

STUDIES ON WELSH ORCHIDS

II. THE OCCURRENCE OF *DACTYLORCHIS MAJALIS* (REICHB.

VERMEUL. IN WALES

By R. H. ROBERTS

ABSTRACT

Two colonies of heavily leaf-marked marsh orchids occur in Wales : one at Ynyslas, Cardiganshire, v.c. 46, and the other in Newborough Warren Nature Reserve, Anglesey, v.c. 52. The plants of both these colonies have been shown to be fully-fertile tetraploids with $2n = 80$. A biometric study has shown that these colonies are practically identical with one another.

Comparison with herbarium material has shown that they are closely allied to the continental *D. majalis* (Reichb.) Vermeul., under which they are placed as a new subspecies *D. majalis* subsp. *cambrensis*.

Some evidence is adduced that the tetraploid marsh orchid species of the subsection *Majales* do not always interbreed freely where they co-exist.

INTRODUCTION

At two localities in Wales there are colonies of marsh orchids which show some affinity with *Dactylorchis purpurella* and, at first sight, might be taken to be hybrids of that species with one of the *D. maculata* aggregate. Both populations consist of heavily leaf-marked plants, with flowers which are, on the whole, considerably larger and of a lighter purple than those of *D. purpurella*.

The first of these, M1, occurs at Ynyslas, Borth, Cardiganshire, v.c. 46, and was included by the Stephensons (1921) under their '*Orchis latifolia*.' The plants grow in the very wet marshy area between the railway and the shore, with some *Phragmites communis*, *Agrostis stolonifera*, *Festuca rubra*, *Hydrocotyle vulgaris* and *Lotus pedunculatus*. The only other species seen in the area was *D. maculata* subsp. *ericetorum*.

The second colony, M2, was discovered by the writer, in 1956, in the marshy tract adjoining the salt marsh of the Cefni estuary, on the north side of the Newborough Warren Nature Reserve, Anglesey, v.c. 52. Associated species here are : *Equisetum palustre*, *Equisetum fluviatile*, *E. variegatum*, *Ranunculus flammula*, *Hypericum tetrapterum*, *Lychnis flos-cuculi*, *Lotus pedunculatus*, *Filipendula ulmaria*, *Parnassia palustris*, *Hydrocotyle vulgaris*, *Oenanthe lachenalii*, *Anagallis tenella*, *Prunella vulgaris*, *Epipactis palustris*, *Dactylorchis fuchsii*, *D. incarnata*, *D. purpurella*, *Festuca rubra* and *Agrostis stolonifera*.

The possibility that the plants of both these populations were hybrids could not be disregarded. This was especially so in the case of the Anglesey plants since *D. fuchsii* and *D. purpurella* also occur in the same area and these two species readily produce hybrids, sometimes in large numbers, wherever they occur together.

It appeared, therefore, to be a matter of some interest to determine the status of these heavily leaf-spotted plants and to ascertain their relationship with one another and with *D. purpurella* as found in Wales. Accordingly, in the seasons 1958 and 1959 the cytology and fertility of the two populations were investigated and a biometric study of them carried out.

METHODS

All measurements of vegetative and floral parts, as well as the counts of the total number of leaves per plant and the number of non-sheathing leaves, have been made in the manner described in a previous study (Roberts, 1961).

Leaf measurements were taken from the second leaf from the base of the stem. In counting the number of leaves per plant, all fully developed leaves were counted, including the upper, non-sheathing ones. A separate count of the latter was also made, every leaf

which did not form a sheath being counted, regardless of its size.

The significance of the differences between means was determined on the basis of 't' tests. All differences significant at $p = .05$.

CYTOLOGY AND FERTILITY

Cytological examination has shown that meiosis in plants from both localities is quite regular and a chromosome number of $n = 40$ was observed.

In several instances pollen mitosis was examined and was also found to be perfectly regular. The chromosomes were frequently sufficiently distinguishable to be counted at this stage also and the same number was again seen.

In addition, and in order to cover as large a sample as possible of the plants in these colonies, the technique of observing pollen fertility described by Heslop-Harrison (1954) was employed. Pollen from 30 plants was examined and showed very high fertility, the cells in all of the tetrads having uniformly two nuclei of slightly different sizes.

