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Biostatistical studies on western European *Dactylorhiza* (*Orchidaceae*) – the *D. maculata* group

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Key words: Angiosperms, *Orchidaceae, Dactylorhiza, D. maculata, D. fuchsii, D. saccifera, D. caramulensis.* – Biostatistics, multivariate analysis, taxonomy, morphology. – Flora of Western-Europe.

Abstract: Multivariate analysis tools are exploited on a data set composed of quantitative characteristics collected on 35 populations of plants of the *Dactylorhiza maculata* (L.) Soó group from Western-Europe. These samples lead to four well-defined clusters; this, together with qualitative, cytological and ecological arguments, allows for the recognition of four specific entities: *D. maculata* s.str., *D. fuchsii* (DRUCE) SOÓ, *D. saccifera* (BRONGN.) SOÓ and *D. caramulensis* (VERMEULEN) TYTECA. It is concluded that the floral characters play an essential role in the taxonomical distinction. It also appears that the set of characters measured, as well as the methods exploited, are especially well-suited and valuable tools for the morphological study of the genus *Dactylorhiza.*

Among the Monocotyledons it is generally admitted that the *Orchidaceae* family has reached the highest evolution level (see, e.g., DARWIN 1862, CAMUS & CAMUS 1921–1928, NELSON 1976, DRESSLER 1981). Parallel to this state the numberless taxonomic studies undertaken have shown the extreme complexity of the structure of this family whose evolution is obviously still under way. Beside the thousands of tropical species, the three hundred European and Mediterranean species do not make an exception to this rule and still set the systematicians a lot of problems. Thus, since the beginning of this century the genera *Epipactis, Ophrys*, and *Dactylorhiza* have undergone many additions, transformations, and amendments. Every year several new species are still described, a phenomenon lying in the present trend to the specific splitting of these genera.

The discussions raised about the *Dactylorhiza* spp. often reveal the actual lack of knowledge and the difficulty to tackle their taxonomy in an objective and practical way. The choice of a methodology adapted to *Dactylorhiza* is unquestionably a conceptually hard task. Many species in this genus are tetraploid ($2n = 80$) and even in some cases polyploid ($2n > 80$) (see, e.g., MOORE 1980, GATHOYE & TYTECA 1989) and able to adapt very quickly to slight changes in the ecological conditions (see, e.g., HESLOP-HARRISON 1968). For all these reasons, their great variability is not a surprising fact.

It thus appears that in the genus *Dactylorhiza*, relevant phylogenetic interpre-

tations are obviously lacking. In such situations, numerical methods are frequently used in order to identify taxa from biometric data. Examples of such morphological approaches abound in the literature for various families and genera; it is out of the scope of the present paper to review them here. For *Dactylorhiza*, very recent approaches have been performed, among others, by van STRAATEN & al. (1988) and VANHECKE (1989) at the individual level, by VÖTH & GREILHUBER (1980), BATEMAN & DENHOLM (1983, 1985, 1988), JAGIEŁŁO (1988) and ourselves (GATHOYE & TYTECA 1987; TYTECA & GATHOYE 1988, 1989) at the population level, and by REINHARD and coworkers (REINHARD 1985, 1990; KALTEISEN & REINHARD 1986; GÖLZ & REINHARD 1984, 1986) at the taxon level.

In our previous papers (GATHOYE & TYTECA 1987; TYTECA & GATHOYE 1988, 1989), though working at a population level somewhat different from GÖLZ & REINHARD's taxon level, we made use of the methods developed by these authors (GÖLZ & REINHARD 1973, 1975) for defining the set of characters to be measured and for quantifying taxonomic distances between pairs of populations. We have already underlined (TYTECA & GATHOYE 1988) the limits and drawbacks of the mathematical methods used in that study. We also felt the need to confirm some of our previously proposed conclusions and taxonomical standpoints with other more classical and powerful numerical methods using multivariate analysis. In this first contribution, we present the results obtained for a set of populations of the *D. maculata* (L.) Soó group studied in Western-Europe, leaving the study of other groups and of the genus as a whole for subsequent papers.

The *D. maculata* group of species (in the sense of Flora Europaea, MOORE 1980) is widely distributed in Europe. It includes diploid ($2n = 40$) as well as tetraploid ($2n = 80$) species. One can say that there are almost as many taxonomical conceptions on the *D. maculata* group (and more generally on the genus *Dactylorhiza*) as there are authors dealing with it, ranging from the single species concept (e.g., SUNDERMANN 1980) to a set of 18 species (AVERYANOV 1989) for Europe and surrounding areas. A majority of authors accept a splitting of the group in 2 subgroups, on the basis of the chromosome number, which is accompanied by a tendency towards morphological and ecological differentiation, not well marked in all instances. The attitude adopted by some German authors (BAUMANN & KÜNKELE 1988, BUTTLER 1986) is somewhat different in this regard: they consider both widely distributed *D. maculata* s.str. (usually known as tetraploid) and *D. fuchsii* (DRUCE) Soó (usually diploid) as one single species, arguing that they are hardly distinguishable in some areas and that various transitional forms occur, whereas they treat other taxa with much more restricted distribution areas as separate species (e.g., *D. saccifera, D. gervasiana, D. maurusia*).

A first important goal of the present study is therefore to point out that, taking into account the adopted set of morphological, quantitative characteristics, it is possible to identify clear taxonomic units and to bring a clear response in the aforementioned controversy. On the one hand, we intend to confirm that *D. maculata* and *D. fuchsii* belong to distinct morphological entities and should be separated in the same way as both are separated from *D. saccifera* (BRONGN.) Soó; on the other hand, we would like to show that a fourth set of populations, referred to as *D. caramulensis* (VERMEULEN) TYTECA, deserves the same taxonomic status as the other three entities. With additional qualitative, ecological, cytological, and distributional arguments, the identified taxa can then be referred to as species or

subspecies. Other goals of the present research are (1) to look for the most significant characteristics with respect to taxon identification and discrimination, and (2) to evaluate the morphological similarities between the populations and taxa under study.

For the sake of clarity, we hereafter allow ourselves to consider the four taxonomic entities (*D. maculata, D. fuchsii, D. saccifera*, and *D. caramulensis*), leaving the discussion on the adopted taxonomical splitting and species rank for a subsequent section.

