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KARYOLOGICAL AND BIOMETRICAL STUDIES ON SOME SPECIES OF THE GENUS *DACTYLORHIZA* NECKER EX NEVSKI SECT. *DACTYLORHIZA* (*ORCHIDACEAE*) OF CENTRAL-NORTHERN ITALY

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Karyological and biometric investigations were carried out on some *Dactylorhiza* taxa, found in Central-Northern Italy. Our results confirm that *D. incarnata* is a well circumscribed species: its morphological and karyological ($2n$ = 40) characteristics appear to be constant. *D. fuchsii s. l.* did not appear so clearly distinguishable as a stable species: its introgressive morphological features have been affected by other species that live in the same places and its ploidy was not constant ($2n$ = 40, 80). The karyological characteristics of the *D. traunsteineri* ($2n$ = 80) - *D. lapponica* ($2n$ = 74–88) group varied. The latter always had an aneuploid complement. The plants that have been commonly identified as *D. majalis s. s.* most probably belong to a different taxon: they were shown to have peculiar ploidy ($2n$ = 60) and slight morphological evidences from *D. majalis s.s*. Even those plants have been identified as *D. praetermissa* should perhaps be reinterpreted as a different taxon. They are characterized by $2n$ = 40 instead of $2n$ = 80, the chromosome number found among all the other populations studied.

Bertolini, V., Prete, C. Del & Garbari, F. (2000). Indagini cariologiche e biometriche su alcune entità del genere *Dactylorhiza* Necker ex Nevski, sect. *Dactylorhiza (Orchidaceae)* dell'Italia centro-settentrionale. *Portugaliae Acta Biol.* **19**: 249-265.

Le specie di *Dactylorhiz*a viventi nell'Italia centro-settentrionale sono state studiate dal punto di vista cariologico e biometrico. *D. incarnata* si conferma come una specie

costante nei caratteri e ben individualbile sia dal punto di vista morfologico che cariologico (2*n* = 40). *D. fuchsii s. l.* non risulta così ben differenziabile a causa dell'introgressione con altre specie viventi nelle medesime stazioni e per il fatto di presentarsi con due differenti citotipi correlabili a situazioni ecologiche diverse (2*n* = 40, 80). Il gruppo *D. traunsteineri* - *D. lapponica* risulta variabile dal punto di vista cariologico; infatti *D. traunsteineri* ha 2*n* = 80 e *D. lapponica* mostra sempre fenomeni di aneuploidia con 2*n* = 74 – 88. Le piante comunemente identificate come *D. majalis s. s.* appartengono probabilmente ad un taxon differente identificabile dal peculiare grado di ploidia (2*n* = 60) e da deboli differenze morfologiche. Anche le piante identificate finora come *D. praetermissa* pongono dei problemi, infatti hanno 2*n* = 40, numero cromosomico che le separa da tutte le altre popolazioni studiate finora che presentano invece 2*n* = 80.

Parole chiave: *Dactylorhiza, D. fuchsii, D. incarnata, D. lapponica, D. maculata, D. majalis, D. praetermissa, D. traunsteineri*, Citotassonomia, Biometria, Italia centro-settentrionale

INTRODUCTION

The genus *Dactylorhiza* Necker ex Nevski was created relatively recently. It was separated from the genus *Orchis* to circumscribe the species that show the following characteristics: presence of elongated rhizotubers (not round or ovoid), more or less 2-4-fid or dentate at apex; non-membranous bracts, generally longer than the ovary; inflorescence not enveloped by a bract which functions as a sheath, before flowering; absence of a basal rosette of leaves.

Dactylorhiza is a monophyletic Eurasian genus. It is found essentially in boreal and temperate zones. It is also present in the new world where it is limited to the Kodiak region in Alaska (*D. aristata*). The indigenous nature of *D. maculata* found in the Ontario region is questionable (LUER, 1975; DELFORGE, 1994). Species of *Dactylorhiza* are generally considered entomophilous, even if some cases of autogamy and apomixis have been reported in the literature (DIANA, 1997).

A great deal of systematic controversy exists over the definition of the *Dactylorhiza* taxonomic units. This has been due to the current lack of knowledge regarding a definite and clear picture of their taxonomy in an objective or practical way. The choice of appropriate methodology for the unequivocal identification of the various taxa which belong to the genus, is undoubtedly difficult.

Many species, which belong to this genus, are tetraploid (2*n* = 80), others are polyploid (2*n* $\geq$ 80) (cf. MOORE, 1980; GATHOYE & TYTECA, 1989). Since all of them are capable of adapting to subtle changes in ecological conditions

(HESLOP-HARRISON, 1968; DELFORGE, 1994), a great deal of inter- and intra-population variability is common. Morphological evidence and cytological studies have indicated that several taxa (at specific or sub-specific level) may have recently appeared, and may be of hybrid origin (ROBERTS, 1966). The allotetraploidy of the species evolved from diploid ancestors with one or more ancestral lines. These lines include both still-existing diploid taxa and autotetraploid taxa (unknown or extinct): fertile allotetraploids most probably arose from them. This suggests that allotetraploidy dominates the process of introgressive hybridisation. It may be some form of speciation within the genus. It is also possible that some allotetraploid species make up local populations. These may have arisen independently from any ancestral line. A relevant and essentially phylogenetic interpretation has not been found regarding the genus *Dactylorhiza*; even if there is no lack of studies, these works have been limited to groups of only a few taxa or to local, circumscribed situations (VERMEULEN, 1947, 1949; HESLOP-HARRISON, 1951, 1956; ROBERTS 1961, 1966; GATHOYE & TYTECA, 1987, 1989; HEDRÉN, 1996; REINHARD, 1985)

There is a great deal of difficulty in defining the various taxa on a karyological or «classic» morphological basis. It is sufficient to note that several authors (BUTTLER, 1986, 1991; BAUMANN & KÜNKELE, 1988) have considered the aggregate of *D. maculata s. s.* (usually a tetraploid unit) and *D. fuchsii* (Druce) Soó *s. l.* (usually diploid) as a single species. They based their conclusions on the belief that these are difficult to distinguish on merely morphological bases in some areas where all forms in transition can be found.

