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Species diversity versus phylogenetic diversity: A practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae)

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ABSTRACT

Setting conservation priorities in taxonomically complex groups such as the orchid genus *Dactylorhiza* is a difficult task. As an alternative to taxonomic diversity, we used here a molecular phylogenetic analysis and the results of a genetic investigation using plastid microsatellites with an extensive geographic sampling to assess in a more objective way the patterns of diversity within this genus. Although western Europe is thought to be the main diversity centre for the genus due to the large number of species found there, we found higher phylogenetic and genetic diversity as well as higher endemicity in the Caucasus and the Mediterranean Basin, two biodiversity hotspots. Species number seems to be correlated with taxonomic effort, tentatively estimated here by the number of herbaria, and is thus biased and not an appropriate measure of diversity. Our results show that phylogenetic analyses and genetic data obtained with molecular tools can offer an alternative measure of biodiversity that is not sensitive to taxonomic inflation. Conservation of allotetraploid taxa is also discussed, and it is recommended that sites in which polyploids are formed should be conserved rather than any specific allotetraploid taxon.

1. Introduction

Members of the genus *Dactylorhiza* Necker ex Nevski (Orchidaceae), the spotted and marsh orchids, are terrestrial orchids from the Northern Hemisphere. They occupy a wide range of open habitats from dune slacks to alpine meadows, including swamps and peat bogs. The subtribe to which they belong, Orchidinae, is most diverse in Eurasia, encompassing the majority of European orchids. According to Averyanov (1990), there are 75 species of *Dactylorhiza* worldwide and 58 in Europe, North Africa and the Near East (hereafter termed Europe and adjacent areas; Delforge, 2001). *Dactylorhiza* has been shown to be monophyletic if the former monotypic genus *Coeloglossum* Hartman is included in synonymy (Pridgeon et al., 1997; Cribb and Chase, 2001).

1.1. *Distribution and diversity*

The distribution of *Dactylorhiza*, including *D. viridis* Bateman, Pridgeon and Chase formerly *Coeloglossum viride* Hartman, covers most of Europe, most of temperate Asia, North Africa, Japan, the Aleutian Islands and northern parts of North America (Fig. 1). Averyanov (1990) distinguished three centres of diversity: western Europe (including the British Isles, Ger-

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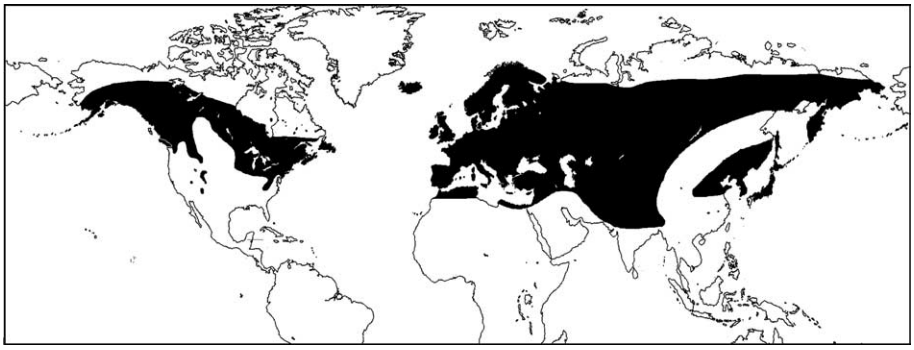

Fig. 1 – World distribution of the genus *Dactylorhiza* (shaded areas), including the former genus *Coeloglossum*, modified from Pridgeon et al. (2001) and Luer (1975).

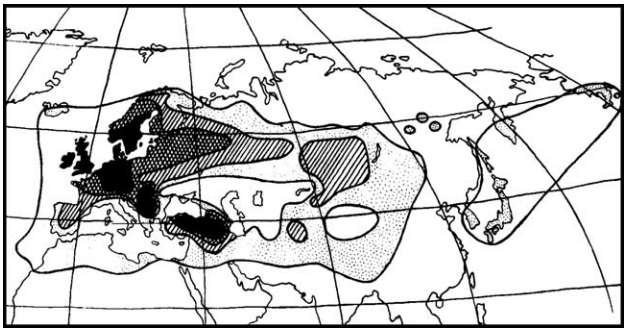

Fig. 2 – Species diversity of *Dactylorhiza* across Eurasia, reproduced from Averyanov (1990). Darker areas are said to contain the greatest number of *Dactylorhiza* species.

many and southern Scandinavia), the Carpathian Balkan area and Asia Minor (Fig. 2). This is in broad agreement with data given in Delforge (2001), according to whom the greatest species richness is found in northwestern Europe. For instance, nine species are endemic to the British Isles according to Delforge's classification (2001). *Dactylorhiza viridis*, the species with the largest range, is the only one to become widespread in the New World (Luer, 1975). *Dactylorhiza* is thus unusual among European orchid genera, most of which show greatest diversity around the Mediterranean Basin.

