

**The Orchid Flora of Portugal - Addendum N. 2 -
Chorological and systematic remarks on *Dactylorhiza
maculata* (L.) Soó s.l. and *Epipactis helleborine* (L.) Crantz
- First report of *E. phyllanthes* G. E. Sm.**

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Summary.

TYTECA, D. & A. CAPERTA (1998): The Orchid Flora of Portugal - Addendum N. 2 - Chorological and systematic remarks on *Dactylorhiza maculata* (L.) Soó s.l. and *Epipactis helleborine* (L.) Crantz - First report of *E. phyllanthes* G. E. Sm. - *Jour. Eur. Orch.* 30 (): - .

Close examination of *Dactylorhiza maculata* s.l. populations in the Serra da Estrela lead to the proposal that all of them are referable to *D. caramulensis* and that *D. ericetorum* is probably absent from this area, although it is well represented in the north of the country. New hypotheses are formulated towards a possible hybrid origin of *D. caramulensis* between *D. ericetorum* and *D. elata*. Three localities with truly typical representatives of *Epipactis helleborine* are reported, two from the Bragança district and one from the Serra da Estrela. A single individual of *E. phyllanthes* was discovered close to Bragança; this is the first mention of an autogamous *Epipactis* in Portugal. Trend towards autogamy was also detected on one plant from the Serra da Estrela. A discussion is initiated on the need to further develop understanding on chromosome structure and mating systems in orchid species, especially in complex genera such as *Dactylorhiza* and *Epipactis*, to adequately fulfil conservation purposes.

Resumo.

TYTECA, D. & A. CAPERTA (1998): As orquídeas de Portugal - Adenda n_ 2 - Comentários sobre aspectos corológicos e sistemáticos de *Dactylorhiza maculata* (L.) Soó s.l. e *Epipactis helleborine* (L.) Crantz - Primera referência a *E. phyllanthes* G. E. Sm. - *Jour. Eur. Orch.* 30 (): - .

Observação cuidada de diversas populações de *Dactylorhiza maculata* s.l. na Serra da Estrela conduziu à proposição de que se referem a *D. caramulensis*, e que *D. ericetorum* encontra-se provavelmente ausente nessa área, embora esteja bem representada no Norte de Portugal. Formulam-se novas hipóteses com respeito a possível origem híbrida de *D. caramulensis*. Referenciam-se 3 localidades com representantes típicos de *Epipactis helleborine*, duas na região de Bragança, e a outra, na Serra da Estrela. Um único exemplar de *E. phyllanthes* foi descoberto perto de Bragança, constituindo a primeira referência a *Epipactis* sp. autogâmica em Portugal. Observou-se também uma tendência para a autogamia numa planta, na Serra da Estrela. Discute-se a necessidade de desenvolver conhecimento sobre estrutura cromossômica e sistemas de reprodução em orquídeas, especialmente em géneros complexos como *Dactylorhiza* sp. e *Epipactis* sp., para responder adequadamente a propósitos de conservação.

Zusammenfassung.

TYTECA, D. & A. CAPERTA (1998): Die Orchideenflora von Portugal - Addendum Nr. 2 - Chorological and systematic remarks on *Dactylorhiza maculata* (L.) Soó s.l. and *Epipactis helleborine* (L.) Crantz - First report of *E. phyllanthes* G. E. Sm. - *Jour. Eur. Orch.* 30 (): - .

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Résumé.

TYTECA, D. & A. CAPERTA (1998): Les orchidées du Portugal - Addendum n_ 2 - Remarques chorologiques et systématiques sur *Dactylorhiza maculata* (L.) Soó s.l. et *Epipactis helleborine* (L.) Crantz - Première observation d'*E. phyllanthes* G. E. Sm. - *Jour. Eur. Orch.* 30 (): - .

