KEW BULLETIN (2023) 78:107–131
DOI 10.1007/S12225-023-10080-5

ISSN 1874-933X (print)
ISSN 0075-5974 (electronic)

Systematic reappraisal of marsh-orchids native to Scotland

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Summary. The intensively studied Eurasian orchid genus *Dactylorhiza* has become a model system for exploring allopolyploid evolution, yet determining the optimal circumscriptions of, and most appropriate ranks for, its constituent taxa remain highly controversial topics. Here, novel allozyme data and detailed morphometric data for 16 Scottish marsh-orchid populations are interpreted in the context of recent DNA sequencing studies. Despite being derived from the same pair of parental species, the two allopolyploid species that currently occur in Scotland can reliably be distinguished using allozymes, haplotypes, ribotypes or sequences of nuclear genes. A modest range of diverse morphological characters are shown to distinguish the two molecularly-circumscribed species, but they have in the past been obscured by equivalent levels of infraspecific variation in characters rooted in anthocyanin pigments; these characters are better employed for distinguishing infraspecific taxa. *Dactylorhiza francis-drucei* (formerly *D. traunsteinerioides*) is confirmed as being distinct from the continental *D. traunsteineri/lapponica*, probably originating through allopatric isolation once the continental lineage reached Britain. All Scottish populations are attributed to the comparatively small-flowered, anthocyanin-rich subsp. *francis-drucei*, which includes as a variety the former *D. 'ebudensis'*; the less anthocyanin-rich subsp. *traunsteinerioides* is confined to Ireland, North Wales and northern England. In contrast with *D. francis-drucei*, only a minority of Scottish populations of *D. purpurella* are attributed to the anthocyanin-rich race, var. *cambrensis*. This species most likely originated through an allopolyploidy event that occurred comparatively recently within the British Isles, as it contains allozyme alleles distinctive of British rather than continental *D. incarnata* (its diploid pollen-parent). In contrast, the rare Scottish population of *D. incarnata* subsp. *cruenta* shares with its Irish counterparts a continental genotype, and is most likely a recent arrival in Scotland through long-distance dispersal. Among all European allotetraploid dactylorchids, *D. purpurella* is the species that most closely resembles *D. incarnata*, both molecularly and morphologically.

Key Words. allopolyploidy, allozymes, conservation priorities, *Dactylorhiza francis-drucei*, DNA sequencing, evolutionary mechanisms, in situ morphometrics, species circumscription, taxonomy.

Introduction

The orchid genus *Dactylorhiza* (Orchidoideae, Orchidinae) has an exceptionally chequered taxonomic history. The genus was segregated from *Orchis* (now known to be only a distant relative) as *Dactylorchis* by Vermeulen (1947), following rigorous morphological and chromosomal studies, but unfortunately his work was bracketed by scientifically trivial nomenclatural publications (Necker 1790 *ex* Nevski 1935; Soó 1960, 1962) that conferred priority on the name *Dactylorhiza*. Within the genus, taxonomic controversies have been even more intense, prolonged, and by no means always rooted in genuine science. Once again, Vermeulen (1938, 1947) was the first author to suggest that allopolyploidy — hybridisation accompanied by chromosome doubling within a single generation — was the primary cause underlying many of these controversies.

Much of the research attention subsequently paid to this genus has been motivated by the desire to better understand the evolutionary significance of both allopolyploidy and autopolyploidy — goals that eventually allowed *Dactylorhiza* to become a model system for the study of whole-genome duplication. The genus features repeated unidirectional allopolyploidisation of the same two diploid ($2n = 40$) parental groups, *D. fuchsii* reliably operating as seed parent and *D. incarnata* as pollen parent. Moreover, the allopolyploidisation events have taken place at contrasting times and between subtly different habitat races of the parental species (e.g. Hedrén *et al.* 2008; Paun *et al.* 2010, 2011; Balao *et al.* 2016; Hawranek 2021; Wolfe *et al.* 2021; Eriksson *et al.* 2022; Thornton 2022). In addition to allopolyploidisation, the search for optimal species boundaries made the genus a pioneering case-study for population-level morphometrics. Early univariate approaches (Heslop-Harrison 1948, 1951, 1953, 1954; Roberts 1961a, 1961b) later gave way to computational multivariate techniques (Bateman & Denholm 1983, 1985, 1989; Dufrene *et al.* 1991; Pedersen 1998;

Accepted for publication 3 October 2022. Published online 18 May 2023

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Shipunov *et al.* 2004; Stahlberg & Hedrén 2008), in one case also employing landmark analysis (Shipunov & Bateman 2005).

Genetic studies of the genus have inevitably reflected the methodologies prevalent at the time each project was pursued. Molecular work began using allozymes in the mid-1990s in both Edinburgh, Scotland and Uppsala, Sweden (Hedrén 1996a, 1996b, 1996c, 2001), closely followed by typological phylogenetic studies spanning the genus that employed nrITS sequences (Pridgeon *et al.* 1997; Bateman *et al.* 2003). The 2000s began with analyses based on nuclear AFLPs (Hedrén *et al.* 2001; De Hert *et al.* 2012) and later plastid RFLPs (Devos *et al.* 2006), generated in parallel with more intensively sampled studies based on a combination of nuclear and plastid microsatellites (Hedrén 2003; Hedrén *et al.* 2007, 2011a; Pillon *et al.* 2007; Nordstrom & Hedrén 2007, 2009; Stahlberg & Hedrén 2008, 2010; Balao *et al.* 2016). During the 2010s, the repertoire of techniques successfully applied to the genus expanded further to include methylation-sensitive AFLPs (Paun *et al.* 2010, 2011) and gene expression patterns (Paun *et al.* 2011; Balao *et al.* 2017). The phylogeny of the genus was eventually established more firmly via the nuclear genome-wide RAD-seq approach (Brandrud *et al.* 2020), which allowed interpretation of the genome to begin to drill down to the level of ecophysiology (Wolfe *et al.* 2021) and genome dynamics (Hawranek 2021), including investigations of small RNAs (Eriksson 2022; Thornton 2022) and transposable elements (Eriksson 2022; Eriksson *et al.* 2022).

Synthesis of this veritable mountain of taxonomically relevant data is most parsimoniously (though by no means universally) interpreted as suggesting the presence of seven native species of *Dactylorhiza* in the British Isles. As determined by Bateman & Denholm (2012) and Bateman (2021, 2022a), these are:

Dactylorhiza viridis (L.) R.M.Bateman, Pridgeon & M.W.Chase (Frog Orchid), reputedly diploid; widespread, but local and decreasing in the south.

Dactylorhiza fuchsii (Druce) Soó (Common Spotted-orchid), diploid; common throughout most of the British Isles.

Dactylorhiza maculata (L.) Soó (Heath Spotted-orchid), autotetraploid; occurs throughout the British Isles but far more commonly in the north, particularly Scotland.

Dactylorhiza incarnata (L.) Soó (Early Marsh-orchid), diploid; widespread but local throughout the British Isles — intolerant of desiccation and divisible into fairly distinct ecotypes.

Dactylorhiza traunsteinerioides (Pugsley) R.M.Bateman & Denholm (Pugsley's Marsh-orchid), allotetraploid; widespread but local, occurring only north of a line connecting mid-Wales with the Humber.

[Note that here, for reasons of nomenclatural priority explained below in the concluding section, titled Nomenclatural Postscript, we employ at species level the epithet *'francis-drucei'* rather than '*traunsteinerioides*', which with regret is demoted to a subspecies of *D. francis-drucei* (Wilmott) Aver.]

Dactylorhiza praetermissa (Druce) Soó (Southern Marsh-orchid), allotetraploid; frequent in England and Wales, absent from Ireland and Scotland but actively expanding northwestward.

Dactylorhiza purpurella (T.Stephenson & T.A.Stephenson) Soó (Northern Marsh-orchid), allotetraploid; frequent, occurring only north of a line connecting the Severn and Humber estuaries.

Dactylorhiza kerryensis (Wilmott) P.F.Hunt & Summerh. (Irish Marsh-orchid, syn. *D. occidentalis*), allotetraploid; confined to Ireland, where it is most frequent in the west.

Evidence has progressively accumulated showing that each of the four allotetraploid species is derived from a member of the *Dactylorhiza fuchsii–maculata* alliance as seed-parent and the *D. incarnata* clade as pollen parent, and that the two parental clades are only moderately closely related (e.g. Hedrén 1996b; Pillon *et al.* 2007; Brandrud *et al.* 2020), having diverged an estimated 8 Myr ago (Brandrud 2019; Hawranek 2021).

Fieldwork for the present study was confined to Scotland, in a focused investigation of 'boreal' dactylorchids that was conceived to address three of the most contentious issues that have long plagued the systematics of British (and indeed European) dactylorchids:

(1) Whether the Gordian Knot of several named taxa collectively known as the narrow-leaved marsh-orchids can ever be satisfactorily untangled (reviewed by Bateman 2011a, 2019; Bateman & Denholm 2012). Three epithets based on Scottish holotypes, *francis-drucei* (Wilmott 1936) and *ebudensis/scotica* (Nelson 1976; Wiefelspütz 1976; Landwehr 1977), have variously been treated as species in their own right or alternatively attributed to *D. 'traunsteinerioides'* (here conversely treated as a subspecies of *D. francis-drucei* — a species that may or may not be a British and Irish endemic), *D. traunsteineri* and/or *D. lapponica* and/or *D. majalis* (each of which may or may not be exclusively continental) (cf. Kenneth *et al.* 1988; Roberts 1988; Allan *et al.* 1993; Lowe 2003; Hedrén *et al.* 2011a; Bateman 2011a; Bateman & Denholm 2012; Eccarius 2016; Hedrén & Skrede 2018; Stace 2019). British populations of small, boldly-marked plants attributed to *'D. lapponica'* (here treated as *D. francis-drucei* subsp.

francis-drucei) were given the maximal conservation protection of being placed on Schedule 8 of the UK's Wildlife and Countryside Act in 1992.

(2) Whether allotetraploid populations of marsh-orchids in North Wales and Scotland that are often comparatively robust and include plants bearing leaf markings, and have been awarded the epithets *cambrensis* and *majaliformis* respectively, (a) represent the same taxon, as argued by Bateman & Denholm (1983, 2012), and if so, (b) whether that taxon is best treated as a full (and most likely endemic) species (e.g. Averyanov 1984) or an infraspecific taxon within either *Dactylorhiza majalis* (e.g. Roberts 1961b, 1966), *D. kerryensis* (e.g. Campbell 1937; Sell & Murrell 1996) or *D. purpurella* (e.g. Nelson 1976; Løjtnant 1979).

(3) Whether, despite its striking overall paucity of genetic variation, *Dactylorhiza incarnata* maintains populations in the British Isles that reliably differ genetically from those already studied in continental Europe, and also whether an unusual population of leaf-marked individuals of this species discovered in the Scottish Highlands in 1982 (Kenneth & Tennant 1984; Bateman & Denholm 1985; Allan *et al.* 1993) has been correctly attributed to *D. incarnata* subsp. *cruenta* (O.F.Müll.) P.D.Sell (Flecked-early Marsh-orchid), a taxon better known from the Alps and Scandinavia. This rare taxon also remains of legislative interest to both the British and Scottish conservation bodies.

Scottish exemplars of these taxa are illustrated in Fig. 1, and recently updated distribution maps are shown for the two allotetraploid species in Fig. 2. Authorites of taxa mentioned in the text are given in Appendix 1.

Materials and Methods

Much of the work for this study was conducted in 1995 and 1996, utilising typical contemporary methods. The project was constructed around a combination of allozymes and character-rich multivariate morphometrics, sampling at the population level individual plants each of which provided data for both morphological and molecular analysis. Although the existence of our results from this project has occasionally received brief mention in earlier publications (Bateman 2001, 2011a; Hedrén 2002; Hédren *et al.* 2011a; Bateman & Denholm 2012), the data have not until now been presented or their implications rigorously explored. Their relevance has not diminished in the intervening years.