Table 1. List and location of the *Dactylorhiza* samples.[1] *B* Belgium, *Cors* Corsica, *F* France, *I* Italy, *P* Portugal. [2] Numbers used in the text and the Figures

Species	Locality and year	Country[1]	No.[2]
D. maculata	Amcomont 86	B	1
D. maculata	Wésomont 86	B	33
D. maculata	Wésomont 88	B	34
D. maculata	Bras 86	B	9
D. maculata	Masbourg 87	B	25
D. maculata	Fourneau-Saint-Michel 88	B	18
D. maculata	Fagne Wallonne 86	B	17
D. maculata	Pisserotte 86	B	27
D. maculata	Bihain 88	B	2
D. maculata	Sévigny-la-Forêt 87	F	31
D. maculata	Kalmthout 88	B	22
D. maculata	Wingene 87	B	35
D. fuchsii	Biron 88	B	3
D. fuchsii	Bomal 86	B	4
D. fuchsii	Bomal 87	B	5
D. fuchsii	Bomal 88	B	6
D. fuchsii	Lanaye 86	B	23
D. fuchsii	Comblain 86	B	13
D. fuchsii	Han-sur-Lesse 87	B	21
D. fuchsii	Branscourt 86	F	8
D. fuchsii	Étang Neuf 87	F	15
D. fuchsii	Causse Noir 87	F	11
D. fuchsii	Boscodon 87	F	7
D. fuchsii	Recco 87	I	28
D. saccifera	Colle di Val d'Elsa 87	I	12
D. saccifera	Gerfalco 87	I	19
D. saccifera	Gusti 87	I	20
D. saccifera	Marzano Appio 87	I	24
D. saccifera	Evisa 88	Cors.	16
D. saccifera	Saint-Georges 88	Cors.	30
D. saccifera	Venaco 88	Cors.	32
D. caramulensis	Parafita 88	P	26
D. caramulensis	Carrazeda de Ansiães 88	P	10
D. caramulensis	São João do Monte 88	P	29
D. caramulensis	Dornes 88	P	14

Material and methods

Collecting data. The biometric data exploited here come from 35 localities visited between 1986 and 1988 (12 for *D. maculata*, 12 for *D. fuchsii*, 4 for *D. caramulensis* and 7 for *D. saccifera*). In each locality, generally 15 to 20 plants randomly sampled were examined (527 plants on the whole); they are supposed to reflect the variability of each of the populations studied. The delimitation of what can be considered as a population with respect to other taxa incidentally present in the same locality will no longer be discussed here; comments on this subject are given in other papers (BATEMAN & DENHOLM 1989, TYTECA & GATHOYE 1989). As a first approximation, we will consider that all plants studied in one location are referable to only one taxon (see subsequent sections for additional comments). The characteristics of the localities were given elsewhere (TYTECA & GATHOYE 1988, 1989) and need not be reproduced here; their geographic situation is given in Table 1 and on the map of Fig. 1. Vouchers are deposited in the Liège Herbarium (LG).

For each individual plant studied, 27 quantitative characters, listed in Table 2 (no. 1 – 8, 10 – 28), are measured in the field. These have been taken from GÖLZ & REINHARD's methodology (1973, REINHARD 1985), initially for the purpose of comparison of our samples with theirs. All parts of the plants are taken into account: leaves, stem, inflorescence, and flowers (bract, ovary, petals, sepals, lip, and spur). One of GÖLZ & REINHARD's characters (length of the uppermost internodium: character 9 in Table 1) could not be taken into account, since it was not measured in the same way on all the samples. The high number of characters (27) can be justified by the hope to describe as completely as possible the plant morphology and, as a consequence, the expected differences between individuals and populations. Room is lacking here to give even a summary of the collected measures; the interested reader is referred to TYTECA & GATHOYE (1988, 1989) for a detailed information on that matter.

Analyzing data. When effective, allometry may yield significant distorsion of statistical

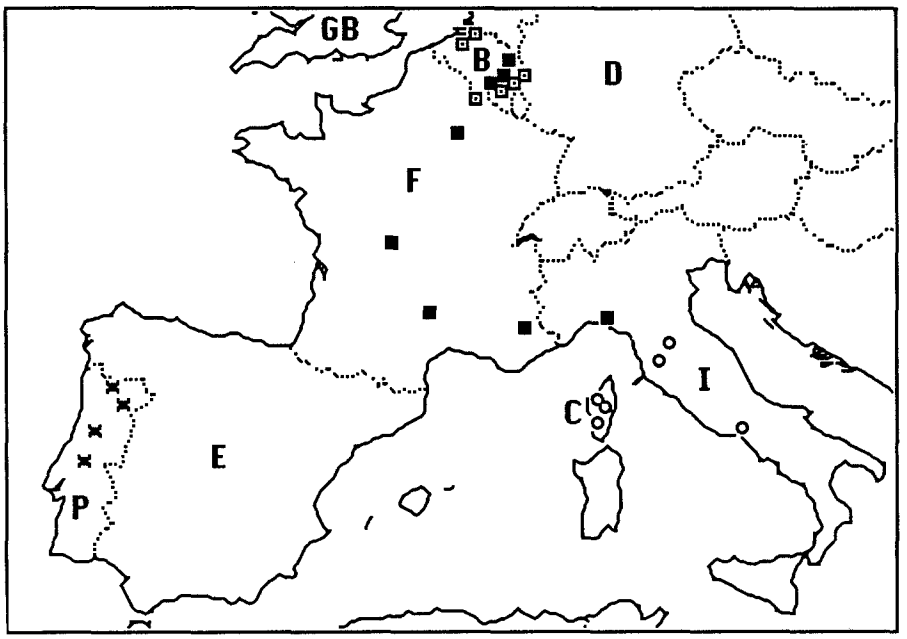

Fig. 1. Origin of *Dactylorhiza* samples (*B* Belgium, *GB* Great Britain, *D* Germany, *F* France, *C* Corsica, *I* Italy, *E* Spain, *P* Portugal). ⊡ *D. maculata*, ■ *D. fuchsii*, ○ *D. saccifera*, × *D. caramulensis*

Table 2. List of quantitative characters used in the biostatistical study of *Dactylorhiza* (after REINHARD 1985)

a) Vegetative and general aspects

1. Plant height (cm)
2. Number of cauline leaves
3. Lowermost leaf length (cm)
4. Lowermost leaf width (cm)
5. Length of second leaf, from the base (cm)
6. Width of second leaf (cm)
7. Position, from the base, of the second leaf greatest width (cm)
8. Uppermost leaf length (cm)