Later in the season well-swollen ovaries were gathered from marked plants and showed seed fertilities of up to 97%. The range of values obtained was similar to that found in *D. purpurella*; in samples of the latter, from two localities in Anglesey, fertilities of up to 95% and 93% respectively were observed.

It is therefore abundantly clear that these heavily leaf-marked plants from both the Cardiganshire and Anglesey colonies are fully fertile tetraploids with a chromosome number of $2n = 80$.

VEGETATIVE CHARACTERS

The sample data for stature, leaf number, number of upper, non-sheathing leaves,

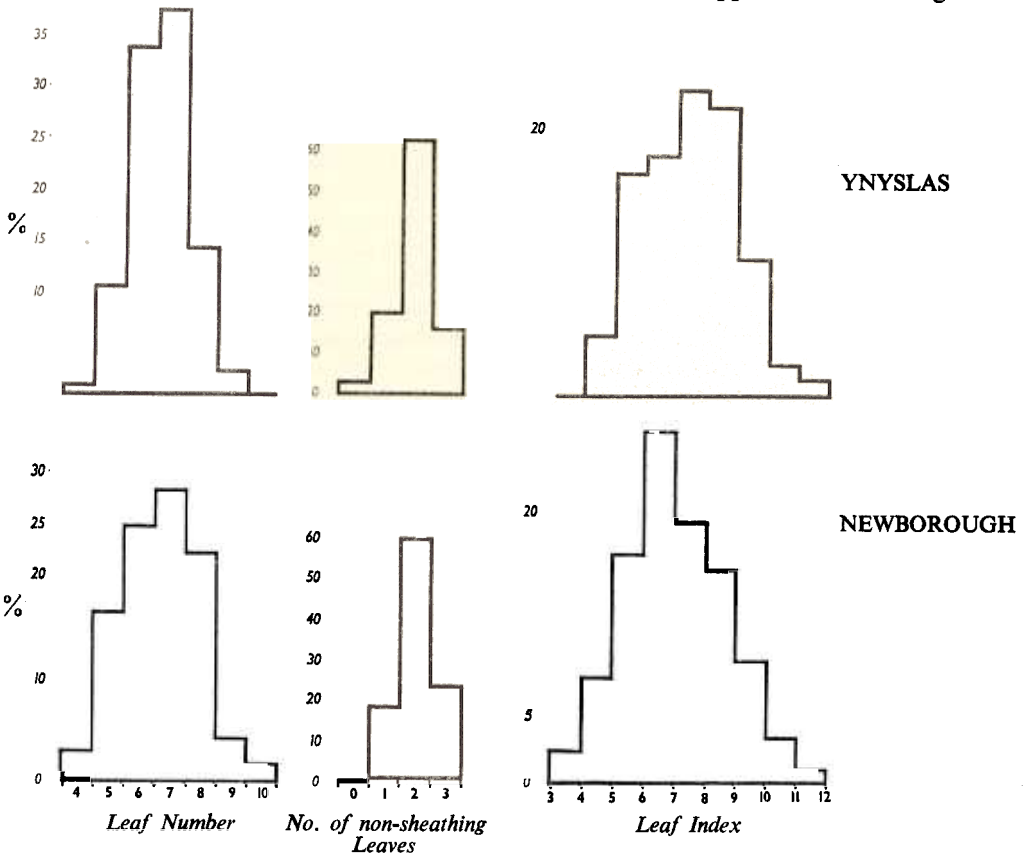


Fig. 1 Histograms of leaf number, number of non-sheathing leaves, and leaf index in the two populations.

and leaf dimensions are given in Tables 1 and 2. It will be seen that the sample means for all of these characters agree very closely in the two populations : in all except leaf ratio the very small differences between them are not statistically significant. The histograms, Fig. 1, indicate the close similarity of the two colonies in vegetative characters.

TABLE 1
Sample data for stature, leaf number, and number of non-sheathing leaves.
N = number in sample; M = mean; S.E.M. = standard error of mean.