According to DUFRÈNE *et al.* (1991), it is possible to clearly identify taxonomic units and to give a clear answer to the aforesaid controversy by taking into account a pool of morphological and quantitative characters,.

Comparative morphology constitutes one of the main tools by which we can arrive at a pertinent definition of the taxa in *Dactylorhiza*. Numerical taxonomy (or mathematical taxonomy) (SNEATH & SOKAL, 1973; DUNN & EVERITT, 1982) offers an aggregate of techniques. It takes into account numerous significant characters regarding numerous samples. In this way, one can try and establish coherent taxonomical units and study the associations that link them.

Numerical methods that use biometrical data have frequently been employed to circumscribe the orchid taxa. In this sense the methodology created by GÖLZ & REINHARD (1973, 1975; etc.) can be considered a precursor. These Authors defined a pool of characters that must be measured for the quantification of the taxonomic distances between pairs of populations in orchids. This pool was elaborated and then refined by other authors (GATHOYE & TYTECA, 1987, 1989; TYTECA & GATHOYE, 1987, 1988). Some other authors have also utilized the biometrical method; they too have always taken a very large number of measurements into consideration (ANDERSSON, 1994; BATEMAN & DENHOLM, 1983; ADCOCK *et al.*, 1983; GÖLZ & REINHARD, 1975; GATHOYE & TYTECA, 1987; REINHARD, 1990).

The techniques in question are collectively known as «cluster analysis». These methods lead to the construction of a phenogram.

The statistical approach to morphological data cannot be the sole means by which the various taxonomic units are interpreted. Any karyological and cytotaxonomic study that could verify genetic compatibility/incompatibility at the ploidy level should not be excluded. Given the almost total lack of techniques for the highlighting and description of the karyotypes in *Dactylorhiza* the point is rather moot.

Even if there are numerous data on the chromosome count in *Dactylorhiza*, they have been insufficient to define the species with any certainty. This has been due to two factors: one is the chromosome count, which is often not totally discriminant (although it is useful for discriminating within populations with extensive morphological variability [JONSELL, 1982; TYTECA & GATHOYE, 1988; DE LARA, 1995]); the other is the subjective interpretation of the different taxonomic hierarchies and their nomenclature, by various authors.

PURPOSE OF THE RESEARCH

Even if the number of the species of *Dactylorhiza* that are present in Italy is much lower than in other areas, such as Central and Northern Europe, both the number of known stations and that of the individual units that make up the population are noteworthy, at least for several of the species under consideration.

By limiting the study to Sect. *Dactylorhiza* (thus excluding Sections *Sambucinae* [Parlat.] Smoljian and *Iberanthus* [Schlechter] Smoljian), we found that the most widespread species - or at least that which is commonly interpreted as such – was *Dactylorhiza fuchsii s. l.* It was distributed in two main, but very different, types of habitats: one was in the Mediterranean hills that are characterized by cool and rather dry soil, at altitudes as high as 800 m a.s.l.; the other was at equal or even higher-altitudes but for the most part in humid or even semi-flooded areas. These plants often grow in very delicate, threatened or transforming environments, such as mountain bogs.

In these swampy habitats, *Dactylorhiza fuchsii* often lives sidebyside with other taxa, such as *D. maculata, D. incarnata, D. praetermissa, D. traunsteineri, D. lapponica* and *D. majalis*. At times it hybridizes with them, giving rise to populations which are often difficult (if not impossible) to identify. It is also difficult to recognise the “pure” single specimens from the F1 hybrids or those which are products of backcrossing or polyploidy.

Various species of *Dactylorhiza* can be found in Central-Northern Italy. This area can be considered a crossroads of the entities that are generically defined as boreo-alpine, and others that have tended to gravitate, mainly, within the Mediterranean area. We considered appropriate to try and define a systematic taxonomical picture of the species present there. An investigation that utilized both cytotaxonomical and biometrical methods for the calculation of Euclidean distances was carried out.

MATERIALS AND METHODS

Area of investigation

The stations with the highest number of samples were identified on the basis of chorological data.

The plants considered were collected mostly from mountain peat-bogs, except for a station (Bosco di Molina di Quosa, in the province of Pisa), which has a Mediterranean environment. About 10 samples (when possible) per station were collected. The stations investigated are listed below

Province of Piacenza: 1 - Passo della Cappelletta 1400 m a.s.l., 2 - Passo Santa Barbara 1400 m a.s.l., 3 - Lamagrande 1200 m a.s.l.
Province of Modena: 4 - Monte Cimone 1850 m a.s.l., 5 - Case Golino 1200 m a.s.l., 6 - San Gimignano 1475 m a.s.l., 7 - Bosco Reale 1300 m a.s.l., 8 - Le Maccherie 1545 m a.s.l.
Province of Lucca: 9 - Prati di San Pellegrino 1650 m a.s.l., 10 - Fociomboli 1100 m a.s.l., 11 - Lamarossa 1430 m a.s.l., 12 - Roggio 800 m a.s.l., 13 - Bardanello 200 m a.s.l., 14 - Pania della Croce 1250 m a.s.l.
Province of Pistoia: 15 - Val di Luce 1450 m a.s.l., 16 - Valle del Sestaione 1350 m a.s.l.
Province of Pisa: 17 - Molina di Quosa 200 m a.s.l.