[9. Uppermost internodium length (cm): not used in this study]

10. Stem diameter under inflorescence (mm)
11. Stem diameter above lowermost leave (mm)
12. Number of flowers
13. Inflorescence length (cm)
14. Length of inflorescence axis between the insertion points of first and fifth flowers (cm)

b) Floral aspects (measures in mm taken on fourth flower from inflorescence base)

15. Bract length
16. Bract width
17. Ovary length
18. Lateral sepals length
19. Lateral sepals width
20. Petals length
21. Petals width
22. Labellum length
23. Labellum lateral lobes length, from base
24. Labellum median lobe length
25. Labellum width
26. Labellum median lobe width, at base
27. Spur length
28. Spur diameter, at base

results (see, e.g., SOMERS 1986, 1989). Therefore a log transformation was carried out on all characters in order to limit the influence of allometry on the results. However, comparison with the results obtained from the original characters indicated only tiny differences with logarithmically transformed variables. It was therefore concluded that allometry had a negligible influence on the treatment of our data. Therefore, we decided to present hereafter the results obtained on original variables.

Firstly, a principal component analysis was used on all measured specimens of the *Dactylorhiza maculata* group (527 specimens) in order to reveal the main relationships between characters in that group and to represent the location of the four species in regard to the main sources of variation between individuals.

Next, clustering methods were carried on data to check whether the obtained groups correspond to the supposed taxonomic entities. Clustering the 527 specimens would be technically difficult to realize and to interpret. We therefore turned to the 35 localities with the assumption that all individuals in each of the local populations always belong to the same single species (this is further discussed in the section on discriminant characters – see Table 4). The Mahalanobis distance was calculated for all pairs of populations. This distance measures the difference between locality centroids and thus, better preserves the heterogeneity of each population than the Euclidian distance which should be calculated between the means. The distance matrix was submitted to five agglomerative clustering methdos (proportional-link linkage with 75% connexity, complete linkage, UPGMA, WPGMA, and WARD's) in order to point out a stable structure which is not dependent of the method choice. A clustering method with reallocation (k-means method: MCQUEEN 1967, SPÄTH 1980) was also used to stabilize the classification. One hundred random initial configurations were given for each step (2, 3, 4, 5, 6 groups). A principal coordinate analysis was also applied on the distance matrix to represent the distances between populations on a graph.

While PCA maximizes the distances between individuals, canonical discriminant analysis looks after principal axes that maximize distances between centroids of species. The canonical axes can be completely different from principal component axes because CDA searches only to point out variation sources which oppose species instead of individuals. The correlations of characters with canonical axes give us their relative significance towards each axis.

The characters revealed by the CDA are correlated with one another (Fig. 8). To search for characters associated with independent information, it is necessary to use discriminant analysis. This method, which proceeds like a multivariate regression but aims at opposing groups of individuals, can be performed by forward selection (one by one character entrance in the model), backward selection (one by one character elimination) and stepwise selection (combination of forward and backward selections).

All these analyses are rather classical (see, e.g., SNEATH & SOKAL 1973). They were performed with the statistical analysis system (SAS 1982), except the clustering methods for which the R package was exploited ("The R package for multivariate data analysis" of LEGENDRE and VAUDOR, referred to in LEGENDRE & FORTIN 1989).

Results

Relationships among variables. As mentioned above, the results reported hereafter were obtained through a principal component analysis (PCA) on the whole set of specimens of the *D. maculata* group. The first three axes account for 39.0, 14.4, and 9.6% of the total variance, respectively. As the variance part explained by other axes decreases rapidly, only the first three will be discussed here. All characters are positively correlated with the first axis. Hence, the latter can be considered as an isometric and allometric size axis (see SOMERS 1989) which represents a morphological continuum (dimensions of the leaves and the flowers) from small and slender individuals to robust and great plants. The 95% confidence ellipses of the four *Dactylorhiza* taxa considered (Fig. 2) show that these are not well separated by Axis 1 and that the great axes of all ellipses are nearly parallel with it: all that can be detected here is that *D. caramulensis* and *D. saccifera* are generally greater plants than *D. maculata* and *D. fuchsii*. Each taxon shows similar patterns of size

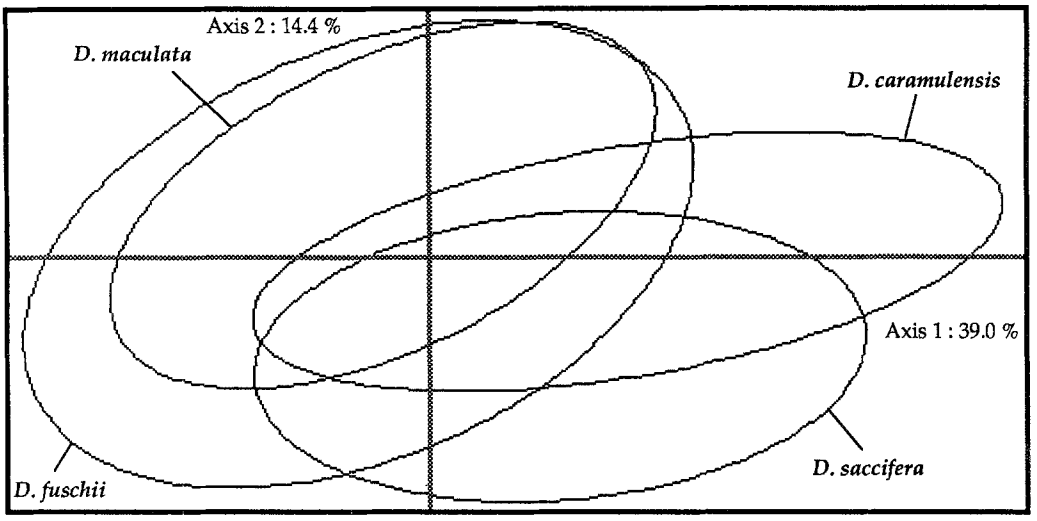

Fig. 2. 95% confidence ellipses for the 4 taxa of the *Dactylorhiza maculata* group in the plane of the first two axes of the PCA

