

On Measuring Marsh-orchids

MORPHOMETRIC PROCEDURE, TAXONOMIC OBJECTIVITY AND MARSH-ORCHID SYSTEMATICS

INTRODUCTION

The marsh-orchids *Dactylorhiza traunsteineri* (Sauter) Soó and *D. lapponica* (Laest. ex Hartman) Soó occur predominantly in montane and submontane habitats of northern Europe and the Alps. They are enigmatic taxa whose distinguishing characteristics (and therefore status and distributions) remain controversial. In an earlier paper, we provisionally omitted *D. traunsteineri* from the British and Irish flora pending further investigation (Bateman & Denholm 1983), and referred plants previously regarded as *D. traunsteineri* to a subspecies of *D. majalis* (Reichenbach) P. F. Hunt & Summerhayes.

A recent issue of *Watsonia* contained consecutive papers that re-instated *D. traunsteineri* (Roberts 1988) and added *D. lapponica* (Kenneth *et al.* 1988) to the list of British and Irish marsh-orchids. Roberts' (1988) arguments focused on a single highly heterogeneous dactylorchid colony at Rhos-y-Gad, Pentraeth, Anglesey, which was sampled independently by Roberts (1966, 1988), Bateman & Denholm (1983) and Jenkinson (1986). All these morphometric studies included an assessment of the range of variation and taxonomic status of Pugsley's Marsh-orchid, a taxon referred to *Dactylorhiza traunsteineri* by Roberts and *D. majalis* subsp. *traunsteinerioides* (Pugsley) Bateman & Denholm by Jenkinson and ourselves. Roberts (1988, Table 1) noted statistically significant differences in three characters between his data (together with those of Jenkinson (1986)) and our own and, though unable to explain the discrepancies, argued that they "raise doubts about the reliability of procedures used in Bateman & Denholm's study".

Roberts (1988, Table 2) then compared his mean values for eight characters of the Rhos-y-Gad population with pooled mean values for eight Alpine populations of *D. traunsteineri* extracted from Reinhard's (1985) Table 3. The "remarkable similarity" between Rhos-y-Gad and Alpine plants was regarded as sufficient evidence to indicate general similarity of British and Alpine populations, and allowed Roberts to re-affirm his previously stated opinions that 1) the correct epithet for the British and Irish plants is *traunsteineri* rather than Pugsley's (1936, 1940) *traunsteinerioides*, 2) "introgression" does not occur between this taxon and *D. majalis* subsp. *purpurella* (T. & T. A. Stephenson) D. M. Moore & Soó and 3) hence, by implication, *traunsteineri* should be regarded as a full species distinct from *D. majalis*.

Kenneth *et al.*'s (1988) arguments for the presence of *D. lapponica* in Britain were also based primarily on comparison of mean values for selected morphometric characters of British populations with means for Scandinavian and Alpine populations in Table 3 of Reinhard (1985).

We believe that the conclusions of Roberts and Kenneth *et al.* highlight several widely-held misconceptions concerning the validity of different morphometric procedures and comparability of the resulting data, which are discussed below.

ACQUISITION ON MORPHOMETRIC DATA

CONSTRAINTS ON THE DATABASE

The configuration of any morphometric database is determined by three criteria: number of populations sampled, number of individual plants measured and number of characters recorded. Given a set period of time for a study, they are mutually antagonistic. We believe that the optimal balance should be determined by the primary objective of the investigation. For example, tests for possible adaptive significance of particular structures require few characters but many populations and individuals per population to detect often subtle but biologically significant variation. However, the most rigorous taxonomic studies result from detailed overall description (i.e. many characters)

of plants from a wide geographical and ecological spread (i.e. many populations). Time constraints therefore dictate that the third criterion (i.e. number of plants measured per population) must be the minimum required for meaningful comparison.

Admittedly, smaller samples incur greater sampling error and provide less precise estimates of the distribution of values about the mean. However, since significance tests take account of the numbers of individuals sampled, there is little theoretical support for Roberts' (1988, p. 44) suggestion that the discrepancies in floral dimensions between his multiple samples and our single sample of the Rhos-y-Gad population could reflect the difference in sample size (30–40 and 10 plants respectively).

SELECTING TAXA, COLONIES, POPULATIONS AND INDIVIDUALS

Any morphometric study requires *a priori* selection of the range of variation to be described and, where appropriate, subsequently partitioned into taxa. Within *Dactylorhiza*, non-random selection of populations is necessary if rarer taxa such as *D. traunsteineri* and *D. lapponica* are to be included in the study. Unfortunately, the subjectivity of this procedure allows discrimination against 'awkward' (i.e. morphologically peripheral) populations; if practised, such prejudice often results in false morphological discontinuities and erroneous taxonomic conclusions.

Random sampling of individual plants is also precluded within dactylorchid colonies (sensu Bateman & Denholm 1983, p. 347) that contain more than one species. Unfortunately, in its characteristic habitat (species-rich, *Schoenus*-dominated fens) Pugsley's Marsh-orchid usually forms highly heterogeneous colonies with several other dactylorchid taxa and hybrids. The consequent need for selectivity can be reduced in some colonies by restricting sampling to areas where individuals of the target population predominate, though these must still be distinguished from individuals of co-existing populations of other taxa. In practice, this is achieved primarily by subjective *a priori* delimitation of the morphological range that is considered acceptable within the target taxon. Different operators undoubtedly prescribe different limits of tolerance.