Fieldwork

During June – July 1995 and 1996, RMB gathered in situ morphometric measurements from 13 tetraploid marsh-orchid populations in Scotland: five populations of Northern Marsh-orchids (*Dactylorhiza purpurella* s.l.) and eight populations of narrow-leaved marsh-orchids (*D. traunsteinerioides* s.l.). The latter were collected under licence from Scottish Natural Heritage. Either 10 or, more often, 20 randomly chosen plants were scored for each population, and single leaves were also collected from each plant and field-chilled for subsequent allozyme analysis by LM and WC in the laboratory of RAE. Three further populations of Northern Marsh-orchid were sampled for allozyme analysis without accompanying morphometric data, whereas conversely, another population yielded morphometric data but no allozyme data. In addition, what was at the time the only known Scottish population of the diploid marsh-orchid *D. incarnata* subsp. *cruenta* was analysed for both allozymes (WC, RAE) and morphometrics (RMB), and compared with five populations of this subspecies sampled in west-central Ireland by RMB and ID between 1981 and 1997. Details of the study populations are given in Table 1.

In addition, small numbers of five species cultivated within the grounds of RBG Edinburgh — two diploids and three putative allotetraploids — were subjected to both allozyme and morphometric analyses in order to provide a broader taxonomic and geographic context for the present study (see also Bateman 2021).

Morphometrics

Character scoring

A complete list of the 52 characters scored by us was detailed by Bateman & Denholm (1985). While in the field we measured in situ 28 vegetative characters plus three floral characters; the remaining 21 characters (C1 – C17, C20 – C21) were recorded on the same data sheet in evening of the same day or subsequently in the microscopy laboratory (C26 – C27). Field measurements were made using a 15 cm steel rule bearing increments of 0.5 mm. A flower–bract unit for subsequent measurement was, wherever feasible, removed from a position one third to halfway from the base of the inflorescence, aiming to minimise the effect of the flower-size decreases from the base to the apex of the inflorescence that are evident in most Eurasian orchid species (Bateman & Rudall 2006). Each flower was initially placed in a numbered vial and later mounted onto double-sided adhesive tape attached to a filing card. Following measurement, these cards acted as compact herbarium vouchers. Metric characters for most floral organs were measured at a resolution of 0.1 mm, using a Leitz ×8 graduated ocular.

Fig. 1. Representative plants of the Scottish marsh-orchid taxa analysed in the present study. **A, B** *Dactylorhiza incarnata* subsp. *cruenta,* Lochdroma, Wester Ross; **C, D** *D. purpurella* var. *purpurella,* Aberlady Bay, East Lothian; **E, F** *D. purpurella* var. *cambrensis,* Thurso, Caithness; **G, H** population previously regarded as *D. francis-drucei* subsp. *traunsteinerioides,* Applecross, Wester Ross; **J, K** *D. francis-drucei* subsp. *francis-drucei* s.s., Raasay, North Ebudes; **L, M** *D. francis-drucei* subsp. *francis-drucei* var. *ebudensis,* North Uist, Outer Hebrides. Enlarged images of flowers are reproduced at a constant scale of 22 mm in image width.

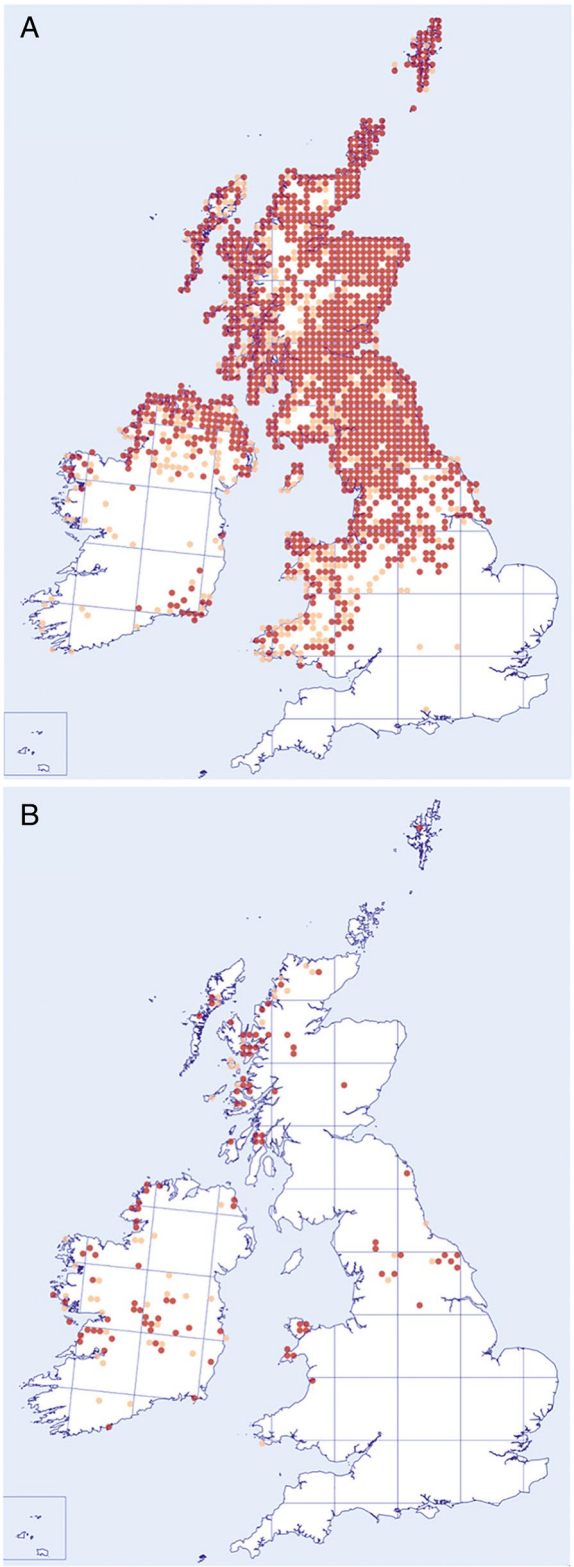

Fig. 2. Distribution maps for **A** *Dactylorhiza purpurella* and **B** *D. francis-drucei* in the British Isles, as revised by the present authors and summarised in the latest British and Irish plant atlas (Stroh *et al.* 2023). Paler hectads have not been recorded since year 2000.

The colour of the distal half of each labellum was matched to the nearest one or two colour block(s) of the Royal Horticultural Society Colour Chart. The colours were later quantified through conversion to three CIE (Commission Internationale de l'Eclairage) coordinates. Two of these ('x' and 'y') define a position on a square grid superimposed onto a near-triangular array of colours that pale toward white at the centre of the triangle. The corners correspond with pure blue, pure green and pure red, respectively. Density of pigment was represented by a third coordinate (reflectivity or luminance, 'Y'), which decreases in value outward from the centre of the triangle (see figs 12 and 13 of Bateman *et al.* 2017; also http://hyperphysics.phy-astr.gsu.edu/hbase/vision/cie.html). A compound microscope was used to count bract marginal cells across three fields of view, each 1.5 mm in diameter, before their mean length was calculated and their average angularity was summarised.

The 52 characters scored describe the stem and inflorescence (5), leaves (12), leaf markings (7), bracts and ovary (7), labellum (14), spur (4) and sepals (3). They can alternatively be categorised collectively as metric (21), meristic (3), multistate-scalar (24) and bistate (4).

Data analysis

Our chosen approach to data analysis and interpretation was both detailed and experimental. Morphometric data for individual plants were summarised on an Excel v15.4 spreadsheet. Mean values, plus sample standard deviations and coefficients of variation for all metric and meristic characters, were calculated for every character in each study population. Univariate and bivariate analyses were summarised and presented using Deltagraph v7.1 (SPSS/Red Rock software 2013).

The morphometric matrix consisted of 209 individuals × 52 characters and contained only 0.3% missing values; no single character incurred more than 5% missing values. The derived matrix of population means consisted of 14 populations × 52 characters and lacked missing values. Both matrices were analysed by multivariate methods using Genstat v14 (Payne *et al.* 2011).

Of the 52 characters scored, four of the seven leaf-marking characters (C46 – C49) were omitted to avoid over-weighting a feature that appears to reflect only a single underlying gene, and the presence or absence of a basal leaf (C36) was omitted because such leaves had proven vulnerable to premature senescence. The remaining 47 characters were used to compute a symmetrical matrix that quantified the similarities of pairs of data sets (i.e. plants) using the Gower Similarity Coefficient (Gower 1971) on unweighted data sets scaled to unit variance. This similarity measure is comparatively effective when presented with a matrix of heterogeneous characters that includes missing values (Gower

& Legendre 1986; Lloyd 2016; Bateman 2022b). The resulting matrix was in turn used to construct a minimum spanning tree (Gower & Ross 1969) and subsequently to calculate principal coordinates (Gower 1966, 1985) — compound vectors that incorporate positively or negatively correlated characters that are most variable and therefore potentially diagnostic. Principal coordinates are especially effective for simultaneously analysing heterogeneous suites of morphological characters and have the additional advantage of comfortably accommodating missing values. Such ordinations have proven invaluable for assessing relationships among orchid species and populations throughout the last four decades, employing a consistent analytical approach that was reviewed in detail by Bateman (2001).

Two separate multivariate analyses were conducted on the putative allotetraploids, the first being based on measurements for individual plants, whereas the second was based on mean values calculated for each analysed variable in each of the 12 study populations. For each of the two multivariate analyses, the first four principal coordinates (PC1 – PC4) were plotted together in pairwise combinations to assess the degree of morphological separation of individuals (and thereby of populations and taxa) in these dimensions, and pseudo-F statistics were obtained to indicate the relative contributions to each coordinate of the original variables.

In addition, the single putative population of *Dactylorhiza incarnata* subsp. *cruenta* then known in Scotland was compared morphometrically with Irish populations that have been attributed to this subspecies since they were first recognised as *cruenta* in 1948 (e.g. Heslop-Harrison 1949); five such populations were measured by RMB and ID between 1981 and 1997 in Cos. Galway and Mayo (Table 1).

Allozymes

Our study benefited considerably from preliminary allozyme studies already performed on the genus *Dactylorhiza* by Mikael Hedrén (published a year later as Hedrén 1996a, 1996b, 1996c). Of seven allozyme loci explored by Hedrén, the three involving phosphogluconate-group substrates (the dimeric *6-pgd* and *pgi* [syn. *gpi*] plus the monomeric *pgm*) had been shown to offer an effective combination of both reliably discriminating between the parents of the western European allotetraploids (*D. fuchsii/maculata* and *D. incarnata*) but also showing some variation among populations of the same putative species of allotetraploids. Also, according to Hedrén (1996b), all three systems were competent to resolve allele dosage levels. We therefore elected to focus on these three loci.

Data were collected in the laboratory of RAE by LM in 1995 and by WC in 1996. Chilled leaf tissues were prepared satisfactorily as a crude buffer extract with no elaborate purification or concentration steps; optimisation of pH values proved to be the most crucial methodological challenge (e.g. Wendel & Weeden 1989).

For each individual analysed, approximately 1 cm^2 of leaf was ground in 80 µl of a Tris-HCl extraction buffer (Soltis *et al.* 1983), modified by replacing ß-mercaptoethanol with dithiothreitol. Extracts were absorbed onto paper wicks and proteins were separated on horizontal starch gels at 60 mV for 30 mins until removal of the wicks, after which current was increased to 70 mV for a further 3 – 4 h. *Pgi* alleles were resolved on the lithium-borate tris-citrate buffer system of Ashton & Braden (1961), as modified according to Lonn & Prentice (1990), whereas a histidine-citrate buffer system (Wendel & Weeden 1989) was used to separate alleles of *pgm* and *6-pgd*. Staining recipes followed Wendel & Weeden (1989) with only minor modifications. Gel patterns were recorded immediately, both graphically and photographically, prior to immersion in a methanol-acetic acid fixative.

Each 20-lane starch gel included extracts from 16 allotetraploid individuals bracketed at either end by extracts from "standard" plants representing the diploid parental genomes. The *fuchsii* standard was derived from a small population maintained in cultivation in RBG Edinburgh (1984/1618: originally gathered in 1984 from a coal bing at Gorebridge, Midlothian), whereas the *incarnata* standard was derived directly from a natural population located 21 km east of RBG Edinburgh in extensive dune-slacks at Aberlady Bay (this population was also subjected to morphometric analysis: Table 1). Numbers of individuals analysed per population ranged from five to (more often) 16.

When scoring the resultant gels (cf. Weeden & Wendel 1989), alleles were designated by lower-case letters, beginning with the most rapidly migrating allele. 'Missing letters' denoted alleles found in populations of *Dactylorhiza* outside the present study, but as summarised for European populations by Hedrén (1996a) rather than later coding employed for a broader spectrum of Eurasian populations by Hedrén (2001). Routine use of the diploid parental 'standards' contributed appreciably to accurate identification of specific alleles; nonetheless, some gels incurred sufficient ambiguity to discourage us from presenting the tentative results (denoted by 'f' in Table 2).

