STUDIES ON WELSH ORCHIDS.

III. THE COEXISTENCE OF SOME OF THE TETRAPLOID SPECIES OF MARSH ORCHIDS.

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ABSTRACT

Studies of mixed populations of some of the tetraploid marsh orchids have shown that in some Welsh localities there are barriers to gene-exchange between the coexisting species.

There is conclusive evidence of a complete sterility barrier between *Dactylorhiza traunsteineri* and *D. purpurella* at four different localities. In a mixed population of *D. majalis* and *D. purpurella* the two species remain distinct, and the evidence suggests that little if any gene-flow has occurred between them. Elsewhere it appears that some introgression may have taken place between *D. praetermissa* and *D. purpurella*, but even there the two species have not become completely merged.

It is suggested that barriers to interbreeding may not be equally effective between all of the species. Possibly they may not everywhere be as complete even between the same two species.

INTRODUCTION

The taxonomic problems of the Dactylorchids in the British Isles have received a great deal of attention over the last fifty years. On occasion they have even been the subject of sharp controversy. A comparison of the treatment of this group by Bentham & Hooker (Ed. 7, 1924) with those of Clapham in the first and second editions of the *Flora of the British Isles* (Clapham, Tutin & Warburg 1952, 1962) gives some indication of the very considerable changes which have been made—many of them within the last twenty years.

The difficulties of this group have been due partly to the critical nature of the forms within it, as well as to the fact that the species themselves display a bewildering variability. This is to be found not only between one population and another, but within the populations themselves. It is only by the recent application of biometric and cytological techniques—mainly by J. Heslop Harrison—that a fuller understanding of the British forms of this genus has come about.

The genus *Dactylorchis* was divided by Vermeulen (1947) into three main sections, but only one of these, the section *Maculatae* (Parl.) Vermln., occurs in the British Isles. This has been further divided by Heslop Harrison (1954) into three sub-sections: *Incarnatae* Vermln., *Eumaculatae* Vermln., and *Majales* (Pugsley) H. Harr. f. Of these the most difficult taxonomically is the last. The variation within the sub-section *Majales* is often extremely localized. It is therefore not surprising that systematists have held widely differing opinions as to the number of species involved, or where the boundaries separating them should be drawn.

In Britain the interest aroused by the description of two new species: *D. praetermissa* by Druce (1914) and *D. purpurella* by T. & T. A. Stephenson (1920), was further increased by Pugsley's announcement of the discovery of two additional new forms in Ireland (Pugsley 1936), and resulted in a period of active field work. While this added considerably to our knowledge of these plants, the orthodox methods of the herbarium taxonomist proved inadequate to deal with the enormous variation found among the forms of the sub-section *Majales* in the field. The result was the making of several new 'species', among which *Orchis pardalina*, *O. kerryensis*, *O. occidentalis*, *O. traunsteinerioides* and *O. francis-drucei* will be recalled.

In the British Isles four main entities are at present recognized in this sub-section:

Dactylorhiza majalis (Reichb.) P. F. Hunt & Summerh.*
D. traunsteineri (Saut.) Soó
D. praetermissa (Druce) Soó
D. purpurella (T. & T. A. Stephenson) Soó

These are treated as species though their relationships with one another are not yet fully understood (Heslop Harrison 1954; Clapham 1962). The occurrence of populations morphologically intermediate between some of them has led to the assumption that they are not separated by inherent sterility barriers, but depend for their continued existence as separate entities on geographical and ecological factors (Clapham 1962). In certain localities there is said to be evidence of complete interfertility, as, for example, in a mixed population of *D. traunsteineri* and *D. praetermissa* in Norfolk (Heslop Harrison 1954), and in some localities in the north of England where *D. traunsteineri* is stated to have been 'hybridized out of existence' by *D. purpurella* (J. W. Heslop Harrison & J. A. Richardson 1953). If this is the case, then it would be reasonable to treat all four entities as subspecies of an aggregate species *D. majalis*—a treatment which Clapham (1962) has already gone some way towards adopting. The possession by all of them of the same chromosome number ($2n = 80$) would also appear to have encouraged the acceptance of such a view.