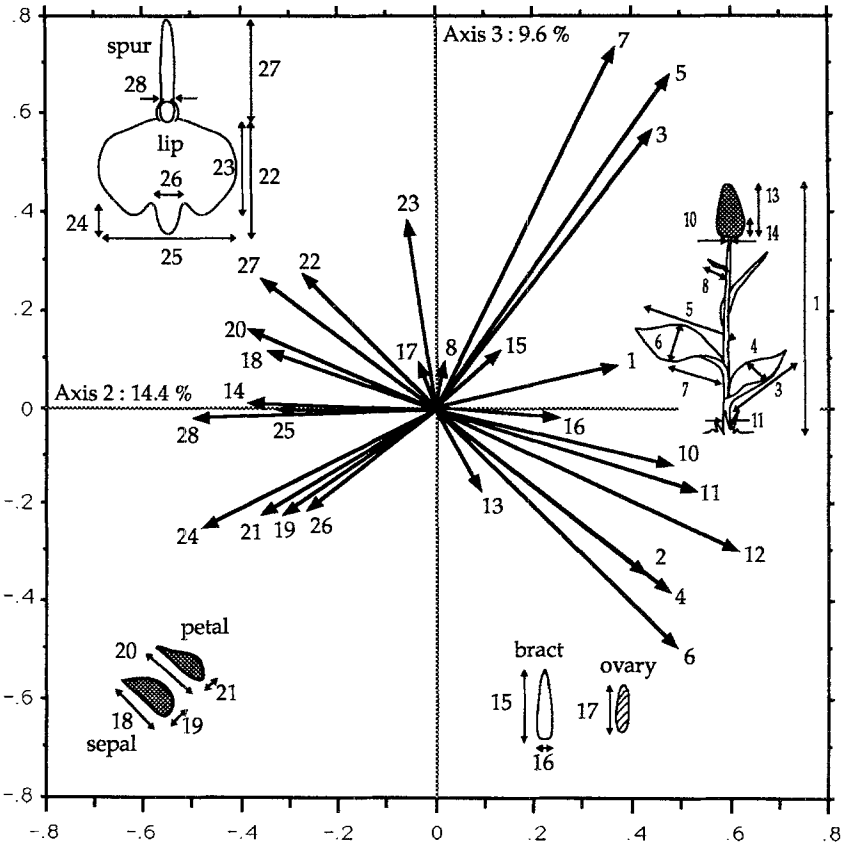

Fig. 3. Correlation circle of Axes 2 and 3 of the PCA of the *Dactylorhiza maculata* group

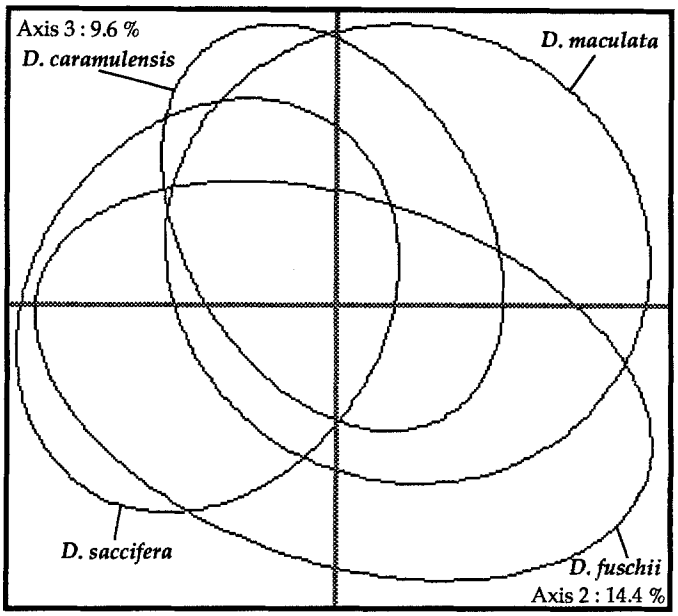

Fig. 4. 95% confidence ellipses for the 4 taxa of the *Dactylorhiza maculata* group in the plane of Axes 2 and 3 of the PCA

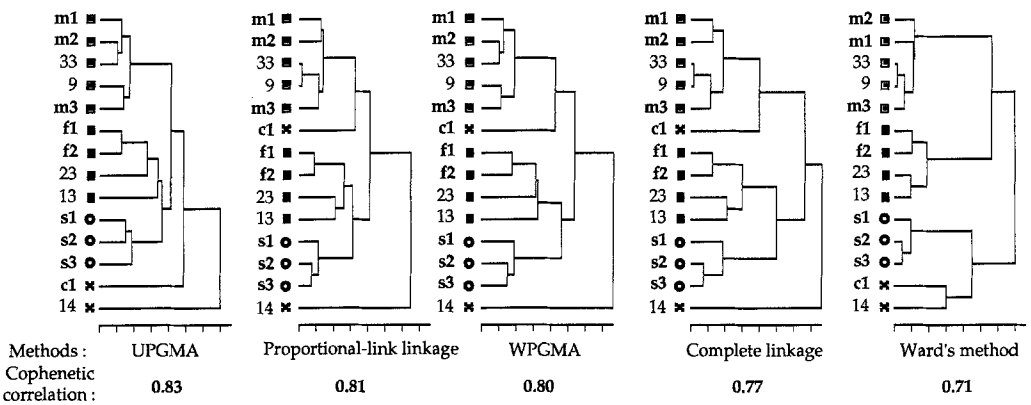

Fig. 5. Dendrograms obtained with 5 clustering methods for the 35 populations of the *Dactylorhiza maculata* group. The populations of the same taxon which are clustered together in all instances are referred to as a groups of populations, labelled m 1, m 2, etc. (see Fig. 6 for the composition of these groups)

variation. As Axis 1 is a size axis, the other ones will be shape axes because they are linearly independent of the first one.