Results

Allozymes

Table 2 details the allele frequencies obtained for the three studied loci for Scottish populations of

Table 1. Details of Scottish and Irish marsh-orchid (*Dactylorhiza*) populations sampled for morphometric and allozyme analysis in the present study. [1] Allozyme data only. [2] Morphometric data only. [3] DNA sequence data available. [4] One week added to compensate for an unusually early flowering season. [5] One week subtracted to compensate for an unusually late flowering season. [6] Names of dactylorchid taxa: F, *fuchsii*; M, *maculata*; II, *incarnata incarnata*; IC, *incarnata coccinea*; ICr, *incarnata cruenta*; IP, *incarnata pulchella*; P, *purpurella*; D, *francis-drucei*. Taxon frequencies: vr, very rare; r, rare; o, occasional, f, frequent.

Taxon	Locality	Habitat	Altitude (m a.s.l.)	Peak flowering	Associated dactylorchid taxa + hybrids[6]
francis-drucei 'subsp. *traunsteinerioides'*	Loch a Mhuilinn, Milton, APPLECROSS[3], Wester Ross	calcareous flush	18	6/3-4[5]	F(r), M(o), ?II(o), IC(r), P(f), F×P(o), F×D(o), M×D(o), I×D(r), P×D(o)
francis-drucei subsp. *francis-drucei*	W Lochan Dobhrain, ACHAHOISH, Knapdale, Kintyre	calcareous flushes	220	6/2-3	M(f)
	S Lochan nam Fiann, Glen BORRODALE, Westerness	calcareous flush	220	6/2-3	M(o), IP(o)
	S Meall Clach an Daraich, ACHNAHA, W Kilchoan, Westerness	calcareous flush	40	6/2-3	M(o), IP(f)
	W Druim an AONAICH[3], N Dun Caan, Raasay, N Ebudes	calcareous flushes	280	6/4[5]	M(vr)
	Loch KERNSARY[3], E Poolewe, NE Gairloch, Wester Ross	calcareous flushes	35	6/4[5]	M(c), IP(o)
francis-drucei subsp. *francis-drucei* var. *ebudensis*	HORNISH[3], Newtonferry, N Uist, Outer Hebrides	damp machair	2	6/2-3	IC(o)
	SUENISH, Newtonferry, N Uist, Outer Hebrides	damp machair	2	6/2-3	IC(o)
purpurella var. *purpurella*	ABERLADY[3] Bay, Gullane, E Lothian	dune slacks	3	6/2-3	?II(c), IC(c), F×P(r), I×P(vr)
	NW KILCHOAN[1], Ardnamurchan, Westerness	rough roadside pasture	25	6/4	?M(o)
	Marsh, ARDNAISH[2] peninsula, Broadford, Skye, N Ebudes	marshy meadow	1	6/4	F(o), M(o), II(f), F×P(f)
	Machair ROBACH[3], Newtonferry, N Uist, Outer Hebrides	damp machair	2	7/1-2	IC(o)
	BORVE[1], Harris, Outer Hebrides	coastal rough pasture	8	7/1	?M(o)
purpurella var. *cambrensis*	AULTBEA[1], Loch Ewe, Wester Ross	coastal rough pasture	4	6/4	None
	DUNNET Links, Castletown, Thurso, W Sutherland	inland dune slack	25	6/4[5]	None
	NW Thurso Castle, THURSO, Caithness	grassy bank	3	6/3[5]	None
	Imm. W SCRABSTER Harbour, NW Thurso, Caithness	grassy scree at foot of coastal cliffs	30	6/3[5]	None
incarnata subsp. *cruenta*	Pavement, S Lough GELAIN, Corrofin, Co. Clare	loughside pavement	35	6/2[4]	F(o)
	E Lough BUNNY[2,3], Gort, Co. Clare	loughside fen	20	6/2[4]	F(f)
	E Lough MASK[2], Augnish, Ballinrobe, Co. Mayo	loughside fen	22	6/4	F(f)

Table 1. (continued)

Taxon	Locality	Habitat	Altitude (m a.s.l.)	Peak flowering	Associated dactylorchid taxa + hybrids[6]
	SW Lough CARRA[3], Ballinrobe, Co. Mayo	loughside fen	25	6/1-2[4]	F(r)
	KEELBRIDGE[2], SW Lough Carra, Ballinrobe, Co. Mayo	loughside fen	30	6/1-2	F(o), M(f),F×M(vr), F×ICr(r)
	NE Lochdum Farm, LOCHDROMA[3], Braemore, Wester Ross	calcareous flush	320	6/4-7/1[5]	?II(r), IP(o)

Dactylorhiza francis-drucei, *D. purpurella*, *D. incarnata* and *D. fuchsii*, together with qualitative results for four additional species held in cultivation at RBG Edinburgh. Letters denoting specific alleles of the three loci follow Hedrén (1996a, 1996b, 1996c).

Diploid species parental to the allotetraploids

Dactylorhiza incarnata yielded greater allozyme diversity, both within and among populations (Table 2A), than was predicted through extrapolation from Hedrén's (1996a) Scandinavian data. Of the four subspecies investigated, three yielded only the *b* allele for the *pdg* locus, whereas all three populations of subsp. *cruenta* included individuals characterised by possession of the *a* allele. Only two populations of *D. incarnata* yielded reliable data for the *pgi* locus, producing the expected result of reliable homozygosity for the slow *e* allele. For the *pgm* locus, subspp. *incarnata* and *coccinea* (including Aberlady, here used as the allelic yardstick for *incarnata*) proved consistently homozygous for the *c* allele, whereas populations of subspp. *pulchella* and *cruenta* yielded mixtures of the *b* and *c* alleles.

Analysis of *Dactylorhiza fuchsii* was confined to the single 'yardstick' accession from Gorebridge (Table 2A). This population provided no surprises; it contained the expected pairing of *b* and *c* alleles for *pgd*, the *b* allele only for *pgi*, and was, after some debate, judged to bear the *d* and *e* alleles for *pgm* (cf. Hedrén 1996a, 1996b).

Allotetraploids

In the case of *Dactylorhiza francis-drucei*, *pgi* profiles were dominated by alleles *a* and *c*, though in some populations a minority of plants replaced the *a* allele with the *b* allele (Table 2A). Results for *pgi* were reliably balanced between the *fuchsii*-derived allele *b* and *incarnata*-derived allele *e*, and *pgm* typically provided equal frequencies of the *b* and *d* alleles, though a few plants in the Aonaich and Kernsary populations replaced the *b* allele with the *c* allele. No allelic patterns distinguished between the named taxa *traunsteinerioides* s.s., *francis-drucei* s.s. (*'lapponica'* sensu Kenneth *et al.* 1988; Stace 1997) and *ebudensis*.

Results for *Dactylorhiza purpurella* were straightforward for the *pgd* and *pgi* systems. Six of the seven populations studied contained only the *b* allele for *pgd*, though the Dunnet population also maintained allele *a* at a frequency of approximately 7% (Table 2A). Balanced heterozygosity of the *b* and *e* alleles was consistent for *pgi* and of the *c* and *e* alleles for *pgm*, thus contrasting with *D. francis-drucei* at this locus.

Other RBG Edinburgh accessions

Of the four non-British species cultivated in RBG Edinburgh, to the best of our knowledge only *Dactylorhiza majalis* s.s. had previously been subjected to allozyme analysis (Hedrén 1996a, 1996b, 1996c). Our results were consistent with previous work on this species, yielding the *a* and *b* alleles for *pgd*, *b* and *e* alleles for *pgi*, and *b* and *d* alleles for *pgm* (Table 2B). *Dactylorhiza alpestris* surprisingly diverged from the morphologically similar *D. majalis* by replacing the *b* allele with the *c* allele for *pgd*. A third allotetraploid species, *D. elata*, matched *D. majalis* and *D. alpestris* in *pgi* and *pgm* profiles, but consistently possessed the *b* and *c* alleles for *pgd*. The Madeiran island endemic *D. foliosa*, a diploid more closely related to *D. fuchsii* than to *D. incarnata* (e.g. Pillon *et al.* 2007), maintained only one allele at each locus: the *c* allele for *pgd*, the *a* allele for *pgi* and, surprisingly, the *b* allele for *pgm* — an allele that is characteristic of *D. incarnata* rather than *D. fuchsii*. Multiple genetic lines with contrasting geographic origins investigated within three of these four cultivated species consistently yielded identical, species-specific results (Table 2B).

Morphometrics

Tetraploid marsh-orchids: individual plants

The first two principal coordinates for 209 individual plants of *Dactylorhiza francis-drucei* s.l. and *D. purpurella* s.l. accounted for 29% of the total variance. We anticipated that the first coordinate would distinguish between the two molecularly circumscribed species, but in fact this role fell to the second coordinate, which permitted only slight overlap of the two species (Fig. 3). This coordinate largely represented

Table 2. Allozyme allele frequencies determined in **A** natural populations and **B** cultivated populations of Scottish dactylorchids (f = gel judged inadequate, * = diploid populations routinely employed as allozyme standards). Allele annotation follows Hedrén (1996). For (B), + indicates the reliable presence of the specified allele, whereas ++ indicates consistent double dosage (i.e. homozygosity) for all three loci in *Dactylorhiza foliosa*.

Locus	**6-pgd**			**pgi**		**pgm**				
Allele	**a**	**b**	**c**	**b**	**e**	**a**	**b**	**c**	**d**	**e**
(A) Natural populations										
D. fuchsii										
Ex Gorebridge*	0	50	50	100	0	0	0	0	50	50
D. incarnata incarnata										
Applecross	0	100	0	f	f	0	0	100	0	0
Kernsary	0	100	0	f	f	0	0	100	0	0
D. incarnata pulchella										
Lochdroma	0	100	0	f	f	0	40	60	0	0
Kernsary	0	100	0	f	f	0	25	75	0	0
D. incarnata cruenta										
Lochdroma	100	0	0	f	f	0	100	0	0	0
Gelain (Co. Clare)	25	75	0	0	100	0	25	75	0	0
Carra (Co. Mayo)	25	75	0	f	f	f	f	f	f	f
D. incarnata coccinea										
Aberlady*	0	100	0	0	100	0	0	100	0	0
Dog's Bay (Co. Galway)	0	100	0	f	f	f	f	f	f	f
D. purpurella purpurella										
Aberlady	0	100	0	50	50	0	0	50	0	50
Kilchoan	0	100	0	50	50	0	0	50	0	50
Borve	0	100	0	50	50	f	f	f	f	f
D. purpurella cambrensis										
Aultbea	0	100	0	50	50	0	0	50	0	50
Dunnet	7	93	0	50	50	0	0	50	0	50
Thurso	0	100	0	50	50	0	0	50	0	50
Scrabster	0	100	0	50	50	0	0	50	0	50
D. f-d. 'traunsteinerioides'										
Applecross	50	0	50	54	46	0	44	6	50	0
D. f-d. francis-drucei										
Achahoish	50	5	45	50	50	0	50	0	50	0
Borrodale	28	30	42	47	53	0	50	0	50	0
Achnaha	50	5	45	50	50	0	50	0	50	0
Aonaich	47	3	50	54	46	0	47	3	50	0
Kernsary	50	0	50	50	50	0	35	15	50	0
D. f-d. ebudensis										
Hornish	31	19	50	50	50	0	50	0	50	0
Suenish	50	0	50	50	50	0	50	0	50	0
(B) RBGE cultivated lines										
D. foliosa 1			++	++			++			
D. foliosa 2			++	++			++			
D. majalis 1	+	+		+	+		+		+	
D. majalis 2	+	+		+	+		+		+	
D. alpestris	+		+	+	+		+		+	
D. elata 1		+	+	+	+		+		+	
D. elata 2		+	+	+	+		+		+	
D. elata 3		+	+	+	+		+		+	

