976) Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution 30: 818–830.

SAS Inst. (1990) SAS/STAT user's guide. SAS Institute, Cary, NC.

Rückbrodt U., Rückbrodt D., Hansen K., Hansen R.-B. (1992) Bemerkungen zu den in der Türkei vorkommenden Orchideenarten und ihrer Verbreitung. Ber. Arbeitskr. Heim. Orchid. 9: 161–176.

Sneath P. H. A., Sokal R. R. (1973) Numerical Taxonomy. Freeman, San Francisco.

Soltis D. E., Haufler C. H., Darrow D. C., Gastony G. J. (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73: 9–27.

Soó R. de (1960) Synopsis generis *Dactylorhiza* (*Dactylorchis*). Ann. Univ. Sci. Budapestinensis, sect. Biol. 3: 335–357.

Soó R. de (1980) *Dactylorhiza*. In: Tutin T. G. et al. (eds.) Flora Europea 5. Cambridge Univ. Press, Cambridge, pp. 333–337.

Vermeulen P. (1938) Chromosomes in *Orchis*. Chron. Bot. 4: 107–108.

Weeden N. F., Wendel J. F. (1989) Genetics of plant isozymes. In: Soltis D. S., Soltis P. S. (eds.) Isozymes in Plant Biology. Dioscorides Press, Portland, pp. 46–72.

Wendel J. F., Weeden N. F. (1989) Visualization and interpretation of plant isozymes. In: Soltis D. S., Soltis P. S. (eds.) Isozymes in Plant Biology. Dioscorides Press, Portland, pp. 5–45.

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