Un examen approfondi des populations de *Dactylorhiza maculata* s.l. de la Serra da Estrela aboutit à proposer que toutes peuvent se rapporter à *D. caramulensis* et que *D. ericetorum* est probablement absent de cette aire, bien qu'il soit bien représenté dans le nord du pays. De nouvelles hypothèses sont formulées quant à l'origine hybride possible de *D. caramulensis* entre *D. ericetorum* et *D. elata*. Trois localités avec des représentants très typiques d'*Epipactis helleborine* sont signalées, deux des environs de Bragança et une de la Serra da Estrela. Un individu unique d'*E. phyllanthes* a été découvert près de Bragança; il s'agit de la première mention d'un *Epipactis* autogame au Portugal. Une tendance à l'autogamie a également été observée sur une plante de la Serra da Estrela. Une discussion est entamée quant à l'opportunité de contribuer à une meilleure compréhension de la structure chromosomique et des systèmes de reproduction des espèces d'orchidées, en particulier dans des genres complexes comme *Dactylorhiza* et *Epipactis*, dans l'optique de répondre adéquatement aux objectifs de conservation.

Interpretation of *Dactylorhiza maculata* s.l. populations

Two taxa have recently been proposed as the only representatives of the *Dactylorhiza maculata* (L.) Soó group in Portugal, namely *D. caramulensis* (Verm.) Tyteca and *D. ericetorum* (E.F. Linton) Averyanov (TYTECA 1997). Starting, among others, from the study of herbar specimens, it was concluded that the distributions of these two species significantly overlap and that both are almost evenly distributed through the northernmost third of Portugal. However, an important remark was made at that time, concerning the representativeness of single individuals taken isolately from their populations, due to the important variability and the considerable overlapping of characters, especially in difficult *Dactylorhiza* groups such as *D. maculata* s.l.

In year 1998 our prospections were, among others, devoted to closer examinations of *D. maculata* s.l. populations in the area under consideration. Starting from the south, the most important distribution center is constituted by numerous populations throughout the Serra da Estrela. Several of them were visited on 4th and 5th July, covering various types of biotopes, i.e., semi-natural mowing meadows, heath moors, acid bogs, spring zones and road verges, at altitudes distributed between 800 and 1550 m. Some of these biotopes are clearly anthropogenic (mowing meadows); others are obviously much closer to primitive natural conditions (heath moors, bogs, spring zones) and may constitute stable habitats for the vegetation, including orchids, in the absence of human interventions. Table 1 gives a list of the populations examined.

An interpretation that would take the individual plant as the analysis unit would allow to identify here at least four (sub-) species, i.e., *Dactylorhiza maculata*, *D. ericetorum*, *D. caramulensis* and *D. elata*. Most populations appear with high levels of variation in morphological characteristics. While within some populations, the influence of *D. elata* is clearly perceivable in several plants, in others, a more important influence of *D. ericetorum* (or *D. maculata*) is exhibited. In most cases, these influences appear as a function of the biotope; i.e., the dominance of *D. ericetorum* phenotype is more obvious in heath moors and even more in turf bogs. A reason for this difference may involve genotypic responses predicated by different edaphic-climatic conditions, for there appears to be a strong correlation between habitat and phenotype expression. However, these tendencies are never clearcut, and most populations show various trends towards the other aforementioned taxa.

Because no clear morphological discontinuity would allow here to detect two (or more) distinct taxa, the field researcher (and more especially the *Dactylorhiza* specialist) would tend to view the Estrela populations as representatives of only one species. The taxon that best integrates the various aforementioned influences appears, as previously advocated, as *D. caramulensis*. While in other regions of northern Portugal, the populations are more stable and less variable (such as, e.g., in the Serras do Caramulo, de Bigorne and do Alvão, or around the high Douro valley), this is not the case in the Serra da Estrela, where the populations exhibit more variations.

These observations may lead to the conclusion that the Estrela populations were heavily influenced by hybridisation events. This is not surprising, since

hybridisation is widespread in the genus, especially among tetraploid species, and even constitutes a important potential speciation factor (Soó 1980; DELFORGE 1994; HEDRÉN 1996a). Indeed, all four taxa considered so far are known to be tetraploid. Moreover, these observations are consistent with VERMEULEN's original proposal (1970) that "the ssp. *caramulensis* seems to be a good example of introgression: characteristics of *sesquipedalis* in *maculata*".