Locality	N	Stature (cm.)		Total number of leaves		No. of non-sheathing leaves		Non-sheathing leaves / Total leaves %
		M	S.E.M.	M	S.E.M.	M	S.E.M.	
M1	85	28.0	.53	6.6	.10	1.9	.06	28.8
M2	92	27.3	.57	6.8	.13	2.0	.06	29.4

TABLE 2
Sample data for leaf dimensions and leaf index. Symbols as in Table 1

Locality	N	Leaf length (cm.)		Leaf width (cm.)		Ratio $\frac{\text{Leaf length}}{\text{Leaf width}}$	
		M	S.E.M.	M	S.E.M.	M	S.E.M.
M1	85	12.4	.24	1.7	.04	7.3	.16
M2	92	12.3	.26	1.8	.05	6.8	.17

Leaf marking in both populations consists of heavy, purple-brown, solid spots or blotches on the upper surface of the leaves and is very similar to that found in the Continental *D. majalis* and its Irish subspecies *occidentalis*. The spots are distributed over the whole of the leaf surface and not restricted to any particular part of it, as is generally the case in *D. purpurella*. There is some variation in the degree of leaf marking, from rather sparse and few spots to very many and intense, so that occasionally they tend to run together and cover most of the leaf surface. But in both of these colonies the size of the spots is fairly uniform and, in general, about 3 mm. diameter.

As in most populations of the tetraploid marsh orchids, a proportion of these plants have no leaf marking. In these colonies, however, the percentage of plants with no leaf marking is low : about 7% in the Ynyslas colony (M1) and about 18% in the Newborough colony (M2).

Leaf shape in the two colonies is also identical, the leaves being narrowly lanceolate, more or less keeled and folded, widest at or just below the middle, with a subacute, very slightly hooded apex. The ratio mean leaf-length/mean leaf-width is considerably higher than in any of the *D. purpurella* populations for which figures are available : 6.8 in the Anglesey colony, M2, and 7.3 in the Cardiganshire colony, M1. In *D. purpurella* this ratio varies from 3.2 to 6.1 (Roberts, 1961). In this respect these heavily leaf-marked colonies show a clear difference from all the *D. purpurella* populations and this feature is one which is quite noticeable in the field.

The habit of the plants on the whole resembles that of *D. purpurella*, with the exception that in a small proportion there is a greater tendency for the lower two leaves to be arcuate-recurved.

FLORAL CHARACTERS

Table 3 gives the sample data for labellum and spur dimensions in these two colonies.

The very slight difference between them in labellum width is not statistically significant. In labellum length, however, they differ by a significant amount, but this is no doubt due to the much more elongated mid-lobe of the labellum in the Cardiganshire plants. The labellum dimensions, particularly of the Anglesey colony, differ very little from those of an Irish colony of *D. majalis* subsp. *occidentalis* (Heslop-Harrison, 1953).

Flower colour and labellum pattern in the two colonies agree very closely. The colour of the flowers is on the whole a lighter red-purple than in *D. purpurella* and the heavy labellum pattern is consequently more conspicuous. In both populations labellum pattern, consisting of irregular dark lines and dots, often arranged more or less in two concentric broken loops, is more variable than in most populations of *D. purpurella* and shows the same range of variation in both of them.

TABLE 3
Sample data for labellum and spur dimensions. Symbols as in Table

Locality	N	Labellum length (cm.)		Labellum width (cm.)		Spur length (cm.)		Spur width (cm.)	
		M	S.E.M.	M	S.E.M.	M	S.E.M.	M	S.E.M.
M1	86	.84	.007	1.04	.010	.79	.007	.33	.003
M2	118	.79	.007	1.06	.009	.87	.008	.32	.004

The basic labellum shape in the two colonies appears to be much the same. In the Ynyslas colony (M1), however, the lateral lobes tend to be more rounded and entire than in the Newborough colony (M2), where they are angled and incised (Plate 2). In this respect the latter approach the labellum shapes found in *D. majalis* both on the Continent and in Ireland. Another feature which can only be seen in the field is that in most individuals the lateral lobes of the labellum are slightly reflexed.

