Journal of the HARDY ORCHID SOCIETY



The Hardy Orchid Society

Our aim is to promote interest in the study of Native European Orchids and those from similar temperate climates throughout the world. We cover such varied aspects as field study, cultivation and propagation, photography, taxonomy and systematics, and practical conservation. We welcome articles relating to any of these subjects, which will be considered for publication by the editorial committee. Please send your submissions to the Editor, and please structure your text according to the "Advice to Authors" (see website <u>www.hardyorchidsociety.org.uk</u>, January 2004 Journal, Members' Handbook or contact the Editor). Views expressed in journal articles are those of their author(s) and may not reflect those of HOS.

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Front Cover Photograph

Hybrid between *O. pauciflora* and *O. mascula* ($O \times colemanii$) (see article on page 66)

Photo by Gianpiero Ferrari

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Editorial Note

This issue has something of a *Dactylorhiza* focus and I am very pleased to include the important observations on the genus in the Outer Hebrides by Frank Horsman. Frank's article is accompanied by an insightful update on the genus from Richard Bateman who as most will know has done much to help understand this complex and taxonomically challenging group of orchids. Mark Smyth's attractive foliage variant adds to the *Dactylorhiza* theme. In addition, it is good to have two new contributors with an interesting account of conservation in Switzerland from Samuel Sprunger and more on Italy from another excellent photographer in Gianpiero Ferrari.

Chairman's Note Celia Wright

We started the year well with an enjoyable meeting at Kidlington recently and have two more meetings to look forward to this year. The first is at St Chad's Parish Centre, Leeds, on Saturday September 3rd. For this we have booked two special speakers - Jean Claessens and Jacques Kleynen from the Netherlands. Their book entitled "*The Flower of the European Orchid*" is due to be published about now. I am assured that the book contains much detail on flower structure and especially pollination. This is supported by excellent photography. We hope that copies of their book will be available to anyone who wishes to purchase a copy and have it autographed. We are bringing Jean and Jacques over specially from The Netherlands for our meeting so I hope that many of you will come to support HOS

and have an enjoyable day. Do bring botanically inclined friends as I'm sure they will find this interesting. There will be plenty of space in the hall at St Chad's, without the previous restrictions on numbers of Harlow Carr.

Another venture is our second orchid seed sowing workshop. This will be held on Sunday 7th August at Hagbourne Village Hall in Oxfordshire. The date has been chosen to be the right time of year for sowing seed with fungus, something that everyone there will have the opportunity to do on the day. Some of last year's participants have written recently on our Forum of their success with seed sowing following the last workshop. Anyone who would like to come should contact Alan Leck at <u>alanleck@alanleck.plus.com</u>.

I hope to see some of you on field trips this summer, but to you all, enjoy your orchid hunting in 2011.

Plant Show 2011

Class 1 Three pots native British orchids, distinct varieties

1st Michael Powell: Orchis anthropophora (Photo 1a), Anacamptis morio, Ophrys sphegodes

Class 2 Three pots native European (not native to Britain) orchids, distinct varieties

1st Richard Manuel: Ophrys sphegodes variants, Ophrys x heraultii (Photo 2b), Ophrys speculum var. orientalis (Photo 2c)
2nd Michael Powell: Neotinia tridentata, Anacamptis morio ssp. caucasica, Ophrys garganica

Class 4 Three pots hardy orchids, distinct varieties, any country of origin

1st Michael Powell: *Ophrys heldreichii* (Photo 4a), *Ophrys heldreichii* \times *tenthredinifera*, *Ophrys lutea* \times *apifera* (Photo 4c)

Class 6 One pot native European (not native to Britain) orchid

1st Richard Manuel: ×Serapicamptis bevilacquae2nd Michael Powell: Serapias lingua "Lemon and Lime"

Class 7 One pot non-European hardy orchid

1st Richard Manuel: Pterostylis pedunculata

2nd Andrew Bannister: *Caladenia latifolia* **3rd** Andrew Bannister*: *Caladenia garnea*

Class 8 One pot Dactylorhiza

1st Michael Powell: Dactylorhiza romana

Class 9 One pot Orchis, Anacamptis or Neotinea

1st Michael Powell: Orchis italica2nd Malcolm Brownsword: Anacamptis papilionacea x morio

Class 10 One pot Ophrys

1st Richard Manuel: Ophrys tenthredinifera** (Photo 10)
2nd Michael Powell: Ophrys tenthredinifera
3rd Malcolm Brownsword: Ophrys lutea

Class 11 One pot Serapias

1st Michael Powell: Serapias olbia
2nd Malcolm Brownsword: Serapias ×demadesii

Class 12 One pot Cypripedium

1st Andrew Bannister: Cypripedium formosanum
 2nd Jeff Hutchins: Cypripedium fasciolatum × candidum

Class 13 One pot *Calanthe*

1st Malcolm Brownsword: Calanthe discolor

Class 14 One pot *Pleione* 1st Malcolm Brownsword: *Pleione Whakari*

(There were no entries in Classes 3, 5 and 15)

****Winner of "Best in Show" Trophy**

Richard Manuel's Ophrys tenthredinifera, the winning entry in Class 10

Winner of RHS Banksian Medal

Michael Powell with 21 points

(Richard Manuel 12 points, Malcolm Brownsword 11 points [3 points for 1st, 2 for 2nd, 1 for 3rd])

(*Second entry in same class does not count towards Banksian medal points)

The judge was Brian Walker

The following pages feature some of the first placed winners in the 2011 Plant Show. A complete set of photographs of the 1st placed winning plant is displayed on the HOS website. Numbers refer to the Class and where multiple plants are involved they are differentiated by a letter (a-c) matching their order in the results list above.

