LANKESTERIANA 7(1-2): 187-189. 2007.

ALLOTETRAPLOID EVOLUTION IN *DACTYLORHIZA* (ORCHIDACEAE)

MARK W. CHASE[1,3], MICHAEL F. FAY[1], RICHARD BATEMAN[1], MIKAEL HEDRÉN[2] & YOHAN PILLON[1]

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, U.K.
[2]Department of Ecology, Plant Ecology and Systematics, University of Lund, Sölvegatan 37, SE-223 62 Lund, Sweden.
[3]Corresponding author: E-mail: m.chase@kew.org

KEY WORDS: *Dactylorhiza*, allopolyploidy, hybridization, ITS rDNA, gene conversion, plastid microsatellites

One of the most perplexing problems in Western European terrestrial orchid taxonomy has been how to deal with the large numbers of taxa that have been described for the allopolyploid taxa, which are the products of hybridization between taxa in the *Dactylorhiza maculata* (L.) Soó group and the *D. incarnata* (L.) Soó group (Table 1). The polyploidy hybrids are lumped under the category of *D. majalis* (Rchb.) P.F.Hunt & Summerh. s.l. (Table 1). By taking their ecology into account, it is clear that offspring from putatively the same parental taxa have different ecological preferences and been given taxonomic recognition as species by many authors. It has also been clear that within each of the parental complexes several distinct entities exist, again differing in their ecologies and morphology. Many of the named allotetraploid taxa are highly restricted, and therefore there are conservation implications if such taxa are foci of efforts to prevent them from disappearing. It is therefore appropriate to study such taxa, both on evolutionary and conservation bases.

To study these problems, we employed a genetic approach using two sets of markers, the nuclear ribosomal spacer regions (nrITS) and plastid microsatellites. These were first sequenced to determine if there were differences in length that could be used as characters, which was discovered to be the case. We then designed primers to amplify short fragments (140-200

TABLE 1. General taxonomy and distribution of Western European species of *Dactylorhiza*.

Ploidy	Species	Distribution
diploid	*D. foliosa*	Madeira
diploid	*D. fuchsii*	Western Europe, North Africa and Western and Central Asia; in the east replaced by *D. saccifera*
diploid	*D. saccifera*	Italy, Greece, the Balkans
diploid	*D. incarnata*	Western Europe, North Africa and Western and Central Asia
diploid	*D. euxina*	Near East
diploid	*D. sambucina*	Sweden to southern France east to Greece and Eastern Europe
diploid	*D. (Coeloglossum) viridis*	North Temperate Zone
auto-tetraploid	*D. maculata*	Western Europe, but difficult to separate from *D. fuchsii* in Central and Eastern Europe and rare in southern Europe
Allo-tetraploid*	*D. majalis* s.l. (including *alpestris*, *elata*, *occidentalis*, *praetermissa*, *purpurella*, *sphagnicola*, *traunsteineri* etc.)	Broadly distributed in Europe, Asia, and with a limited distribution in North America and Iceland ; some *s. s.* taxa with localized distribution (e. g. *occidentalis* only in western Ireland)

*Thought to have originated as crosses between the *D. incarnata* group and the *D. maculata* group, but exact parentage highly speculative and the major focus of this study.

base pairs) that contained the length-variable regions, and then these differences could be assessed by the length of the amplified fragments. This made the process quick. The short length of the amplified fragments also meant that it was possible to use DNA extracted from herbarium specimens; DNA from such sources was typically found to be highly degraded. Plastid DNA can demonstrate which of the parental taxa is the maternal parent of the hybrids. ITS rDNA is part of the nuclear genome and inherited biparentally, but hybrids soon begin a process of gene conversion and lose one of the two parental copies that they initially possessed. For fairly recently synthesized hybrids, both parental ITS alleles are present, but for older hybrids only one of these alleles remains. We have used these two sets of markers to dissect the complex patterns of morphology and ecology; the process of gene conversion in nrITS provides a relative timescale for the ages of the various groups of allopolyploids (Pillon *et al.* 2007).

It was relatively easy in western Europe to find genetic differences between the two most common species of spotted orchids, *D. fuchsii* (Druce) Soó and *D. maculata*. These species and the *D. incarnata* complex are also easily distinguished by both sets of markers. These are also ecologically and morphologically easily separated, but in eastern Europe *D. fuchsii* and *D. maculata* are difficult to distinguish on morphological grounds. All material labelled as *D. maculata* from Austria and Germany that we examined are recent hybrids; they exhibit nearly equal amounts of the two ITS alleles found in western Europe in *D. fuchsii* and *D. maculata*. We do not know the ploidy of these plants, but we suspect that they will turn out to be allotetraploids, as Shipunov *et al.* (2004) found in similar plants in northern Russia. These plants grow in acid sites, which in western Europe would contain *D. maculata*, and the morphology of these plants is intermediate between those of the two parents. We do not consider that *D. maculata* occurs in central and eastern part of Europe.

Allotetraploids that originated from crosses between *D. fuchsii* and *D. maculata* and members of the *D. incarnata* group are some of the most common and conspicuous orchids in Europe, and these too exhibit morphological and ecological differences. In addition to *D. incarnata* (almost always the paternal parent), one set of these was parented by an unknown diploid species, most likely found in southern Europe and similar to *D. foliosa* (Rchb.f.) Soó from Madeira and *D. maculata*. The correct name for these allotetraploids is *D. elata* (Poir.) Soó. These are older allotetraploids, and they almost always contained just the *D. maculata* allele without any remaining copies of *D. incarnata*. In Ireland, we found another allotetraploid, *D. occidentalis* (Pugsley) P.Delforge, that had exactly this same parentage, but these accessions contained both parental alleles, sometimes exhibiting conversion toward the *D. incarnata* allele. This, then, is a more recently formed allotetraploid than *D. elata*. Another set of allotetraploids was formed from a species similar to extant *D. fuchsii*, with older, *D. majalis* s.s., and younger, *D. traunsteineri* (Saut. ex Rchb.) Soó, forms.