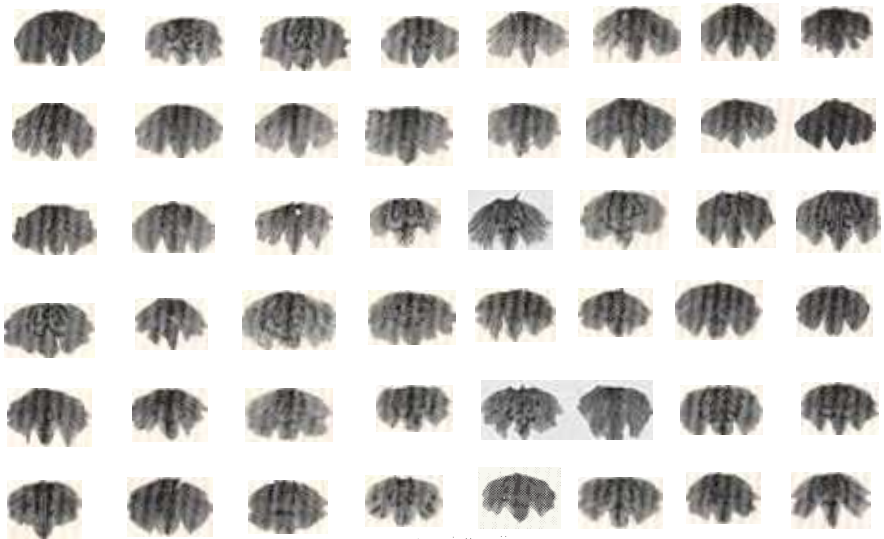
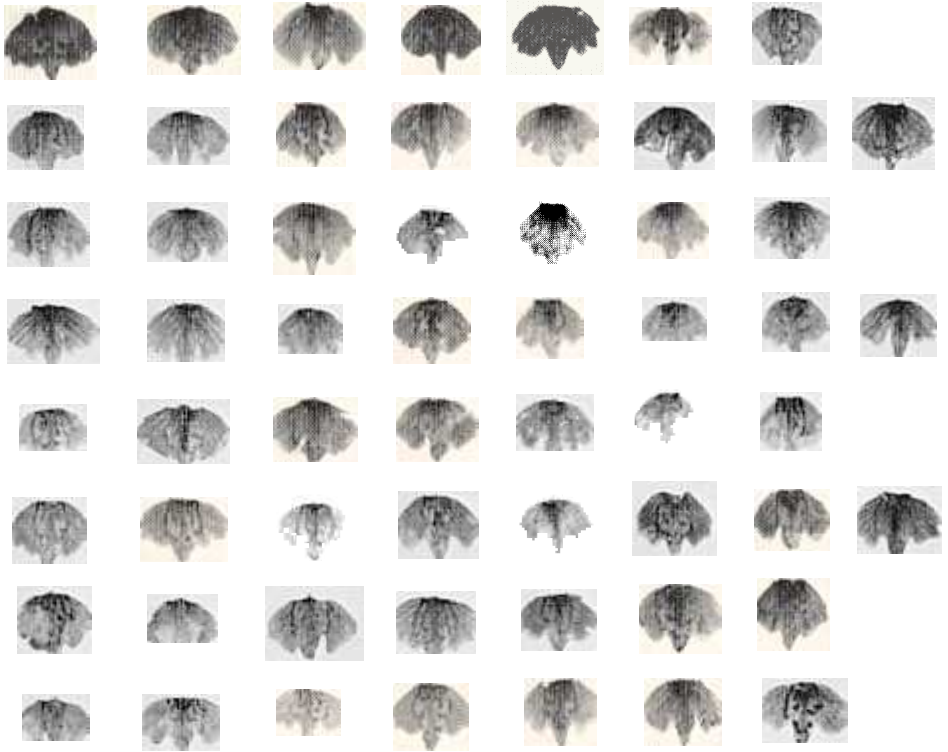
Mean spur width in the two colonies differs by a small but statistically significant amount, but there is a large difference in spur length. However, there are just as large differences in mean spur length between some populations of *D. purpurella* (Roberts, 1961) and it seems that this is a normal result of population divergence. Indeed, it is clear that in spur dimensions the plants of these two colonies differ very little from the range of variation found in *D. purpurella*. The Irish subsp. *occidentalis*, on the other hand, has a much slenderer spur, which, in some plants, is curved about a third of its length from the tip (Heslop-Harrison, 1953). In the majority of herbarium specimens of Continental *D. majalis* examined at the British Museum and at Kew there was no evidence of the slenderness or of the curvature of the spur shown by the Irish plants. Where spur width in the herbarium material could be measured it was around .30 cm in most instances. If an allowance of only 5% is made for shrinkage during drying it will be seen that this is well within the range of spur width in the two Welsh populations.