On the contrary, at several localities in Wales two of these species coexist in close association, but appear to remain specifically distinct: *D. traunsteineri* with *D. purpurella* at five separate places in Anglesey and Caernarvonshire, *D. majalis* with *D. purpurella* at one locality in Anglesey (Roberts 1961b), and *D. praetermissa* with *D. purpurella* at a Merionethshire locality (Benoit & Richards 1963). These observations were not in accord with the complete interfertility of the various species, and biometric studies were made in an attempt to clarify some aspects of this problem.

Methods

Several of the morphological characters which are used to differentiate the species were studied by the usual biometric methods. The mean values for each sample were then plotted along five axes, and the points were joined to form a polygon, combining the data from each species population into a single visual expression. Comparison of the polygons then shows at a glance how the various populations resemble or differ from one another.

Results

D. traunsteineri *and* D. purpurella

D. traunsteineri and *D. purpurella* are found growing intermixed or in closely adjacent habitats at four separate places in Anglesey (v.c. 52) and at one in Caernarvonshire (v.c. 49). At two of the Anglesey localities both species occur in such close proximity as to afford every opportunity for cross-pollination to have taken place. They also grow in sufficiently large numbers to make a biometric study worth while; and, moreover, the boundaries of the selected populations coincide closely with the limits of the sampling areas.

The vegetative characters studied were mainly those which have been used to separate these two species, namely, the total number of leaves, the number of non-sheathing leaves, and the ratio of leaf length to leaf width. The flower characters chosen were the length and width of the labellum, the product of these two being used as an approximate index of labellum size. Since in many populations of *D. traunsteineri* the number of flowers in the inflorescence is remarkably low, a comparison of this character in the two species was also made.

In order to eliminate the possibility that the sample data might not be sufficiently representative of these populations, sampling was repeated during a second season, but no

*This name is taken here in a wider sense than that of Hunt and Summerhayes (1965), to include *Orchis kerryensis* Wilmott, *O. occidentalis* Pugsl. and *Dactylorchis majalis* subsp. **cambrensis** R. H. Roberts, *Watsonia* **5**, 37–42 (1961). This last plant now becomes *Dactylorhiza majalis* (Reichb.) P. F. Hunt and Summerh. subsp. **cambrensis** (R. H. Roberts) R. H. Roberts **comb. nov.**

significant differences were found between the two lots of data so obtained. The results can therefore be regarded with some confidence.

From the sample data (Tables 1 and 2) the large and highly significant differences in these characters between the two species are clearly evident, despite their close association

TABLE 1. Sample data for vegetative characters of *D. traunsteineri* and *D. purpurella* from two mixed populations.

	N	*Total number of leaves*		*No. of non-sheathing leaves*	*Leaf length (cm)*		*Leaf width (cm)*		*Mean length / Mean width*
		mean	*s.e.m.*	*mean*	*mean*	*s.e.m.*	*mean*	*s.e.m.*	
PENTRAETH									
D. traunsteineri	50	3·7	·08	0·6	8·4	·22	1·0	·03	8·4
D. purpurella	54	6·1	·13	1·5	9·1	·23	1·6	·05	5·7
CORS ERDDREINIOG									
D. traunsteineri	101	3·9	·06	0·7	10·4	·21	1·0	·02	10·4
D. purpurella	50	6·0	·14	1·3	9·9	·25	1·9	·06	5·2

TABLE 2. Sample data for labellum dimensions, labellum-size index, and number of flowers in the inflorescence from two mixed populations.

	N	*Labellum length (cm)*		*Labellum width (cm)*		*Labellum-size index*	*No. of flowers in inflorescence*
		mean	*s.e.m.*	*mean*	*s.e.m.*		*mean*
PENTRAETH							
D. traunsteineri	40	·79	·014	1·02	·018	·81	7·5
D. purpurella	55	·70	·006	·88	·011	·62	20·6
CORS ERDDREINIOG							
D. traunsteineri	72	·89	·015	1·10	·017	·98	9·7
D. purpurella	35	·71	·009	·93	·014	·66	23·0

within these two areas. Histograms (Fig. 1) for leaf number, number of non-sheathing leaves, and number of flowers in the inflorescence for the two species at these localities are shown as examples of how distinct the biometric data prove them to be.