For example, two of our study plants of Pugsley's Marsh-orchid from Rhos-y-Gad possessed unusually broad labella and could have been hybrids with co-existing *D. maculata* (L.) Soó. However, they lacked the most characteristic features contributed by spotted-orchids to hybrids with marsh-orchids: relatively narrow spurs, large numbers of non-sheathing leaves and, most reliable of all, the presence of leaf-markings. After some debate they were included in our sample, though they would probably have been excluded by many other workers. Interestingly, the difference between our mean labellum dimensions for Rhos-y-Gad and those of Roberts (1988) remains statistically significant when these two morphologically extreme plants are excluded. Additional explanations for the discrepancy must therefore be sought.

SELECTING THE TIMING OF MEASUREMENT

Dactylorchids undergo substantial morphological changes during annual growth. For example, inflorescence length can triple during anthesis, and flowers from the base of an inflorescence are appreciably larger than those at the apex. Such ontogenetic variations impair compatibility of samples taken on different dates during the same season. Other factors change the phenotypic composition of populations from year to year. We examined Rhos-y-Gad on four occasions during the last eight years (1980, 1981, 1982, 1987) and noted substantial variations in both the habitat and the population of Pugsley's Marsh-orchid. Some of the variations were non-directional (e.g. selective grazing of larger plants in some years), but others can be directional (e.g. progressive reduction of soil moisture content tends to eliminate less drought-tolerant individuals). Given these factors, the consistency of Roberts' (1988) repeated measurements (1963, 1984, 1986) is perhaps more surprising than their difference from ours.

SELECTING DEFINITIONS OF CHARACTERS

Despite Roberts' (1988) claim for "remarkable similarity" of his population means for *D. traunsteineri* from Rhos-y-Gad to Reinhard's (1985) data from the Alps (mean values for eight pooled populations), there are statistically significant differences ($p < 0.05$) in means for two of the eight characters listed (Tables 1 and 2). The most notable, a substantial (by dactylorchid standards) 2 mm difference in mean spur lengths, was ignored by Roberts (1988). However, it highlights

TABLE 1. COMPARISON OF DATA FOR PUGSLEY'S MARSH-ORCHID AT RHOS-Y-GAD (THREE OPERATORS), AND EIGHT COMBINED ALPINE POPULATIONS OF *D. TRAUNSTEINERI*. Includes all eight characters listed by Roberts (1988, Table 2), with three additional vegetative characters susceptible to environmental modification.

Character number and name	Bateman & Denholm (1983)		Jenkinson (1986)		Roberts (1988)		Reinhard (1985)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1. Total number of leaves ^a	3.60	0.52	3.20	0.42	3.82	0.53	3.99	0.63
2. Leaf width (cm) ^a	1.36	0.49	1.05	0.17	1.00	0.21	1.03	0.23
3. Inflorescence length (cm)	4.32	1.24	3.35	0.71	4.51	0.84	4.73	1.13
4. Number of flowers	11.50	5.00	10.10	3.98	9.40	3.62	8.43	2.34
5. Labellum max. width (mm)	12.10	1.95	10.15	1.00	10.50	1.29	10.63	1.12
6. Labellum max. length (mm)	8.91	0.78	8.00	1.33	8.20	0.84	7.74	0.76
7. Labellum, length of central lobe (mm)	2.64	0.91	2.20	0.48	2.25	0.62	2.38	0.67
8. Spur length (mm) ^a	9.02	1.39	9.10	0.74	8.92	0.90	10.90 ^b	1.24
9. Plant height (cm)	16.7	5.2	10.8	1.7	—	—	24.7	4.6
10. Stem diameter (mm) ^a	3.94	1.19	2.20	0.48	—	—	3.28	1.00
11. Length of longest leaf (cm) ^a	8.54	1.70	6.37	0.99	—	—	9.03 ^c	2.07
Year sampled	1981		1986		1986		1984	
Populations studied	Rhos-y-Gad		Rhos-y-Gad		Rhos-y-Gad		8 Alpine populations	
Number of plants measured	10		10		30		75	

^a Data probably not fully compatible, as character was sometimes ambiguously defined.

^b Mean = 8.30 with spur diameter subtracted (see text).

^c Length of second lowest sheathing leaf.

TABLE 2. SIGNIFICANCE OF PAIRWISE COMPARISONS OF MEAN VALUES LISTED IN TABLE 1 Determined by 't' tests with degrees of freedom modified, where appropriate, to account for significant differences between sample variances. ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Characters numbered as shown in Table 1.

Character no.	Character type ^b	Comparison ^a					
		A × B	A × C	A × D	B × C	B × D	C × D
1	V	ns	ns	ns	**(C)	*** (D)	ns
2	V	ns	*(A)	ns	ns	ns	ns
3	V	*(A)	ns	ns	*** (C)	*** (D)	ns
4	V	ns	ns	ns	ns	ns	ns
5	F	*(A)	*(A)	*(A)	ns	ns	ns
6	F	ns	*(A)	*** (A)	ns	ns	*(C)
7	F	ns	ns	ns	ns	ns	ns
8	F	ns	ns	*** (D)	ns	*** (D)	*** (D)
9	V	** (A)	—	*** (D)	—	*** (D)	—
10	V	** (A)	—	ns	—	*** (D)	—
11	V	** (A)	—	ns	—	*** (D)	—

^a A = data from Bateman & Denholm (1983), B = Jenkinson (1986), C = Roberts (1988), D = Reinhard (1985). For statistically significant differences, the letter in parentheses shows the sample having the higher mean value.

^b V = vegetative character, F = floral character.

another major constraint on the compatibility of data generated by different research groups: inconsistencies or misconceptions in the definition of ostensibly identical characters.