1.2. *Taxonomic issues in the genus*

Dactylorhiza is universally recognized as a taxonomically challenging genus (Bournérias et al., 1998; Pedersen, 1998; Delforge, 2001; Hedrén, 2001), as demonstrated by the differences in the number of species recognized by different authors (reviewed by Pedersen, 1998), from 12 to 75 worldwide and from 6 to 58 in Europe. There can even be important differences between treatments by the same author. Delforge (1995) for example, added nine species between his monographs of 1995 and 2001. This taxonomic complexity can largely be explained by the frequency of hybridization, and nearly all hybrid combinations are possible (Averyanov, 1990). Most *Dactylorhiza* species belong to the *D. incarnata/maculata* polyploid complex, which is composed of three broad groups: *D. incarnata* s.l., *D. maculata* s.l. and allotetraploids that are hybrids between the first two groups (Hedrén, 2001). The *D. maculata* group is itself composed of diploid and tetraploid species, delimitation of which is often difficult.

1.3. *Threats and conservation status*

As with many other terrestrial orchids, populations of *Dactylorhiza* have decreased due to habitat loss. Many wetlands in Europe have been drained, and changing agricultural practices have led to the degradation of their habitats through use of fertilizers, early haymaking, etc. More recently, the decrease in agricultural pressure has had a counterintuitive effect: abandonment of grassland leads to forest expansion and fewer suitable habitats. However, a few species such as *D. fuchsii* and *D. praetermissa* have shown some ability to colonize human disturbed environments, but generally transiently. Another threat to *Dactylorhiza* is the collection of their tubers to make salep, used as food and medicine. This is a particularly important threat in the Himalayas (Srivastava and Mainera, 1994), where *D. hatagirea* or "panch aunle" is judged critically endangered (Biodiversity Conservation Prioritisation Project, 1998) due to over-collection. Thus, several species of *Dactylorhiza* are declining, and some are already protected at a national scale, e.g., in Belgium, Luxembourg, Nepal, and the UK.

Setting conservation priorities in taxonomically complex groups is an essential but especially difficult task because these species tend to be over-represented in red lists (Pilgrim et al., 2004). Hybridization has often made decision-making difficult in conservation (Rieseberg and Gerber, 1995; Wayne and Gittleman, 1995), and neglecting taxonomy can have disastrous effects on the conservation of a particular group, e.g., the tuatara (Daugherty et al., 1990). In the case of *Dactylorhiza* such problems have already been encountered; *D. lapponica*, formerly classed as a threatened species in Britain, proved to be indistinguishable from *D. traunsteineri* (a more frequent species) after morphological and molecular investigations (Bateman, 2001; Pillon et al., in press; Bateman, submitted). Thus, caution should be applied before setting taxon priorities, and molecular systematics can aid in this task (Soltis and Gitzendanner, 1999).

1.4. *Alternative measures of biodiversity*

The aim of conservation biology is to preserve biodiversity: "the variety of life in all its manifestation" (Gaston and Spicer, 1998). Species richness is by far the most commonly used measure of biodiversity, but many others also exist (Purvis and Hector, 2000). Some approaches have proposed giving different weight to species because some species are more distinctive and genetically isolated than others, e.g., one species of apomictic *Taraxacum* may not deserve the same attention as the single species of *Welwitschia* (Vane-Wright et al., 1991). Because *Dactylorhiza* species are unequally distant from each other, some being barely distinguishable genetically and others relatively isolated, we thought that evolutionary history or phylogenetic diversity could be a better measure of the diversity of a region (Faith, 1992; Mace et al., 2003) than purely taxonomic diversity. A hierarchical taxic weighting approach (Vane-Wright et al., 1991) cannot be applied to *Dactylorhiza* because of reticulate evolution. Thus, we propose here to use neutral molecular markers to assess global diversity distribution within *Dactylorhiza*. Rate of evolution in DNA sequences is known to vary among lineages (e.g., Soltis et al., 2002), but genetic distances between taxa are nevertheless correlated with the time of their divergence and thus can act as a surrogate for genetic, morphological and biochemical distinctiveness. Here, the taxonomic status of the taxa in questions is not clear, so we do not know if we should be considering the variation we detect to be inter-or intraspecific. Previous studies have shown than molecular markers such as DNA sequences, plastid microsatellites and AFLPs are often linked with morphological (Barraclough and Savolainen, 2001; Rodríguez et al., 2003; Shipunov et al., 2004) or ecological (Kelly et al., 2003) diversity of species, but see e.g., Bonnin et al. (1996) and Hamrick et al. (1991) for situations in which historical change is a better predictor of genetic diversity within populations.

2. Methods

Our sampling of more than 600 accessions covers a large part of Europe and adjacent areas and comprises taxa that represent all sections, subsections and 15 of the 19 aggregates in Averyanov's system (1990) and 37 species in Delforge's classification (2001). A more detailed view of the geographical and taxonomic coverage of our sampling is given elsewhere (Shipunov et al., 2004; Pillon et al., in press). All unsampled aggregates of Averyanov (1990) are represented by species related to *D. incarnata* that occur in the Caucasus or extend from Asia Minor to India.