Polygons derived from the sample means are shown (Fig. 2) together with those for a population of each of the two species growing in the absence of the other: one from a colony of *D. traunsteineri* (Hellifield, M.–W. Yorks., v.c. 64) in a locality where *D. purpurella* has not been found (Roberts & Gilbert 1963), and the other from a colony of *D. purpurella* (near Bangor, Caerns., v.c. 49) in which *D. traunsteineri* does not occur. The essential similarities of the three populations of *D. traunsteineri* are evident, as are those of the three populations of *D. purpurella*. On the other hand the conclusion is unavoidable that at the two localities where they coexist there is no suggestion of the contamination of either species by the other. On the contrary, all the data indicate the complete absence of hybridization or of introgression between these two species.

In view of the conclusive evidence afforded by the biometric data, a thorough search was made on several occasions during the flowering seasons in 1962, 1963 and 1964 for plants which could reasonably be suspected of being the F_1 hybrid. Not one such plant was seen, however, in either of these areas, nor at two other places in Anglesey where these species also grow within a few yards of one another. This confirmed previous observations

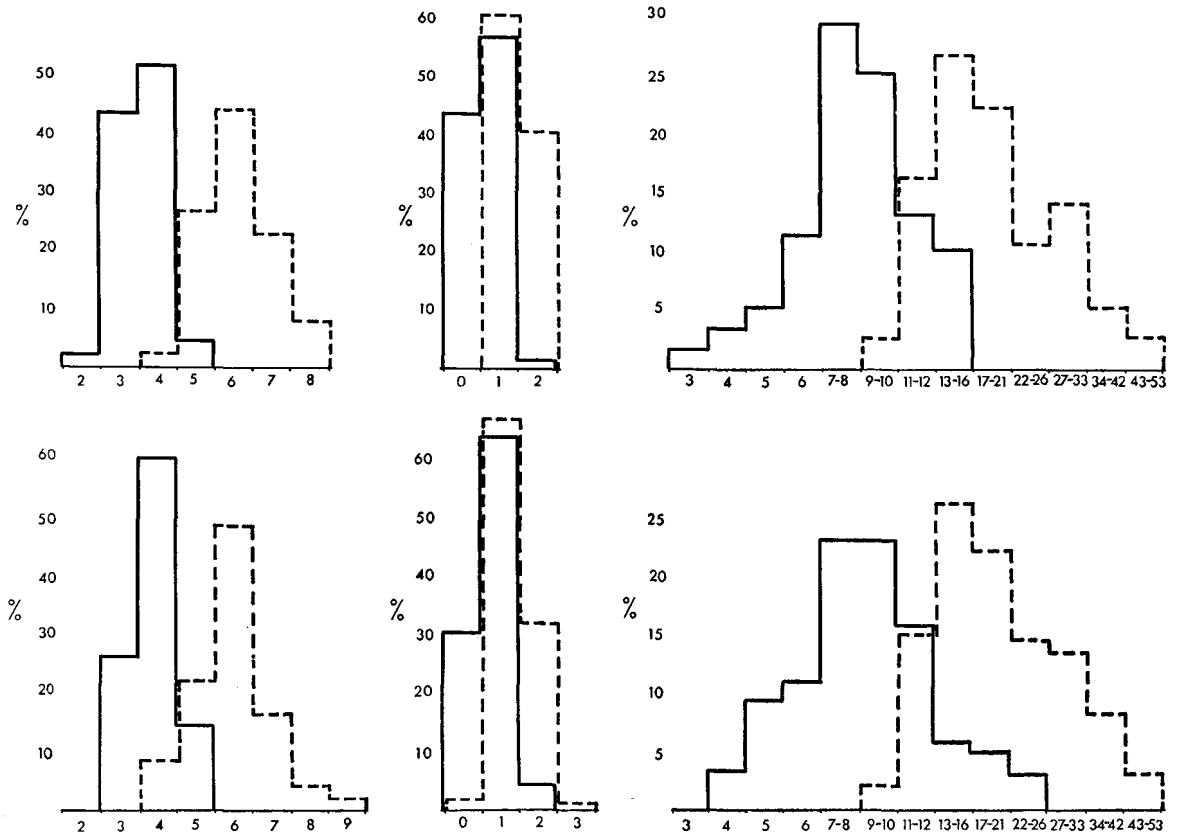

Fig. 1. Histograms of (from left to right) leaf number, number of non-sheathing leaves, and number of flowers per inflorescence in two mixed populations of *D. traunsteineri* (solid lines) and *D. purpurella* (broken lines); top row, Pentraeth; bottom row, Cors Erddreiniog.