Photos by Mike Gasson





Observations on the Hebridean Marsh-orchid in the Outer Hebrides Frank Horsman

Dactylorhiza ebudensis (Wief. ex R.M. Bateman & Denholm) P. Delforge, the Hebridean Marsh-orchid (Figs 1 & 2), is endemic to the machair of the island of North Uist in the Outer Hebrides. Until recently, the plant was thought to be confined to a wide strip of machair running from just west of Machair Robach to Sudhadnais near Newton Ferry in the east. This strip contains several thousand plants. However, two new sites have recently been discovered, one further west on the North Uist machair and another on the island of Berneray, just north-east of North Uist. The first was discovered at Loch Sollas in 2006 by Steve Duffield of South Uist and later confirmed by Richard Bateman. I visited the site in 2007 and found a core of some 200 flowering plants surrounded by several outliers.

The second new site was found by me nearby, on the small island of Berneray, in 2007. There were just three flowering plants growing together in a single dune slack. Material from one plant was subjected to a DNA test which proved positive for the *traunsteinerioides* group (R.M. Bateman & M. Hedrén, pers. comm., 2009). The plants were growing near a relatively new fence. It occurred to me that seed from the orchid may have been translocated from the main population on North Uist by fence erectors (installation of new fencing is not uncommon in the Outer Hebrides).



Figures 1 & 2. *Dactylorhiza ebudensis* on the North Uist machair, Outer Hebrides, Scotland.

Photos by Steve Duffield.

The most important observation I have made in connection with *D. ebudensis* since I moved to the Outer Hebrides from Yorkshire some eight years ago is my discovery of D. traunsteinerioides (Pugsley) Landwehr ex. R.M. Bateman & Denholm, Narrow-leaved Marsh-orchid (Figs 3 & 4), growing in one of the many dune slacks that support D. ebudensis at Clachan Sands, though not actually occurring among D. ebudensis. In 2008, there were approximately 50 flowering plants, of which five would previously have been identified as D. lapponica auct. non. (Laest. ex. Hartm.) Soó (Bateman, 2006, pp. 96-97). The plants were growing in a Schoenus flush, surrounded by D. ebudensis. This was a definite flush, the high density of Schoenus nigricans L. contrasting strongly with the odd single plants of Schoenus grazed by sheep in the dune slacks occupied by D. ebudensis. Clearly the geology of this flush was different from that of the surrounding dune slack. Dactylorhiza traunsteinerioides appears to prefer more calcareous sites than D. ebudensis. Bateman (pers. comm.) comments: "My pH measurements and field observations suggest (but by no means confirm) that it is the movement of the groundwater rather than pH that may distinguish the subtly different habitat preferences of ebudensis from the rest of the traunst group. Certainly, ebudensis has broken away from 100% co-occurrence with Schoenus nigricans." Was D. traunsteinerioides one parent of D. ebudensis and D. incarnata (L.) Soó subsp. coccinea (Pugsley) Soó the other, one of several possibilities put to me by Richard Bateman? This record for D. traunsteinerioides is the first for the Southern Isles in the Western Isles (i.e. the Outer Hebrides excluding Harris and Lewis). In 2009 Jamie Boyle, the senior RSPB warden for the Southern Isles, gave me the details of a site for an orchid he had found on the small tidal Island of Oronsay several years earlier but had not been able to identify. When I visited the site I found just ten flowering plants of D. traunsteinerioides, including a few plants of the "D. lapponica" type. This is only the second record for D. traunsteinerioides in the Southern Isles.

Thus, we have two sites for *D. traunsteinerioides* on the North Uist machair. It would appear to be "hanging on" on North Uist. *Dactylorhiza incarnata* subsp. *coccinea* is ubiquitous on the machair. I even had it growing in my (sandy) lawn when I lived in North Uist! Whether the three *D. ebudensis* sites on North Uist were originally contiguous is not known.

In 2008 I visited Luskentyre and Borve on South Harris, just north of North Uist. Luskentyre is a long-established site for "*D. lapponica*" (*D. traunsteinerioides*), though I was looking primarily for *D. ebudensis*. The Luskentyre site is a stonewalled field, apparently normally grazed by sheep. However, this grazing does not appear to disturb the orchids. There is a SSSI immediately adjacent to this field. When I asked Scottish Natural Heritage when they intended to include this field in the SSSI they told that they had no plans to do so, which I simply don't understand! This is one of the best orchid sites known to me. The upper part of the field is some-