Phylogenetic diversity was obtained from a phylogenetic tree (Fig. 3) based on a combined analysis of the sequences of the nuclear ribosomal internal transcribed spacers (ITS nrDNA) and the intron of the plastid gene *rpl16* (see e.g., Rønsted et al., 2005, for sequencing procedures). Representative sequences were submitted to GenBank (Accession Nos. DQ022863 to DQ022926). Only a few accessions representing the full range of diversity observed for these two loci were included in the final analysis. Phylogenetic analysis was performed in PAUP*4.01b10 using maximum parsimony; heuristic searches employed 200 replicates of random taxon entry order with tree bisection reconnection (TBR) swapping and no tree limit per replicate. Clade support was assessed with 1000 bootstrap replicates. DELTRAN optimization was used for branch length measures because of known problems with ACCTRAN in PAUP*4.01b10. No particular short or long branches that could result in substantial under- or overweighting of a lineage were observed. All DNA sequences have been submitted to GenBank and have Accession Nos. DQ022863 to DQ022926 (DQ022863 to DQ022894 for ITS and DQ022895 to DQ022926 for the *rpl16* intron).

Using the distributions given in Delforge (2001), we listed for each country or region the species occurring there and mapped them on the tree. Phylogenetic diversity was measured for each region as the sum of branch lengths (i.e., number of nucleotide substitutions) between the first node within *Dactylorhiza* and the tips of the tree whenever the corresponding terminal occurred in the area, as recommended by Rodrigues and Gaston (2002).

Several assumptions were made to calculate phylogenetic diversity. We made the approximation that allopolyploid species, which are generally not genetically divergent from their parents (Hedrén, 1996; Hedrén, 2001; Shipunov et al., 2004; Pillon et al., in press), have the evolutionary history of both of their parents. This will have little effect on the results because most allotetraploids still co-occur with their parents. Some diploid species displayed variability in their sequences, most often for ITS and rarely for the *rpl16* intron. Specimens from a given species could differ by up to three substitutions for one locus but generally fewer. Because we did not detect any particular geographical or habitat-related structure in this variability (this is particularly clear for *D. fuchsii* and *D. maculata* s.s.; Pillon et al., in press), we considered these polymorphic taxa to be a single terminal. We gave to such terminals the average length between the shortest and longest branch within these taxa. Thus, extra phylogenetic diversity due to intraspecific variability was taken into account. Due to their substantial divergence, European and Chinese *D. viridis* were considered as different taxa, although we lack evidence to determine whether or not they are conspecific. Consequently, we recognized 11 terminals on our phylogenetic tree corresponding to the number of phylogenetically distinguishable diploid and autotetraploid species: *D. fuchsii*, *D. maculata/D. foliosa*, *D. saccifera*, *D. aristata*, *D. sambucina*, *D. romana*, *D. iberica*, European *D. viridis*, east Asian *D. viridis*, *D. euxina* and *D. incarnata*. At least 11 other diploid or autotetraploid taxa recognized as species by Delforge (2001) are so far indistinguishable from one of the species cited above according to molecular studies, for instance most taxa segregated from *D. fuchsii*, *D. maculata* and *D. incarnata* (Bullini et al., 2001; Hedrén et al., 2001; Shipunov et al., 2004; Pillon et al., in press). Others are suspected to be hybrids between the taxa listed above (Pillon et al., in press).

Considering the level and representativeness of our sampling, we believe that we have sampled all the evolutionary history present in Europe using these types of moderately variable markers, i.e., we have probably already detected all the sequence variation (except a few minor variants) that can be found in *Dactylorhiza* across Europe. For the species we did not investigate, we assumed that they had the same phylogenetic diversity as their close relatives.