The species investigated and their relative collection localities were as follows

D. maculata (L.) Soó (3, 5, 10)
D. fuchsii (Druce) Soó (1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17)
D. incarnata (L.) Soó (3, 5, 10)
D. cf. *majalis* (Rchb.) P. F. Hunt & Summerhayes (3)
D. traunsteineri (Sauter ex Rchb.) Soó (3)
D. lapponica (Laestadius ex Rchb. fil.) Soó[1] (2)
D. cf. *praetermissa* (Druce) Soó (3)
D. fuchsii x D. lapponica (2)
D. fuchsii x D. incarnata (5, 8)

Karyological analysis

Immature ovaries were collected in a flowering state, from 1 to 4 blooming flowers. A longitudinal incision was made on each one and the floral bud was removed. Each ovary was placed in 0. 03% colchicine solution for approximately 3 hours at room temperature (18-20°C). This was followed by rapid washing with distilled water. The samples were fixed in Carnoy solution (ethyl alcohol and cold acetic acid, 3:1) for at least one hour and were then rapidly washed with

[1] It is often difficult to make a definite identification within the framework of the *D. traunsteineri* - *D. lapponica* complex. Intermediate characteristics have been noted at many stations (cf. KALTEISEN & REINHARD, 1986; PERAZZA & DECARLI-PERAZZA, 1988 among others). At times, these can also depend on the seasonal climatic trend.

distilled water and preserved in 70% ethyl alcohol, at a temperature of 3-4°C. They were then hydrolyzed in HCl 1N for about 6-7 minutes at 60°C. They were placed in Schiff's solution (leuco-basic fuchsin) for staining for about 2 hours. Once the excess dye had been eliminated the material was washed in distilled water. The material (the megaspores mother cells obtained by scraping the inside tissue of the ovary) was squashed in acetic orceine to reinforce the dye. Permanent slides were prepared.

Biometry

The biometric methods used during the study were those elaborated by GATHOYE & TYTECA (1987), with slight modifications. We measured 47 characters of each of the samples: 25 were direct measurements, called *absolute characters*; 17 were termed *relative characters* (Table 1). Among these latter the "*lip index*" was calculated according to the formula drawn up by TYTECA & GATHOYE (1988). We added other measurements to these that could be represented by two-state characters to further evidence the possible distance. The presence/absence of the fistular stem, the presence/absence of spots on the leaves and the basal/apical position of the same spots on the basal leaf were noted. The result was a matrix made up of 111 individual units and 47 characters. The matrix was elaborated using the «SYNTAX» program.

Table 1 - List of quantitative characters (REINHARD, 1985 and GATHOYE & TYTECA 1987, modified)

a) Absolute characters - general aspects

1. Height of plant
2. Number of cauline leaves
3. Length of lowest leaf (in cm)
4. Width of lowest leaf (in cm)
5. Length of second leaf (in cm)
6. Width of second leaf (in cm)
7. Position, starting from base, of maximum width of second leaf (in cm)
8. Length of upper flower (in cm)
9. Length of upper internode (in cm)
10. Diameter of trunk under inflorescence (in mm)
11. Diameter of trunk above the lowest leaf (in mm)
12. Number of flowers
13. Length of inflorescence (in cm)
14. Length of inflorescence axis between the points of insertion of the first and fifth flowers (in cm)

b) Absolute characters - floral aspects (measurements in mm, made on the 4th flower starting from the base of inflorescence)

15. Length of lateral sepals
16. Width of lateral sepals
17. Length of petals
18. Width of petals
19. Length of the labellum
20. Length of the lateral lobes of the labellum starting from the base
21. Length of median lobe of the labellum
22. Width of the labellum
23. Width of median lobe of the lip at the base
24. Length of the spur
25. Diameter of the spur

c) Relative characters (relations between absolute characters)

26. 1/10	31. 12/13	36. 17/18	41. 20/21
27. 3/4	32. 13/19	37. 19/20	42. 21/23
28. 5/6	33. 15/16	38. 19/21	43. 22/23
29. 5/7	34. 15/17	39. 19/22	44. 24/25
30. 8/9	35. 16/18	40. 19/24	45. Lip index

d) Two-state characters[2]

46. Presence/absence of fistulous trunk
47. Presence/absence of spots on the first two leaves, and an apical/basal position of the same.

[2] Characters not utilized by REINHARD (1985), and evaluated by arbitrarily assigning a value. For character 46, the value «0» was assigned to the «presence» character, the value «1» to the «absence» character. For character 47, the value «00» was assigned to the «presence of spots in apical position» character, «01» to the «presence of spots in the basal position» character, «11» was assigned to the «no spots» character. «*Lip index*» is a character not utilized by REINHARD (1985). For the character, see HESLOP-HARRISON (1951) or ZADOKS (1954).