FLOWERING TIME

The Continental *D. majalis* and its Irish subspecies are both early flowering, reaching a peak in late May and early June (Pugsley, 1935; Vermeulen, 1949), though it should be noted that the flowering period of the Irish plants may extend into July (Heslop-Harrison, 1954). The plants of the Welsh populations flower with *D. purpurella* in June and July: the first plants of the Ynyslas colony (M1) were coming into flower on 7th June, 1958, and those of the Newborough colony (M2) on 3rd June, 1959. This is about a fortnight later than *D. traunsteineri*, the earliest of the marsh orchids to flower in Anglesey. In the seasons 1958 and 1959 this has been found to commence flowering around the 15th of May.

It appears, therefore, that the Welsh plants are later flowering than Continental and Irish *D. majalis*. There are, however, populations in western Scotland and the Hebrides, practically indistinguishable from some of *D. majalis* subsp. *occidentalis* in western Ireland,

PLATE



Random samples of labella from homologous flowers in the two populations.
(a) 64 plants from the Cardiganshire colony, M1;
(b) 48 plants from the Anglesey colony, M2. Both $\times 1$.

which, like the Welsh plants, also flower with *D. purpurella* in June and July (Hall, 1937; Campbell, 1937; Heslop-Harrison, 1954).

CONCLUSIONS

It is clear from the sample data that these two colonies of heavily leaf-spotted plants agree remarkably well with one another in vegetative characters; while the small differences in labellum dimensions, in the average expression of labellum shape, and in spur dimensions, are no greater than between several of the *D. purpurella* populations in North Wales (Roberts, 1961).

They also show close agreement in flower colour, labellum pattern, spur shape and in the position of the lateral sepals, which are well reflexed.

A comparison with herbarium material of Continental *D. majalis* (Reichb.) Vermeul. and of its Irish subspecies *occidentalis* (Pugsley) H.-Harr. f., preserved in the British Museum and at Kew, justifies the inclusion of the Welsh plants under *D. majalis*. From the commonest Continental form of *D. majalis* the Welsh plants differ in possessing very much longer and narrower leaves, denser spikes of rather smaller flowers, and shorter bracts, only the lowest of which are longer than the flowers. They also differ in their habit, which more nearly resembles that of *D. purpurella*, the leaves being for the most part less spreading and more rigid. But there is some variation in this character and a small proportion of the Welsh plants, especially in the Anglesey colony, have more spreading, arcuate-recurved leaves reminiscent of some of the Irish specimens.

They differ from the Irish subsp. *occidentalis* not only in having much longer, narrower and more rigid leaves, but also in having much thicker spurs.

Among the Continental material in the British Museum and at Kew there are gatherings from the Valley of Durance, Hautes Alpes, made by M. S. Campbell in 1937, and from Thorenc, in the Alpes Maritimes, collected by D. P. Young in 1949. Two sheets of the former gathering had been labelled by H. W. Pugsley "cf. *majalis*" and "*O. majalis* (narrow leaved)" respectively; the latter gathering had been named "*O. majalis* Rchb." All of these show a very close approach to the Welsh plants, from which they seem to differ only in having longer bracts and laxer spikes of somewhat larger flowers.

Because of the clear, though minor, differences in morphological characters from *D. majalis*, both on the Continent and in Ireland, and because they occur in a separate geographical area, the most appropriate category for these Welsh plants seems to be that of a new subspecies of *D. majalis*. The name *Dactylorchis majalis* subsp. *cambrensis* is proposed for them.

Dactylorchis majalis* subsp. *cambrensis R. H. Roberts *subsp. nov.* A subsp. *majalis* foliis multo longioribus (c. 12.4 × 1.7 cm.), bracteis brevioribus infimis quam flores longioribus, spicis densioribus, floribus minoribus et aetate florendi (Iunio vel Iulio) differt. Praeterea a subsp. *occidentali* (Pugsley) H. Harr. f. foliis longioribus angustioribus erectioribus, nectariis pinguioribus et aetate florendi distinguitur. Chromosomata : 2n = 80. Habitat : paludes udissimae prope litus Cambriae.

Holotype : marsh along the Cefni estuary, Newborough Warren Nature Reserve, Anglesey, v.c. 52, June, 1959, R. H. Roberts, in Herb. Kew.