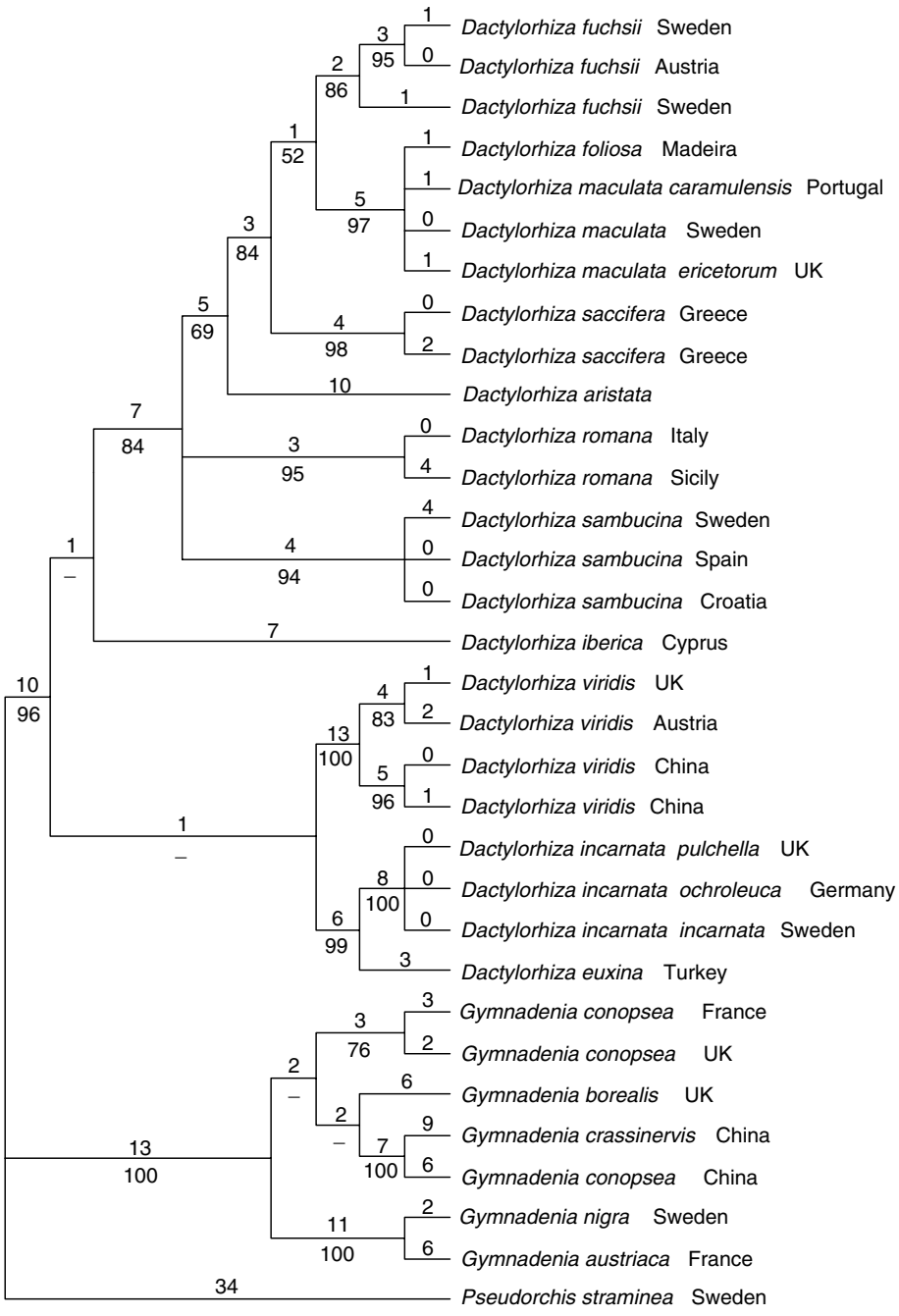

Fig. 3 – One of the most parsimonious phylogenetic trees of *Dactylorhiza* obtained using a combined dataset of ITS and *rpl16* intron sequences. Numbers above branches are branch lengths (DELTRAN optimization), numbers below branches are bootstrap percentages (1000 replicates).

Here is an example of how phylogenetic diversity was measured for one region. According to Delforge (2001), the following species occur in the Netherlands: *D. fuchsii*, *D. maculata*, *D. ericetorum* (genetically identical to *D. maculata*), *D. incarnata*, *D. viridis*, *D. praetermissa* and *D. majalis* (both allotetraploids resulting from the cross of *D. fuchsii* and *D. incarnata*). Therefore, we considered only the following terminals: *D. fuchsii*, *D. maculata*, *D. incarnata* and European *D. viridis*. When considering the infraspecific variability in *D. fuchsii*, *D. maculata* and European *D. viridis* as described above, the sum of branch lengths gives 60.5 (rounded to 61).

To evaluate consistency between species diversity and phylogenetic diversity within an area we used a numerical index that is the ratio of the number of species present in this area according to Delforge (2001) relative to the number of terminals (or sequence types), i.e., a ratio of taxonomic diversity to molecular diversity. Using Spearman's rank test, we tested correlation between the number of species, the number of ter-

minals, the index, the number of herbaria and the surface area of 30 countries or regions. Correction for multiple tests was done using Bonferroni sequential correction (Rice, 1989). Numbers of herbaria were taken from the Index Herbariorum (2003) and were used as a potential surrogate for taxonomic effort in a given region.

Because plastid microsatellites are generally variable between and within species (Provan et al., 2001), they are useful markers to assess both the differences between species and the variability present in each species and to reveal some geographical patterns. We used here a large dataset of more than 600 samples that yielded 38 different plastid haplotypes across the range of *Dactylorhiza* (Shipunov et al., 2004; Pillon et al., in press).

3. Results and discussion

3.1. *Geography and evolutionary history*

The distribution of phylogenetic diversity across Europe is given in Table 1 and Fig. 4. The greatest amount of phylogenetic diversity was observed in Greece, the Caucasus and the Crimea, with up to 90% of the total phylogenetic diversity found throughout Europe concentrated in these regions. High diversity was also found in Bulgaria, France (the Mediterranean part), Italy, Turkey, the former Yugoslavia, and to a lesser extent Albania, Romania and Spain. All phylogenetic diversity of *Dactylorhiza* occurring in Europe (85% of the total in the genus) can be found in the Mediterranean basin and the Caucasus, two of the 25 biodiversity hotspots recognized by Myers et al. (2000). *Dactylorhiza saccifera* and *D. romana* are both Mediterranean species, *D. iberica* is found only in the eastern Mediterranean basin and the Caucasus, and *D. euxina* is found only in the Caucasus. The high phylogenetic diversity observed in the eastern Mediterranean Basin and the Caucasus can thus probably be explained by the fact that the genus *Dactylorhiza* first diversified there and some species migrated to other areas, as hypothesized by Averyanov (1990).