The correlation circle for Axes 2 and 3 (Fig. 3) shows an opposition on Axis 2 between vegetative (1 – 10) and floral (18 – 28) characters. On Axis 3, the characters are clustered by types of measurements (and not by parts of the plant). Among the 14 characters measuring lengths, only two are below Axis 2 with all width characters. Axis 3 suggests an opposition, more pronounced for leaves, between the shapes of the plant parts. Robust plants (with low length/width ratios) lie in the lower part of the graph while slender plants (with high length/width ratios) are

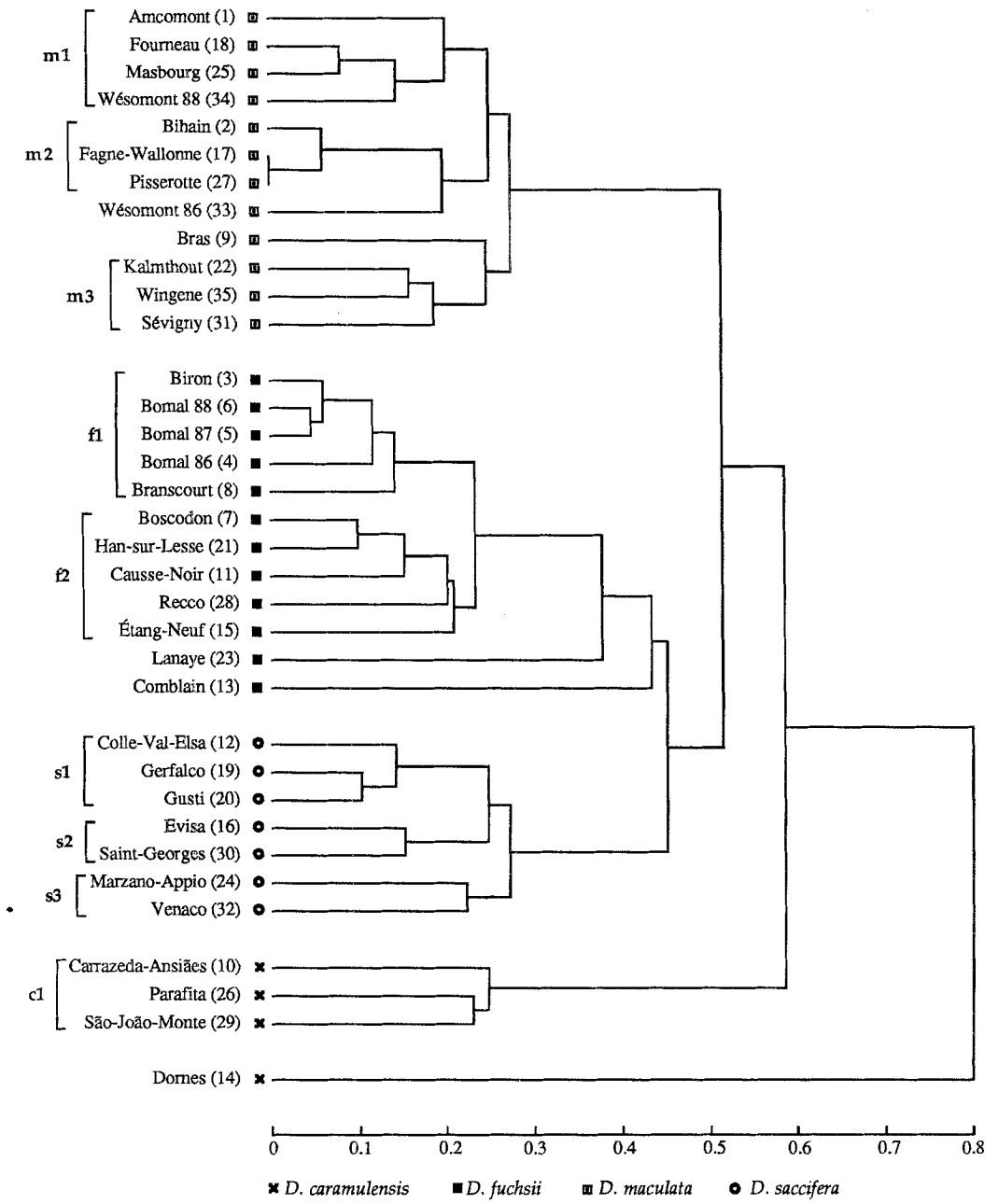

Fig. 6. Detailed dendrogram obtained with the UPGMA clustering method of the *Dactylorhiza maculata* group

located above Axis 2. The overlapping of 95% confidence ellipses (Fig. 4) is relatively important. However, Axis 2 shows a slight opposition between *D. maculata* and *D. saccifera*. These species can be separated on the basis of distinct ratios between vegetative and floral parts. On Axis 3, *D. fuchsii* plants show a trend to possess proportionally wider parts than *D. caramulensis* and *D. maculata*. This graph suggests that the main variation sources in the *D. maculata* group show only a small trend towards connection with taxonomical classification. More elaborated

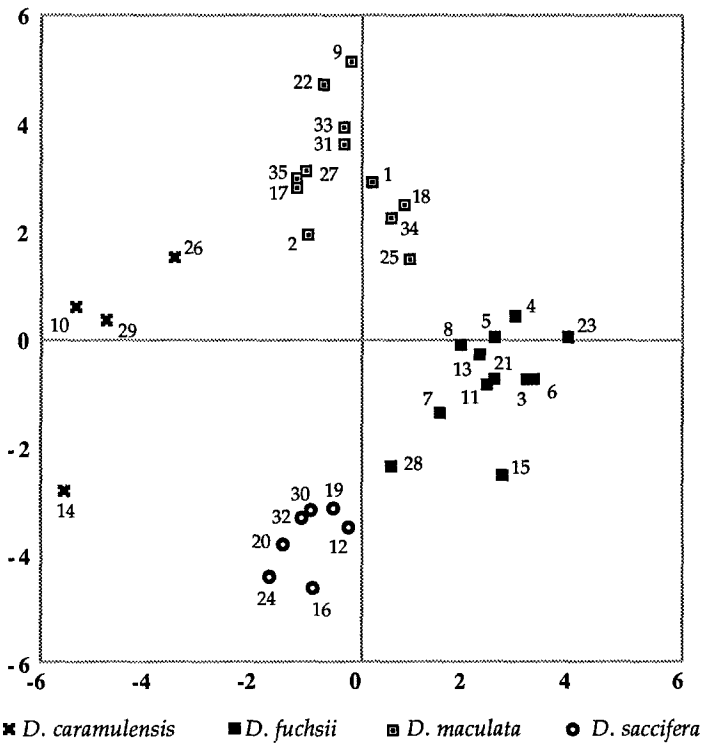

Fig. 7. Principal coordinates diagram for Axes 1 and 2, computed from the Mahalanobis distances between the 35 *Dactylorhiza maculata* populations. The numbers refer to Table 1

analyses are therefore needed to search discriminant characters between the species of the *D. maculata* group.