Roberts (1961, 1988) apparently followed the 'British School' (e.g. Heslop-Harrison 1948 *et seq.*) in excising the spur from the labellum prior to measurement (Fig. 1b). Reinhard (1985, Fig. 6) followed the 'Continental School' (e.g. Vermeulen 1947 *et seq.*) in mounting the labellum and spur as an integral unit (Fig. 1a). Thus, spur lengths given by Reinhard (1985) are equivalent to the mean spur lengths given by Roberts (1988) *plus the approximate diameter of the spur*; subtraction of the

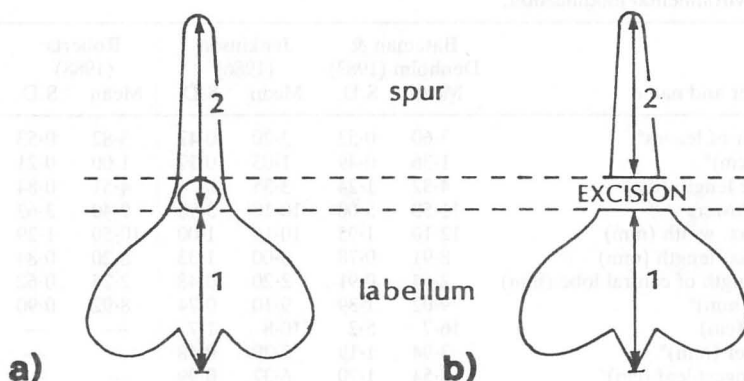


FIGURE 1. Comparison of methods of mounting dactylorchid flowers prior to measurement used by a) the Continental School and b) the British School. The two methods yield identical values for character 1 (labellum length to apex of central lobe) but substantially different values for character 2 (spur length).

mean spur diameter (2.6 mm) brings Reinhard's mean spur length for Alpine *D. traunsteineri* much closer to that of Roberts for the Rhos-y-Gad population. We cannot assess whether this error was also perpetuated by Kenneth *et al.* (1988) because their methods of measurement are not stated. In addition, some readily quantified characters (notably the potentially valuable 'heavy' leaf markings of *D. lapponica*) were only qualitatively described by both Kenneth *et al.* (1988) and Reinhard (1985), thus preventing valid comparison. Such misconceptions emphasize the importance of precisely defining and quantifying every character; several other characters listed in Tables 1–3 are ambiguously defined and potentially incompatible.

SELECTING DATA A POSTERIORI FOR PUBLICATION

Some selectivity of characters is inevitable when comparing sets of population means, as full comparison is restricted to characters common to all of the data sets under scrutiny. Thus, Roberts (1988) was obliged to omit from his Table 2 twenty of Reinhard's (1985) 28 characters. Moreover, Roberts' assertion of similarity between his mean values for Rhos-y-Gad and those of Jenkinson (1986) was achieved by further selecting two of these eight characters (labellum length and labellum width) that yielded similar values for the two samples. Comparison of a larger number of characters (Tables 1 and 2) demonstrates highly significant differences in vegetative characters such as total number of leaves and inflorescence length. Furthermore, comparison of mean values obtained by Jenkinson (1986) with our own (including additional vegetative characters not measured by Roberts) demonstrates that Jenkinson's plants had on average shorter stems and shorter leaves. In fact, the diminutive mean stature of Jenkinson's plants reflects sampling within a small, relatively dry area of the meadow at Rhos-y-Gad (M. N. Jenkinson pers. comm. 1988). Consequently, our mean values for vegetative characters are closer to those of Roberts (1988) than to those of Jenkinson (1986), even though Roberts' and Jenkinson's measurements were taken during the same season (1986) and post-dated our published survey by five years.

Thus, we attribute the difference between mean values obtained by ourselves, Roberts (1988) and Jenkinson (1986) from Rhos-y-Gad largely to differences in *a priori* perception of the range of variation encompassed by Pugsley's Marsh-orchid and in the area of the habitat sampled, compounded by inconsistencies between workers in the precise definition of characters. We conclude that internal consistency is likely within any one project but that, in the absence of detailed consultation, the comparison of data collected by different workers is fraught with hazard.

TABLE 3. COMPARISON OF MEAN VALUES FOR ALL COMPATIBLE METRIC CHARACTERS OF SELECTED MARSH-ORCHID POPULATIONS IN WESTERN SCOTLAND AND YORKSHIRE

Character	Western Scotland ^a	Ha Mire Wood, Yorkshire ^b
Plant height (cm)	7.0–21.0	15.4
Number of expanded sheathing leaves	2.3–3.0	3.7
Number of non-sheathing leaves	0.8–1.7	1.0
Length of longest leaf (cm)	5.0–8.9	8.5
Width of longest leaf (mm)	1.1–1.5	1.3
Length of basal bracts (mm)	13–19	17
Length of inflorescence (cm)	3.0–4.6	2.7
Number of flowers	8.9–12.8	9.9
Labellum, length to apex of central lobe (mm)	6.3–7.8	6.3
Labellum, maximum width	(6.4–)7.3–9.4	7.7
Spur length (mm)	7.5–9.2 ^c	7.2
Spur, maximum width when flattened (mm)	2.6–3.4	2.9

^a Data from Kenneth *et al.* (1988, Table 1). Figures given are ranges of mean values for seven populations (4–14 plants/population).

^b Data from Bateman & Denholm (1983, Table 2). Figures are mean values for ten plants in one population.

^c Figures are difficult to interpret, as the method of measurement was not described (see Fig. 1).

TAXONOMIC INTERPRETATION OF MORPHOMETRIC DATA

POPULATION MEANS AND INTRA-POPULATION VARIATION

In our studies of the tetraploid and diploid marsh-orchids (Bateman & Denholm 1983, 1985), we applied the same multivariate algorithms to data sets for both 1) individual plants and 2) population means (each of 52 characters). Considerable overlap of individuals of different taxa provided crucial evidence that species could not be delimited within either *D. majalis* or *D. incarnata* (L.) Soó, though assignment of populations to subspecies was based primarily on the multivariate analysis of population means. Once an optimal intraspecific classification was achieved by this method, data for all measured individuals of each taxon were pooled to allow its description using character states selected by univariate analyses. There are therefore three levels in the analytical hierarchy: 1) individual plants, 2) populations and 3) specific or intraspecific taxa.