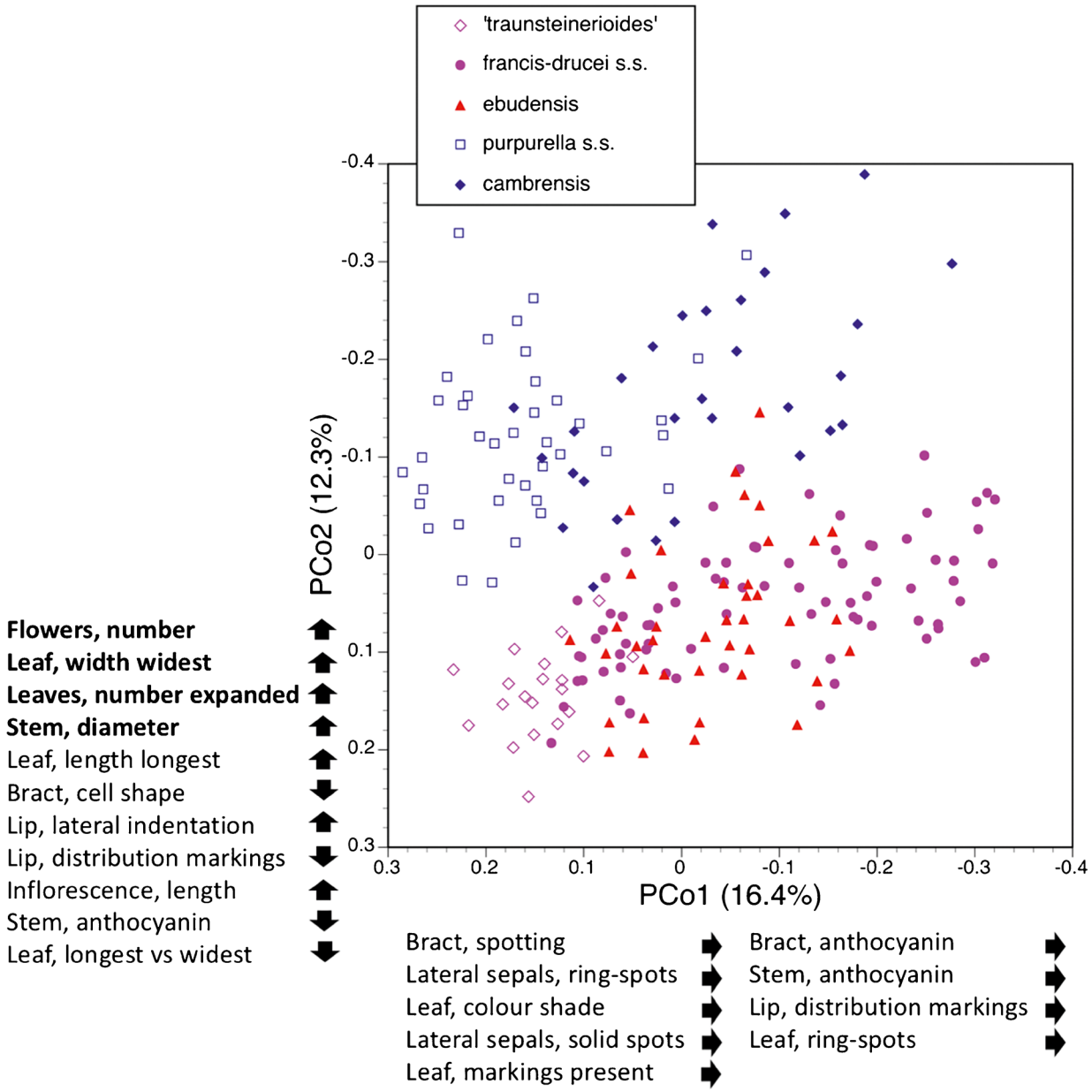

Fig. 3. Plot of the first two principal coordinates for 47 diverse morphological characters measured in 209 plants of 14 Scottish populations of the tetraploid marsh-orchids *Dactylorhiza francis-drucei* and *D. purpurella*. Parenthetic percentages represent the proportion of the total variance accounted for by each coordinate. Characters contributing significantly to each coordinate are listed in order of decreasing importance, with arrows indicating the direction of increase in value; boldface characters were dominant.

the greater vegetative vigour of *D. purpurella*, being dictated by flower number, the number, length and especially width of the sheathing leaves, and the diameter of the stem. Subsidiary contributors to this coordinate that favoured *D. francis-drucei* included more angular bract-margin cells, markings more widely distributed across the labellum, a greater frequency of diffuse anthocyanins below the inflorescence, and the widest leaf also being the longest (in the majority of *D. purpurella* plants the widest leaf was that located immediately below the longest leaf).

The first coordinate reflected the extensive variation evident within both species in a wide spectrum of discrete anthocyanin markings on both floral and vegetative organs. Discrete spots on the bracts and leaves are often

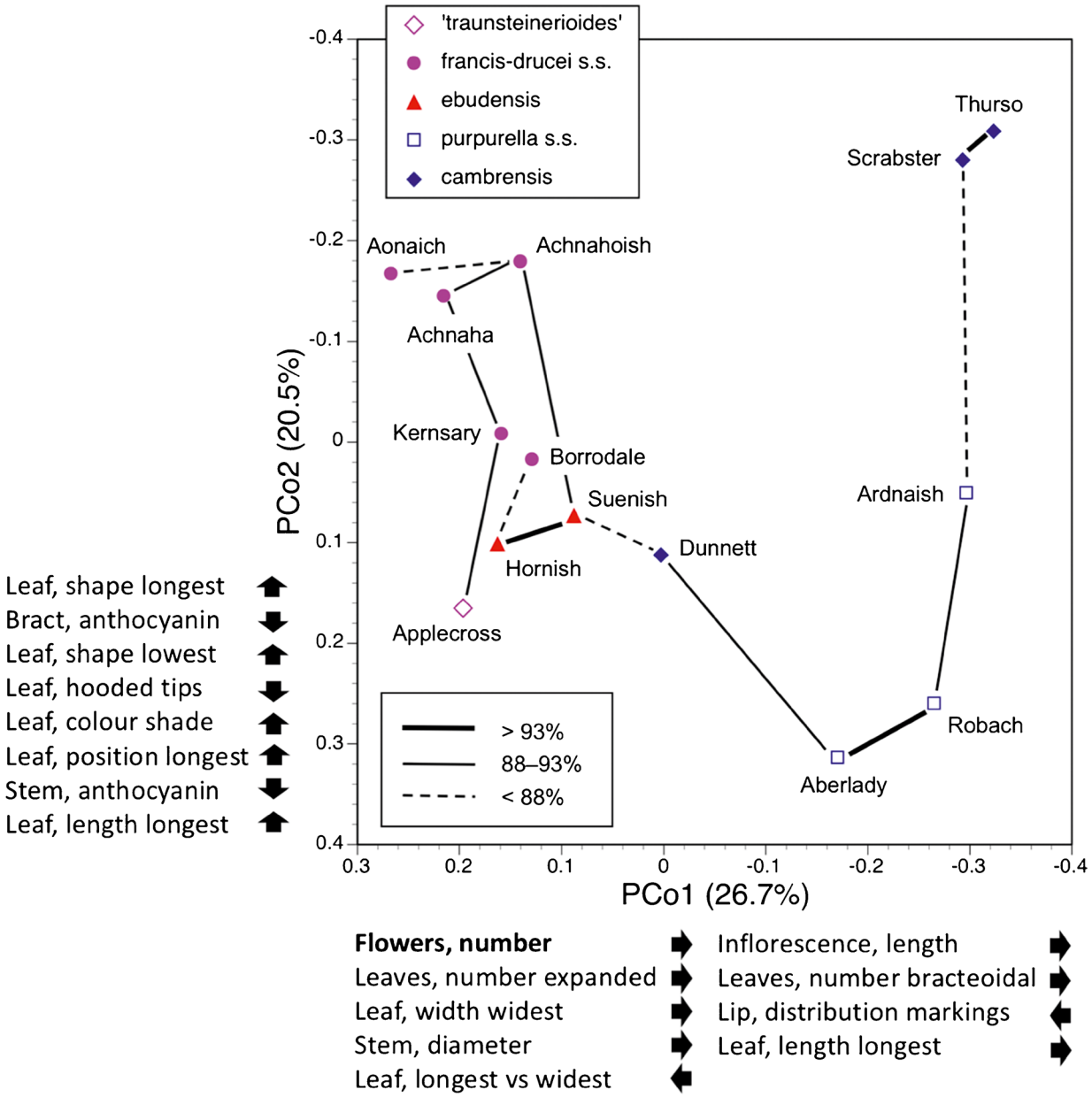

Fig. 4. Plot of the first two principal coordinates for 47 diverse morphological characters measured in 14 Scottish populations of the tetraploid marsh-orchids *Dactylorhiza francis-drucei* and *D. purpurella,* analysed as population mean values. Parenthetic percentages represent the proportion of the total variance accounted for by each coordinate. Characters contributing significantly to each coordinate are listed in order of decreasing importance, with arrows indicating the direction of increase in value; boldface characters are dominant. Populations are linked by a minimum spanning-tree representing maximum Gower Similarity values.

accompanied by leaves that tend to be a slightly darker, bluer green, and by bold loop markings (both solid and annular) on the lateral sepals. Weaker positively correlated characters included greater frequency of diffuse anthocyanins on both bracts and stem. The main effect of this axis was to present both species as marginally overlapping horizontally elongate ellipses in Fig. 3, each grading from anthocyanin-poor on the left to anthocyanin-rich on the right. Interestingly, flower colour was far less variable than the remaining anthocyanin-based characters and played no meaningful part in dictating positions of plants on the plot. Taxonomically, the first coordinate was surprisingly effective at largely separating var. *cambrensis* (Fig. 1E, F) from *Dactylorhiza purpurella* s.s.

(Fig. 1C, D). Less surprisingly, PCo1 also placed Applecross — the only study population of *D. francis-drucei* to wholly lack leaf-marked individuals (Fig. 1G, H) — at the anthocyanin-low extreme of the *francis-drucei* ellipse, though it was not resolved as a discrete entity. The third and fourth coordinates were much weaker and lacked taxonomic structure. Significantly, none of the first four coordinates suggested any distinction between *ebudensis* (Fig. 1L, M) and the far more geographically widespread *D. francis-drucei* subsp. *francis-drucei* (Fig. 1J, K).

Tetraploid marsh-orchids: population means

Reducing individual-level data-sets to population means inevitably decreases dimensionality within the data and so allows the first two coordinates to encompass a greater proportion of the total variance — in this case, 47% (Fig. 4). The two coordinates from the individual analysis are essentially transposed at population level, PCo1 being a "vigour" coordinate; it represents most of the characters that contributed to PCo2 in the plot of individuals (Fig. 3). The second coordinate resembles the first coordinate from the individual plants plot in that it represents each of the two species as an elongate ellipse and distinguishes between *Dactylorhiza purpurella purpurella* and *D. purpurella cambrensis*. However, the spectrum of characters underlying the axis is somewhat altered, those representing diffuse anthocyanins being promoted at the expense of those representing localised anthocyanin markings. More importantly, in outline the longest and lowest leaves tended to be more rounded in the Thurso and nearby Scrabster subpopulations of *cambrensis* and hence had more-or-less planar rather than hooded apices. These plants grew in taller vegetation and therefore bore their leaves roughly evenly spaced along the stem (Fig. 1F), but showed unusually low levels of diffuse anthocyanins on stem and bracts. In contrast, the nearby Dunnet population of *D. purpurella cambrensis*, which occupied exposed and grazed stabilised dune-slacks and so was environmentally dwarfed, is placed close to the *D. francis-drucei* cluster on Fig. 4, though the two species are connected by a reassuringly weak link on the minimum spanning tree. The strongest links in the tree connect pairs of sampled populations that were either subpopulations of what was effectively one extensive metapopulation (the Scrabster and Thurso populations of *cambrensis*, and the Suenish and Hornish populations of *ebudensis*) or occupied near-identical habitats (the dune-slack populations of *D. purpurella* s.s. from Aberlady and Robach).

The third and fourth coordinates were weak and offered only very limited discriminatory power among populations of *Dactylorhiza francis-drucei*. The third coordinate separated the Borrodale population from the remainder on account of its longer, slightly more curvaceous spurs and the light spotting observed on the underside of the leaves of some plants. The fourth coordinate weakly separated *ebudensis* according to its spurs, which were slightly more saccate than those of *francis-drucei* s.s.; spur widths measured halfway along spur length were only slightly less than the comparable widths obtained at the spur mouth.