when deliberate searches for the F_1 hybrid had also failed (Roberts 1960). A previous record of the putative hybrid (Lacey & Roberts 1958) from Caernarvonshire must now be regarded with doubt, and re-examination of the herbarium specimens on which it was based suggests that they are more probably aberrant forms of *D. traunsteineri*.

One of the characteristics of *D. traunsteineri* in the British Isles is that it is among the earliest of the marsh orchids to come into flower (Heslop Harrison 1953). In Anglesey it has been found to commence flowering as early as 5 May, reaching a peak around the end of May and continuing until the third week of June. Rarely are any flowers to be seen after 20 June. *D. purpurella*, on the other hand, starts to flower usually during the first week of June, reaching a peak around 20 June, and continuing well into the first half of July. Thus, although the peak flowering periods of the two species are separated by about three weeks, there is still a considerable overlap, and it appears that, in these localities at least, these species are isolated by an inherent sterility barrier.

D. majalis *and* D. purpurella

At three places in Wales there are colonies of a heavily leaf-spotted marsh orchid which has been placed as a subspecies under *D. majalis* (Roberts 1961b, 1962). In one locality—near Newborough, Anglesey—it grows intermixed with *D. purpurella*, and observations on this population had indicated that, despite cohabiting, the two forms remained specifically distinct.

With one exception the same characters have been used as for the mixed populations of *D. traunsteineri* and *D. purpurella*. However, the number of flowers in the inflorescence