RESULTS

Karyology

The following chromosome numbers were counted (Table 2):

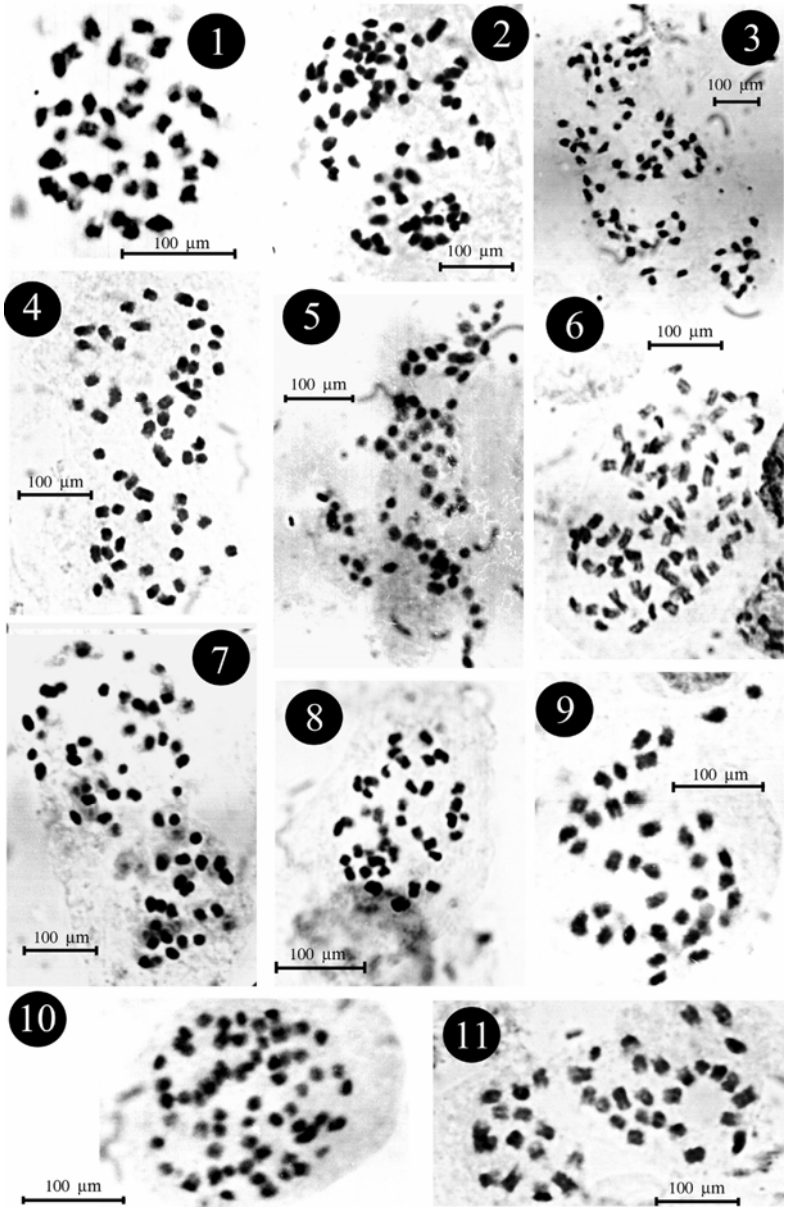
D. cf. *praetermissa*	2*n* = 40 (fig. 1)
D. maculata	2*n* = 80 (fig. 2)
D. fuchsii (mountain biotype)	2*n* = 80 (fig. 3)
D. cf. *majalis*	2*n* = 60 (fig. 4)
D. traunsteineri	2*n* = 80 (fig. 5)
D. lapponica	2*n* = 74-88 (figs. 6, 7)
D. fuchsii (Mediterranean hill biotype)	2*n* = 40 (fig. 8)
D. incarnata	2*n* = 40 (fig. 9)
D. fuchsii x *D. incarnata*	2*n* = 60 (fig. 10)
D. maculata	2*n* = 40 (fig. 11)
D. fuchsii x D. lapponica	2*n* = 80 (no photo available)

Biometric evidence

Biometric analysis produced 2 clusters. Cluster 1 described only the measurable morphological differences and not those that were defined as two-state characters. Cluster 2 included the data of cluster 1 along with the data that could be defined as two-state characters.

In cluster 1 (simplified in Table 3), the taxa with a lower number of chromosomes, *D. fuchsii* (Mediterranean biotype) and *D. incarnata*, were located on the two far sides of the cluster. *D. lapponica*, which was myxoploid (see below), was clearly separated from the others units. The hybrids were scattered within the clusters with the species that were their parents (as, for example, *D. fuchsii* x *incarnata*).

In cluster 2 (simplified in Table 4) the plants referable to *D. fuchsii* (mountain biotype) had a random position within the entire dendrogram. This was due to the insertion of the two-state characters, which made them homogeneous to other species of *Dactylorhiza*. The species that were differentiated from the others because of their chromosome numbers are arranged at the ends of the cluster. *D. incarnata* and *D. lapponica*, which both showed myxoploidy were placed there.



Figs. 1-11 - 1 *D.* cf. *praetermissa* 2*n* = 40; 2 *D. maculata* 2*n* = 80; 3 -*D. fuchsii* (montane biotype) 2*n* = 80; 4 *D.* cf *majalis* 2*n* = 60; 5 *D. traunsteineri* 2*n* = 80; 6 *D. lapponica* 2*n* = 74; 7 *D. lapponica* 2*n* = 84; 8 - *D. fuchsii* (Mediterranean hill biotype) 2*n* 40; 9 *D. incarnata* 2*n* = 40; 10 *D. fuchsii x D. incarnata* 2*n* = 60; 11 *D. maculata* 2*n* = 40.