The data presented by Roberts (1988) are level 2 (population means), whereas those presented by Reinhard (1985) for *D. traunsteineri* are level 3 (taxon means). Data published by Kenneth *et al.* (1988) for *D. lapponica* (ranges of population means) lie uncomfortably between levels 2 and 3. Hence, these data sets are not strictly comparable and although Roberts (1988) and Kenneth *et al.* (1988) stress the similarity of their respective data sets to those of Reinhard (1985), the true concordance of the data cannot be adequately assessed.

The drawbacks of this type of comparative, univariate approach are emphasized by Table 3, which compares Kenneth *et al.*'s (1988, Table 1) range of means for Scottish populations of *D. lapponica* with our means (Bateman & Denholm 1983, Table 2) for a population of *D. majalis* subsp. *purpurella* (corresponding to form 'A' of Stephenson & Stephenson (1920); see also Roberts (1961)) from Ha Mire Wood, Malham, Yorkshire. The Ha Mire Wood population lies within the range for Scottish *D. lapponica* in nine of the twelve characters listed. The three exceptions are spur length (0.3 mm outside, possibly due to differences in method of measurement), inflorescence length (3 mm) and total number of leaves (0.7, again possibly due to differences in the definition of a dactylorchid leaf). This "remarkable similarity" (*sic*) allows only three possible interpretations: 1) the Ha Mire Wood population is *D. lapponica*, 2) at least some of the populations identified as *D. lapponica* by Kenneth *et al.* (1988) are actually *D. majalis* subsp. *purpurella* or 3) the identification of taxa by visual comparison of population means for a small number of characters is irredeemably conceptually flawed and should not be practised. We prefer explanation (3), and regard the case for the presence of both *D. lapponica* and *D. traunsteineri* in the British Isles as unproven (though by no means unlikely).

CLASSIFICATION AND NOMENCLATURE

Roberts (1988) used differences between his populations and ours, together with Reinhard's (1985) recent biometric data on Alpine *D. traunsteineri*, as tools to undermine our broader conclusions concerning the status of Pugsley's Marsh-orchid in the British Isles. Unfortunately, two separate issues have become confused: 1) should populations of Pugsley's Marsh-orchid be treated as a subspecies of *D. majalis* or as a separate, distinct species, and 2) should they bear the epithet *traunsteineri* or *traunsteinerioides*?

In our 1983 paper, we confidently argued that the substantial morphological overlap between populations such as Rhos-y-Gad and populations of other subspecies of *D. majalis* clearly precludes recognition of Pugsley's Marsh-orchid as a distinct species. Much additional morphometric data collected subsequently has increased the extent of the overlap and reinforced this conclusion.

Our use of the epithet *traunsteinerioides* rather than *traunsteineri* was much more tentative, and prompted by the necessity to label a taxon if it is to remain acceptable botanical currency. Roberts (1988) quoted our (admittedly weak) reasons for this nomenclature decision, but omitted our subsequent statement that biometric measurements should be taken from Alpine populations to test this hypothesis (Bateman & Denholm 1983, p. 373). Reinhard's (1985) data are valuable but insufficient to resolve this issue due to 1) constraints on the comparability of data collected by different research groups working in isolation (see above) and 2) the presentation of data as taxon (level 3) and/or population (level 2) means, preventing essential comparison of individual plants (level 1).

Moreover, the possible resolution of the nomenclatural controversy in favour of sinking *traunsteinerioides* into synonymy with *traunsteineri* would not in any way affect the arguments for treating the amalgamated taxon as a subspecies of *D. majalis*. The correct name for Pugsley's Marsh-orchid would then be *D. majalis* subsp. *traunsteineri* (Sauter) Sundermann (1980, p. 40). Similarly, *D. lapponica* may be more appropriately treated as *D. majalis* subsp. *lapponica* (Laest. ex Hartman) Sundermann (1975, p. 45).

CONCLUSIONS

Current evidence is insufficient to determine whether certain problematic marsh-orchid populations in the British Isles should be referred to the predominantly Continental 'species' *Dactylorhiza traunsteineri* (Sauter) Soó and *D. lapponica* (Laest. ex Hartman) Soó. In contrast, there is strong morphological evidence that British and Irish populations referred by some botanists to *D. traunsteineri* are conspecific with *D. majalis* (Reichenbach) P. F. Hunt & Summerhayes. Thus, if future studies demonstrate that some British and Irish dactylorchid populations cannot be distinguished from Continental populations referred to *D. traunsteineri*, they should be included within *D. majalis* subsp. *traunsteineri* (Sauter) Sundermann. If they prove to differ significantly, the British and Irish populations should be maintained separately as *D. majalis* subsp. *traunsteinerioides* (Pugsley) Bateman & Denholm (Pugsley's Marsh-orchid). The controversies surrounding these taxa highlight several commonly encountered methodological and conceptual pitfalls in morphometric studies.

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ERRORS AND MISCONCEPTIONS IN THE STUDY OF MARSH-ORCHIDS

In a former paper (Roberts 1988) I pointed out that the evidence put forward by Bateman & Denholm (1983) was insufficient to warrant the removal of *Dactylorhiza traunsteineri* (Sauter) Soó from the British and Irish flora. Furthermore, some of their data from the British and Irish plants referred to this taxon were unreliable and failed to provide a sufficiently sound basis for the taxonomic changes they proposed. As a result I suggested a return to the *status quo* before the publication of their paper.