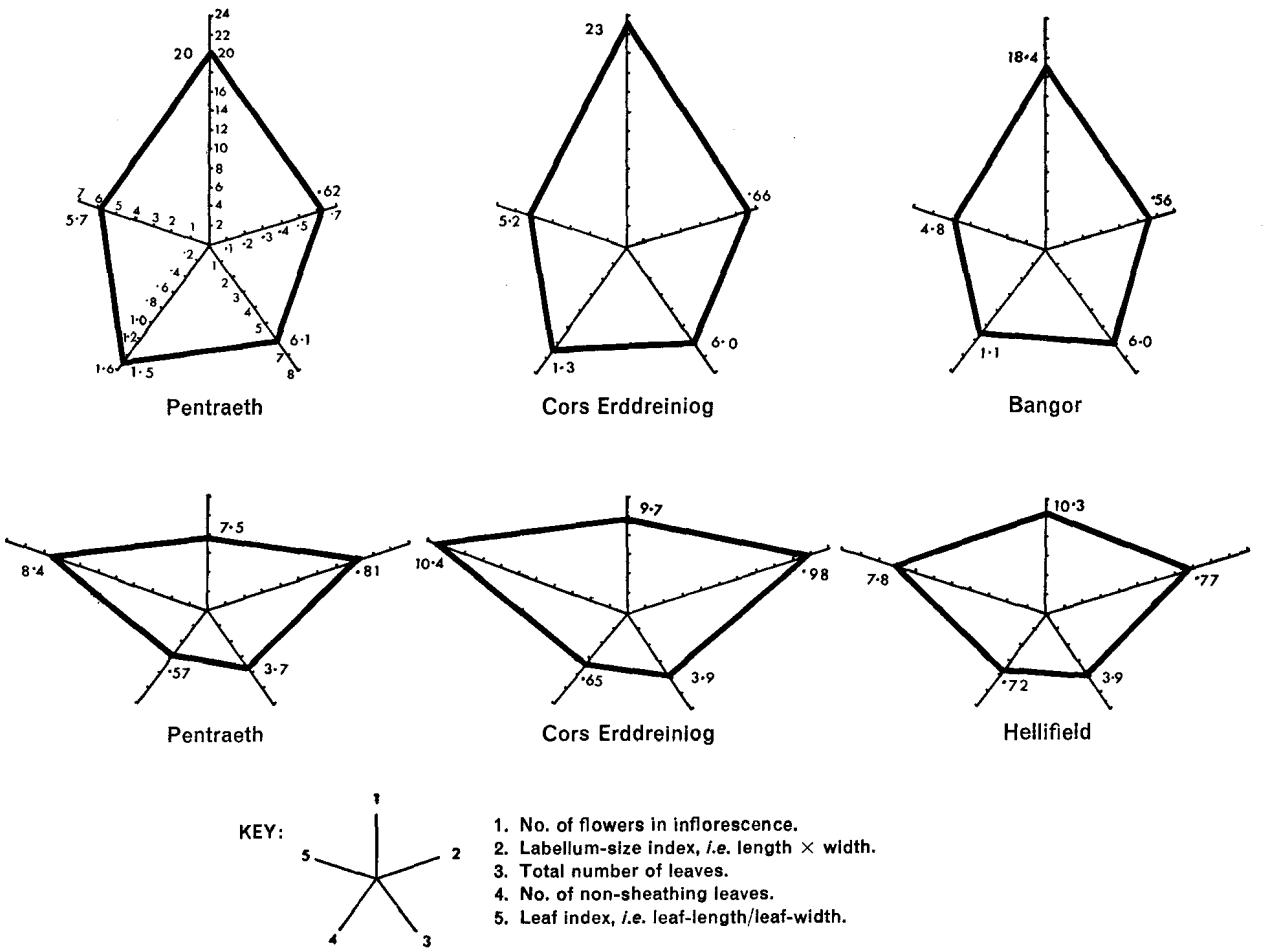

Fig. 2. Polygraphs from two mixed and two 'pure' populations of *D. traunsteineri* (bottom row) and *D. purpurella* (top row). Scales as shown at top left.