Table 3 - Simplified cluster (no two-state carachters)

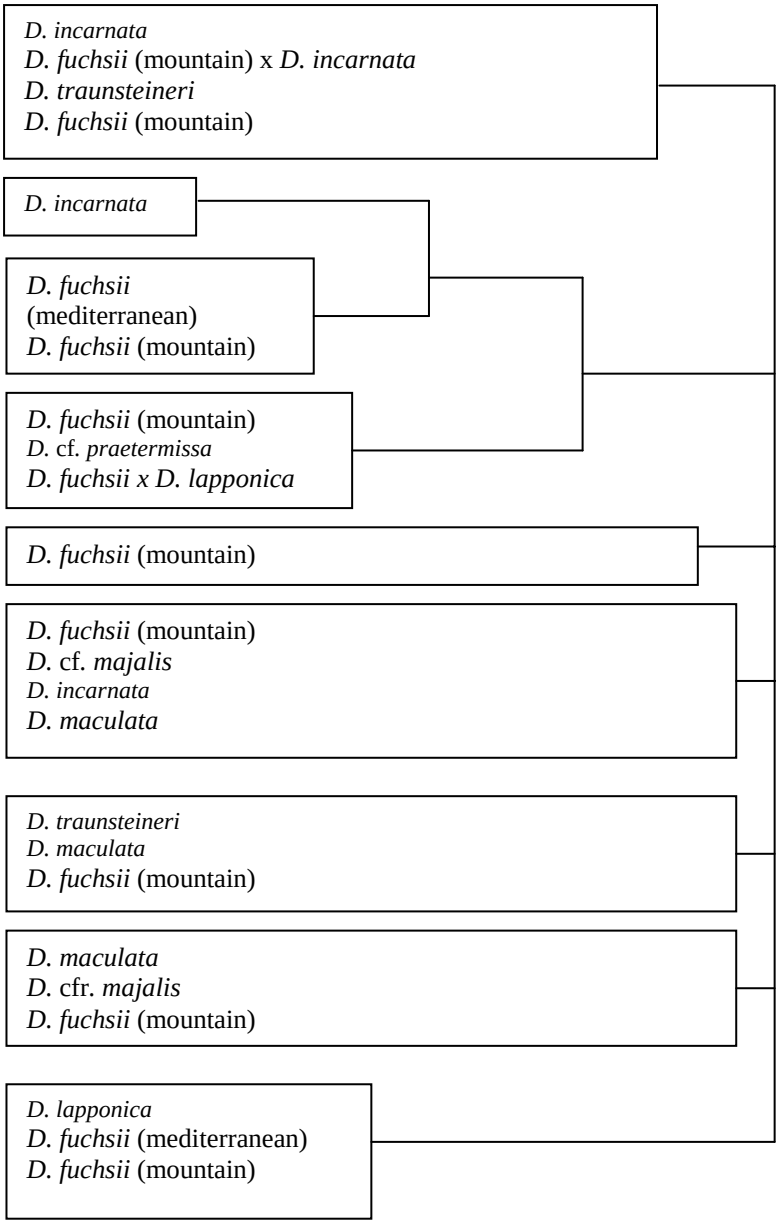
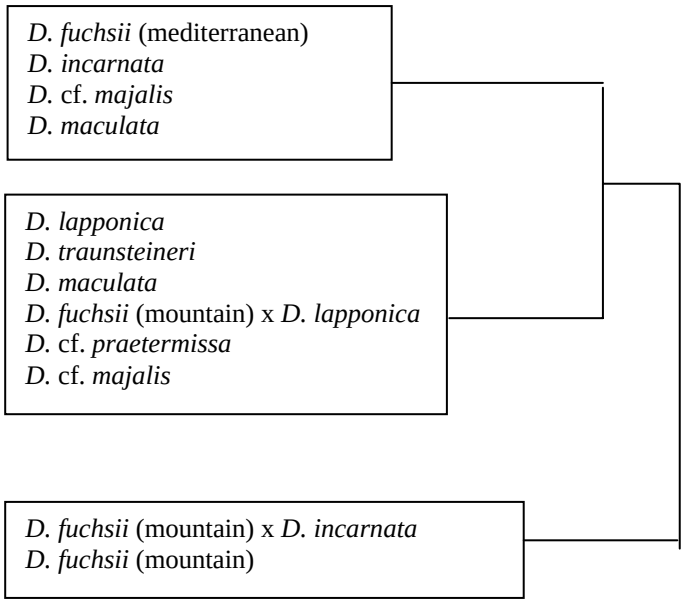


Table 4 - Simplified cluster (two-state carachters considered)



DISCUSSION

Karyology[3]

The results of the karyological investigation on *Dactylorhiza incarnata* indicated that it is diploid (2*n* = 40). This chromosome number is in agreement with that reported in numerous stations all over Europe by other authors.

Dactylorhiza traunsteineri was characterized by tetraploidy (2*n* = 80). This was in accordance with previous data in literature (GÖLZ & REINHARD, 1985, etc.).

Dactylorhiza lapponica was characterized by considerable aneuploid phenomena as evidenced by its chromosome numbers (2*n* = 74-88). These results can be hypothetically attributed to myxoploidy in the nucella: the populations of cells of the nucella differ among themselves as to the chromosome somatic number. According to this hypothesis, *D. lapponica* with myxoploidy of the nucella would reproduce asexually by apomixis, giving rise to

[3] For exhaustive bibliographic reference see: DARLINGTON & WYLIE (1955); FEDOROV (1969); GOLDBLATT (1981, 1984, 1985, 1988); GOLDBLATT & JOHNSON (1990); HEUSSER (1938); KUZMANOV (1993); MOORE (1970, 1972, 1973, 1977); LÖVE & LÖVE (1974); MRKVICKA (1992); ORNDUFF (1968, 1969).

adventitious embryos, as has already been reported in *D. insularis* (DIANA, 1997).

An alternative hypothesis is based on the phenomenon known as agmatoploidy. This determines an increase in the number of chromosomes, due to their fragmentation, as a consequence of chromosomal deletion (GARDÉ & MALHEIROS-GARDÉ 1953).

The chromosome number in *Dactylorhiza fuchsii* was mostly 2*n* = 80; this result was limited to the populations of the higher stations. The chromosome number of the plant populations of the more Mediterranean-like environments, such as Molina di Quosa (Pisa), was 2*n* = 40.