Diploid marsh-orchids: individual plants

The ordination of 60 plants of six populations of *Dactylorhiza incarnata cruenta* (Fig. 5) yielded a strong first coordinate that largely reflected positive correlation between three characters likely to share expression of a single set of genes: discrete markings on the upper and lower surfaces of the leaves and on the bracts. This axis generated two crude clusters, one notably richer in these vegetative markings and annular markings on the lateral sepals. Five of the six populations sampled contributed individuals to each of the two clusters; for example, of the ten plants sampled at Lochdroma, eight are placed in the markings-rich category and two in the markings-poor category (these plants were later tentatively reassigned to subsp. *pulchella*). Only the comparatively markings-deficent Bunny population is confined to a single cluster, reflecting a more general trend within Ireland for plants of subsp. *cruenta* from Co. Clare to be less likely to be anthocyanin-rich than are plants from Co. Mayo (distinguished by blue vs purple symbols respectively in Fig. 5).

The Scottish (i.e. Lochdroma) plants of subsp. *cruenta* are placed toward the negative end of the appreciably weaker second coordinate, which otherwise discriminates poorly among the five Irish populations. It is largely a vigour coordinate, dictated by several characters that reflect the sizes of both floral and vegetative organs. Lochdroma features labella that are unusually narrow and thus longer than wide; the majority of plants have labella that are entire rather than shallowly three-lobed and are sufficiently small that the markings cover most of the labellar surface rather than leaving an unmarked border (Fig. 1A). Spurs are small, lateral sepals are dominated by annular markings, and most plants also have spotted bracts. Stems and inflorescences are short and narrow (Fig. 1B), and compared with the Irish populations, Lochdroma plants have on average one fewer sheathing leaf. In addition, Lochdroma leaves are only half the length and two-thirds the width of the Irish populations, and most Lochdroma plants have leaves that are spotted on both surfaces (as they are in the Irish population from Keelbridge, which resembles Lochdroma on PCo1). The even weaker third and fourth coordinates served only to largely separate the three study populations from the Irish "Lake District" of Co. Mayo (Mask, Carra and nearby Keelbridge).

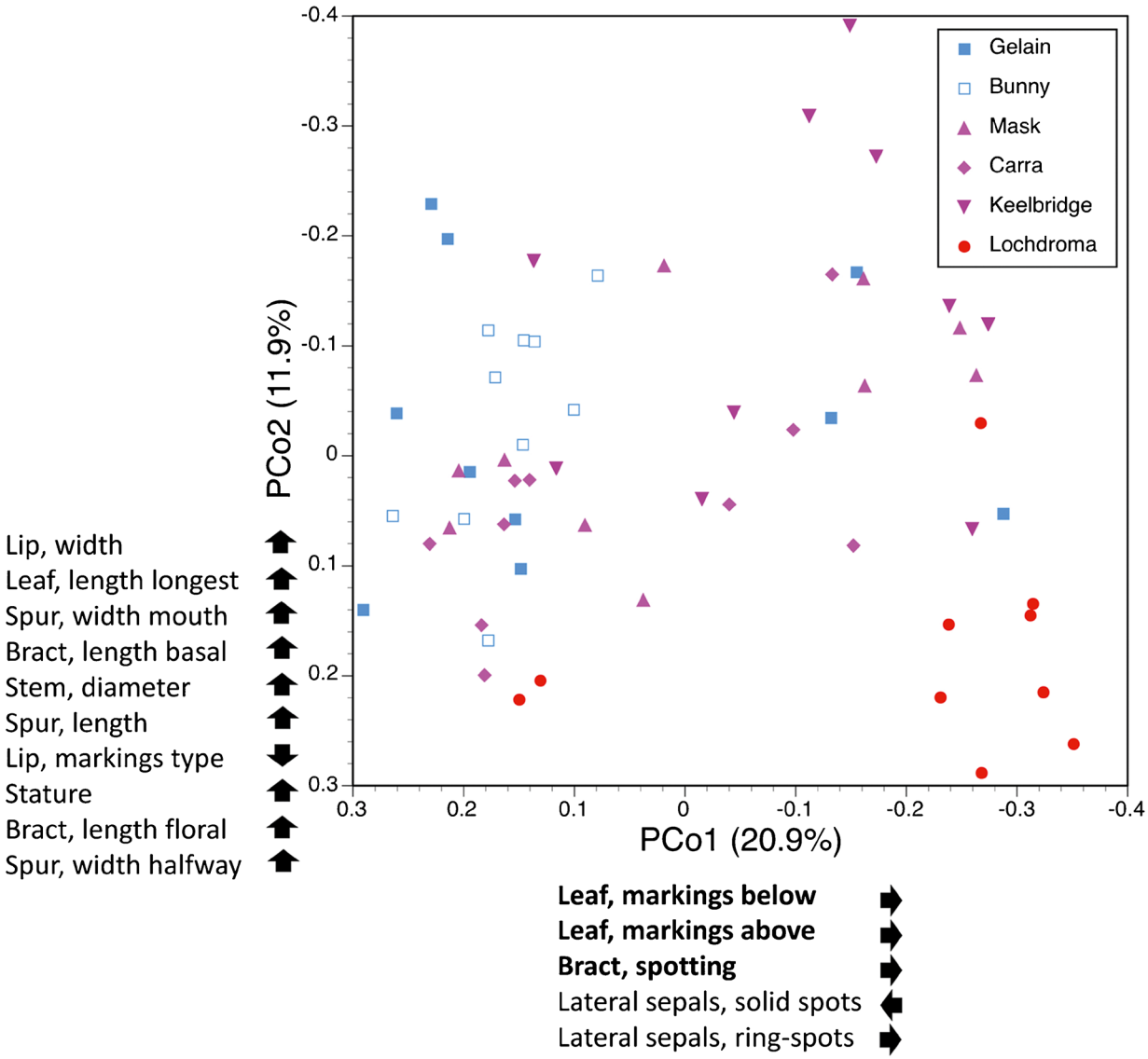

Fig. 5. Plot of the first two principal coordinates for 47 diverse morphological characters measured in a total of 60 plants representing one Scottish population (Lochdroma) and five Irish populations of the Flecked-early Marsh-orchid, *Dactylorhiza incarnata* subsp. *cruenta*. Parenthetic percentages represent the proportion of the total variance accounted for by each coordinate. Characters contributing significantly to each coordinate are listed in order of decreasing importance, with arrows indicating the direction of increase in value; boldface characters are dominant.

Discussion

Comparison of British allozyme profiles with their Scandinavian equivalents

Our limited allozyme study was inspired by extensive research using seven loci conducted by Hedrén (1996a, 1996b, 1996c), primarily sampling Scandinavian dactylorchid populations. On the basis of his results (as summarised in the present Table 3), we selected our three study loci to fulfil two contrasting purposes.

In Hedrén's data, the *pgi* locus reliably distinguishes the diploid *Dactylorhiza fuchsii* and its autotetraploid derivative, *D. maculata* (both dominated by allele *b*) from *D. incarnata*, dominated by allele *a*, and from the six allotetraploid species that possess approximately equal frequencies of alleles *a* and *b* — alleles that they acquired through hybridisation between paternal *D. incarnata* and maternal members of the *D. fuchsii* clade. The *pgd* locus was selected because of its apparent ability to subdivide, through contrasting frequencies of the *a*, *b* and *c* alleles, the six allotetraploids analysed by Hedrén

Table 3. Allozyme allele frequencies presented in previous studies of western European dactylorhizas. Sources: Hedrén (1996b) for *Dactylorhiza purpurella,* Hedrén (1996a) for the remaining taxa; data were derived from Scandinavian populations, except those for *D. praetermissa* (two populations from SC England). A few minor alleles have been omitted to facilitate comparison with data for the present project summarised in Table 2.

Locus	**6-pgd**			**pgi**		**pgm**				
Allele	**a**	**b**	**c**	**b**	**e**	**a**	**b**	**c**	**d**	**e**
D. fuchsii	0	47	52	99	0	0	0	3	24	61
D. incarnata (3 subspp.)	100	0	0	0	100	0	100	0	0	0
D. purpurella	14	76	10	50	50	0	6	44	3	47
D. traunsteineri	51	2	36	48	50	0	50	3	36	10
D. lapponica	50	0	23	38	50	0	50	9	41	0
D. praetermissa	49	43	7	47	49	2	45	0	23	15
D. majalis s.s.	51	38	9	48	51	4	46	0	40	10
D. sphagnicola	49	1	26	56	38	1	50	0	43	7
D. maculata	0	27	70	85	0	0	0	6	51	38

into three groups: *traunsteineri* plus *lapponica* plus *sphagnicola* (dominated by *a* + *c*), *majalis* s.s. plus *praetermissa* (dominated by *a* + *b*), and *purpurella* (predominantly *b*). Lastly, the *pgm* locus was selected primarily because of its reputed ability to distinguish *purpurella* from the remaining allotetraploids through favouring *c* plus *e* alleles over the faster pairing of *b* plus *d* alleles (Table 3).

Perhaps the most striking feature of Hedrén's (1996a, 1996b, 1996c) data for *Dactylorhiza incarnata* was the fact that analysis of several populations each of three subspecies nonetheless consistently yielded fixed homozygosity of a single allele for all three of the loci used here (Table 3), helpfully simplifying its identification as the paternal parent of the allopolyploids (though precluding identification of the paternal **sub**species). Features of Hedrén's data that were least readily explained primarily involved *D. purpurella*; specifically, its apparent enrichment in the *b* allele of *pgd* and its co-dominance of the *c* allele of *pgm*, which on the basis of Scandinavian material was assessed as absent from *D. purpurella*'s supposed pollen-parent *D. incarnata* and rare in its supposed seed-parent *D. fuchsii* (Table 3: Hedrén 1996a).

Our results for the *pgi* locus are entirely consistent with those of Hedrén, usefully serving to demonstrate the allopolyploid nature of both *Dactylorhiza francis-drucei* and *D. purpurella*. This locus need detain us no further. However, both the *pgd* and *pgm* loci yielded results that were both surprising and informative.

Arguably the greatest surprise was that British and Irish populations of *Dactylorhiza incarnata* proved to be capable of maintaining allelic diversity, which was observed in two of the four subspecies analysed by us. With the exception of subsp. *cruenta*, all of the British and Irish populations proved to be characterised by the *b* allele for *pgd*, rather than the *a* allele reported by Hedrén (1996a, 1996b) as being the one and only allele in all Scandinavian populations (this important allelic distinction was checked by running a mixture of British and Scandinavian plants of *D. incarnata* on the same electrophoretic gel in the laboratories of both RAE and M. Hedrén). The only exceptions to this British–Scandinavian dichotomy were the three populations of subsp. *cruenta*, each of which contained both the *a* and *b* alleles. In the case of the Scottish population of *cruenta* (Lochdroma), all plants with leaf markings contained the Scandinavian *a* allele, whereas admixed plants lacking leaf markings (hence provisionally allocated to subsp. *pulchella*) contained the characteristically British and Irish *b* allele. Unfortunately, we do not know whether the same correlation between leaf markings and specific *pgd* alleles characterised the Irish populations of subsp. *cruenta* at Carra and Gelain; although these populations similarly contained both leaf-marked and unmarked plants, the two morphs were not distinguished reliably when analysed for allozymes.

Similarly, the *pgm* locus showed the presence of both *b* and *c* alleles in both of the populations of *Dactylorhiza incarnata* subsp. *cruenta* successfully analysed by us for this locus, but in this case, both of these alleles were also detected in our two sparsely sampled populations of subsp. *pulchella*. At Lochdroma, the putative *pulchella* plants were admixed among bona fide plants of subsp. *cruenta*, but this was not true of the single *pulchella* plant from Kernsary that reputedly bore the *b* allele.

Moving on to consider the allotetraploids, our results for the three named morphs of Scottish *Dactylorhiza francis-drucei* (formerly *D. traunsteinerioides* s.l.) were consistent with the results for the closely related Scandinavian taxa *D. traunsteineri* and *D. lapponica* for all three loci (cf. Tables 2, 3); in both geographic regions, a minority of populations contained a minority of plants bearing the *b* allele of *pgd* or the *c* allele of *pgm*. In the case of *D. purpurella*, a minority of Scandinavian

plants included the *a* or *c* alleles at the *pgd* locus, but Hedrén (1996b) received a greater surprise from his results at the *pgm* locus; Scandinavian populations of *D. purpurella* proved to be uniquely dominated by the *c* and *e* alleles of *pgm*. We found that this pattern was consistently mirrored in all six *pgm* data-sets for Scottish populations of *D. purpurella*, irrespective of whether they were attributed to var. *purpurella* and var. *cambrensis*. Among the four additional dactylorchid species cultivated at RBG Edinburgh, only *D. majalis* was also analysed by Hedrén (1996a) from natural populations, the two sets of allozyme results proving congruent.