Similarities among populations. As shown in Figs. 5 – 7, almost all localities are clustered according to their taxonomical classification. In all agglomerative methods (Figs. 5 and 6) except WARD's, the *D. caramulensis* population of Dornes (no. 14) is outside the cluster formed by other *D. caramulensis* populations. In Fig. 7, the singularities of this population appear clearly. The dendrograms of Fig. 5 show differences only in the way that the different species are clustered. Except with Ward's method, populations of *D. fuchsii* and *D. saccifera* are always associated. *D. caramulensis* populations show some affinities with *D. maculata,* although the method with the highest cophenetic correlation (UPGMA: see Fig. 6) does not cluster it with anyone of the other species. The K-means method gives similar results. For the different trials, this method produced several local minima except for four groups. At this step, the clusters also correspond perfectly to the four assumed taxonomical entities (e.g., the *D. caramulensis* population of Dornes is associated with the other *D. caramulensis* populations), with only one exception: the *D. fuchsii* population of Recco (no. 28) is associated to *D. saccifera* samples.

A few more detailed comments about the composition of clusters (see Fig. 6) can be made at this stage. Some clusters are stable regardless of the clustering method used. In the *D. maculata* group, Cluster m 1 includes populations from semi-natural hay meadows, while Cluster m 2 is composed of populations from acid peat moors, which tend to yield more slender plants. The composition of

Cluster m 3 is rather mixed in this regard, while the isolated samples no. 9 and 33 come from habitats similar to those of Cluster m 1. A similar kind of ecological distinction appears for *D. fuchsii:* Cluster f 1 mainly includes populations from open, dry chalk grasslands, while Cluster f 2 gathers populations from more shaded and/or fresh habitats (meadows, scarce woods); the former have been shown to possess significantly smaller flower parts than the latter (TYTECA & GATHOYE 1989). The isolated populations no. 13 and 23 are characteristic of more robust and flower-bearing plants growing in relatively humid and eutrophicated habitats (TYTECA & GATHOYE 1988). For *D. saccifera*, two groupings are parallel with the geographical position: Cluster s 1 is only composed of populations from continental Italy, while Cluster s 2 includes plants from Corsica. Cluster s 3 brings together Italian and Corsican populations, without any clear ecological explanation. The populations of Cluster c 1 all come from open meadows of northern Portugal, while the isolated sample no. 14 originates from C. Portugal and includes only 5 plants living in a somewhat different habitat (clear wood). Finally, let us briefly comment the shift of the Recco sample (no. 28) from the *D. fuchsii* cluster into the *D. saccifera* cluster, observed when using the k-means method (see above). The intermediate position of this sample is well illustrated on the graph of Fig. 7 and can be put in parallel with the intermediate geographical position of this sample, between the *D. fuchsii*

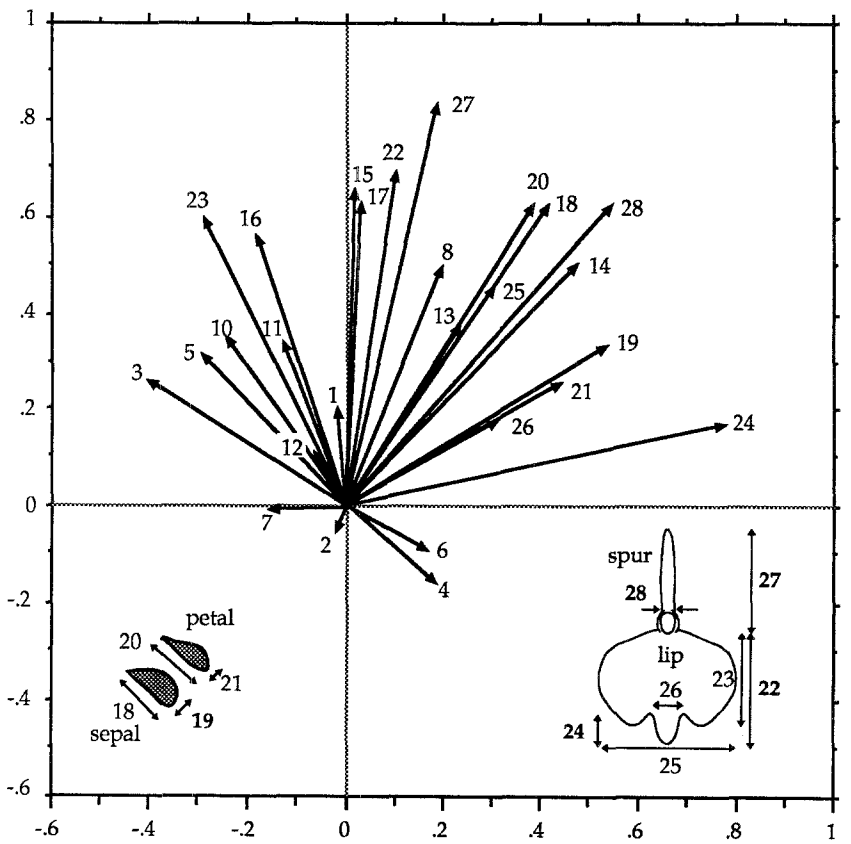

Fig. 8. Correlations of the characters with the first two axes of the canonical discriminant analysis of the *Dactylorhiza maculata* group

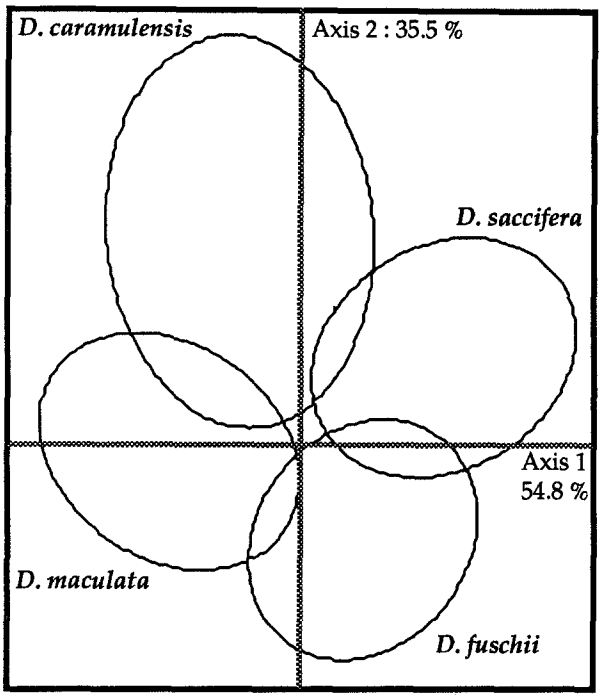

Fig. 9. 95% confidence ellipses for the 4 taxa of the *Dactylorhiza maculata* group in the plane of the first two axes of the DCA

populations of central Europe and the *D. saccifera* populations of the Mediterranean area (Italy, Corsica).