Its present known distribution is confined to two vice-counties : 46 and 52.

Its occurrence in other localities in Wales and possibly in England is by no means improbable. There are specimens in the herbarium of the British Museum, from a Hampshire locality, which are intermediate in many respects between the Welsh plants and *D. praetermissa* var. *junialis*. Pugsley had examined these plants and said that they were not exactly his "*Orchis pardalina*," but near it. This was also appreciated by P. M. Hall and A. J. Wilmott, who, between themselves, referred to these Hampshire plants as "*pardalinoidea*."

The labella in these are broader in relation to their length than they are in *D. praetermissa*, with lateral lobes which are notched and angled, and a labellum pattern consisting of broken loop markings rather than the continuous double loops characteristic of

var. *junialis*. Leaf marking, too, mostly consists of solid spots and blotches as in *D. majalis*, though in some specimens these are mixed with larger, ringed spots.

Whether or not the Welsh plants are connected by intermediates on the one hand with the Continental *D. majalis*, and on the other, through the var. *junialis*, with *D. praetermissa*, they are themselves morphologically distinct enough to merit the status given to them in this paper.

HYBRIDITY

Within the same area as one of the colonies described here another tetraploid marsh orchid, *D. purpurella*, also occurs. This raises the question of the interfertility of the tetraploid members of the subsection *Majales*. Heslop-Harrison (1954) has shown that they are inter-fertile to the extent of producing a viable F1, but the fertility of the F1 generation itself has not been demonstrated.

The evidence available for this Anglesey colony (M2) suggests quite clearly that complete intergradation with *D. purpurella* does not occur here. The fact that this colony (M2) agrees so well morphologically with the Cardiganshire colony (M1), where *D. purpurella* does not occur, is, presumably, sufficient indication that it is not influenced to any great extent by introgression with *D. purpurella*.

That all the tetraploid species of marsh orchids are not capable of completely intergrading is also suggested by the co-existence at three localities in Anglesey of *D. purpurella* and *D. traunsteineri*. No plants of an intermediate character between these two species occur at any of these localities and a biometric study of the two species in these stations gives no indication that introgressive hybridisation has taken place.

It has been suggested that the tetraploid marsh orchid species interbreed freely in many areas where they meet (Heslop-Harrison, 1954), but the evidence from these Welsh localities certainly suggests that this is not always the case.

ACKNOWLEDGEMENTS

I would like to thank Dr. A. D. Bradshaw of the University College of North Wales, Bangor, for his generous help; Dr. W. S. Lacey, also of the University College, Bangor, for his interest in the work and for his helpful criticism of the manuscript; and my colleague Miss A. E. Williams for drawing my attention to the Cardiganshire plants.

I am also indebted to the authorities of the herbaria at the British Museum (Natural History) and at the Royal Botanic Gardens, Kew, for facilities to study herbarium material of British and Continental dactylorchids.

REFERENCES

- CAMPBELL, M. S. (1937). Three weeks botanising in the Outer Hebrides. *Rep. Bot. Soc. & E.C.*, **11**, 304-318.
- HALL, P. M. (1937). The Irish Marsh Orchids. *Rep. Bot. Soc. & E. C.*, **11**, 330-354.
- HESLOP-HARRISON, J. (1953). Studies in *Orchis* L. II. *Orchis traunsteineri* Saut. in the British Isles. *Watsonia*, **2**, 371-391.
- HESLOP-HARRISON, J. (1954). A Synopsis of the Dactylorchids of the British Isles. *Ber. Geobot. Forsch. Inst. Rübel, Zurich*.
- PUGSLEY, H. W. (1935). On Some Marsh Orchids. *J. Linn. Soc. Bot.*, **49**, 553-592.
- ROBERTS, R. H. (1961). Studies on Welsh Orchids. I. The variation of *Dactylorchis purpurella* (Steph.) Vermeul. in North Wales. *Watsonia*, **5** (1).
- STEPHENSON, T. & T. A. (1921). *Orchis latifolia* in Britain, *J. Bot. Lond.*, **59**, 1-7.
- VERMEULEN, P. (1949). Varieties and forms of Dutch orchids, *Ned. Kruidk. Archief*, **56**, 225-242.