Bateman & Denholm (1989) have now gone to great lengths to show that my arguments are not valid: that because of inconsistencies between different workers in their definitions of characters, their data are not compatible; and, moreover, that I have misunderstood the procedures employed by other workers. In some instances they have even attributed to me statements and claims that I have not made.

Below I have dealt with some of the points they have raised in the order in which they occur in their present paper, and, for ease of reference, under the section headings used by them.

CONSTRAINTS ON THE DATABASE

In this section Bateman & Denholm (1989) state that “there is little theoretical support for Roberts’ (1988) suggestion that the discrepancies in floral dimensions between his multiple samples and our single sample . . . could reflect the difference in sample size (30–40 and 10 plants respectively).” What I actually said was: “The possibility was considered that the small size of their sample may account for the poor estimates of population means.” This sentence contains no reference, either directly or by implication to the significance of the differences between their mean values and mine, but simply states that smaller samples are liable to give less precise estimates of population means, which is a well-known fact of elementary statistical theory. It is another way of saying that “smaller samples incur greater sampling error” as Bateman & Denholm (1989) themselves have put it. It seems that these authors have taken ‘discrepancy’ to be synonymous with ‘significant difference’, but these terms are not interchangeable and Bateman & Denholm have attributed to me a statement I have not made. In fact, what I said has full theoretical support.

SELECTING TAXA, COLONIES, POPULATIONS AND INDIVIDUALS

Bateman & Denholm have quite properly underlined the importance of distinguishing individuals of the target population from those of co-existing populations of other taxa. By their own admission, however, two plants which could have been hybrids between *D. traunsteineri* and the co-existing *D. maculata* (L.) Soó were included in their sample. The decision to include them was made on the grounds that such hybrids are characterized by relatively narrow spurs, large numbers of non-sheathing leaves and the presence of leaf-markings. However, they have overlooked the possibility that, in addition to F₁s, the situation is often complicated by the presence of F₂ or backcross plants, or even some of subsequent origin, as was shown by Lord & Richards (1977) in mixed populations of *D. fuchsii* (Druce) Soó and *D. majalis* subsp. *purpurella* (T. & T. A. Steph.) Soó. In such plants the morphological characters of narrow spurs, large numbers of non-sheathing leaves, and the presence of leaf-markings are often not nearly so pronounced, and it is such plants that can pose problems. Their inclusion in a sample is not only the most likely source of the supposed "different limits of tolerance" set by different workers, but will also have a disastrous effect on the conclusions, for the procedures employed by Bateman & Denholm have no in-built mechanism which can identify and reject spurious data.

Observations of pollen fertility, as described by Heslop-Harrison (1954), provide a much more reliable criterion of the status of doubtful plants. The test is readily applied and I have found it to be of the utmost value in the determination of plants whose status on morphology alone would have remained obscure. Bateman & Denholm's failure to use this test must certainly be regarded as a serious weakness in their sampling procedures.

SELECTING THE TIMING OF MEASUREMENT

Bateman & Denholm's statement requires some qualification. Firstly, many characters such as number of leaves, number of flowers per inflorescence, presence or absence of leaf-marking, etc. do not exhibit ontogenetic variation. These authors give two examples of such variation but only one of them, inflorescence length, is correctly cited. The second example they give is that the flowers from the base of an inflorescence are appreciably larger than those at the apex, from which they infer that flowers at the base, having opened some days before those at the apex, have enlarged appreciably in the interval. This assumption is erroneous. Once dactylorchid flowers have opened, floral dimensions remain unchanged throughout anthesis. A simple experiment demonstrates this very clearly.

All the flowers were removed in sequence from the lower two-thirds of the inflorescence of two plants of *D. majalis* subsp. *praetermissa* (Druce) Moresby Moore & Soó, so as to provide two samples at different dates from each of them. The first sample was taken from one side of the inflorescence, leaving the flowers on the other side to be removed a week or so later. These made up the second sample. Labella and spurs from both samples were mounted in the usual way and the data obtained from them are shown in Table 1, a glance at which is sufficient to show that floral dimensions are not subject to ontogenetic variation. This was the basis on which I compared Jenkinson's data for labellum dimensions from *Rhos-y-gad* with mine.

The difference in size between flowers at the base of an inflorescence and those at the apex is a

TABLE 1. DATA FOR FLORAL DIMENSIONS FROM TWO SAMPLES TAKEN FROM OPPOSITE SIDES OF THE INFLORESCENCE AT DIFFERENT TIMES IN THE SAME SEASON FROM TWO PLANTS OF *D. MAJALIS* SUBSP. *PRAETERMISSA*

Date of sample Number in sample	Plant A				Plant B			
	26.5.87 15		6.6.87 8		29.5.87 10		6.6.87 10	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Labellum width (mm)	14.2	0.06	14.1	0.09	13.2	0.07	13.1	0.13
Labellum length (mm)	9.2	0.06	9.2	0.09	8.4	0.08	8.4	0.15
Spur width (mm)	3.7	0.07	3.8	0.09	3.9	0.09	3.5	0.08
Spur length (mm)	9.3	0.08	9.1	0.07	9.2	0.17	8.9	0.21

well-known phenomenon in dactylorchids, but it is not the result of ontogenetic variation: those at the apex are innately smaller.

Bateman & Denholm further claim that environmental factors have brought about visible changes in the phenotypic composition of the Rhos-y-gad population in the course of a few years; and that it has undergone substantial directional variation as a result of the gradual drying-out of the habitat.