Figures 3 & 4. *Dactylorhiza traunsteinerioides*. Calcareous flush around a marshy depression, Applecross, Wester Ross, Scotland. Photos by Richard Bateman

what acid and the lower part calcareous, with tufa present. Hundreds of flowering plants of "D. lapponica" grow alongside D. fuchsii (Druce) Soó subsp. hebridensis (Wilmott) Soó, D. maculata (L.) Soó and Orchis mascula (L.) L. Isolated specimens of D. purpurella (T. & T.A. Stephenson) Soó var. cambrensis (R.H. Roberts) R.M. Bateman & Denholm also occur in this area. For a period they are all in flower together so hybrids were sought and duly found. Although I did not find D. ebudensis here, the existence of such an isolated but strong population of D. traunsteinerioides might indicate that it was more widespread in the past. This possibility is supported by my finding a new, but very small, site for D. traunsteinerioides (previously "D. lapponica") at Borve, not far south of Luskentyre. This site looked very interesting as I drove by, so I obtained permission from the crofter to examine the site. There were very few specimens of D. traunsteinerioides growing within a larger population of D. purpurella var. purpurella. This is a problem inherent in dealing with these dactylorchids. The difficulty of DNA testing arises which means, for the amateur, paying someone to do them - if you can find someone, that is. In 1983 D.J. Tennant found D. traunsteinerioides at Borve in a calcareous flush above a coastal

marsh (R.J. Pankhurst, Botanical Society for the British Isles joint recorder for vicecounty 110, pers. comm.). This site is just south of my Borve locality. Local botanist George MacLeod and I searched the Tennant site in 2008 but there was no sign of *D. traunsteinerioides*. Thus, one Borve site is precarious and the other may have been lost.

In 1941 J.W. Heslop Harrison *et al.* (1942) recorded *D. traunsteinerioides* at two sites in North Harris east of Tarbert. The first was: "... in Lingadale [NB 1601] on the right bank of the stream", and the second: "... as a continuation of this, on the roadside near Oban, N. Harris." These "Forms" were described as: "... answering exactly to Wilmott's description and figures [Wilmott, 1936] ..." George MacLeod and myself explored Lingadale independently in 2008 looking for this plant, but without success. I also examined the Oban site in 2008, again without success. It should be pointed out that both these records were accepted by the Botanical Society of the British Isles Maps Scheme, despite being J.W. Heslop Harrison records.

So all the recorded sites for *D. traunsteinerioides* in the Outer Hebrides are on the machair, with the exception of Heslop Harrison's two sites in Lingadale and nearby at Oban. The machair constitutes a linear (though in places broken) link. It is interesting to compare the large population of *D. traunsteinerioides* at Luskentyre with the very large population of *D. ebudensis* on the North Uist machair. Were the Loch Sollas, Clachan Sands and Berneray populations of *D. ebudensis* originally contiguous, linked by the machair? Similarly, were the Borve and Luskentyre populations of *D. traunsteinerioides* originally linked by the machair? *Dactylorhiza traunsteinerioides* grows with *D. ebudensis* at Machair Robach in North Uist. Perhaps *D. traunsteinerioides* was once more commonly found growing with the progenitor(s) of *D. ebudensis*? It is interesting to compare and contrast the evolution of the very large North Uist *D. ebudensis* population with that of the large Luskentyre population of *D. traunsteinerioides* ("*D. lapponica*").

I should like to thank Richard Bateman for commenting on my article.

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Two Steps Forward, One Step Back: Deciphering British and Irish Marsh-orchids Richard Bateman

Background

I submitted my first ever peer-reviewed paper on the orchids of the British Isles in 1980 – an overview of the orchid flora of Hertfordshire based on three years of frantic fieldwork, conducted in my spare time by way of a series of decrepit motorcycles. Casting around for a more scientifically rigorous follow-up project, my eyes alighted on the genus that had caused me the greatest problems during my field surveys – *Dactylorhiza*. Of course, only the naiveté of youth could possibly have permitted so reckless a choice for an orchidological novice. Mind you, my collaborator, Ian Denholm, should have known better; unlike me, he was already in possession of a doctorate in population genetics.

One objection that could immediately have been raised against our blundering where most angels had wisely feared to tread was that the genus had already been tackled head-on by two notable British botanists: Jack Heslop-Harrison (who later became Director of Kew) in the 1950s and Dick Roberts in the 1960s. Ian and I followed closely in the footsteps of our exalted predecessors by similarly applying morphometric and karyotypic techniques; in other words, we consistently measured the plants and, where feasible, counted their chromosomes, deriving data from a representative portion of each study population. The one innovation that we were able to deploy was to quantify a much larger range of characters per plant and then use multivariate statistics to analyse the resulting large bodies of data, thanks in part to mathematical advice provided by multivariate guru John Gower. However, we could not realistically aspire to acquiring genetic data, which at that time lay well beyond the pockets of two 'hobbyist' botanists.

On the other hand, our timing was good, in that the genus had just become more topical. British botanists were still reeling from being told by *Flora Europaea* (Soó 1980) that three of the four then widely recognised species of tetraploid marshorchids native to the British Isles were actually mere subspecies of the Continental *Dactylorhiza majalis*. The primary goal selected by Ian and myself, pursued by us with laudable scientific 'objectivity', was to prove *Flora Europaea* wrong! Exhibiting behaviour typical of 'young men in a hurry', we concluded our fieldwork in a single year, rampaging over much of the British Isles in the summer of 1981 (my quixotic journey from Skipton to Malham Tarn in order to measure *D. purpurella*, riding a folding bicycle devoid of brakes in torrential rain, being particularly memorable for the ensuing near-death experience at Malham Cove).