Both results have been documented, although under various nomenclature combinations (LÖVE, 1951; GATHOYE & TYTECA, 1989; VÖTH & GREILHUBER, 1980; SOÓ, 1980). Unfortunately, it is not possible to deduce the ecological location of the various cytotypes from the information available in these studies. This is true even in relation to the splitting of *D. fuchsii* into numerous units of uncertain taxonomical rank and significance (cf. BUTTLER, 1986, 1991; DELFORGE, 1994).

At the station examined, the only one in the Apennine chain, which represents the entire plant population of those mountains, the individuals attributed to *Dactylorhiza praetermissa* had a chromosome number of 2*n* = 40. This result is not in agreement with previous works (GATHOYE & TYTECA, 1989, etc.), 2*n* = 80 has consintently been cited

The populations of *D. maculata* showed 2*n* = 80 except in one sample (2*n* = 40). This is in agreement, with most of the data found in the literature (GATHOYE & TYTECA, 1989, etc.). This last finding (2*n* = 40)is not discordant with some other results, even if they have been sporadically reported (DEL PRETE *et al.*, 1980; VÖTH & GREILHUBER,1980).

The populations referred to as *D. majalis* showed a chromosome number of 2*n* = 60; this is in total disagreement with previously reported data. For example 2*n* = 80 was noted by several authors (GATHOYE & TYTECA, 1989; ROBERTS, 1961, etc.) while 2*n* = 40 was found by others (DE LARA, 1995, etc.).

D. incarnata x *D. fuchsii* (mountain morphotype) showed 2*n* = 60. In the literature, this hybrid has been reported to have 2*n* = 40 (JAGIELLO *et al.*, 1989).

Biometry

The plants examined belong to different species that have very similar morphological characters. These features are sometimes difficult to describe morphologically, given that hybrids occur very frequently, and that these plants have highly compatible gene pools.

Clusters basically agreed with both karyological and morphological evidence. They emphasise the isolation of some populations of *D. fuchsii* (2*n* = 40), *D. incarnata* (2*n* = 40) and *D. lapponica* (2*n* = 74-88), in comparison with all the

other groups. This was expressed in cluster 1 (simplified in Table 3) for all three species. In cluster 2 (Table 4) *D. fuchsii* (Mediterranean hill biotype) showed the same morphological (but not morphometrical) characteristics as the other *D. fuchsii* (mountain biotype). These data suggest that a common origin may exists for all the *incertæ sedis* plants. We can attribute a common origin to *D. traunsteineri, D. lapponica* and *D. praetermissa*, that are included in the above-mentioned interval. The biometric position of the hybrids in the clusters was the same as at least one of their two ancestors (Table 3, 4).

CONCLUSIONS

Our data indicate that the older taxa may be represented by populations with $2n = 40$; this is in accordance with what was proposed by CORRIAS *et al.* (1998) for other species of the genus. *D. fuchsii* (Mediterranean biotype or cytotype) and *D. incarnata* (high-mountain type), most probably came in contact with each other as a result of Quaternary phenomena, giving rise to the allotetraploid species with $2n = 80$. CORRIAS *et al.* (1998) suggested that *D. incarnata* is most probably one of the ancestors of many groups of the *Dactylorhiza* genus. The existence of *D. fuchsii* x *incarnata* hybrids with $2n = 80$ (tetraploids, instead of diploids) would support this hypothesis. GATHOYE & TYTECA (1989) found evidence that supports this hypothesis. They reported that in some stations two parent species were absent. They based their results on the biometrical examination of samples of the hybrid and on numerous similarities with the parental species.

The presence of *D. maculata* in the Apennine mountain chain was definitively confirmed by our survey. This species very likely has a hybridogenous origin due to allopolyploidy. This phenomenon most probably caused the plants to have a tetraploid somatic level, which may represent some adaptation to climatic changes (GATHOYE & TYTECA, 1989). Alternatively, the origin of *D. maculata* may be explained in terms of autotetraploidy which originated from individuals with $2n = 40$, even if this chromosome number is extremely rare. Another possibility is that *D. maculata* arose from *D. fuchsii* as a consequence of autopolyploidy: this condition may have developed during the post-glacial era. The phenomenon seems to be limited to the edges of the areas that were recolonized after the Würm glacial epoch, similarly to that which had been hypothesized for other Arctic-Alpine species (KÜPFER, 1974).

According to CAUWET-MARC & BALAYER (1984), polyploidy could have favoured the evolution of the original chromosome number, $x = 10$, towards those known at present for the genus *Dactylorhiza*. Autopolyploidy and allopolyploidy are the main evolutionary mechanisms for *Dactylorhiza* diversification. The genetic isolation that derives from this condition is unquestionably the basis for the formation of many *taxa*.

Our biometrical and cytotaxonomical analyses reconfirmed the close relationship between *D. traunsteineri* and *D. lapponica*. The latter is in fact

considered a subspecies of the former [*D. traunsteineri* subsp. *lapponica* (Laest. ex Hartmann) Soó] by some authors. The two units can be distinguished from each other by their phenanthesic periods and, above all, by the myxoploidy discovered almost consistently in *D. lapponica*.

The karyological characteristics of the Apennine population, ascribed to *D. praetermissa* as a consequence of its morphological features, pose some taxonomic problems. The $2n = 40$ chromosome number isolates it completely from other populations. It also suggests that these plants belong to a population at the edge of *D. incarnata* variability rather than to *D. praetermissa s. s*. This local form could have differentiated because of geographic isolation. The distribution area of *D. praetermissa s. s.* is located at the northern tip of Europe and does not even reach the Alpine chain. *D. incarnata* is isolated and found in so very few stations in the Apennine chain that it may be considered a relict.