There are important inconsistencies between the distribution of species (Fig. 2) and phylogenetic diversity (Fig. 4). From the distributions given in Delforge (2001), the regions where the highest species diversity in *Dactylorhiza* is found are France, Germany, the former USSR, Britain, Norway and Sweden (Table 1). Phylogenetic diversity in Germany and Scandinavia was only 77% of the total and in Britain only 70%. In species-rich regions globally, phylogenetic diversity is lower than we would expect if there were a roughly linear relationship between number of species and phylogenetic diversity. This contrasts with the results of Rodrigues and Gaston (2002), who reported a near perfect linear correlation between

Table 1 – Number of species, number of different sequences (terminals), phylogenetic diversity and index (ratio of the number of species/number of terminals) for countries and regions of Europe, North Africa and the near East

	Number of species	Number of sequence types	Phylogenetic diversity	Index
Britain	17	4	61	4.3
Germany	20	5	67	4.0
Norway	17	5	67	3.4
Sweden	17	5	67	3.4
Ireland	13	4	61	3.3
France	20	7	77	2.9
Denmark	14	5	67	2.8
Poland	11	4	61	2.8
ex-USSR	19	7	87	2.7
Switzerland	13	5	67	2.6
Finland	12	5	67	2.4
Austria	11	5	67	2.2
ex-Czechoslovakia	11	5	67	2.2
Spain	13	6	72	2.2
Italy	15	7	77	2.1
Belgium	8	4	67	2.0
Turkey	13	7	75	1.9
Netherlands	7	4	61	1.8
Romania	7	4	72	1.8
Greece	12	7	78	1.7
ex-Yugoslavia	11	7	77	1.6
Portugal	6	4	49	1.5
Bulgaria	9	7	77	1.3
Hungary	6	5	67	1.2
Albania	6	6	71	1.0
Cyprus	2	2	20	1.0
Syria + Lebanon	3	3	33	1.0
Luxemburg	3	3	56	1.0
Morocco	3	3	43	1.0
Algeria	2	3	42	0.7
Iceland	2	3	47	0.7
Tunisia	1	2	38	0.5

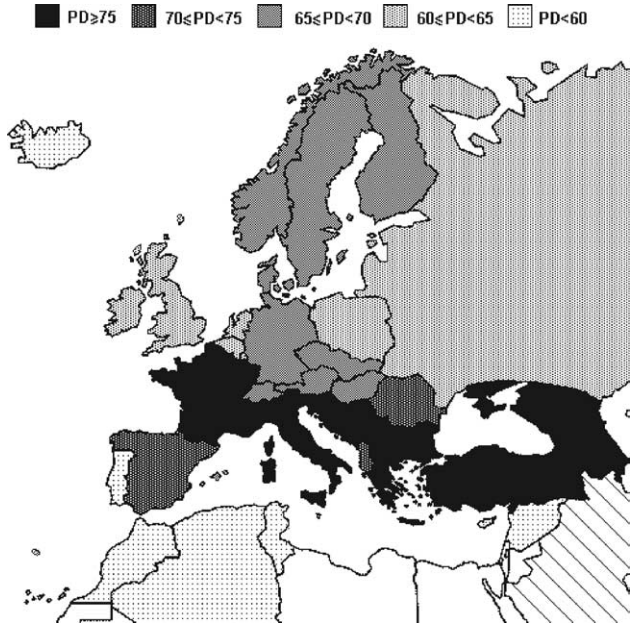

Fig. 4 – Distribution of the phylogenetic diversity of *Dactylorhiza* across Europe and adjacent areas. Phylogenetic diversity (PD) was measured as the number of substitutions on a phylogenetic tree based on ITS and *rpl16* intron sequences. No *Dactylorhiza* species occur in the white areas according to Delforge (2001). The cross-hatched area was not assessed.

number of bird genera and phylogenetic diversity in a grid of southern Africa. Values of our numerical index take into account the incongruence observed between the number of species recognized in a region and the number of different sequences found or the number of terminal taxa on the tree (Table 1). Low values were observed for most regions adjacent to the Mediterranean Basin for which the index gave values lower than 2.0. The highest values were found in Britain, Germany, Norway, Sweden and Ireland. This variation in index value could be explained by the type of markers used, which were only two DNA sequences that may inappropriate to distinguish closely related species and thus could underestimate diversity. However, as described below the use of more variable markers such as plastid microsatellites does not show higher diversity in northern Europe (see Fig. 5).

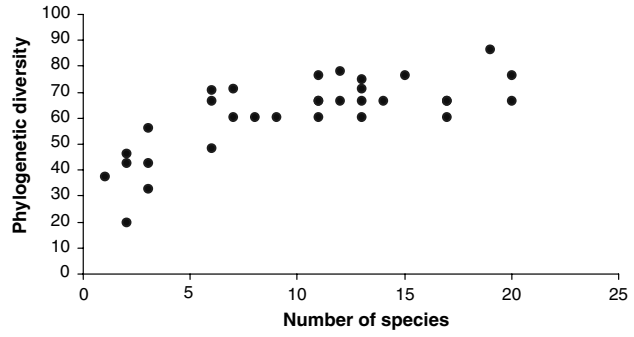

Fig. 5 – Relationship between the number of species and phylogenetic diversity (in base pairs) across regions of Europe and adjacent areas.