The result of our hyperactive endeavours was not quite what I anticipated; on the basis of measuring 14 populations, we not only reconfirmed the existence of the four main taxa but also reluctantly treated them as subspecies of D. majalis! The key fact that we could not surmount was that the four taxa -D. praetermissa, D. purpurella, D. occidentalis and D. traunsteinerioides - overlapped with each other in overall morphology, rather than being separated by distinct discontinuities. This means that a morphologically extreme plant of taxon A will look more like an average plant of taxon B than an average plant of taxon A – a recipe for the absence of truly diagnostic characters (whatever contemporary floras might say!) and hence precluding reliable identification of individual plants, let alone of any hybrids. This rationale remains valid so our results and major conclusions still stand, even in the wake of our subsequent sampling of many more populations. A nice fat, painfully detailed but still scientifically robust paper rapidly ensued (Bateman & Denholm 1983). Case seemingly closed. So why today am I arguing that our tetraploid marsh-orchids are four bona fide species? Indeed, why am I still researching dactylorchids at all, three decades after concluding my original project?

Hybridisation and instant speciation

Most casual observers believe that many of the problems encountered when attempting to identify dactylorchids are caused by hybridisation. And they are fundamentally right, though hybridisation causes problems in two radically different ways. Firstly, there is no doubt that dactylorchids are basically over-sexed; put any two taxa in close proximity and progeny will soon ensue – progeny who often have few reservations about subsequently interbreeding with their parents. But similar behaviour is exhibited by many other genera of flowering plants, orchid and non-orchid. Rather, dactylorchids have an additional reproductive proclivity that separates them from the botanical crowd – relatively frequently, when two dactylorchids with normal chromosome complements hybridise, they also duplicate the entire genome of the hybrid plant, thereby doubling its size (and thus the number of genes that it contains). When this process, termed allopolyploidy, is successfully completed, it effectively results in instant speciation; two diploid individuals combine to generate a novel tetraploid.

Moreover, because polyploidy is implicated in the origin of many crop plants, notably cereals, and possibly also in the origin of the flowering plants themselves, research interest in the process of genome doubling is now widespread and lucrative. Exactly how each gene responds to suddenly discovering that it must henceforth share its genome with its (initially identical) twin has become of more than academic importance. Consequently, we have moved beyond genetic studies that simply describe the sequence of bases in the orchid's DNA to 'epigenetic' studies that consider how the genetic code combines with environmental influences to dictate the appearance of the resulting plant. The 'esoteric' information that has gradually

accrued on polyploid dactylorchids through the last few decades has suddenly taken on a greater import, allowing *Dactylorhiza* to step forward as a potential 'model organism' for major experimentation. Thus, dactylorchids now travel in the wake of the fruit fly and that most tedious of flowering plants, the thale-cress (*Arabidopsis*).

That D. praetermissa, D. purpurella, D. occidentalis and D. traunsteinerioides have allotetraploid origins would not have surprised Heslop-Harrison, who inferred this evolutionary scenario in the middle of the last century (e.g. Heslop-Harrison 1954). Nor would he have been surprised to learn that all of the allotetraploids found in western Europe were derived from the two main diploid lineages: spotted-orchids of the D. fuchsii aggregate and marsh-orchids of the D. incarnata aggregate (Figure 1). What would, I think, have excited him more would be learning that, in every case, the 'mother' who provided the ovules was D. fuchsii and the father who provided the pollen was D. incarnata, never vice versa - knowledge that came to light only in recent years (e.g. Bateman, 2006; Pillon et al. 2007). One day, we may even learn why this is the case - I suspect that it may have some connection with the extraordinarily low levels of genetic variation observed within D. incarnata (cf. Hedrén 1996; Hedrén et al. 2001). I also suspect that Heslop-Harrison would have supported the idea that the degree to which each resulting complex genome has stabilised and resumed 'normal service' in the tetraploids could be used to infer how long ago, at least in relative terms, each genome doubling event took place. Some allopolyploids, such as D. majalis s.s. and the genetically similar D. praetermissa, apparently originated significantly earlier than other allopolyploids, such as D. purpurella, D. occidentalis and D. traunsteineri(oides) (Pillon et al. 2007; Paun et al. 2010; Hedrén et al. 2011). The younger lineages are hypothesised to have originated since the most recent glacial period ended - that is, within the last 18,500 years, and most likely within the last 11,500 years, following the most recent periglacial period (Bateman 2011).

So, the last 15 years or so of research into dactylorchid genetics (e.g. Hedrén 1996; Hedrén *et al.* 2001; Bateman *et al.* 2003; Bateman 2006; Pillon *et al.* 2007; Hedrén *et al.* 2011) and epigenetics (e.g. Paun *et al.* 2010, 2011) have given us more reliable tools to distinguish among the named taxa, notably so-called DNA 'bar-coding'. They have also given us a much better understanding of not just how, but also how many times, tetraploid lineages originated. Surely now we can easily circumscribe the named taxa and unequivocally decide whether species or subspecies status is most appropriate for each taxon?

Well, not so fast. Some observers, including myself, wish to treat each independent evolutionary origin – that is, each case of genome doubling – as a separate species (Bateman, 2006, 2011; Pillon *et al.* 2007). This would allow confident assignment of species status to both *D. purpurella* and *D. occidentalis*, and probable species sta-

tus to both *D. praetermissa* (admittedly, its DNA closely resembles that of the exclusively Continental *D. majalis*: Pillon *et al.* 2007; Nordström & Hedrén 2009) and *D. traunsteinerioides* – a taxon that does appear to be subtly genetically distinct from the morphologically similar *D. traunsteineri* in the Alps and from *D. lapponica* in Scandinavia. Other observers counter by arguing that, because each of these tetraploid taxa has the same diploid species as mother and the same diploid species as father, they should be treated as different subspecies of the same aggregate species, *D. majalis* (e.g. Pedersen 2007; Nordström & Hedrén 2009; Hedrén *et al.* 2011).