Discriminant characters. For discriminant analyses, the variables should have an approximate multivariate normal distribution within each class with a common covariance matrix in order for the probability levels to be valid. As in many similar studies, the covariance matrices are heterogeneous. However, demonstrative results are already obtained with these heterogeneous matrices. Therefore, one can expect that results would only be better if these matrices were homogeneous.

The first two axes produced by the CDA explain more than 90% of the variance on differences between species. The correlations of characters with these axes (Fig. 8) show that there are several discriminant characters. The 95% confidence ellipses (Fig. 9) show that the overlapping between species is similar for each of them. At 80%, the confidence ellipses are completely disjoined. Axis 1 opposes *D. maculata* and *D. saccifera* while Axis 2 separates *D. caramulensis* and *D. fuchsii.* These oppositions are similar to those obtained with PCA axes. Characters which contribute to Axis 1 are in a decreasing order: 24, 28, and 19. For Axis 2, characters 27 and 22 are the main ones, followed by several others (15, 16, 17, 18, 20 23, 28, ...).

The results of discriminant analysis reveal that characters 27 and 24 are the best to explain the differences between the four species (Table 3), as was expected from the CDA. As discriminant analysis also produces discriminant functions, we use them to evaluate the performances of our characters (Table 4). When a discriminant analysis is performed with only character 27, about 60% of the individuals

Table 3. Discriminant order of the characters produced by three different models of discriminant analysis. *A.S.C.C.* Average squared canonical correlation

Step	Characters	A.S.C.C.	Proportion (%)
1	27	0.186	27.6
2	+24	0.347	51.9
3	+28	0.382	57.5
4	+22	0.422	62.7
5	+19	0.475	70.6
6	+16	0.508	75.5
7	+6	0.558	82.9
8	+14	0.576	85.6
27	all	0.673	100.0

Table 4. Specimens proportion belonging to each *Dactylorhiza* species which are well classified by the discriminant functions calculated using different combinations of discriminant characters (in %)

Species	27	+24	+28	+22	All
D. caramulensis	51.3	71.8	84.6	82.0	100.0
D. fuchsii	74.7	78.2	78.0	83.0	97.8
D. maculata	41.8	79.9	85.6	87.6	99.5
D. saccifera	68.8	77.7	75.8	85.7	97.3
Sum	59.6	78.2	80.8	85.2	98.5

are already well classified. With characters 27 and 24, the proportion reaches more than 75%. Other characters bring less independent discriminant power. With all measured characters, only 8 specimens (out of 527) are still misclassified. These are always isolated individuals in samples composed of about 15 plants: 2 plants identified as *D. fuchsii* (among which 1 plant in the Recco sample) show more similarities with *D. saccifera*; 2 other *D. fuchsii* plants rather point to *D. maculata*; 1 plant from a *D. maculata* sample would be classified as *D. fuchsii*; finally, 3 plants identified as *D. saccifera* show greater similarities with *D. fuchsii*. Thus, no *D. caramulensis* plant appears to be misclassified (as indicated in Table 4).

Discussion

Correlations among characters. We only seek here to point out morphological differences that can be put in relation with taxonomic classification. Measures of morphological characters are often correlated. These correlations can be induced by size effects (isometry) but also by shape variations which are correlated with size (allometry). For VANHECKE (1989), the correlations between morphological

characters in the genus *Dactylorhiza* are so high that they have only a poor discriminant power at infraspecific and interspecific levels. However, his sample only included 32 individuals belonging to six clones of *D. praetermissa* on which 43 morphological characters (including 11 ratios) were measured. This species generally shows also great variations in size and aspect (NELSON 1976, TYTECA & GATHOYE 1989). In those conditions, it would be astonishing to observe differences between clones. VANHECKE (1989) explains the overlapping between clones by a high level – 45% – of significant correlations ($P \leqslant 0.01$).

In our study, although this proportion is larger (83.8%), the information or variance shared by all characters only amounts to 39.0%, i.e., the variance explained by the first axis of the PCA on the *D. maculata* group. Size and shape correlated with size can explain this shared information, which is not without interest, because confidence ellipses show that *D. caramulensis* and *D. saccifera* are generally greater than *D. maculata* and *D. fuchsii*. This is already one morphological feature that is related with the taxonomical criterion. But PCA deals only with variation between individuals and this variation can mask differences between species.

Our results show that vegetative characters are opposed to floral characters. This is one of the correlation parts that is not related to the first axis. This opposition is apparently independent of the taxonomic level because it is observed at the genus level (to be shown in subsequent papers), at the group level (as shown herein), at the species level and even at the population level (not shown here). These correlations show also that a high number of correlated characters does not induce that they are correlated together. Differences or affinities between several characters may exist.

Discriminant characters. The discriminant analysis indicates which characters have the best discriminant value: the length of the spur (27) for the *D. caramulensis-fuchsii* couple and the length of the central lobe (24) for the *D. saccifera-maculata* couple. However, the correlation circle of the canonical discriminant analysis (Fig. 8) shows that other characters are correlated with the second axis. The same discriminant characters, though sometimes in a different order, are produced by discriminant analyses performed without the Recco and/or Dornes populations, which are sometimes excentric in the clustering procedures (see above). Hence, it will be safer to consider several more or less correlated characters (e.g., 22, 28, ...), in addition to the previous ones, to recognize the affinities of an unknown population. In some cases, one of them will be more efficient than another one according to environmental conditions.

In the *D. maculata* group, discriminant characters are chiefly floral. Indeed, these characters are surely important for an entomogamous species. Differences in flower sizes and shapes between species may suggest that insects are responsible for isolation between species. Nowadays, too little information exists on specificity of pollination by insects on *Dactylorhiza* to confirm their significance in the taxonomic isolation.

Taxonomic units. Our results confirm the well-known fact that within any Dactylorchid population or species, the observed variability is considerable. As VAN STRAATEN & al. (1988) conclude, the "genetic diversity (between isolated populations) as well as the phenotypic plasticity of the individuals are the main causes" of variability. The ecoclimatic conditions are evident factors influencing the morphology of *Dactylorhiza* species (see, e.g., HESLOP-HARRISON 1968). Without ques-

tioning this variability and the adaptative capacity of these orchids we intended to show that it is possible, using biometrical data, to obtain an objective view of the morphological groups in the genus.