Most species found in northern Europe belong to the *D. incarnata/maculata* polyploid complex, in which speciation through hybridization is commonplace. This explains a part of the discrepancy between species richness and phylogenetic diversity. Also, many of these taxa are artificial; for example most of the allotetraploid taxa have multiple origins and are not readily distinguishable from each other genetically (Pillon et al., in press). Species delimitation within the *D. incarnata* complex, comprising diploid taxa, has not found support so far in molecular studies (e.g., Hedrén et al., 2001). Moreover, there is at least one case of such artificial taxa outside the polyploid complex; according to allozyme data, there is free genetic exchange between *D. romana* and *D. markusii*, which should consequently be regarded as a single species (Bullini et al., 2001). *Dactylorhiza* has been widely subjected to splitting due to the supposedly exceptional morphological variability of the taxa, and thus we suspect that the number of *Dactylorhiza* species recognized in an area may better be correlated with the level of taxonomic effort carried out in the vicinity.

Results of correlation tests between the numbers of species, terminals, herbaria, index and surface area are given in Table 2. No correlation was found between the surface area of a region and any other parameter except the number of species, for which we found a moderate correlation ($p = 0.041$). The last correlation was no longer significant after a correction for multiple tests had been applied. In contrast, we found positive and highly significant correlations between the number of species, the number of herbaria and the index. Britain and Germany are both in the top five regions for these parameters. Similar results were obtained if we substituted the number of herbaria with the number of taxonomists recorded in the World Taxonomist Database (data not shown). The number of herbaria was used here as a possible surrogate for taxonomic effort. We are conscious that this is only a rough estimator, but it does distinguish western European countries with a long tradition in systematics from less developed countries, where less taxonomic work has been done. Thus, the number of taxa reported in a region may be better correlated with taxonomic effort, leading to species-level recognition of minor entities of doubtful distinctiveness (taxonomic "splitting").

3.2. *Phylogeography inferred by plastid microsatellites*

Among the 38 plastid haplotypes found in the genus *Dactylorhiza*, 34 occur in Europe and adjacent areas and are distributed as follows: nine haplotypes occur only in the Mediterranean Basin, eight only in the Caucasus, one is confined to both hotspots, eight never occur in either of the two hotspots and eight occur in at least one of the two hotspots and elsewhere (Fig. 6). Much genetic diversity is found in the hotspots (76% of the total diversity found in Europe and adjacent areas), and a majority of this is endemic to these regions (53%). Although we did not sample all regions of Europe and all taxa recognized in the genus (such a task will be difficult to achieve), our sampling is well spread across latitude and longitude. We have sampled extensively in some northern regions such as the British Isles, Sweden and European Russia, but our sampling in most Mediterranean regions is

Table 2 – Tests of correlations between the number of species and terminals and herbaria and index and surface area in 30 countries or regions using Spearman's rank test

	Index	Number of sequence types	Number of herbaria	Surface
Number of species	$p = 3.3 \times 10^{-6***}$	$p = 2.0 \times 10^{-4***}$	$p = 2.5 \times 10^{-4***}$	$p = 0.041^{*}$
Index	–	$p = 0.024^{*}$	$p = 4.4 \times 10^{-4***}$	$p = 0.25$ ns
Number of sequence types	–	–	$p = 0.0047^{**}$	$p = 0.059$ ns
Number of herbaria	–	–	–	$p = 0.073$ ns

Figures given here are without correction. After Bonferroni sequential correction, only the correlations between number of species and surface and between index and number of sequence types became non-significant (correlations with a single star *).

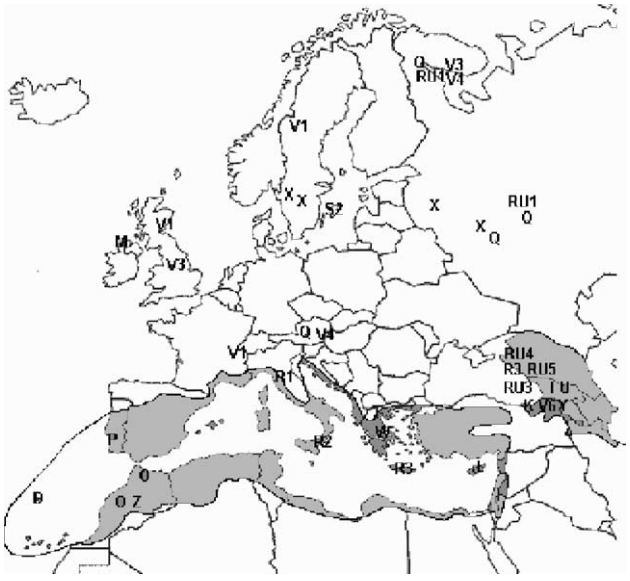

Fig. 6 – Distribution of the rare haplotypes of *Dactylorhiza* in Europe and adjacent areas. We show on this map only the 18 haplotypes that were only found in the two biodiversity hotspots, the Mediterranean Basin and the Caucasus (shaded areas): D, I, J, K, O, P, T, U, W, Y, Z, R1, R2, R3, RU3, RU4, RU5, V6 and those (eight) that were never found in either of the hotspots: M, Q, X, RU1, S2, V1, V3, V4. Data from Shipunov et al. (2004) and Pillon et al. (in press).

relatively sparse. We predict that further sampling could only increase the relative genetic diversity found in the Mediterranean Basin. Furthermore, several of the haplotypes not yet found in either of the two hotspots are widespread elsewhere, which means they are likely to be found at least in one of the two hotspots if sampling is substantially increased. This pattern of diversity is consistent with previous observations of extensively sampled groups such as the fern genus *Asplenium* (Trewick et al., 2002), white oaks (*Quercus* spp.: Dumolin-Lapègue et al., 1997), black alder (*Alnus glutinosa*: King and Ferris, 1998) and a grasshopper (*Chorthippus parallelus*: Cooper et al., 1995). However, using a large sampling of 22 species of trees and shrubs, Petit et al. (2003) found that Mediterranean populations were more divergent (as found here), but populations at intermediate latitudes had higher diversity, possibly due to admixture during recolonization after glaciation.