A further argument in favour of recognising multiple species of tetraploid marshorchids is provided by the subtle to strong differences in ecological tolerances evident among the allopolyploids, which suggest that they have already acquired genuinely independent evolutionary histories. But even here there has developed a crucial debate. I suspect that much of the ecological specialisation of contrasting allotetraploids reflects those of the strains of *D. fuchsii* and *D. incarnata* from which they are derived: for example, an allotetraploid hybrid formed between the heath/bogloving orchids *D. maculata* and *D. incarnata* ssp. *pulchella* would inherit their greater tolerance of acidic soils. In contrast, other observers believe that much of the ecological specialisation evident in the allotetraploids emerged *after* their respective origins, reflecting recent adaptive adjustments to local environmental conditions. Despite the sophistication of the analytical techniques applied to these taxa in recent years (e.g. Paun *et al.* 2010, 2011), both scenarios remain credible.

By now, readers will have realised that deciding optimal ranks for named taxa on the basis of the underlying evolutionary processes is far from straightforward, even in rare cases where the relevant conceptual issues are well understood and several contrasting sources of scientific data are available. There is much truth in the old saw that each scientific question successfully answered spawns a minimum of two new questions.

The Hebridean Marsh-orchid as a case-study

In the mid-1990s, having recently moved from Oxford to Edinburgh, I began to look for a Scottish case-study that might usefully simplify the complexities of deciphering the tetraploid marsh-orchids. The obvious answer lay in the Hebridean Marsh-orchid, *D. ebudensis*, which was at that time considered to be confined to a single extensive dune/machair system on the Outer Hebridean island of North Uist (for a definitive account of the distribution and habitat preferences of this intriguing taxon see Horsman 2011 – this issue).

This population is highly restricted not only in space but also in time; relative changes in land and sea levels mean that the dune system that it presently occupies



D. fuchsii fuchsii



D. incarnata pulchella



D. purpurella cambrensis



D. maculata ericetorum



D. incarnata ?pulchella



D. occidentalis



D. incarnata ?coccinea



?hebridensis



D. fuchsii



D. ebudensis

is unlikely to have existed for more than the last 2,500–3,000 years. My working hypothesis was that the Hebridean Marsh-orchid was an allotetraploid that had originated in its present location, and hence within the last 3,000 years. The most obvious parents would be as father *D. incarnata* ssp. *coccinea*, with which it co-occurs in quantity, and as mother *D. fuchsii* var. *hebridensis*, which resides in slightly drier regions of the adjacent machair (Figure 1). However, the large co-occurring population of *D. purpurella* also presents a credible parent of allopolyploids, as well as near-certain source of annoying hybrids – annoying because *D. ebudensis* and *D. purpurella* are too similar morphologically to permit ready identification of any hybrids that might form between them.

By the time I first visited the North Uist locality in 1995, I had solved only the most trivial of the many controversies surrounding the Hebridean Marsh-orchid – that of its nomenclature. When first reported by M. S. Campbell in 1936, the population was simply assigned to *D. majalis*. Many years passed before the population was formally used as the basis of *D. majalis* ssp. *scotica* by Nelson (1976) in his majestic monograph, but in the same year it was also described as *D. majalis* ssp. *occidentalis* var. *ebudensis* by Wiefelspütz (1976), who had examined the population *in situ*. Both descriptions contained serious errors, and neither author correctly designated a type specimen (cf. Bateman 2009). However, none of the protagonists seriously considered the possibility that the Hebridean Marsh-orchid had an evolutionary origin separate from those of *D. majalis* or *D. occidentalis*.

Our first serious attempt to apply science to the Hebridean Marsh-orchid remains largely unpublished. The morphometric data that we gathered from the population showed that it differed substantially from the Continental *D. majalis* and the Irish *D. occidentalis*. It more closely resembled, but could be distinguished from, the co-occurring *D. purpurella*. The same subtle distinction was evident relative to populations of *D. traunsteinerioides* from northern England, Wales and Ireland. However, it was not possible to use morphology to confidently separate *D. ebudensis* from *Scottish* plants of *D. traunsteinerioides*, especially those previously mis-assigned to the Scandinavian *D. 'lapponica'* (Bateman 2006, 2011; Hedrén *et al.* 2011).

Our first attempt to gather genetic data used old-fashioned allozyme analysis. This technique relies on the differential movement of selected proteins across starch gels and requires very fresh material – a serious challenge when the samples must be transported from the Outer Hebrides to Edinburgh for analysis. Thus, our 1995

Figure 1. Hypotheses of origin of three allotetraploid marsh-orchids that have received differing degrees of support from recent (epi)genetic data; putative mother, father and offspring are listed from left to right. Photos by Richard Bateman

results were equivocal, though they tentatively suggested a strong similarity to *D. purpurella.* Fortunately, a fresh set of samples gathered in 1996 gave a clear result, which both demonstrated that *D. ebudensis* is indeed a tetraploid (three copies of some genes were present, whereas diploids can maintain only two) and showed that its proteins could not be distinguished from those of Scottish *D. traunsteinerioides.* A close relationship between *D. ebudensis* and *D. traunsteinerioides* was also suggested by a genome-fragmentation technique called AFLP that was applied to a single plant of *D. ebudensis* by Hedrén *et al.* (2001).