This shows a complete lack of understanding of conditions at this locality, where the habitat is a calcareous mire irrigated by calcium-rich ground water derived from springs and seepages. The main part of the population of *D. traunsteineri* occurs on the spring line and is not affected to any great extent by drought or drainage, and there is no support for the view that the Rhos-y-gad mire is gradually drying out. Neither is there any evidence for the supposition that this population of *D. traunsteineri* is gradually changing in its phenotypic composition. On the contrary the consistency of my sample data repeated after an interval of over 20 years (Roberts 1988) lends considerable support for this view.

Moreover, if Bateman & Denholm's observations were correct, the changes in the Rhos-y-gad population would be an outstanding example of rapid evolutionary adaptation, and it would be reasonable to expect *D. traunsteineri* to have become adapted to drier, grassland habitats in some parts at least of its distributional area. However, as such a phenomenon has not been observed, there is no support for this hypothesis.

SELECTING DEFINITIONS OF CHARACTERS

According to Bateman & Denholm (1989) a major cause of incompatibility between sample data produced by different workers is inconsistency in the definition of morphological characters, or misconceptions of how such characters are defined by others. As an example they cite the different methods of mounting labella and spurs, prior to measurement, by Reinhard (1985) and myself.

Reinhard mounts the labellum and spur in one piece, as shown in Fig. 1a. My method is to separate the labellum from the spur by making a cut, as shown at C in Fig. 1b, the flower being inverted to facilitate the operation. The ovary and column are then separated from the spur and the latter mounted on card, as shown in Fig. 1c. There is no excision as suggested by Bateman & Denholm (1989) in their Fig. 1b.

Reinhard's measurement of spur length is made as shown at A in Fig. 1a; mine as shown at D in Fig. 1c, not as shown at B in Fig. 1a, as Bateman & Denholm have assumed. Consequently the values given by Reinhard and myself are reasonably compatible. Bateman & Denholm's assertion that mean spur lengths given by Reinhard (1985) are equivalent to those given by me (Roberts 1988) "plus the approximate diameter of the spur" is erroneous: it is not necessary to subtract mean spur diameter (2.6 mm) from Reinhard's value to make it equivalent to that from the Rhos-y-gad population. The misconception in this instance is thus shown to be on the part of Bateman & Denholm, and in fact the spur length of the Alpine plants is considerably greater than in the Anglesey ones, as was clearly shown in Table 2 of my paper (Roberts 1988).

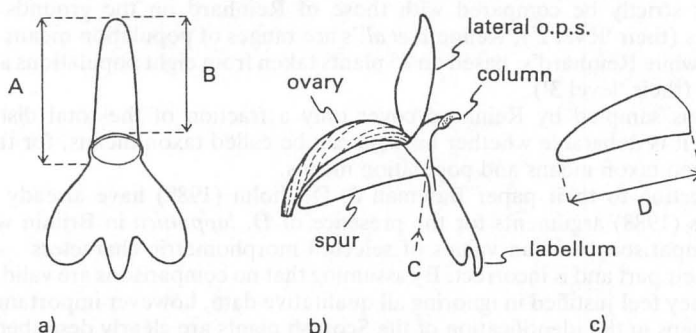


FIGURE 1. Comparison of methods of mounting floral parts prior to measurement: (a) labella and spurs mounted together by Reinhard; (c) spurs mounted separately by Kenneth *et al.* and Roberts. The two methods give identical values for spur length. o.p.s. = outer perianth segment. (Not to scale).

It will be recalled that one of the discrepancies cited by Bateman & Denholm (1983) to justify the removal of *D. traunsteineri* from the British and Irish flora was that the Alpine plants had *smaller spurs*. Yet Reinhard's data show that the opposite is the case. No adjustment of these figures, e.g. by subtracting 2.6 mm from the spur length given by Reinhard, can alter the fact that most of the discrepancies quoted by Bateman & Denholm (1983, p. 373) have no basis in reality.

SELECTING DATA A POSTERIORI FOR PUBLICATION

Bateman & Denholm are once again mistaken when they assert that I have claimed a general similarity between my mean values from the Rhos-y-gad population and those of Jenkinson (1986) and that this was achieved "by further selecting two . . . characters (labellum length and labellum width) that yielded similar values for the two samples." A glance at their Table 2 (Bateman & Denholm 1989) will show that, in fact, six of the eight characters show similar values for the two samples, i.e. Bateman & Denholm have contradicted the facts shown in their own Table. Moreover, my only reference to Jenkinson's (1986) paper was made when discussing labellum dimensions. These authors have thus distorted the facts to further their own argument.

Jenkinson's data for these two characters were quoted to show that although a small sample (10) gives less precise estimates of population means, his values do not differ from mine to the same extent as those of Bateman & Denholm (1983, Table 2). Consequently it seemed unlikely that their large mean values for these characters were attributable to sample size alone.

Although my data were taken six days after Jenkinson's, his mean value for length of labellum mid-lobe (2.2 mm) also compares well with mine (2.25 mm), as do his means for spur length (9.10 mm and 8.92 mm) and number of flowers per inflorescence (10.10 and 9.40 respectively). All of these are characters which are not subject to ontogenetic variation. On the other hand, the highly significant difference in inflorescence length is only to be expected, given the difference in the dates of sampling. Jenkinson's mean value of 3.2 for the total number of leaves can be ascribed to the small and unrepresentative sample measured by him, for it now appears that he confined his sampling to a small, comparatively dry part of the meadow at Rhos-y-gad (Bateman & Denholm 1989).

There is thus no need to invoke "differences in *a priori* perception of the range of variation encompassed by Pugsley's Marsh-orchid" as Bateman & Denholm have done to explain some of the differences between the mean values obtained by them (Bateman & Denholm 1983), Jenkinson (1986) and myself (Roberts 1988) from this population. As I have shown, simpler and more rational explanations are available.