There are at least two explanations for this unbalanced distribution of the genetic diversity of *Dactylorhiza* across Europe. The first is that several species are endemic or near-endemic to the Mediterranean Basin or the Caucasus (*D. romana*, *D. saccifera*, *D. iberica* and *D. euxina*) as are their specific markers. Another factor that probably increased the relative genetic diversity found in the Mediterranean Basin is Pleistocene glaciation (Hewitt, 1996). Because northern Europe was repeatedly covered with ice, the Mediterranean Basin hosted refugia for most European species during the cold periods. Genetic variation suggests potential refugia for *Dactylorhiza* in Greece, the Iberian Peninsula and North Africa (Pillon et al., in press). Thus, the genetic diversity carried northward by recolonizers is only a subset of the southern diversity, i.e., a proportion of southern diversity was not carried northward. Consequently, according to our results, 76% of the genetic diversity found in Europe and adjacent areas and 68% of the genetic diversity found worldwide in *Dactylorhiza* still occurs in the Mediterranean Basin and the Caucasus. We estimate that losing all populations in the two hotspots would result in the irrevocable loss of 53% of the European and 47% of the world genetic diversity of *Dactylorhiza*.

3.3. *Conservation of allopolyploid taxa*

Allotetraploids, although often considered new taxa, do not have any genetic distinctiveness, at least at the time of their formation. If polyploids are formed repeatedly, Hedrén et al. (2001) suggested that conservation of the parental lineages should be prioritized because allotetraploids could be regenerated from them. As is commonly observed in other groups of plants (Soltis and Soltis, 1999), allotetraploids in *Dactylorhiza* have multiple origins, spread across space and time (Pillon et al., in press). Furthermore, many of the allotetraploid taxa are poorly defined, and thus their current taxonomy is not appropriate for setting conservation priorities (Bateman and Denholm, 1983; Bateman, 2001; Bateman, submitted). Although most allopolyploids in *Dactylorhiza* carry some genetic markers that are commonly found in their putative parents (Hedrén, 1996; Shipunov et al., 2004; Pillon et al., in press), they sometimes carry alleles that are rare or absent in the other groups (Bullini et al., 2001; Pillon et al., in press), even though these alleles are always closely related to parental ones. Therefore, some older allopolyploids contain diversity that has become rare or no longer exists in their parental lineages, and thus these taxa have some conservation value. Some of them may be old enough to contain new alleles. However, current taxonomies will not be the best tool to prioritize this allotetraploid group possessing rare markers. Because many allotetraploids have multiple origins, their morphology is not a good predictor of their markers. For

instance, the widespread allotetraploid *D. majalis* s.s. has the common plastid haplotype of the diploid *D. fuchsii* in Sweden, but in France it more often has a haplotype that is rare in diploid lineages. However, there is generally a local consistency, i.e., within a particular region an allotetraploid taxon has a single origin (Hedrén, 2003; Shipunov et al., 2004; Pillon et al., in press). Consequently, conservation priorities may be determined in thoroughly investigated areas, such as the British Isles or Sweden, without extrapolation to other areas.

In the British Isles, most individuals of *D. traunsteineri* (including *D. lapponica*) and some individuals of *D. praetermissa* carry the plastid haplotype C (Pillon et al., in press). This haplotype has only once been found in a diploid species but is frequently found in allotetraploids. Although this C haplotype is close to the A haplotype commonly found in the widespread diploid species *D. fuchsii*, some conservation consideration should be given to *D. traunsteineri*, which has a fragmented distribution in the British Isles. In contrast, most allotetraploids occurring in Sweden or northern Russia have widespread haplotypes that are also present in the local parental species (Hedrén, 2003; Shipunov et al., 2004; Pillon et al., in press). Most of the Swedish allotetraploids would not need special treatment because any future allopolyploidization event would presumably result in genetically similar plants.

Studies of rDNA ITS (Shipunov et al., 2004; Pillon et al., in press) revealed that northern allotetraploids were generally younger than the southern ones, probably reflecting postglacial formation. Thus, the process of allopolyploidization may be currently more active in northern Europe, although probable recent events have also been recorded in alpine regions (Tyteca et al., 1991; Delforge, 2001), in spite of the presence of older allotetraploids in this region (Pillon et al., in press). Preserving this process should not be disregarded because generation of new combinations in allotetraploids is a source of variation. Recent studies have also shown that allotetraploids can no longer be considered as a simple sum of two genomes (Otto, 2003). Although northern populations are less diverse and contain no unique markers, conservation of *Dactylorhiza* would not be pointless. Preservation of habitats where parental lineages occur and co-occur and allotetraploids could originate and become established is a way to preserve the evolutionary process. In this case, ecologically based rather than taxon-based prioritization would be advisable.