Comparison of allozyme profiles with DNA sequencing profiles

Hedrén *et al.* (2011a) sampled allotetraploids widely across the British Isles, though material from Scotland was limited to single populations each of *Dactylorhiza francis-drucei traunsteinerioides*, *francis-drucei* s.s. and *ebudensis*, together with four populations of *D. purpurella* s.s. confined to the Outer Hebrides plus the Applecross population located on the west coast of the mainland. As with the present allozyme data, *D. purpurella* proved to be the most genetically variable of the four British and Irish allotetraploid species in both nuclear and especially plastid microsatellites, whereas all three named forms of *D. francis-drucei* proved similar in terms of nuclear microsatellites and virtually indistinguishable using plastid microsatellites. For both genomes, *D. francis-drucei* was shown to be more similar to *D. praetermissa* than to *D. purpurella*, a pattern mirroring that evident in allozyme results. In terms of nrITS sequences, its approximately equal frequencies of ribotypes III, V and X led to an intermediate placement of *D. francis-drucei* between *D. purpurella* (ribotypes X and V) and *D. praetermissa* (ribotypes III and V). These three ribotypes are readily sourced in putative diploid parents of the allopolyploids: *D. incarnata* is characterised by ribotype X whereas *D. fuchsii* commonly features ribotypes III and V (Pillon *et al.* 2007).

Within *Dactylorhiza purpurella*, no obvious genetic differences were observed by either Pillon *et al.* (2007) or Hedrén *et al.* (2011a) between *D. purpurella cambrensis* (represented largely by Welsh rather than Scottish material) and *D. purpurella* s.s. — conclusions that are congruent with the allozyme data presented here. Similarly, within *D. francis-drucei*, Hedrén *et al.* (2011a) failed to find any meaningful genetic distinction between *ebudensis*, *francis-drucei* s.s. and *traunsteinerioides* s.s. However, they did detect possible introgression into *ebudensis* from either admixed *D. purpurella* or *D. incarnata*, perhaps explaining the unfortunate recovery of an atypical plastid microsatellite profile from the single plant of *ebudensis* that was analysed by Pillon *et al.* (a topic discussed in detail by Bateman 2011a, 2019; Bateman & Denholm 2012). Subsequent in-depth exploration of RAD-seq data confirmed a relatively high frequency of gene flow into *ebudensis* from intermingled plants of *D. purpurella* on North Uist (Hawranek 2021).

The data-rich RAD-seq-based phylogenetic study of Brandrud *et al.* (2020) encompassed mostly continental samples and included only five Scottish plants: single representatives of the diploids *Dactylorhiza fuchsii* and *D. incarnata* from the Outer Hebridean island of North Uist, plus a single plant of *D. purpurella* s.s. from Suenish (also North Uist), single plant of supposed *D. francis-drucei traunsteinerioides* from Applecross, and a single plant of undoubted *D. francis-drucei francis-drucei* from Kernsary, close to the type locality for the species. The main conclusions to be drawn from their study were that both *D. purpurella* and *D. francis-drucei* s.l. are fairly similar genetically but nonetheless each was resolved as monophyletic, as were *D. praetermissa* and the wholly continental *D. majalis*. One conclusion that Brandrud *et al.* (2020) did not emphasise, but that is clearly evident from their Fig. S3 (Bateman 2019), is that the British and Irish *D. francis-drucei* is also monophyletic and potentially sister to *D. traunsteineri* s.s., a species now arguably better viewed as incorporating the former *D. lapponica* and as being confined to continental Europe (Bateman 2019). This conclusion is also supported by the epigenetic data of Paun *et al.* (2010) and by extensive nuclear microsatellite data acquired by Balao *et al.* (2016, their fig. 2), who showed *D. francis-drucei* to be approximately as genetically distinct from *D. traunsteineri* as it is from *D. majalis*.

Overall, the more detailed recent genetic investigations (e.g. Brandrud *et al.* 2020; Hawranek 2021) have downplayed the relatively distinct and diverse genetics found in *Dactylorhiza purpurella* during the present study and earlier by Hedrén *et al.* (2011a). In contrast, RAD-seq has enhanced present and past allozyme data in more reliably distinguishing *D. francis-drucei* from *D. praetermissa* within the British Isles, and more importantly, has proved more successful than allozymes and microsatellites in discriminating British and Irish *D. francis-drucei* from continental *D. traunsteineri*.

Comparison of morphometric with genetic divergence levels

In summary, the many diverse sources of genetic data now available, including allozymes (Tables 2, 3), are able to readily distinguish and reliably circumscribe both *Dactylorhiza purpurella* and *D. francis-drucei*, but they fail to identify any meaningful genetic structure among Scottish populations of either of these allopolyploids. It is therefore of considerable interest that the ordination of individual allotetraploid plants (Fig. 3) shows morphological variation to be marginally greater within these species than between them. The first coordinate effectively separates infraspecific taxa primarily on a range of characters that are dependent on anthocyanin pigments, separation of the two

species being relegated to the second coordinate using 'vigour' characters, supported by features such as more angular bract cells and more widely distributed lip markings but less frequent notching of the lateral lobes on the lip in *D. francis-drucei*. Two similar axes, supported by broadly similar characters, were found in the corresponding morphometric analysis conducted at the population level (Fig. 4), but here the first two axes are transposed in their respective strengths, species distinction taking precedence over distinctions among infraspecific taxa. Nonetheless, the wide scatter of points across the plot, particularly of *D. purpurella* populations, emphasises both the considerable morphological diversity present within the two allopolyploids and the comparative subtlety of the morphological distinction between the two species. In this particular case, detailed molecular data are more discriminatory at the species level than are detailed morphological data, helping to explain why earlier taxonomic circumscriptions based only on morphology tended to become seduced into over-weighting anthocyanin-based characters, thereby incurring an unacceptably high risk of circumscribing artificial taxa (Bateman 2011a, 2019; Hedrén *et al.* 2011a; Bateman & Denholm 2012).

The dominance of pigmentation characters predictably extends into the morphometric analysis of six Scottish and Irish populations of *Dactylorhiza incarnata* subsp. *cruenta* (Fig. 5), where it dictates the first coordinate to an extent where one might speculate that two morphologically distinguishable taxa are present. Only the fact that five of the six populations have placed at least two individuals in each of the two clusters shows that all of these plants are conspecific. However, it is less clear that they are genuinely con**sub**specific, because some evidence has accumulated to suggest that there exists genetic structure within this group, in contrast with the inexplicably low levels of genetic variation that characterise the remaining subspecies of *D. incarnata* (e.g. Hedrén 1996a, 2001, 2003; Pillon *et al.* 2007; Balao *et al.* 2016).

Likely origin of Scottish populations of *Dactylorhiza incarnata* subsp. *cruenta*

Focusing on the classic Scottish population of *cruenta* (Lochdroma), all eight plants present in the anthocyanin-rich cluster proved to have the *pgd-a* allele and *pgm-b* allele that characterise Scandinavian *Dactylorhiza incarnata* (Hedrén 1996a), whereas the two admixed plants placed in the comparatively anthocyanin-deficient cluster had the *pgd-b* allele and *pgm-a* allele that are here shown to characterise *D. incarnata* in the British Isles. Similar mixtures of plants bearing either Scandinavian or British/Irish allele profiles, and of plants bearing or lacking discrete leaf markings, were found in the two Irish populations analysed here for allozymes (Carra and Gelain: Table 2A). These results are elegantly congruent with the nuclear and plastid microsatellite-based study of the Gelain *incarnata* population conducted a decade later. Specifically, Hedrén *et al.* (2011b) detected strong genetic differentiation between plants with and without leaf markings, the rarer leaf-marked plants showing limited gene-flow with the admixed unmarked plants and greater evidence of inbreeding.

Thus, leaf-marked plants of *Dactylorhiza incarnata* and the *pgd* and *pgm* alleles that are dominant in continental Europe are also positively correlated in the British Isles, but here both this phenotype and this genotype are rare. These results are consistent with comparatively recent (presumably post-glacial) arrival of seed of leaf-marked *D. incarnata* subsp. *cruenta* from mainland Europe, followed by limited introgression into pre-existing populations of unmarked subsp. *pulchella*. If so, it is likely that colonisation of west-central Ireland, where *cruenta* is now locally frequent, occurred earlier than establishment of the isolated outpost at Lochdroma in west-central Scotland. Admittedly, there exists a potential source of the characteristically continental *incarnata* alleles *pgd-b* and *pgm-c* in the form of *D. francis-drucei*, but this species would also have been obliged to donate the *pgd-c* and *pgm-d* alleles, yet these alleles are absent from all analysed populations of *D. incarnata*. Also, the closest known locality of *D. francis-drucei* to Lochdroma is situated 25 km to the south (BSBI DDb 2022). The Lochdroma *cruenta* population was first found in 1982 (Kenneth & Tennant 1984). Two further supposed populations have since been discovered in Scotland: a small population in West Sutherland (recorded 1998 – 2002) and a larger population on Hoy in the Orkney Islands (2019 onward), though improved images sent to RMB suggest that the latter may actually represent depauperate plants of *D. francis-drucei* — a species that also occurs within a kilometre of the Sutherland *cruenta* site. On balance, we consider highly improbable an origin of the *cruenta* populations through gene-flow from *D. francis-drucei*; certainly, the Lochdroma plants show no morphological evidence of hybridity (Fig. 1A, B) of the kind observed by Aagaard *et al.* (2005) in Scandinavia.

More broadly, it might prove instructive to compare allozyme profiles with plastid and nuclear microsatellites for populations assigned to *Dactylorhiza incarnata* subsp. *cruenta* across Europe, because Hedrén (2009) showed that Alpine *cruenta* share with most British plants of *D. incarnata* plastid haplotype A, whereas *cruenta* populations in Scandinavia (the type region for *cruenta*) are dominated by the typically continental B haplotype. It is therefore possible that neither British and Irish nor Alpine leaf-marked populations should strictly be assigned to *cruenta*.

Likely origin of *Dactylorhiza purpurella*

The discovery that the *pgd-b* and *pgm-c* alleles dominate *Dactylorhiza incarnata* in the British Isles has even more profound implications for our understanding of the origin of *D. purpurella*, which is not only uniquely dominated by, but is also homozygous for, *pgd-b*. Even more tellingly, the data presented in Table 2A suggest that *D. purpurella* is stably heterozygous for *pgm-c* and *pgm-e*, which characterise *D. incarnata* and *D. fuchsii* respectively within the British Isles. Admittedly, *pgm-b* is present at low frequencies in some Scandinavian populations of *D. purpurella* (Hedrén 1996b), where a ready souce for the *b* allele can be found in the typical Scandinavian genotype for *D. incarnata* (Table 3).

Following its original description (Stephenson & Stephenson 1920), *Dactylorhiza purpurella* was initially regarded as endemic to the British Isles, until suspicions were raised that sporadic populations along the North Sea coasts of northern Denmark (together with the Faroe Islands) and southern Norway might also be attributable to this species (e.g. Pedersen 2007; Eccarius 2016). A dactylorchid population on the Dutch Frisian island of Schiermonnikoog also briefly masqueraded as *D. purpurella* before being awarded its own highly questionable species epithet, *D. vadorum* (cf. Kreutz & Dekker 2016a, 2016b).

Significantly, a well-sampled RAD-seq survey of European *Dactylorhiza incarnata* by Brandrud (2019) revealed a strong separation of British populations from all continental populations, mirroring our allozyme results. Within the British Isles, the degree of genetic divergence from continental populations increased from southeast to northwest. The one exception to this rule was western Norway, where typically British genotypes were detected in *D. incarnata* using RAD. There is thus an almost perfect coincidence between the geographic distribution of the British/Irish genotype of *D. incarnata* and the distribution of *D. purpurella* which, uniquely among the allotetraploids, shares the same distinctive alleles.