The unique karyological data on *D. majalis* could be the cause of some rethinking on the origin of the Apennine populations that have been attributed to it. They might be hybrids or backcrossing-hybrids, rather than units belonging to the species *D. majalis* "tout court". The biometric data evidenced that this plant is dispersed in various clusters. This finding may bend support to the hypothesis that these populations are the result of cross-breeding, most probably between *D. incarnata* and *D. traunsteineri*, given that they were always found near each other in the stations examined.

The characteristics of the genus *Dactylorhiza* confirm some very close relationships and common origins among numerous different taxa or at least among those that can be differentiated on a morphological basis. A large-scale cytotaxonomic, biometrical and allozymatic investigation is necessary in order to clarify the processes of introgressive hybridization, polyploidization and the stabilisation of hybrids that give rise to the various taxonomic units.

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LITERATURE

ADCOCK E. M., GORTON E. & MORRIES G. P. (1983). A study of some Dactylorhiza populations in Greater Manchester. *Watsonia*, 14: 377-389.

ANDERSSON E. (1994). On the identity of orchid populations: a morphometric study of the Dactylorhiza traunsteineri complex in eastern Sweden. *Nord. J. Bot.*, 14 (3): 269-275.

BATEMAN R. M. & DENHOLM I. (1983). A reappraisal of the British and Irish dactylorchids, 1. The tetraploid marsh-orchids. *Watsonia*, 14: 347-376.

BAUMANN H. & KÜNKELE S. (1988). *Die Orchideen Europas*. Kosmos. Stuttgart.

BUTTLER K. P. (1986). *Orchideen. Die wildwachsenden Arten un Unterarten Europas, Vorderasiens und Nordafrikas*. Steinbachs Naturführer, Mosaik Verlag. München.

BUTTLER K. P. (1991). *Field guide to Orchids of Britain and Europe*. The Crowood Press. Swindon.

CAUWET-MARC A. M. & BALAYER M. (1984). Les genres Orchis L., Dactylorhiza Necker ex Nevski, Neotinea Reichenb.: caryologie et proposition phylogénique et d'évolution. *Bot. Helvetica*, 94 (2) :391-406.

CORRIAS B., ARDUINO P., BULLINI L., CIANCHI M., DE BONIS L., MOSCO M. C. & ROSSI W. (1998). Speciation by hybridization in Mediterranean orchids. *Act. Coll. Optima Paris 11/17 Maggio 1998* (Abstract).

DARLINGTON C. D. & WYLIE A. P. (1955). *Chromosome atlas of Flowering plants*. University Press, Aberdeen.

DE LARA R. (1995). Rapports 555-589 in KAMARI G., FELBERF. & GARBARI F. (Eds.) Mediterranean Chromosome number reports. *Flora Mediterranea*, 5: 363-373.

DEL PRETE C., GARBARI F. & GIORDANI A. (1980). Numeri cromosomici per la flora italiana. *Inform. Bot. Ital*., 12: 117-120.

DELFORGE P. (1994). *Guide des Orchidées d'Europe D'Afrique du Nord et du Proche-Orient*. Delachaux et Niestlé. Paris/Lausanne.

DIANA S. (1997). Sulla presenza di poliembrionia in Dactylorhiza insularis (Sommier) Landw. (Orchidaceae). *Boll. Soc. Sarda Sci. Nat*., 31: 201-205.

DUFRÈNE M., GATHOYE J. L. & TYTECA D. (1991). Biostatistical studies on Western European Dactylorhiza (Orchidaceae) - The D. maculata group. *Pl. Syst. Evol*., 175: 55-72.

DUNN G. & EVERITT B. S. (1982). *An introduction to mathematical taxonomy*. Cambridge University Press, Cambridge.

FEDOROV A. N. (Ed.) (1969). *Chromosome numbers of flowering plants*. Komarov Botanical Institute Acad. Sci., U. S. S. R. Leningrad.

GARDÉ A. & MALHEIROS-GARDÉ N. (1953). Comportamento nucleolar na meiose de Luzula campestris (L.) DC. *Broteria*, 22: 115-130.

GATHOYE J. L. & TYTECA D. (1987). Etude biostatistique des Dactylorhiza (Orchidaceae) de Belgique et des territoires voisin. *Nat. Belg*., 57: 389-424.

GATHOYE J. L. & TYTECA D. (1989). Contribution à l'étude cytotaxonomique des Dactylorhiza d'Europe occidentale. *Mem. Soc. Roy. Bot. Belg*., 11: 30-42.

GÖLZ P. & REINHARD H. R. (1973). Biostatistische Untersuchungen an europäischen Orchideen. *Ber. Schweiz. Bot. Ges*., 83: 93-105.

GÖLZ P. & REINHARD H. R. (1975). Biostatistische Untersuchungen über Ophrys bertoloniiformis O. et E. Danesch. *Ber. Schweiz. Bot. Ges*., 85 (1): 31-56.

GÖLZ P. & REINHARD H. R. (1985). Scandinavische und Alpine Dactylorhiza Arten (Orchidaceae). *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ*., 17 (3): 321-416

GOLDBLATT P. (1981). *Index to plant chromosome numbers 1975-1978*. Monogr. Syst. Missouri Bot. Gard.

GOLDBLATT, P. (1984). *Index to plant chromosome numbers 1979-1981*. Monogr. Syst. Missouri Bot. Gard.

GOLDBLATT, P. (1985). *Index to plant chromosome numbers 1982-1983*. Monogr. Syst. Missouri Bot. Gard.

GOLDBLATT P. (1988). *Index to plant chromosome numbers 1984-1985*. Monogr. Syst. Missouri Bot. Gard.