Closer to the extreme north of Portugal and the Spanish border, a trend towards morphological separation seems to parallel ecological differences, and populations from heath moors and bogs are generally more satisfactorily identified as *D. ericetorum* (without any perceivable influence of *D. elata*), while in wet semi-natural meadows, populations can be more adequately viewed as *D. caramulensis*, with generally less variation than in the Serra da Estrela. On the other hand, the distribution of *D. elata* is now well documented (TYTECA 1998), and covers areas in western coastal regions as well as in north-east Portugal and surrounding Spain. New prospections in 1998, close to Montalegre and the Serra do Larouco, as well as in neighbouring Galicia (Province of Ourense) and Castilla-y-León (Province of León), are consistent with the above interpretation regarding the *ericetorum/caramulensis* separation. However, generally speaking, in Spain, as one proceeds further north, the morphological/ecological separation seems to fade away, and *D. ericetorum* constitutes the dominant type in all biotopes, as reflected by the populations examined in 1998 and listed in Table 2.

The discussion made above, concerning the interpretation of the populations in the Serra da Estrela, can yield two types of consequences. First, the distribution map of *D. ericetorum* should probably be redrawn, without any locality from that region. The same might also be true for *D. elata*, which seems to lack completely in and around the Serra da Estrela. It is plausible that the latter was completely absorbed in successive introgressions into a taxon from the *D. maculata* group, namely *D. ericetorum*. Indeed, the observations gathered in Table 1 show that, whereas a phenotype similar to *D. ericetorum* can be largely dominant, this is much less the case for *D. elata*, whose influence can be perceived, most often, in isolate individuals. This leads to the second issue that should be addressed, that is, the populational heterogeneity of the so-called *D. caramulensis*, a possible neopolyploid with heterozygous origins, involving hybridizations between the other two taxa, followed by cross-fertilizations within and between individuals of the former. This should be a good argument

towards testing the possible hybrid origin of that taxon. More specifically, the aforementioned hypothesis of introgression should be substantiated.

Such a research should be conducted not only on morphometric bases (TYTECA & GATHOYE 1989; DUFRÊNE *et al.* 1991), but also at genetic and molecular levels. For morphologically similar species, cytogenetic methodologies constitute an important tool to investigate biosystematic relations between them. In fact, several factors have been considered important in orchid speciation, such as chromosome diversity and high incidence of polyploidy (GOLDBLATT 1980; GILL 1989; MEI SUN pers. comm.). Polyploidy is well documented in *Dactylorhiza* spp. (HESLOP-HARRISON 1953; SCHEGEL *et al.* 1989; HEDRÉN 1996a, b). Because many changes in the genome of a particular species may not result in visible phenotypic change, genetic and molecular procedures can provide a means for examining the total divergence between related genomes, for the study of genetic variation in natural populations, genome structure and genome origins, and processes involved in hybridization and speciation (BENNETT 1995).

Only this way, will we gain deeper knowledge of the role polyploidy and other cytogenetic mechanisms play in the extremely complex and dynamic evolution of ecosystems.

New observations on *Epipactis helleborine* (L.) Crantz

In our recent survey (TYTECA 1997), one of the four Portuguese *Epipactis* taxa had been indicated with some hesitation, namely *E. helleborine*, because the plants identified as such were generally much slender than what is usually observed in median Europe. During field prospections in 1998, three of the localities visited in northern Portugal showed plants and populations that could more obviously be associated with that species. Their characteristics appear in Table 3.

The two localities from Trás-os-Montes exhibited tall, truly typical plants in "medio-European" standards, with numerous large, broad, soft and relatively flat leaves, and multiflowered inflorescences. In one of the biotopes (Grandais), the medio-European character is even more perceivable by the simultaneous presence of hundreds plants of *Neottia nidus-avis*, a very rare orchid in Portugal, for which this is one of the only two localities known so far, and

perhaps the only one truly indigeneous (see the comments in TYTECA 1998). In the third locality (Serra da Estrela) the plants were a little smaller but still typical; one of them with a clearly distinct habit showed a trend towards autogamy (see next section).