Many authors consider *D. maculata* and *D. fuchsii* to be subspecies (of *D. maculata* s.l.), but nearly all authors agree that *D. foliosa* is distinct from *D. maculata* s.l. on morphological and ecological grounds. Although it is true that numerous hybrids occur between these two throughout their ranges (and in eastern and central portions of Europe this not pure *D. maculata*), this appears to be a recent phenomenon. None of the allotetraploids we examined exhibited mixtures of the *D. maculata* and *D. fuchsii* ITS alleles. These two entities are easily distinguished morphologically, and *D. fuchsii* is a calcicole whereas *D. maculata* is a calcifuge. Our results do not distinguish genetically between *D. foliosa* and *D. maculata*, so if authors wish to consider *D. maculata* and *D. fuchsii* as subspecies, then *D. foliosa* must also be considered as a subspecies of *D. maculata* s.l. Results from analysis of nuclear, low-copy chalcone synthase (L. Inda and M. Chase, unpubl.) indicate that the entity that gave rise to *D. elata* was closer to extant *D. foliosa* than to *D. maculata*. This finding points again to the fact that there must have been (or perhaps still is) a diploid species of the *D. maculata/foliosa* type somewhere in southern Europe and that *D. maculata* s.s. is not a parent of the *D. elata* allotetraploids that populate modern Europe. We have not yet examined the parentage of *D. occi-*

TABLE 2. Recommended framework classification of European members of the *D. incarnata* and *D. maculata* groups and their derived polyploid complex. The plastid haplotype and ITS allele(s) given here are considered typical of each taxon. This summary focuses on well-established species, incorporating regional endemics but excluding many local endemics.

Taxon	Ploidy and parentage	Plastid haplotype	ITS allele(s)
D. fuchsii (inc. *cornubiensis*, *okellyi*)	2X	A	V, IIIb
D. maculata (inc. *ericetorum*, *elodes*)	4X (autotetraploid)	B	I
D. saccifera	2X	C, G, W	VI
D. incarnata s.l. (all W European taxa)	2X	E	Xa
D. euxina	2X	Y, K	Xb
D. elata (North Africa)	*maculata* × *incarnata*	O	IIIa, completely converted
D. elata (Europe)	*maculata* × *incarnata*	B	I, most accessions completely converted
D. occidentalis (inc. *kerryensis*)	*maculata* × *incarnata*	B	I dominant, X in 1/3 or fewer copies
D. sphagnicola	*maculata* × *incarnata*	B	Xa dominant, I in 1/3 or fewer copies
D. majalis (inc. *alpestris*)	*fuchsii* × *incarnata*	A, C	V, IIIb, most accessions completely converted
D. praetermissa (inc. *junialis*)	*fuchsii/saccifera* × *incarnata*	A, C	V, IIIb, VI, often with VI dominant
D. traunsteineri (inc. *lapponica*)	*fuchsii* × *incarnata*	A, C	V, IIIb, rarely with Xa dominant
D. purpurella (inc. *cambrensis*)	*fuchsii* × *incarnata*	A	V, IIIb, rarely with Xa dominant

dentalis in the same detail as for *D. elata*, but this is underway. We present (Table 2) a provisional taxonomy for Western European *Dactylorhiza*, based on the results of this and other studies.

All across Europe, there are many sites where these species, diploids and tetraploids coexist, and in many cases researchers have been tempted to think that the hybrids arose locally. However, all of our evidence indicates that the hybrids arose elsewhere, further south in Europe, and migrated along with the diploid progenitors to their current localities. There is no context for studying any of these species on a regional scale to understand better their origins – they must be studied broadly.

Their conservation also calls for a unique strategy. Again, since they did not arise where they now grow and they arose repeatedly from the same parental taxa, the process should be the focus of conservation efforts. Rather than conserving taxa, in *Dactylorhiza* it seems more appropriate to preserve the habitats where hybridization has been occurring, but knowing that few hybrids are currently being formed in northern Europe means that more attention must be focused on appropriate sites in southern Europe where in general conservation efforts have not been as successful in the past.

LITERATURE CITED

Pillon, Y., M.F. Fay, M. Hedrén, D.S. Devey, A. Shipunov, M. van der Bank, R.M. Bateman & M.W. Chase. 2007. Insights into the evolution and biogeography of western European species complexes in *Dactylorhiza* (Orchidaceae). Taxon: in press.

Shipunov, A.B., M.F. Fay, Y. Pillon, R.M. Bateman & M.W. Chase. 2004. *Dactylorhiza* (Orchidaceae) in European Russia: combined molecular and morphological analysis. Amer. J. Bot. 91: 1419-1426.

Mark Chase received his undergraduate degree from Albion College, Michigan and his Ph.D. was from the University of Michigan (Ann Arbor) in 1985. His thesis was a monograph of *Leochilus* (Orchidaceae). He carried out post-doctoral research in molecular biology with Jeff Palmer at the University of Michigan. He then moved to the University of North Carolina and then after four year to the Royal Botanic Gardens, Kew, where he set up the program in molecular systematics. He became a member of the Royal Society in 2003 and Keeper (Director) of the Jodrell Laboratory in 2006.