Conservation of actively diversifying groups is now often considered a priority (e.g., Mace et al., 2003). Polyploids have been once considered as dead ends and thus may not deserve any attention for conservation, but in recent years they have been shown to be evolutionarily dynamic (e.g., Soltis and Soltis, 1999). Polyploids show higher heterozygosity than their diploid parents and consequently have reduced inbreeding depression (Soltis and Soltis, 2000). Recently formed polyploids can be remarkably successful. For example, *Spartina anglica* (Baumel et al., 2001), an allotetraploid formed less than 200 years ago, is now considered to be one of the world's 100 worst invasive species (Invasive Species Specialist Group, 2004). Many species are now recognized as ancient polyploids, including *Zea mays* (Gaut and Doebley, 1997), some *Brassica* species (Lagercrantz, 1998) and *Arabidopsis* (Bowers et al., 2003). Several species-rich lineages such as whole angiosperm families probably have an ancient polyploid origin (Soltis and Soltis, 2000). Similarly, according to molecular phylogenetic studies, allotetraploid *Nicotiana* section *Suaveolentes* (approximately 25 species native to Australia and Africa) have a single origin (Chase et al., 2003) and subsequently radiated to form the largest section in the genus. Thus, polyploid species should not be neglected solely on the basis of their ploidy; their reproducibility and reproductive potential should also be taken into account.

4. Conclusions

The taxonomic complexity of *Dactylorhiza* has so far made conservation activity difficult. Using molecular markers to provide an objective assessment of diversity in the genus, we showed the greatest genetic diversity to be in the Mediterranean Basin and the Caucasus, two of the 25 biodiversity hotspots defined by Myers et al. (2000). Further sampling is desirable, especially in the Mediterranean Basin (e.g., Italy) and the Near and Middle East. Some species were not sampled, but we expect that many of them will prove close to if not indistinguishable from those already sampled. Some of the aggregates from Asia described in Averyanov (1990) were not sampled, including *D. hatagirea*, a critically endangered Himalayan species. Such taxa will be a priority in our future work. However, we believe that our sampling in Europe is reasonably representative and that the concentration of diversity in the two biodiversity hotspots will remain, if not increase, with further sampling. The Mediterranean Basin and the Caucasus can thus be considered as major targets for the conservation of *Dactylorhiza* at both European and world scales. *Dactylorhiza aristata*, a non-European species and the only representative of its section can be considered relatively safe, given its wide distribution in Japan, the Aleutian Islands and Alaska.

We are aware of the discrepancy of the scale used here: the conservation of a genus at a continental scale. However, this study presents a major step forward as we have moved the perceived diversity centre of *Dactylorhiza* from northwestern Europe to the Mediterranean, as for other European orchid genera. The Mediterranean Basin is still a large area (the largest of the 25 hotspots) covering many countries. However, studies have already revealed particular regions within this hotspot with greater richness and endemism (Médail and Quézel, 1997). Although further work at a finer scale is needed to determine which of these smaller regions host high diversity and originality for *Dactylorhiza*, we can retain Greece, Madeira and the Atlas Mountains as regional hotspots for *Dactylorhiza* due to endemicity and diversity (particularly Greece).

Because they used a large group, the vascular plants, as a surrogate for terrestrial organisms, Myers et al. (2000) identified the main regions of biodiversity, which also appear to be good candidates as centres of diversity of particular groups. We think that this is more defensible than conclusions reached previously for *Dactylorhiza* by traditional taxonomists. This case study shows that evolutionary history can be a good alternative to measure biodiversity (Mace et al., 2003) because it correlates well with genetic diversity and can differ significantly from species richness, contrary to previous observations (Polasky et al., 2001; Rodrigues and Gaston,

2002). This approach permits researchers to measure biodiversity without necessarily knowing the appropriate species concept in a particular group, although it does require a substantial amount of laboratory work. As has been observed for primates and carnivores across the world (Sechrest et al., 2002), most of the evolutionary history of *Dactylorhiza* is concentrated in the hotspots. Considering that many of the genera related to *Dactylorhiza*, members of the subtribe Orchidinae, have a dominantly European or Mediterranean distribution (Pridgeon et al., 2001), this pattern can probably be extrapolated to other orchids. Because most European orchids belong to subtribe Orchidinae (83% of the species recognized in Delforge, 2001), the Mediterranean Basin and the Caucasus should then be considered as priority areas for orchid conservation at the European scale, with orchids having potential as a flagship group. Unfortunately, the biodiversity hotspots were not only designated on the basis of their important diversity but also on the degree of alteration of primary vegetation. After millennia of human civilization, the Mediterranean Basin has lost 95% of its primary vegetation, the second most altered of the 25 hotspots (Myers et al., 2000). The Mediterranean ecosystem is also judged to be one that is likely to suffer the most dramatic changes in the next century (Sala et al., 2000). Global warming will tend to modify the range of many species, potentially pushing them northwards, after they have been repeatedly pushed in the other direction by the Quaternary ice ages. Thus, a partial loss of the genetic diversity that has accumulated in the southern refugia could have a more dramatic effect than another glaciation.

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