Clustering on population centroids, canonical discriminant analysis and discriminant analyses show that the four studied taxonomical units are morphologically different. The PCA shows that *D. saccifera* and *D. caramulensis* are larger plants than *D. maculata* and *D. fuchsii*. The labellum of *D. fuchsii* and *D. saccifera* is deeply trilobated with a median lobe whose length often exceeds that of the lateral lobes; by *D. maculata* and *D. caramulensis* the median lobe is much shorter and less clearly loose of the lateral lobes. The deepness of the trilobation and the relative importance of the median lobe with respect to the lateral lobes are well reflected by the labellum shape index (HESLOP-HARRISON 1951): its value oscillates around 1.2 for *D. maculata* – *D. caramulensis* and around 1.45 for *D. fuchsii* – *D. saccifera* (TYTECA & GATHOYE 1989). The dimensions of the spur are also primordial for separating the four taxa: it appears clearly that *D. caramulensis* has a longer spur than *D. fuchsii*; the spur is thicker for *D. saccifera* than by *D. maculata*. In general, the whole flower characters of *D. caramulensis* and *D. saccifera* are greater than for the two other taxa.

These morphological differences are of course not sufficient to claim that the four taxa are four different species. However, joined with other observations, they suggest that these taxons are well isolated. For example, *D. fuchsii* and *D. saccifera* are diploid ($2n = 40$) while *D. maculata* and *D. caramulensis* are tetraploid ($2n = 80$; GATHOYE & TYTECA, forthcoming). There also exist qualitative characters allowing to separate the four taxa (see, e.g., TYTECA & GATHOYE 1989). Among these, the shape of the labellum is well reflected by the aforementioned labellum shape index. The ecological characteristics are generally well distinct, *D. maculata* and *D. caramulensis* growing preferably in wet meadows, in turf moors and more rarely in fresh woods, while *D. fuchsii* is often found in chalky, dry grasslands or open woods, and *D. saccifera* typically grows in chestnut groves and in alcaline (or acid in Corsica) meadows and fens. On the other hand, *D. maculata* and *D. fuchsii* are widely distributed throughout Europe. Towards southern and south-western Europe, they seem to be replaced (with in some instances intermediate populations), respectively, by *D. caramulensis* in Portugal, Spain, and south-western France, and *D. saccifera* in Corsica, Sardinia, Sicily, Italy, Yugoslavia, the Balcanic Countries, Greece, and Turkey (GÖLZ & REINHARD 1984, TYTECA & GATHOYE 1988, TYTECA 1989). The distribution areas known so far thus suggest that *D. caramulensis* and *D. saccifera* are robust, large-flowered meridional vicariants of *D. maculata* and *D. fuchsii*, respectively. With the above considerations, the subgroups formed by *D. maculata* and *D. caramulensis* on one the hand and by *D. fuchsii* and *D. saccifera* on the other hand, are clearly separable. Other factors to be taken into account for taxonomical research mainly include research on the pollinators, but information is largely lacking until now.

Three of the four discussed taxa correspond to species generally well accepted in the literature, according to classical taxonomic concepts (*D. maculata, D. fuchsii, D. saccifera*). The fourth taxon, *D. caramulensis*, was described (VERMEULEN 1970) and raised to the specific level (TYTECA 1989) only recently. The multivariate analysis exploited here gives, on morphological bases, an unambiguous additional support to its validity as a taxon at a level equal with the other three.

On the other hand, our results could hardly be exploited to support the recognition of additional infraspecific taxa, with perhaps at most one exception. The analyzed *D. saccifera* and *D. caramulensis* samples are insufficient to distinguish subspecies or varieties. The various ways in which the *D. maculata* samples are clustered (see Fig. 5) and the relative position of each sample as illustrated in both Figs. 6 and 7 do not provide support to the separation of subsp. *elodes*, since the only population considered as typical for that subspecies (Kalmthout – no. 22) is stably clustered with two other populations, one of which (Wingene – no. 35) is quite typical for subsp. *maculata*. Populations no. 2 (Bihain), 17 (Fagne Wallonne) and 27 (Pisserotte) are always clustered together; they can all be considered as transitional to subsp. *elodes*. However, the five clustering methods used (Fig. 5) associate them in quite different ways to the other *maculata*-clusters. Perhaps the most significant trend in this context is to be found in the *D. fuchsii* clusters: the clear and stable separation between clusters f1 and f2 (Fig. 5) corresponds to an obvious morphological difference: the flowers of the plants in cluster f1 have been found to possess clearly smaller dimensions than those of cluster f2 (TYTECA & GATHOYE 1988, 1989). In contrast with *D. maculata* subsp. *elodes*, none of the existing subspecies or varieties of *D. fuchsii* seems to correspond to these morphological features; our results could yield an argument to creating such a new taxon.

Conclusions

It can be concluded that the measured morphological characters provide a sufficient description of the taxa under consideration, and allow for a clear taxonomical separation of the samples studied in the *D. maculata* group. Among these characters, only four are sufficient for correctly classifying more than 80% of the individuals in each of the identified taxa (as reported in Table 4). It also appears that the floral characters play an essential role in the taxonomical distinction.

The samples collected in 35 populations of plants in the *D. maculata* group lead to four well-defined clusters, at an equal level from morphological standpoints. These correspond to four specific entities: *D. maculata* s.str., *D. caramulensis, D. fuchsii*, and *D. saccifera*. The position of a few critical samples is slightly varying with the exploited method, and can be interpreted from geographical or ecological standpoints. With the additional evidence provided by qualitative characters (such as the labellum shape), and ecological and caryological analyses, the subgroups formed by the former two and the latter two of these species are clearly separable. It can be submitted that *D. caramulensis* and *D. saccifera* are robust, large-flowered meridional vicariants of *D. maculata* and *D. fuchsii*, respectively. With the exception of a subset of *D. fuchsii* populations showing smaller flowers, no clear trend exists towards the separation of the four species into subspecies or varieties.

Future research should be oriented in the following directions. First, the remaining groups of *Dactylorhiza* of western Europe should undergo the same analysis as the *D. maculata* group studied herein, in order to provide the same kinds of arguments for clarifying their taxonomy, and to give an insight into the taxonomy of the genus as a whole. Second, beside the caryological and ecological analyses briefly mentioned in the above lines, additional analyses are needed to support the adopted taxonomical assumptions: these include the study of the relationships with pollinating insects, and more elaborate technique such as DNA analysis.

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