I was not able to publish DNA sequences of *ebudensis* until the benchmark phylogenetic paper of Bateman *et al.* (2003), which was based on the nuclear-chromosomal ITS region that is inherited equally from both parents. Like every other taxon included in the analysis, *ebudensis* was represented by just one plant. And the result was a surprise – that plant clustered with *D. purpurella* and *D. incarnata*, rather than with *D. traunsteinerioides*, *D. occidentalis* or *D. majalis*. An additional plant of *ebudensis* was subjected to a wider-ranging set of DNA analyses by Pillon *et al.* (2007). This suggested equal contributions of ITS alleles from the *D. fuchsii* and *D. incarnata* aggregates, a pattern also found in both *D. purpurella* and *D. traunsteinerioides*.

However, this sole analysed plant of *ebudensis* delivered a surprise regarding the plastid genome, which is inherited only from the mother. The plastids yielded a sequence type characteristic of *D. incarnata*. Taken together, these observations suggested that, uniquely, *D. ebudensis* had *D. incarnata* as its mother and *D. fuchsii* as its father, rather than vice versa (Bateman 2006). If true, this observation would strongly support the hypothesis that *D. ebudensis* had a unique, relatively recent origin in the dunes of North Uist. Applying my criteria for species recognition, this would in turn mean that, despite its restricted distribution and habitat and lack of clear morphological distinguishing features, *D. ebudensis* is a *bona fide* species. My argument was considered sufficiently strong by Clive Stace to encourage him to treat *ebudensis* as a fifth allotetraploid species in the third edition of his definitive flora of the British Isles (Stace 2010).

Of course, the most foolish action that we could have taken at that point would be to muddy these ostensibly clearing waters by analysing further samples of *D. ebudensis*. Unfortunately, that is exactly what we did. Further DNA sequencing demonstrated that the majority of the plants of *ebudensis* had plastids most typical of *D. traunsteinerioides*, suggesting that the presence of an *incarnata* plastid in the *ebudensis* plant analysed by Pillon *et al.* (2007) reflected either hybridisation between *ebudensis* and *incarnata* or even a rare laboratory error. Application of a more subtle DNA-based technique termed microsatellite analysis yielded a consistent result from both nuclear and plastid genomes: *ebudensis* closely resembled plants of *D*.

traunsteinerioides from Scotland and northern England (Hedrén *et al.* 2011). The most recent molecular data to be generated were not genetic but epigenetic, describing the degree of methylation experienced by the nuclear genome (methylation influences the action of genes; although it has a strong heritable component, it is also subject to environmental modification). These data initially appeared to distinguish between *ebudensis* and *traunsteinerioides*, but when examined more closely, it became clear that this property is population-specific; populations of *traunsteinerioides* differ substantially from each other in methylation levels (Paun *et al.* 2010). Indeed, the results suggest strong local adaptation to climatic conditions such as aspects of rainfall and temperature (Paun *et al.* 2011) – an interpretation that could usefully be explored further via the under-used, low-tech approach of experimental hybridisation and cultivation of the progeny under controlled conditions (cf. Haggar 2003–7).

Thus, the present 'best guess' for the nature of *ebudensis* is that it is an ecologically specialised, locally adapted 'super-population' of *D. traunsteinerioides*, and therefore perhaps best treated as a subspecies (Bateman 2011). Indeed, those who prefer to view *traunsteinerioides* itself as a subspecies (of *D. majalis*) would be obliged to relegate *ebudensis* to a mere variety. But of course, we have not necessarily reached the end of the story. So far, I have failed to persuade any of my molecular collaborators to analyse all of the populations of dactylorchid taxa that co-occur with *ebudensis* (*D. purpurella*, *D. incarnata coccinea*, *D. fuchsii hebridensis* and, in small numbers, *D. traunsteinerioides* s.s.: Horsman 2011) in sufficient quantities to rule out the possibility that *ebudensis* really did originate in the North Uist dunes as a result of hybridisation between *D. incarnata coccinea* and *D. fuchsii hebridensis*, followed by genome doubling. Were that origin to be demonstrated in the future, *ebudensis* would once again acquire a strong case for recognition at species level.

Classifying the tetraploid marsh-orchids: evolution or revolution?

In Figure 2, I have attempted to summarise the classification of British and Irish tetraploid marsh-orchids at four pivotal points in their recent history, beginning with the influential *Flora Europaea* (Soó 1980) and then moving on to the second edition of Stace's (1997) equally influential flora of the British Isles, which effectively dictated the nature of the taxa mapped in the subsequent national plant atlas (Preston *et al.* 2002). Stace differed from Soó primarily in treating *praetermissa* and *purpurella* as fully fledged species rather than subspecies of *D. majalis*, and in arguing that the previously exclusively Continental *D. lapponica* occurred in Scotland.

In contrast, Stace's (2010) third edition was strongly influenced by my earlier species-level classification of British and Irish orchids (Bateman 2006) – the first classification to pay serious attention to the implications of molecular data. The same four basic entities – *praetermissa*, *occidentalis*, *purpurella* and *traunsteineri*-



Figure 2. Classifications of British and Irish allotetraploid marsh-orchids advocated by various influential authors in 1980, 1997 and 2006, together with the most recent modifications suggested in the present article (red and green arrows)

oides – extend through these various classifications, but differ in important details of their circumscription. Most of these differences reflect contrasting taxonomic treatments of anthocyanin-rich populations that bear well-developed markings on their leaves and flowers – visually striking characteristics that naturally attracted the attention of traditional taxonomists. The single most important message conveyed by the now impressive spectrum of molecular data at our disposal is that each species of tetraploid marsh-orchid contains both anthocyanin-poor and anthocyanin-rich morphs: respectively, *praetermissa* versus *junialis*, *kerryensis* versus *occidentalis*, *purpurella* versus *cambrensis* (= *majaliformis*), and *traunsteinerioides* versus 'lapponica'.