These observations suggest that *Dactylorhiza purpurella* originated within the British Isles, through allopolyploidy between the British/Irish genotypes of *D. fuchsii* and *D. incarnata*. Given that the climate of the British Isles was periglacial as little as 11,500 calibrated years ago, it seems likely that *D. purpurella* originated more recently and was pre-adapted for life in the post-glacial landscape of the glaciated northern and western regions of the British Isles (Bateman 2011a; Hedrén *et al.* 2011a). Given this timescale, emigration to the Faroes, Norway and Denmark is likely to have occurred very recently, presumably through wind-borne or bird-borne seed. The unusual distribution of *D. purpurella* in Ireland, concentrated in the north and the southeast (Bateman & Denholm 2023) but "inexplicably missing from the Midlands" (Curtis & Thompson 2009: 75), could also indicate relatively recent emigration from mainland Britain. It potentially represents two separate migrations, the first from southwest Scotland to northern Ireland and the second from South Wales to southeast Ireland.

One further aspect of *Dactylorhiza purpurella* that is particularly intriguing is the fact that, irrespective of the kind of genetic analyses being performed, it is reliably resolved as the allotetraploid that most closely resembles its pollen parent, *D. incarnata*, rather than its seed parent, *D. fuchsii*. This outcome is mirrored in morphometric comparisons (Bateman & Denholm, unpublished) and remains in need of a cogent explanation.

Likely origin of *Dactylorhiza francis-drucei*

Bateman (2006, 2019, 2020) argued that several categories of molecular evidence conspired to suggest that populations commonly assigned to *Dactylorhiza francis-drucei* s.l. (as *D. traunsteinerioides* s.l.) had separate evolutionary origins in the Alps, Scandinavia and the British Isles, and should therefore be treated as distinct species. However, recent modelling of RAD-seq data for the allopolyploids in the *D. traunsteineri* s.l. and *D. majalis* s.l. groups, performed separately against the ancestral *fuchsii* and *incarnata* subgenomes, suggested otherwise (Brandrud 2019). As expected, RAD-seq data indicate that these two allopolyploid lineages had separate origins, *majalis* emerging first (estimated at 3,000 – 10,000 yr, compared with 2,000 – 5,000 yr for *traunsteineri*), but a subsequent modelling exercise comparing British vs continental populations of *D. traunsteineri* s.l. concluded that the *traunsteineri* group had a single allopolyploid origin, presumably somewhere within continental Europe.

However, this conclusion appears to contradict the unrooted tree generated from the same body of RAD-seq data (fig. S3 of Brandrud *et al.* 2020), which suggests that British *Dactylorhiza francis-drucei* populations constitute a separate species from continental *D. traunsteineri* that may even be marginally more closely related to *D. majalis* or *D. praetermissa*. Moreover, ordination of the RAD data showed *D. traunsteineri* s.s. to be more closely similar to *D. majalis* than either is to the more discrete cluster of plants representing British *D. francis-drucei* (Brandrud 2019, fig. 2.3), echoing results obtained earlier from analyses of nuclear microsatellites (fig. 2 of Balao *et al.* 2016), small non-coding RNAs (Thornton 2022) and methylation (Paun *et al.* 2010). More recently, a STRUCTURE analysis performed within a study that regrettably assumed monophyly of the narrow-leaved marsh-orchids suggested greater RAD-seq divergence between *D. francis-drucei* and continental *D. traunsteineri/lapponica* than was evident between *D. francis-drucei* and *D. purpurella*, subsp. *francis-drucei* populations typically showing greater "purity" than those of subsp. *traunsteinerioides* (fig. 4

of Hawranek 2021). In contrast, the RAD-seq data of Brandrud *et al.* (2020) and Hawranek (2021) failed to convincingly distinguish the Scandinavian *lapponica* (sampled by them in Norway, Sweden, Finland and Estonia) from Alpine *traunsteineri* (sampled in Switzerland, Austria and Germany) (see also the microsatellite study by Nordström & Hedrén 2008).

We summarise these various studies as strongly suggesting that *Dactylorhiza francis-drucei* is best treated as a species separate from *D. traunsteineri* (including the former *D. lapponica*), and that it speciated more recently than *D. majalis* (and *D. praetermissa*) but earlier than *D. purpurella* (and, we suspect, much earlier than the Irish endemic *D. kerryensis*: Bateman & Denholm, unpublished). However, it remains uncertain whether *D. francis-drucei* is an allopatric derivative of *D. traunsteineri* or arose through a separate allopolyploidy event, both speciation events congruent with *D. fuchsii* as 'mother' and *D. incarnata* as 'father'.

The present allozyme data are consistent with either hypothesis, but do usefully contradict the suggestion put forward by Bateman (2006, 2011a) that *Dactylorhiza francis-drucei* could have originated through an allopolyploidy event that occurred within the British Isles; they also weaken (though not fatally) his argument that *D. francis-drucei* may never have occurred south of the line demarking the glacial maximum at approximately 20,000 yr. Nonetheless, there is little doubt that populations formerly attributed to *D. francis-drucei* (as *D. traunsteinerioides*) but occurring south of the glacial maximum have correctly been reassigned to *D. praetermissa* as subsp. *schoenophila* (Bateman & Denholm 2012; Bateman 2019).

Dactylorhiza francis-drucei reliably yields the *pgd* and *pgm* alleles that are characteristic of *D. incarnata* populations in mainland Europe rather than those in the British Isles, and thus most likely had a continental 'father'. On balance, an allopatric origin in Britain from within *D. traunsteineri* soon after its own origin in mainland Europe currently appears to be the most likely scenario. The presence among the plants analysed through RAD-seq by Brandrud *et al.* (2020) and Hawranek (2021) of a single Norwegian plant attributed by them to *D. traunsteineri* that bore a genotype typical of British *D. francis-drucei* suggests the possibility of secondary migration of this lineage from Scotland to Scandinavia (Bateman 2019, 2022a), thus mirroring geographically the likely emigration to Norway of *D. purpurella* discussed above. If confirmed, the presence of *D. francis-drucei* in Norway would challenge its current status as strictly endemic to the British Isles (Bateman 2022a; Bateman & Denholm 2023).

Conclusions

(1) In the case of Scottish marsh-orchids, a series of genetic studies of increasing technological sophistication has both optimised their taxonomy and deepened our understanding of their evolutionary patterns and processes.

(2) The present results arguably endorse all of the taxonomic conclusions put forward for the diploid marsh-orchids by Bateman & Denholm (1985) and for the tetraploid marsh-orchids by Bateman & Denholm (2012). During the last decade, all four bona fide tetraploid marsh-orchid species native to Britain and Ireland have been re-circumscribed taxonomically in the light of molecular and, to a lesser degree, morphometric reappraisal as certain genotypes, phenotypes and/or regional ecotypes were transferred from one named species to another. The resulting taxonomic circumscriptions have largely been followed by subsequent authors in both Britain (e.g. Harrap & Harrap 2009; Stace 2019; Cole & Waller 2020; Stroh *et al.* 2023) and continental Europe (e.g. Delforge 2016; Eccarius 2016). Nonetheless, we predict that debates will continue regarding whether the species concept applied to the allopolyploids should prioritise having broadly the same parental species (thus yielding an exceptionally broadly circumscribed *Dactylorhiza majalis*) or, as here, we should give priority to multiple origins from different ecological races evident within the two parental species. In our opinion, also still undecided is the important question of whether *D. francis-drucei* emerged relatively recently from within *D. traunsteineri/lapponica* or alternatively represents an independent polyploidy event.

(3) Scotland supports a single diploid species, *Dactylorhiza incarnata*, containing four formally named infraspecfic taxa: subspp. *incarnata*, *coccinea*, *pulchella* and *cruenta*. Whether the first three taxa should be viewed as subspecies or varieties remains debatable (cf. Haggar 2004; Cole & Waller 2020), but the combination of genetic and morphological distinctiveness definitely justifies subspecies status for *cruenta*.

(4) Scotland currently hosts two tetraploid marsh-orchid species, *Dactylorhiza purpurella* and *D. francis-drucei*, which are genetically distinct, morphologically separable, differ in ecological preferences, and have separate evolutionary origins. According to Swainbank (2022), the two species can even be distinguished when in fruit, through the comparatively elongate pods and longer seeds of *D. purpurella*. Bateman & Denholm (2012) ascribed relatively anthocyanin-rich populations of *D. purpurella* to var. *cambrensis* (syn. *majaliformis*, encompassing only a minority of Scottish populations of the species) and those of the species then named *D. traunsteinerioides* to subsp. *francis-drucei*

(a taxon encompassing all Scottish populations of the species: Bateman 2022a; Bateman & Denholm 2023). In both cases, the anthocyanin-rich mode forms a morphological continuum with the less anthocyanin-rich race. In both cases, the two taxa together span an approximately equal range of morphological variation. And in neither case do the anthocyanin-rich and anthocyanin-poor populations appear readily distinguishable genetically. A legitimate argument could therefore be put forward for re-equilibrating var. *cambrensis* and subsp. *francis-drucei* to equal rank. However, if *D. francis-drucei* subsp. *traunsteinerioides* (and thereby the equivalent nominate subspecies, *francis-drucei*) were to be demoted to varietal status, we would then also be obliged to demote the North Uist 'endemic' *ebudensis* — viewed as a full species by Bateman (2006) but shown here to clearly be a morphological subset of subsp. *francis-drucei* — from a variety to a mere forma. Given that the narrow-leaved marsh-orchids lie at the epicentre of this perennial taxonomic Gordian Knot, we do not propose to engineer further taxonomic changes (preferably driven by science rather than mere nomenclatural priority) until a broader morphometric survey, spanning all taxa and the whole of the British Isles, has been completed (Bateman & Denholm, unpublished).

(5) Similarly, the equally complex taxonomic and nomenclatural issues surrounding *Dactylorhiza incarnata cruenta* (cf. Vermeulen 1947; Heslop-Harrison 1949; Summerhayes 1951; Bateman & Denholm 1985; Haggar 2004; Curtis & Thompson 2009; Hedrén *et al.* 2011b; Eccarius 2016) will not be solved until high-throughput sequence data and detailed morphometric data are gathered from populations scattered across Europe. For the present, we prefer to continue using the long-recognised epithet *cruenta*.

(6) Combining genetic approaches with detailed in situ morphometrics has proved to be an especially powerful approach to taxonomic circumscription, offering the opportunity to assess robustly the all-important degree of congruence between genotype and phenotype.

(7) There has been much recent discussion of 'cryptic speciation', when genotypic divergence is hypothesised to precede phenotypic divergence (e.g. Monro & Mayo 2022). However, in circumstances when speciation occurred fairly recently and involved closely similar parental lineages, it is alternatively possible that relative levels of genetic divergence will generate more accurate taxonomic circumscriptions than will relative levels of phenotypic divergence. In the case of *Dactylorhiza purpurella* and *D. francis-drucei*, infraspecific variation in anthocyanin-related characters is approximately as great as phenotypic differences that genuinely distinguish the two species and reflect their independent origins through allopolyploid speciation.

(8) Deciding whether to award pre-eminence to genotypic or phenotypic data is especially relevant to determining whether or not *Dactylorhiza francis-drucei* should be treated as a species separate from continental *D. traunsteineri*. The two taxa can be genetically circumscribed but our ongoing morphometric comparisons (Bateman & Denholm, unpublished) indicate that there are no reliable morphological features competent to distinguish British and Irish *D. francis-drucei* from Alpine *D. traunsteineri* or Scandinavian populations often attributed to *D. lapponica*.

(9) From the viewpoint of conservation, application of IUCN criteria has meant that both *Dactylorhiza francis-drucei* (as *D. traunsteinerioides*) and *D. purpurella* have consistently, and correctly, been designated Least Concern in both the UK (e.g. Cheffings *et al.* 2005) and Ireland (Wyse Jackson et al. 2016). This statement also applies to *D. incarnata* subsp. *cruenta* in Ireland, whereas in Scotland *cruenta* rightly returned to its original designation of Endangered in 2010 (Leach 2010) after spending the previous five years languishing in the bureaucratic doldrums of Data Deficiency. Despite its Least Concern rating, *D. francis-drucei* should be taken seriously as a good indicator species for relatively biodiverse habitats in Scotland, particularly for slopes that support calcareous flushes featuring reliable groundwater movement (Cowie 1999).