POPULATION MEANS AND INTRA-POPULATION VARIATION

One of the most important taxonomic changes made by Bateman & Denholm (1983) was based on a comparison of data comprising population means (their 'level 2') with data taken from descriptions of *D. traunsteineri* by Vermeulen (1949) and Nelson (1976) based on individual plants (their 'level 1'). It is therefore difficult to accept their suggestion that my data (and likewise those of Kenneth *et al.* 1988) cannot strictly be compared with those of Reinhard on the grounds that mine are population means (their 'level 2'), Kenneth *et al.*'s are ranges of population means (between their 'levels 2 and 3'), while Reinhard's, based on 75 plants taken from eight populations and aggregated, are taxon means (their 'level 3').

The populations sampled by Reinhard cover only a fraction of the total distribution of *D. traunsteineri* and it is debatable whether his data can be called taxon means, for there is no clear distinction between taxon means and population means.

In the Introduction to their paper Bateman & Denholm (1989) have already suggested that "Kenneth *et al.*'s (1988) arguments for the presence of *D. lapponica* in Britain were . . . based primarily on comparison of mean values of selected morphometric characters. . .". This is an assumption on their part and is incorrect. By assuming that no comparisons are valid unless the data are quantified, they feel justified in ignoring all qualitative data, however important these may be.

The primary steps in the identification of the Scottish plants are clearly described in Kenneth *et al.*'s (1988) paper, where they state that the initial positive determination was made by H. R. Reinhard after examining numerous photographs and some biometric data sent to him. The comparisons made in Table 1 of Kenneth *et al.*'s paper consist of a mixture of quantitative data, in

the form of ranges of population means, and qualitative data, making a total of 15 pairs of characters. However, a number of additional characters such as the presence of stem anthocyanin, the distribution of markings on the leaf surface, the presence of markings on the bracts, the second inflorescence and several additional characters of the labellum and spur are given in the description on p. 39 of Kenneth *et al.*'s (1988) paper. Altogether, well over 20 pairs of characters were involved in the comparison of these Scottish plants with the Scandinavian and Alpine ones studied by Reinhard (1985).

In an attempt to show that Kenneth *et al.*'s data could equally well apply to *D. majalis* subsp. *purpurella*, Bateman & Denholm (1989, Table 3) have selected twelve characters of the Scottish plants to compare with those from a population of this taxon studied by them (Bateman & Denholm 1983). On finding that three of these pairs do not agree, they attempt to minimise (a) the difference in spur length by attributing it to the different methods of measurement employed by them and Kenneth *et al.*; and (b) the difference in the number of expanded sheathing leaves as being possibly due to differences in the definition of a dactylorchid leaf.

Kenneth *et al.* measure the spur in the same way as I do (D. J. Tennant pers. comm.) and it has already been shown (above) that Bateman & Denholm's description of how I measure spur length is incorrect. There are no grounds, therefore, for assuming that the difference in mean values shown in Bateman & Denholm's Table 3 (1989) is not, in fact, a real one.

In (b), however, the differences in the definition of a dactylorchid leaf are important and require some clarification. In a former paper Bateman & Denholm (1983) divided dactylorchid leaves into three categories which are treated as separate characters: no. 34, defined as the "number of sheathing leaves (excluding basal leaf if present)"; no. 35, number of non-sheathing leaves; no. 36, "presence or absence of a basal leaf", which is defined as "ranging from a chlorophyllose sheath above ground level to a leaf up to half the length of the sheathing leaf immediately above."

Kenneth *et al.* (1988, Table 1) also list three categories of leaves: (a) the number of expanded sheathing leaves, (b) the number of non-sheathing leaves, and (c) the total number of leaves.

These two groups of workers differ in their definitions of some of these categories. For example, character no. 34 in Bateman & Denholm's (1983) paper, Table 2, comprises leaves nos. 3, 4 and 5 in Fig. 2. Kenneth *et al.*'s 'number of expanded sheathing leaves', on the other hand, includes leaf no. 2 in addition to nos. 3, 4 and 5 (D. J. Tennant, pers. comm.). These two characters are therefore not compatible at all, as Bateman & Denholm (1989, Table 3) have assumed. This misunderstanding has led them to adopt the term 'Number of expanded sheathing leaves' for Table 3 in their present paper and, by doing so, they have committed a serious error. While Kenneth *et al.*'s means of 2.3–3.0 are correctly placed in this category, the mean of 3.7 is not, for this value has been taken from Table 2 of Bateman & Denholm's (1983) former paper, where it can be seen under character no. 34. As shown above, this character and Kenneth *et al.*'s 'number of expanded sheathing leaves' are not identical and it is meaningless to compare the means 2.3–3.0 with 3.7 as Bateman & Denholm (1989) have now done in their Table 3.

In their count of 'Number of expanded sheathing leaves' Kenneth *et al.* (1988, Table 1) include leaf no. 2 (Fig. 2) but omit no. 1. In their separate count of 'Total number of leaves', however, no. 1 is included, along with nos. 2–6.

The total number of leaves from the Ha Mire Wood population can be found by adding the means for characters nos. 34, 35 and 36 in Bateman & Denholm's (1983) Table 2, i.e. 3.7 plus 1.0 plus 1.0 making 5.7. This value falls within the range of means, 5.1–9.2, for this character in *D. majalis* subsp. *purpurella* in North Wales (Roberts 1961), but is well outside the ranges in Continental and Scottish *D. lapponica*, 3.04–3.52 and 3.3–4.3 respectively (Kenneth *et al.* 1988, Table 1).