However, molecular data gathered since Bateman's (2006) progress report was published require yet further taxonomic modifications (highlighted in Figure 2). As discussed above, *ebudensis* presently appears better treated as a subspecies of *traunsteineroides*. Also, British *traunsteinerioides* can be distinguished from Continental *traunsteineri*, and British *'lapponica'* from Scandinavian *lapponica*. And most recently, the few sparsely distributed populations attributed to *traunsteinerioides* by Preston *et al.* (2002) that occur south of the line from the Wash to the Severn estuary have proven to have DNA profiles that are more consistent with a poorly differentiated, ecologically specialised taxon that evolved within *D. praetermissa* rather than within *D. traunsteinerioides* (Bateman 2011; Hedrén *et al.* 2011). This insight creates an interesting dichotomy between an early-formed allotetraploid that is largely confined to an area south of the margin of the last ice sheets (*D. praetermissa*) and three later-formed northern allotetraploids that are confined to the area within the maximum extent of the last ice sheet (*D. traunsteinerioides*, *D. purpurella*, *D. occidentalis*, the latter being restricted to Ireland: Bateman 2011).

It is just possible that these latest advances in our knowledge will finally stabilise the taxonomy of the British and Irish dactylorchids ... but I wouldn't bet on it! The most important lesson that the last three decades have taught me is that evolutionary – and thus taxonomic – interpretations inevitably 'evolve' themselves as hard-won datasets progressively accumulate.

Acknowledgements

I thank the many dactylorchid enthusiasts who have been generous with their time, information and ideas over the last three decades, not least Ian Denholm, John Haggar, Mikael Hedrén, Lindsey McLeod, Sofie Nordström, Ovidiu Paun and Yohan Pillon.

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From maize fields to orchid meadows – the restoration of meadows in the Ajoie in the Swiss Jura Samuel Sprunger

Introduction

When a new motorway is constructed in Switzerland, land is purchased to compensate for the loss of habitat for wildlife as part of an ecological compensation programme. A new motorway being constructed in the Swiss Jura passes through countryside that has a rich flora and fauna. As compensation two parcels of land were purchased with a total acreage of approximately 25,800 m² in an area behind a pinewood near to the town of Courgenay by the A16 road. The area had a rather shallow 5 - 15 cm thick mineral soil over limestone rock, covered with about 10 - 30 cm humus. The land faces south and it is normally wet in winter and spring, and arid in summer and autumn when it is usually dry. Before their acquisition the areas were utilised as grazing meadows or maize fields. During their agricultural use the soils were enriched with manure or chemical fertilizer. Furthermore, herbicide, insecticide and fungicide were liberally applied to the maize.

During the work on the A16 between Delémont and Porrentruy and the construction of the Mont Terri tunnel, a concrete road was built adjacent to the building site of



Fig. 1: Map of the flowering meadows





Fig.2: Meadow at flowering times for the wild flowers Photo by Samuel Sprunger

the A16 and the quarry of Sur Serroye, leading through these two land parcels. After the opening of the Delémont to Porrentruy motorway the road was removed and replaced by a marl path. Currently, the land is accessible by the rue de Cras d'Hermont and the concrete road from Bas d'Hermont.

The start of the transformation in 1998

One parcel of land (Lot 1248) has an acreage of 7,166 m² and is surrounded to the north, east and west by a path. In the south and south-west, the land adjoins a pinewood. A hedge and trees were planted on a 4 m wide strip along the path, and on this strip a wild flower mix called "UFA special Brache Mischung" was sown. In the upper half of the parcel the native soil and humus was removed and the wild flower mix "UFA Wiesenblumen CH" was sown at a concentration of 10 grammes per m². The southern part was left in its original state and the wildflower mix "UFA Standard 451" plus 10% *Salvia pratensis* seed was sown with a strength of 2.5gr/m².

Since 1998 the lot has been rented out to a local farmer with the contractual stipulation that he cuts the grass for hay after the 15th June. A second cut or grazing by cows is allowed in September. The strip with the hedge and the fallow is cut only every second year. The contract furthermore forbids any use of fertilizer. The result has been positive for nature in every way. Flowering of the newly introduced plants





occurs from early spring until late autumn, so that the insects can collect nectar almost the entire year. In the hedges numerous birds nest while subsisting on the many different insects, earthworms and berries found on the site.

Transformation of lot 1031 in the year 2004

The second parcel of land (Lot 1031) has an acreage of 18,657 m². To the north it is partially surrounded by a hedge, belonging to the community of Courgenay. In the north-east it adjoins private property and in the east a path separates it from the other parcel of land. In the south the lot is bounded by the pinewood of Courgenay. The lower part of the area along the residential zone and the pine wood is used as pasture land. In the upper part, about one third of the area was stripped of its topsoil. On the entire area the wild-flower mixture "UFA Trockenwiese" was sown at a sowing strength of 10 grammes per m². To shield the area from the houses of the residential zone, a hedge measuring 35m by 3m was planted. The existing hedge in the north of the area, belonging to the community of Courtenay, was extended to a length of 25m and a width of 3m. Since 2004 this parcel has been rented out on the same contractual basis as the other parcel to a local farmer. The results for wildlife have been similarly gratifying. Both lots have a diverse flora and fauna and numerous visitors and walkers, especially between May and June. The lots are a prototype for successful re-establishment of flowers on formerly intensely used agricultural land.