These observations provide us with an additional incentive to pursue new research, in order to substantiate the separations within the *Epipactis helleborine/tremolsii/lusitanica* complex. The latter indeed had been distinguished so far mainly on ecological and morphological grounds; clearly, additional arguments, genetic and molecular, should help to clarify the links between the taxa from this complex. As referred above, other approaches could play an important role in plant biosystematic research, more especially as regards orchids.

The discovery of *Epipactis phyllanthes* G. E. Sm.

On the 7th July 1998 one of us (DT) prospected a site close to Vinhais (1,3 km W Fresulfe, UTM coordinates PG-73-19, prov. Trás-os-Montes e Alto Douro), which is one of the two Portuguese sites where *Cephalanthera rubra* (L.) Rich. had been observed. The biotope is a *Quercus pyrenaica* wood on a west-north-west slope at an altitude of 650 m (PINTO DA SILVA & TELES 1971). Although many plants growing there are mostly acidophilous (e.g., *Digitalis purpurea*, *Erica arborea*, *Pteridium aquilinum*, *Ulex europaeus*), locally calciphilous species are observed (such as *Helleborus foetidus* or *Ornithogallum pyrenaicum*). Probably it is in such spots with alkaline elements that *Cephalanthera rubra* had been found. The species did not show up this year, as seemed to be the case in others years as well.

A unique specimen of an *Epipactis* was found on that day, that obviously showed characteristics of an autogamous plant (see below). The fact was very surprising, because until now no autogamous *Epipactis* had been observed in Portugal, and the closest localities with such plants are to be found in northern Spain, very far from the border. These autogamous species, present in Spain, are *E. campeadorii* Delforge in the Province of Burgos and in Cantabria (DELFORGE 1995), *E. phyllanthes* G. E. Sm. in Cantabria (AEDO et al. 1984, 1985) and *E. muelleri* Godf. in the Province of Tarragona (TYTECA 1992).

The Portuguese plant exhibits the following characters: plant height 59.5 cm; four rather soft leaves, shining green, rather short (the largest leaves are the third and fourth starting from the stem basis, 5.7 x 3.7 cm and 7.0 x 3.2 cm, respectively); first leaf located high on the stem; stem completely green and glabrous, even uppermost; lower bracts large and much longer than the flowers. Inflorescence dense, compact, with 32 flowers, all vertically hanging almost against the stem, barely open, completely green when seen from outside. Ovary green, with very short and loose pubescence, with a yellowish green stalk. Lateral sepals green, $\pm 9 \times 4.5$ mm; petals greenish white, $\pm 8 \times 3$ mm. Rostellum gland absent, pollinia pulverulent; unflattened lip ± 8 mm long, with a well developed hypochilium, with greenish nectar zone; epichilium white, with two small basic humps, pointed, with the tip facing forwards. Clinandrium flat; stigma unretracted, with a \pm "normal" position.

We will not discuss here the identification at length. All enumerated characters are consistent with the identification as *Epipactis phyllanthes* (see, e.g., DELFORGE 1994, 1997). A character that we were unfortunately unable to check was the micro-denticulation of the leaf borders, because we didn't have the required material for adequate examination, being not prepared to such a discovery ! The question may arise as to the origin of that plant: *E. phyllanthes* is well-known for its considerable variability, probably associated with degenerescence processes (starting from *E. helleborine* s.l.), that may have occurred independently in various locations, which is associated with the description of many varieties [or even (sub-) species]. *Epipactis helleborine* is well present in neighbouring localities (see the previous section), but it seems unlikely that the degenerescence process occurred recently from these plants, because the morphological discontinuities are considerable. A possible explanation may therefore come from reproductive assurance. As BAKER (1955) stated, reproductive assurance plays a particularly important role in the establishment of new populations after long-distance dispersal. Species that are self-compatible are more likely to be successful long-distance dispersers than those that are not (HOLSINGER & STEINBACHS 1997). More generally, this factor may provide for a potential explanation of the very scattered distribution of *E. phyllanthes* (and other autogamous *Epipactis* species) in Europe.