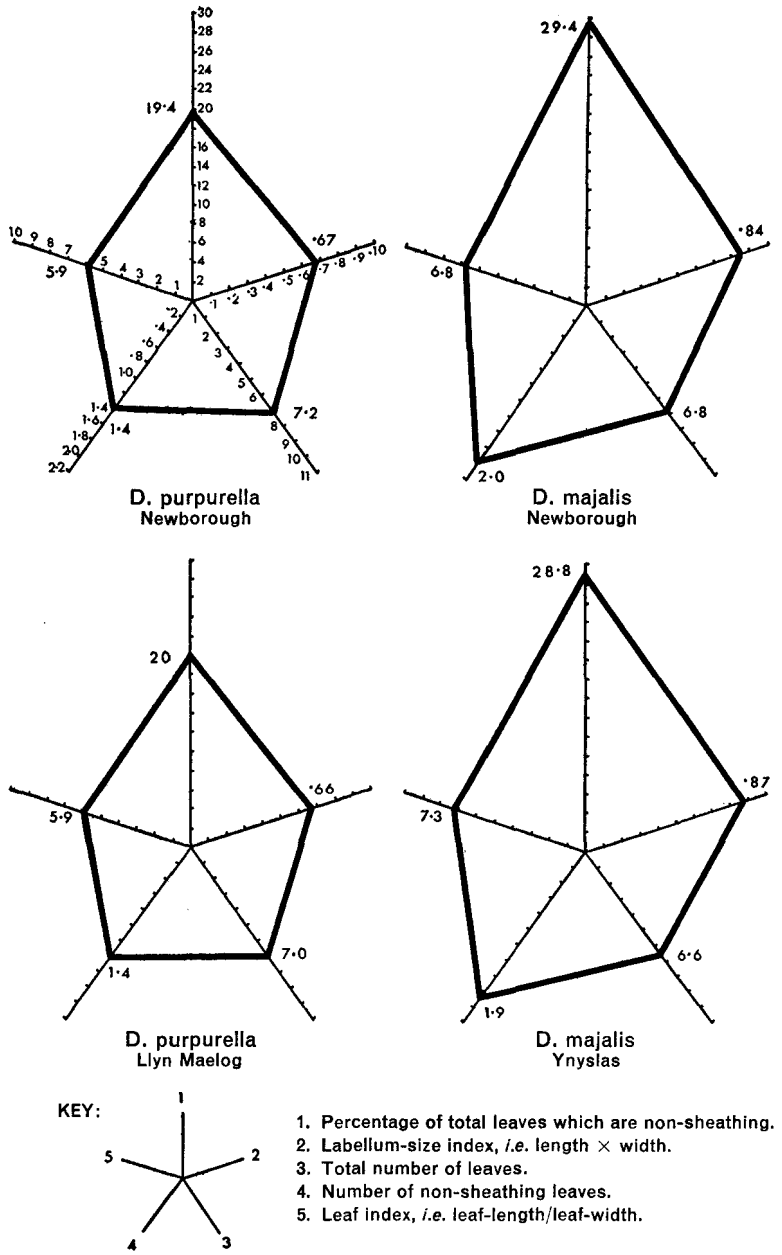

Fig. 3. Polygraphs from one mixed and two 'pure' populations of *D. majalis* and *D. purpurella*. Scales as shown at top left.

is not used to separate *D. majalis* and *D. purpurella*. The percentage of the total number of leaves which are non-sheathing has been used instead as a fifth variable, because these two species had previously been found to differ significantly in this respect.

The polygraphs (Fig. 3) for *D. majalis* and *D. purpurella* in the mixed population, as well as for *D. majalis* growing without *D. purpurella*, at Ynyslas, Cardiganshire, are based on data which have already been published (Roberts 1961a, 1961b). The fourth is from unpublished data for a pure population of *D. purpurella* growing near Llyn Maelog, Anglesey.

These polygraphs show the considerable differences between the populations of the two species where they coexist. Moreover, the close identity of the *D. majalis* colony in Anglesey with that in Cardiganshire, as well as the similarity of the colony of *D. purpurella* growing with it to another of this species growing in the absence of *D. majalis*, are both apparent. From this it seems reasonable to conclude that there has been little or no modification of either species by the presence of the other in the mixed population.

So far it has not been possible to decide whether F_1 hybrids between these species occur in the Newborough population. On the other hand it is not possible to state categorically that they do not occur, but even if they do, these results suggest that gene-exchange between the two species in either direction is at least extremely restricted, if not completely prevented.

Discussion

In the mixed populations of *D. traunsteineri* and *D. purpurella* which have been studied it has been shown that there is no evidence of hybridization or of introgression between the two species. Although their flowering periods differ, there is sufficient overlap to provide ample opportunity for cross-pollination to take place. Furthermore, it does not seem probable that the insect pollinators are able to discriminate between the flowers of the two species. It is well known that cross-pollination of *D. purpurella* with *D. fuchsii* occurs frequently, as is shown by the presence of their hybrid in most of the places where they grow together. Since the differences between the flowers of these two species are greater than between those of *D. traunsteineri* and *D. purpurella*, it is unlikely that flower specificity on the part of the pollinating insects is the isolating factor in the latter instance. But it must be stressed that there is, as yet, no positive information on this point.

This suggests that there are internal barriers to hybridization, and the absence of F_1 hybrids indicates that the isolating mechanism is effective at an early stage: either the prevention of fertilization through pollen incompatibility, the degeneration of the incompatibly fertilized ovules, the inviability of the F_1 hybrid, or some even more obscure factor.

In the case of the mixed population of *D. majalis* and *D. purpurella* the continued coexistence of the two species without merging seems to show at least that they are not completely interfertile. In this instance, however, it has not been possible to obtain conclusive evidence of whether hybrids are present or not, and there are difficulties in the way of recognizing them if they do occur.