Total leaf number is one of the key characters which separates *D. lapponica* (and *D. traunsteineri*) from *D. majalis* subsp. *purpurella*. The difference between the means from the Scottish plants, 3.3–4.3 (Kenneth *et al.* 1988, Table 1), and the mean from the Yorkshire population, 5.7, is therefore sufficient on its own to preclude any possibility of identifying the Ha Mire Wood population as *D. lapponica*, or any of the Scottish populations as *D. majalis* subsp. *purpurella*. Moreover, the rest of Bateman & Denholm's arguments and conclusions in this section become completely untenable.

The presence of *D. lapponica* in Britain is thus seen to be established on reliable and convincing evidence. Kenneth *et al.* (1988), however, have not expressed an opinion on the taxonomic status of these plants, but have accepted Reinhard's arguments for the treatment of *D. lapponica* at species level, which are fully detailed in his paper (Reinhard 1985).

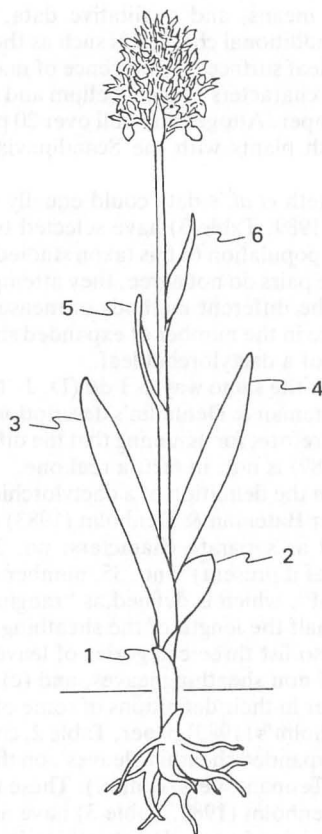


FIGURE 2. Diagram of a dactylorchid plant to explain the different definitions of the leaves. (This diagram only applies to a proportion of the plants in a population.)

CLASSIFICATION AND NOMENCLATURE

In an earlier paper Bateman & Denholm (1983) stated their belief that "gene flow between subspecies is at most only partially restricted". This implies that at Rhos-y-gad, where *D. traunsteineri* co-exists with *D. majalis* subsp. *purpurella*, hybridization between the two should be common and presumably result in a hybrid swarm. However, over the last 30 years I have had ample opportunity to examine the marsh-orchids at this locality and have searched for possible hybrids between these two taxa without success. Furthermore, there is no difficulty in identifying plants as one or the other in the field.

The evidence for introgression of *D. traunsteineri* from *D. majalis* subsp. *purpurella* would be an extension of the range of variability within the population of the former. A useful measure of variability in the characters for which morphometric data are available is the Coefficient of Variation. When this coefficient is calculated for the eight pairs of data in Table 2 of my paper (Roberts 1988), most of them agree closely. For example, for the characters 'total number of leaves' and 'leaf width' from the Rhos-y-gad plants it is 13.9% and 21.0% respectively, and for the Alpine plants 15.7% and 22.3%. The means of this coefficient for the two sets of data are 19.0% and 18.8% respectively and the small difference of 0.2% between them is not significant.

These observations do not support Bateman & Denholm's belief, nor do they provide evidence for the introgression of *D. traunsteineri*. It is also worth noting that (1) where they state that "The supposed British and Irish *D. traunsteineri* show morphological overlap with *D. majalis* subsp. *praetermissa* and subsp. *occidentalis*" (Bateman & Denholm 1983, p. 373) they do not mention *D.*

majalis subsp. *purpurella*. Yet it is this subspecies which occurs with *D. traunsteineri* at all of the three sites where Bateman & Denholm sampled it; (2) they do not record any hybrids between *D. traunsteineri* and *D. majalis* subsp. *purpurella* at any of them; (3) they did not study the co-existing population of subsp. *purpurella* at any of these places, when it seems logical that they should have done so.

In their earlier paper Bateman & Denholm (1983) recommended that biometric data should be taken from Alpine populations of *D. traunsteineri* "to quantify their differences from *D. majalis* subsp. *traunsteinerioides*", that is, not to show whether they differ or not, but by how much they do so. They now appear to have shifted their argument and say that the purpose of taking biometric data from Alpine plants was "to test this hypothesis", the hypothesis being, presumably, whether the British and Irish plants differ at all from the Alpine ones. Their claim that Reinhard's data, which were cited in my Table 2 (Roberts 1988), cannot be used to resolve this issue because of the inconsistencies or misconceptions in the definition of characters, has already been shown to be without foundation because it was based on a misconception on their part (see 'Selecting definitions of characters' above).

CONCLUSIONS

Bateman & Denholm (1989) are quite correct when they state that the controversies over *D. traunsteineri* and *D. lapponica* draw attention to some of the pitfalls encountered in morphometric studies. A fruitful source of error is the complication introduced when a seemingly simple character such as the 'Total number of leaves' is subdivided into three separate characters, as we have seen above. One example of such an error has already been seen in Bateman & Denholm's (1989) Table 3. A second and equally important one occurs in their Table 1, where their mean (3.60) for the 'Total number of leaves' from Rhos-y-gad has been obtained by extracting the means of characters no. 34 (2.6) and no. 35 (1.0) from Table 2 of their 1983 paper, and adding them. Unfortunately, they have omitted character no. 36, which consists of leaves nos. 1 and 2 in my Fig. 2. This is a serious omission, for the value 3.60 is thus equivalent only to leaves nos. 3-6 in Fig. 2 and does not give the true mean for the total number of leaves from this population, as it purports to do. Moreover, this is not the only mistake they have made in compiling the data in Table 1. Such errors are not only an unwitting misuse of morphometric data, but yet further examples of the pitfalls which these authors have been at such pains to warn us against and into which they themselves have fallen.

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(This matter is now closed—Eds.)