Whatever the origin, this constitutes an addition to the Portuguese Flora, as well as the 56th indigeneous orchid species and the fifth Portuguese *Epipactis* (TYTECA 1997).

We end this section on autogamous *Epipactis* with the observation of an isolate plant in an *E. helleborine* population, in the Serra da Estrela as already mentioned in the previous section. The plant under consideration was rather small, with small leaves and few flowers opening slightly, with a poorly functioning rostellum and rather pulverulent pollinia. These aspects may remind of *E. gracilis* B. & H. Baumann, another European autogamous species, growing in similar mountain woody biotopes. However, the plant grew in the vicinity of a "normal" *E. helleborine* population, and due to its uniqueness and the absence of clear discontinuity, we will incorporate it in the variability of the local population. The fact was worth pointing out, however, at least to draw the attention of botanists to the possible discovery of other autogamous *Epipactis* taxa in Portugal. Possibly, there are many more autogamous *Epipactis* sp. in this country, especially in areas where the availability of pollinators is low. Changes in floral morphology that allow more self pollen to be deposited autonomously, for example, provide substantial assurance and could be favoured by natural selection, provided they do not significantly reduce opportunities for outcrossed pollination (HOLSINGER 1996). Any fitness advantage to be found in self fertilization will be found in its immediate impact on differences in individual survival and reproductive success (HOLSINGER & STEINBACHS 1997).

Conclusions: suggestions for protection

From the prospections reported in this paper, it results that an important biotope type would deserve active protection, namely the *Quercus pyrenaica* woods located in the north-east of the country (Trás-os-Montes). Indeed, these are now well documented as the habitats of three orchid species that are very rare in Portugal, i.e., *Cephalanthera rubra*, *Neottia nidus-avis* and *Epipactis phyllanthes*. It is also in this type of biotope, with clear medio-European characteristics very little represented in Portugal, that a species like *E. helleborine* exhibits characteristics mostly typical of populations found in median Europe.

There is an increasing need for research on genetics to allow efficient conservation and sustainable land use. As MARTINEZ & PARKER (1995) stated, there is also an acute requirement to define variation at the population level, with an emphasis on release on such variation. In difficult groups, such as *D. maculata* s.l., *Epipactis helleborine* and others, studies of the two components

of the genetic system *sensu* DARLINGTON (chromosome system and breeding system) (DARLINGTON 1937; MARTINEZ & PARKER 1995; HOLSINGER & STEINBACHS 1997), can be useful and give practical responses to the management problem of incorporating evolutionary processes into conservation.

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Legends of the pictures (slides D. TYTECA)

Plate 1: Four vertical slides

- A - *Dactylorhiza caramulensis* with influence of *D. elata*, Serra da Estrela, Lagoa Comprida, Seia, Beira Alta, 6/7/96.
- B - *Dactylorhiza ericetorum*, Serra do Larouco, Montalegre, Trás-os-Montes e Alto Douro, 12/7/98.
- C - *Epipactis helleborine*, Serra de Nogueira, Vinhais, Trás-os-Montes e Alto Douro, 8/7/98.
- D - *Epipactis helleborine*, Grandais, Bragança, Trás-os-Montes e Alto Douro, 7/7/98.

Plate 2: Four vertical slides

- A - *Epipactis* cf. *helleborine* with autogamous trend, Serra da Estrela, Manteigas, Beira Alta, 5/7/98.
- B, C, D - *Epipactis phyllanthes*, Fresulfe, Vinhais, Trás-os-Montes e Alto Douro, 7/7/98.

Plate 3: One horizontal slide

- Epipactis phyllanthes*, Fresulfe, Vinhais, Trás-os-Montes e Alto Douro, 7/7/98.