As has been mentioned already, the complete interfertility of the tetraploid marsh orchids has become generally accepted in Britain in recent years. Although the conclusions arrived at here do not agree with that view, it must be emphasized that the scope of the present study is too limited to enable wider generalizations to be drawn from it. The fact that *D. traunsteineri* is incapable of interbreeding with *D. purpurella* in Anglesey does not necessarily imply that it may not be able to do so elsewhere, or with other members of the sub-section *Majales*. Nevertheless, the remarkable uniformity displayed by *D. traunsteineri* in certain morphological characters such as the extremely small number of very narrow leaves, as well as in its specialized ecological preferences, makes one suspect that this species, at any rate, is genetically isolated from the other tetraploid species over most of its British range.

A rather different situation seems to prevail in a mixed population of *D. praetermissa* and *D. purpurella* at Harlech, Merioneth. Limited observations suggest that here a certain amount of hybridization and introgression may have taken place, as has already been indicated by Benoit & Richards (1961). On the other hand Mr. V. S. Summerhayes (personal communication) has failed to find any evidence of hybridization in a mixed population of these two species in a Hampshire locality. The apparent introgression in the Harlech population may, in fact, be due partly to the presence of extreme variants of the very variable population of *D. praetermissa*. But the evidence obtained so far from this population is incomplete, and more sensitive methods than those used here may be required to deal with the problems involved.

It may be that the inter-relationships of the tetraploid species of marsh orchids are more complex than has been suspected, and that barriers to gene-exchange may not be equally strong between all of the species, or even between the same two species in different localities. Even if hybridization occurs when two of the tetraploid species, having evolved in geographical isolation, first come into contact, it is possible that the barriers to inter-breeding may subsequently become intensified. In this connection it is pertinent to recall that the isolating mechanisms between some allopatric species are most effective in those areas where the species actually meet.

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REFERENCES

BENOIT, P. & RICHARDS, M. (1963). *A Contribution to a Flora of Merioneth.* Ed. 2. Haverfordwest.

BENTHAM, G. & HOOKER, J. D. (1892). *Handbook of the British Flora,* Ed. 7, revised A. B. Rendle, 1924. Ashford.

CLAPHAM, A. R. (1952), in Clapham, A. R., Tutin, T. G. & Warburg, E. F. *Flora of the British Isles,* Ed. 1. Cambridge. Ed. 2, 1962.

DRUCE, G. C. (1914). Notes on the marsh orchids. *Rep. Bot. Soc. Exch. Club,* **3,** 340.

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.

HESLOP HARRISON, J. W. & RICHARDSON, J. A. (1953). The Magnesian limestone area of Durham and its vegetation. *Northern Naturalists' Union Transactions,* **2,** 1–28.

HUNT, P. F. & SUMMERHAYES, V. S. (1965). *Dactylorhiza* Nevski, the correct generic name of the Dactyl-orchids. *Watsonia,* **6,** 128–133.

LACEY, W. S. & ROBERTS, R. H. (1958). Further notes on *Dactylorchis traunsteineri* (Saut.) Vermeul. in Wales. *Proc. B.S.B.I.* **3,** 22–27.

PUGSLEY, H. W. (1936). New British marsh orchids. *Proc. Linn. Soc.* Session **148,** 121–125.

ROBERTS, R. H. (1960). The Wicklow marsh orchid in Anglesey. *Nature in Wales* **6,** 14–17.

ROBERTS, R. H. (1961a). Studies on Welsh orchids. I. The variation of *Dactylorchis purpurella* (T. & T. A. Steph.) Vermeul. in North Wales. *Watsonia* **5,** 23–36.

ROBERTS, R. H. (1961b). Studies on Welsh orchids. II. The occurrence of *Dactylorchis majalis* (Reichb.) Vermeul. in Wales. *Watsonia* **5,** 37–42.

ROBERTS, R. H. (1962). *Dactylorchis majalis* in Caernarvonshire. *Nature in Wales,* **8,** 43–46.

ROBERTS, R. H. & GILBERT, O. L. (1963). The status of *Orchis latifolia* var. *eborensis* Godfery in Yorkshire. *Watsonia* **5,** 287–293.

STEPHENSON, T. & T. A. (1920). A new marsh orchis. *J. Bot. Lond.* **58,**164–170.

VERMEULEN, P. (1947). *Studies on Dactylorchids.* Utrecht.