GOLDBLATT P. & JOHNSON D. E. (1990). *Index to plant chromosome number 1986-1987*. Monogr. Syst. Missouri Bot. Gard.

HEDRÉN M. (1996). Genetic differentiation, polyploidization and hybridization in northern European Dactylorhiza (Orchidaceae): evidence from allozyme markers. *Pl. Syst. Evol*. 201: 31-55.

HESLOP-HARRISON J. (1951). A comparison of some Swedish and British forms of Orchis maculata L. sens. lat. *Svensk Bot. Tidskr*. 45: 608-635.

HESLOP-HARRISON J. (1956). Some observations on Dactylorchis incarnata (L.) Vermln. in the British isles. *Proc. Linn. Soc. Lond*. 1: 50-82.

HESLOP-HARRISON J. (1968). Genetic system and ecological habit as factors in dactylorchid variation. *Jahresber. Naturw. Ver. Wuppertal* 21/22: 20-27

HEUSSER C. (1938). Chromosomenverhaltnisse bei schweizerischen basitonen Orchideen *Ber. Schweiz. Bot. Ges*. 48: 562-605.

JAGIELLO M., KUUSK V. & LANKOSZ-MROZ M. (1989). Cariological investigation on orchids of the Estonian SSR. Part I Genus Dactylorhiza Necker ex Nevski (Orchidaceae). *Fragm. Flor. Geobot*. 34: 315-326.

JONSELL B. (1982). Ängsnycklar och sumpnycklar i nordligaste Uppland. *Svensk Bot. Tiddskr*., 76: 103-111.

KALTEISEN M., REINHARD (1986). Orchideen im zentralen italienischen Südalpenraum. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ*., 18 (1): 1-135.

KÜPFER P. (1974). Recherches sur les liens de parenté entre la flore orophile des Alpes et celles des Pyréneés. *Boissiera*, 23: 1-322.

KUZMANOV, B. (1993). Chromosome numbers of Bulgarian angiosperms: An introduction to a chromosome atlas of the Bulgarian flora. *Flora Mediterranea*, 3: 19-264.

LÖVE A. (1951). Taxonomical evaluation of Polyploids. *Caryologia*, 3: 263-284.

LÖVE, A. & LÖVE, D. (1974). *Cytotaxonomical atlas of the Slovenian Flora*. Cramer.

LUER C. A. (1975). The native Orchids of the United States and Canada excluding Florida. New York Botanical Garden. New York.

MOORE D. M. (1980). *Chromosome numbers references* In Tutin, T. G., *et al*., (Eds.): Flora Europaea 5: 325-350. Cambridge University Press. Cambridge.

MOORE J. (1970). *Index to plant chromosome numbers for 1968*. Regnum Veg. 68.

MOORE J. (1972). *Index to plant chromosome numbers for 1970*. Regnum Veg. 84.

MOORE J. (1973). *Index to plant chromosome numbers for 1967-1971*. Regnum Veg. 90

MOORE J. (1977). *Index to plant chromosome numbers for 1973-74*. Regnum Veg. 96.

MRKVICKA A. C. (1992). Liste der Chromosomenzahlen europaischer Orchideen. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.*, 24 (1): 125-140.

ORNDUFF R. (1968). *Index to plant chromosome numbers for 1966*. Regnum Veg. 55.

ORNDUFF R. (1969). *Index to plant chromosome numbers for 1967*. Regnum Veg. 59.

PERAZZA G. & DECARLI PERAZZA M. (1988). Orchidee rare o interessanti tra cui Epipactis muelleri ed E. leptochila, specie nuove per il Trentino-Alto Adige. *Ann. Mus. Civ. Rovereto*, 4: 241-253.

REINHARD, H. R. (1985). Skandinavische und alpine Dactylorhiza-arten (Orchidaceae*). Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.*, 17: 321-416.

REINHARD H. R. (1990). Ammerkungen zu einigen Dactylorhiza- Arten (Orchidaceae) Europas. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ*., 22 (1): 1-72.

ROBERTS R. H. (1961). Studies on Welsh orchids. II. The occurrence of Dactylorchis majalis (Reichb.) Vermeul. in Wales. *Watsonia*, 5: 37-42.

ROBERTS R. H. (1966). Studies on Welsh orchids. III The coexistence of some of the tetraploid species of marsh Orchids. *Watsonia*, 6: 260-267.

SNEATH P. H. A., SOKAL R. R. (1973). *Numerical taxonomy. The principles and practice of numerical classification*. Freeman W. H. and Company, San Francisco.

SOÓ. R. V. (1980). Dactylorhiza Neker ex Nevski in TUTIN T. G. *et al.* (Eds.). *Flora Europaea* 5: 333-337. Cambridge University Press. Cambridge.

TYTECA D. & GATHOYE J. L., (1987). Apercu biostatistique des Dactylorhiza de Belgique et du nord de la France. *Orchidophile*, 79: 1386-1392.

TYTECA D. & GATHOYE J. L. (1988). Les Dactylorhiza d'Europe occidentale: approche biostatistique. *Nat. Belg.*, 69 (2): 65-97.

VERMEULEN P. (1947). *Studies on Dactylorchids*. Schotanus & Jens, Utrecht.

VERMEULEN P. (1949). Varieties and forms of Dutch orchids. *Nederl. Kruidk. Arch.*, 56: 203-242.

VÖTH W. & GREILHUBER J. (1980). Zur karyosystematik von Dactylorhiza maculata s. l. und ihrer verbreitung, insbesondere in Niederösterreich. *Linzer Biol. Beitr.*, 12 (2): 415-468.

ZADOKS J. C. (1954). Quelques observations sur les dactylorchideès du Grand Duchè de Luxembourg. *Bull. Soc. Nat. Luxemb.*, 59: 101-132.