(10) April 2022 witnessed submission to the UK government of recommendations for the seventh quinquennial review of Schedule 8 of the Wildlife and Countryside Act. In total, 16 orchid species and subspecies figured among the 306 vascular plants that were considered for Schedule 8 status (JNCC 2022). As a result, the only orchid 'species' recommended for removal from Schedule 8 is *Dactylorhiza francis-drucei* subsp. *francis drucei* (a taxon formerly mis-assigned to *D. lapponica*) — a decision that we support in the light of greatly improved knowledge of its distribution within Scotland. It will also end the irony of offering protection to a species that in truth does not occur in Britain and that, in any case, may not be a valid species, either biologically or nomenclaturally. Unfortunately, the application of the single Scottish population of *D. incarnata* subsp. *cruenta* to join this exclusive club has been recommended for rejection, in stark contrast with the recommended acceptance into Schedule 8 of the two East Anglian populations of the less genetically distinct *D. incarnata* subsp. *ochroleuca* (Bateman 2022a). There is much to be said for consistency of decision-making.

(11) Comparison of the 2000 and 2020 plant atlases for Britain and Ireland (cf. Preston *et al.* 2002; Stroh *et al.* 2023) reveals not only improved mapping of some species, particularly *Dactylorhiza francis-drucei*

(Fig. 2), but also considerable distributional changes among *Dactylorhiza* species. Most notable is the rapid and inexorable northward march of the Southern Marsh-orchid, *D. praetermissa*, which recently reached Cumbria and Northumbria and now appears poised to invade Scotland (Bateman 2022a; Bateman & Denholm 2023). Migration of *D. praetermissa* seed across the Irish Sea to Ireland (perhaps also of *D. kerryensis* seed outwards from Ireland) might also be predicted in the near future. Thus far, although adaptable, *D. praetermissa* does not seem to be invading those habitats preferred by *D. francis-drucei*, but limited evidence suggests that it is an enthusiastic hybridiser with *D. purpurella* (e.g. Stace *et al.* 2016). It will therefore be interesting to see how rapidly these current genetic boundaries become blurred between these two closely related species as the contact zone expands into Scotland. Credible predictions of the likely consequences of migration will require detailed study of existing multi-species colonies using modern technologies.

Nomenclatural Postscript

In his 20-year review of research into British and Irish orchids, Bateman (2022b, p. 362) re-asserted his regret that a purely nomenclatural law, rather than any scientific argument, had forced a nomenclatural change of the Irish endemic *Dactylorhiza occidentalis* to the less well-known epithet *D. kerryensis*, a taxon formerly generally viewed as an infraspecific taxon of *D. occidentalis* (Bateman & Denholm 2009, 2012). Previously, a detailed case for nomenclatural conservation, co-authored by most of the authorities then working on this genus in Europe (Bateman *et al.* 2010), had been summarily rejected by the ultra-conservative panel appointed to police such requests on behalf of the International Code of Nomenclature (Brummitt 2011).

Bateman's (2022b) published comment encouraged orchid enthusiast Felix Benoit to contact him in August 2022 in order to point out the existence of a parallel situation that regrettably afflicts *Dactylorhiza traunsteinerioides*. This unfortunate taxon has long been treated as the ultimate 'taxonomic football', having incurred changes of epithet and/or rank on a regular basis; some of these name changes were motivated by scientific advances but others were purely legalistic. As reviewed by Bateman (2011a), formalised by Bateman & Denholm (2012) and subsequently updated by Bateman (2022b), Pugsley's Marsh-orchid, *D. traunsteinerioides*, was perceived as a tetraploid species endemic or near-endemic to Britain and Ireland that shows sufficient morphological variability to warrant division into two subspecies: a more southerly nominate subspecies inhabiting Ireland, North Wales and northern England, and a typically smaller-bodied, smaller-flowered, often more intensely marked subspecies that is characteristic of Scotland and is named subsp. *francis-drucei*. However, this hierarchical relationship between the epithets *traunsteinerioides* and *francis-drucei* has now been subjected to a purely nomenclatural challenge.

The epithets *traunsteinerioides* (Pugsley 1936) and *francis-drucei* (Wilmott 1936) were formally established in successive papers published in the same issue of the *Proceedings of the Linnean Society*, the former epithet preceding the latter by just four pages. Unfortunately, *francis-drucei* was established by Wilmott at species level, whereas *traunsteinerioides* was established at subspecies level; Pugsley only raised *traunsteinerioides* to species level four years later, a decision taken under implicit pressure from fellow contemporary dactylorchid enthusiasts, not least Wilmott himself (Pugsley 1940). Thus, as noted in litt. by Benoit, the presently accepted relationship between the epithets *traunsteinerioides* and *francis-drucei* should strictly be reversed; as a bona fide species, Pugsley's Marsh-orchid should strictly be attributed to the epithet first employed at species level, namely *D. francis-drucei* (Wilmott) Aver. (Averyanov 1984).

Accepting the blanket dictat that is the (often infuriating) law of nomenclatural priority therefore requires the well-known epithet *traunsteinerioides* to be demoted to a southerly subspecies of a newly promoted species, *Dactylorhiza francis-drucei*. As currently religiously applied, the law of priority overrides all scientific and pragmatic arguments that favour the epithet *traunsteinerioides* over the epithet *francis-drucei* — that *traunsteinerioides* has finally achieved stability for this most unstable of taxa, that it helpfully indicates its closeness of relationship to the Alpine *D. traunsteineri* (and without employing an irritating internal hyphen), that its protologue was arguably more competently prepared, and that its holotype is more typical of the morphology of the species as a whole (the morphologically extreme holotype of *francis-drucei* was illustrated by Bateman & Denholm 2012, p. 42).

Moreover, the most popular flora of the British Isles (Stace 2019), the accompanying hybrid flora (Stace *et al.* 2016), and the latest UK plant atlas (Stroh *et al.* 2023) — a tome that is set to summarise the distribution of that flora for the next 20 years — all employ the previous nomenclature, and so will inevitably provide passive resistance against the altered names. We would certainly be grateful if the venerable custodians of the International Code of Nomenclature would consider developing a more liberal approach to scientifically-based applications for nomenclatural conservation (cf. the discussions of *Dactylorhiza occidentalis* vs *D. kerryensis* in Bateman 2011b, 2022b; Bateman & Denholm 2012).

Dactylorhiza francis-drucei (*Wilmott*) *Aver.*, *Bot. Zhurn.* 69: 875 (Averyanov 1984).
Basionym: *Orchis francis-drucei* Wilmott, *Proc. Linn. Soc. London* 148: 128 (1936).
Type: 'West Ross; slopes above Loch Maree, 23 June 1935, coll. A.J. Wilmott' (Wilmott, 1936, p. 128) (holotype BM: fig. 1 of Bateman & Denholm 2012).
Synonyms (selected): *Orchis traunsteinerioides* (Pugsley) Pugsley, *J. Bot.* (*London*) 78: 179 (1940); *Dactylorhiza traunsteinerioides* (Pugsley) Landwehr, *Orchideeën* 37: 79 (1975), ex R.M.Bateman & Denholm in *List Vasc. Pl. Brit. Isles* (*D. H. Kent*) Supp. 3: 20 (2006); *Dactylorhiza traunsteineri* (Saut.) Soó subsp. *traunsteinerioides* (Pugsley) Soó, Nom. Nov. Gen. *Dactylorhiza* 6 (1962).

Dactylorhiza francis-drucei subsp. **francis-drucei** var. **ebudensis** (*Wief. ex R.M.Bateman & Denholm*) *R.M.Bateman & Denholm*, **comb. nov.**

http://www.ipni.org/urn:lsid:ipni.org:names:77308347-1

Basionym: *Dactylorhiza majalis* (Rchb.) P.F.Hunt & Summerh. var. *ebudensis* Wief. ex R.M.Bateman & Denholm, *Edinburgh J. Bot.* 52: 57 (1995). Type: Scotland, North Uist, Lingay Strand, in dunes near Newton Hotel, 4 June 1974, *W. Wiefelspütz*, DM 37 (lectotype HEID).
Synonyms (selected): *Dactylorhiza majalis* (Rchb.) P.F.Hunt & Summerh. subsp. *scotica* E.Nelson, *Mon. Ikon. Orch. Dactylorhiza*: 90 (1976), nom. nud.; *D. majalis* (Rchb.) P.F.Hunt & Summerh. subsp. *ebudensis* (Wief. ex R.M.Bateman & Denholm) M.R.Lowe, *Eurorchis* 15: 81 (2003); *D. ebudensis* (Wief. ex R.M.Bateman & Denholm) P.Delforge, *Naturalistes Belges* 81: 397 (2000).

Dactylorhiza francis-drucei subsp. **traunsteinerioides** (*Pugsley*) *R.M.Bateman & Denholm*, **comb. et stat. nov.**

http://www.ipni.org/urn:lsid:ipni.org:names:77308335-1

Basionym: *Orchis majalis* Rchb. subsp. *traunsteinerioides* Pugsley, *Proc. Linn. Soc. London* 148: 124 (1936). Type: Ireland, Co. Wicklow, Newcastle, coll. *H. W. Pugsley* (#530).
Synonym (selected): *Dactylorhiza majalis* (Rchb.) P.F.Hunt & Summerh. subsp. *traunsteinerioides* R.M.Bateman & Denholm, *Watsonia* 14: 372 (1983).

The formal descriptions of these taxa given in Bateman & Denholm (2012) remain adequate to support the two subspecies and the variety, pending a future morphometric analysis richer in data and broader in taxonomic scale; although the status of their respective names has changed, their circumscriptions have not.

Appendix 1. Taxonomic authorities of binomials and trinomials employed in the text. Asterisked taxa are considered biologically valid.

Dactylorchis (Klinge) Verm.
Dactylorchis majalis (Rchb.) Verm.
Dactylorhiza Neck. ex Nevski*
Dactylorhiza alpestris (Pugsley) Aver.
Dactylorhiza elata (Poir.) Soó*
Dactylorhiza foliosa (Rchb.f.) Soó*
Dactylorhiza francis-drucei (Wilmott) Aver.*
Dactylorhiza fuchsii (Druce) Soó*
Dactylorhiza incarnata (L.) Soó*
  subsp. *coccinea* (Pugsley) Soó*
  subsp. *cruenta* (O.F.Müll.) P.D.Sell*
  subsp. *ochroleuca* (Wüstnei ex Boll) P.F.Hunt & Summerh.*
  subsp. *pulchella* (Druce) Soó*
Dactylorhiza kerryensis (Wilmott) P.F.Hunt & Summerh.*
Dactylorhiza lapponica (Laest. ex Hartm.) Soó
Dactylorhiza maculata (L.) Soó*
Dactylorhiza majalis (Rchb.) P.F.Hunt & Summerh.*
  var. *ebudensis* Wief. ex R.M.Bateman & Denholm
  subsp. *scotica* E.Nelson
  subsp. *cambrensis* R.H.Roberts
Dactylorhiza occidentalis (Pugsley) P.Delforge
Dactylorhiza praetermissa (Druce) Soó*
Dactylorhiza purpurella (T.Stephenson & T.A.Stephenson) Soó*
  var. *cambrensis* (R.H.Roberts) R.M.Bateman & Denholm*
  subsp. *majaliformis* E.Nelson
Dactylorhiza sphagnicola (Höppner) Aver.*
Dactylorhiza traunsteineri (Saut. ex Rchb.) Soó*
Dactylorhiza traunsteinerioides (Pugsley) R.M.Bateman & Denholm
Dactylorhiza viridis (L.) R.M.Bateman, Pridgeon & M.W.Chase*
Orchis Tourn. ex L.*
Orchis francis-drucei Wilmott

Acknowledgements

Paula Rudall, an anonymous reviewer and Associate Editor André Schuiteman kindly critiqued earlier versions of this manuscript, and Mikael Hedrén offered helpful advice on interpreting allozyme patterns. Felix Benoit kindly wrote in August 2022 to point out the regrettable fact that, at species level, *Dactylorhiza francis-drucei* has nomenclatural priority over *D. traunsteinerioides*. We thank the then Scottish Natural Heritage (now NatureScot) for issuing in 1995 licence SP02-95 to collect leaves from populations then ascribed to *Dactylorhiza 'lapponica'*, and the Nuffield Foundation for providing an undergraduate research bursary (AT/100/96/0022) that funded an eight-week summer studentship salary for WC in 1996.

Declarations

Conflicts of Interest The authors declare that they have never entertained even the slightest hint of any actual or potential conflict of interest of any kind.

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