Educational Trails – Parcour Nature Courgenay

To explore the revitalised area in the context of the ecological compensation for the construction of the motorway, different locations (Combe Vatelin, Etang de la Creule, Ancienne Carrière, Courgenay) have been linked by educational trails, which are also part of the hiking path network of the canton. An information leaflet is available for visitors at the the quarry carpark (Ancienne Carrière) and on the Etang de la Creule.

The spontaneous establishment of orchids

During a visit to both areas at the beginning of March 2009, I was surprised to find about 50 rosettes of *Ophrys apifera* and some of *Anacamptis pyramidalis*. The orchids grew on the area where the soil has been removed, on the areas where it had been left, as well as in the hedge planted in 1998. It is amazing, that orchids could grow in areas where maize was cultivated only six years before!

A possible explanation for this discovery may be that the endophytic fungi, which

Fig. 3: *Ophrys apifera* in flower Fig. 4: Rosettes of *Ophrys apifera* Fig.5: *Anacamptis pyramidalis* in flower Fig. 6: Rosettes of *Anacamptis pyramidalis* Photos by Samuel Sprunger live symbiotically with other orchids in the nearby pine wood, have colonized the soil of the area. As the seeds of the orchids have no endosperm, they absolutely need a specific fungus to germinate. The mycelia of the fungus enters the seed to nourish the embryo. Fed this way, the embryo develops a protocrom which, after having formed the first green leaves, will become a more or less autotrophic seedling. But how did the seed of the two orchids species get on these areas? To my knowledge, neither *Ophrys apifera* nor *Anacampis pyramidalis* were growing in the pine wood of Courgenay beside the two parcels of land or in the direct neighbourhood. The nearest sites for these orchids can be found between 5 to 10 km away. The seed must have been transported to the area by the wind. Considering the number and the size of the leaves of the found plants, germination probably took place in the years 2006 or 2007.

Flowering of the orchids in 2010

During the winter 2009/2010 the orchid rosettes were counted (see plan). In all, five rosettes of *Anacampis pyramidalis* and 153 of *Ophrys apifera* were found on the two lots. *Anacamptis pyramidalis* was, however, only found on the area of lot 1248 that was stripped of humus. Thanks to good weather conditions, the majority of the plants that were counted bloomed in June and July 2010. The majority of the flowering plants were pollinated and formed seed capsules. As a result of the development of the orchids on the land, it would be sensible to delay mowing until between the 15th July and 1st August, to allow the formation of a maximum number of seeds.

Conclusion

Since the inventory of the dry meadows and pastures of the Canton of Jura by Ritter (1985), 50 - 70 % of the dry pasture of the Ajoie and the Canton Jura have disappeared because of intensive agriculture or the natural succession of these biotopes to fallow land and woodland. What has happened in the Jura is also true for all regions of Switzerland. The scenario we see everywhere is as follows: fewer biotopes = less biodiversity = fewer ecosystems = depauperization of flora and fauna = loss of species. The example given above, of the transformation of lots 1248 and 1031 in Courgenay, shows that it is possible to reverse the disappearance of the native flora and fauna of our country. For any new initiatives to turn cultivated areas into biodiverse ones, political will is necessary at national, cantonal and communal levels. If all Swiss communes follow the example of the Canton of Jura, as in the Combe Vatelin, the Etang de la Creule, the pine wood of Courgenay and the newly established meadows, the condition of the biodiversity and the ecosystems of Switzerland could be ameliorated and improved. Currently, approximately a quarter of the Swiss flora (Lauber & Wagner 2000) is growing in areas that have been improved. The list of the plants has been published by Sprunger (1999).

Acknowledgments

The original documents (Sprunger, 1998) on the Combe Vatelin, Pinède de Courgenay, Etang de la Creule are deposited in the office of the community of Courgenay and the department of water and conservation at St. Ursanne. I thank Mr. J.-K. Kohler, the former mayor of Courgenay, who requested my help to rescue the pine wood of Courgenay and enabled the project to go ahead. A big thank you also to Mr. Vincent Challet, Mayor of Courgenay, for his support and encouragement to continue to ameliorate the environment in his community. I would also like to thank the constructors of the Transjura motorway who realised the activities would be beneficial to nature and to man.

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The list of the plants of the mixtures of UFA seeds, mentioned in the text, can be found on the internet at <u>www.ufasamen.ch</u>

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A Visit to Abruzzo Gianpiero Ferrari

Together with John Wakely and Martin Withers, I spent a week in Abruzzo, central Italy, from 18th to 25th May 2009. For the first 4 days we stayed in the area of Mount Gran Sasso D`Italia (Great Stone of Italy) and then moved to the Abruzzo National Park. Gran Sasso d'Italia forms the centerpiece of the Gran Sasso e Monti della Laga National Park which was established in 1993 and holds the highest mountains in continental Italy south of the Alps. It is part of the Apennines, the mountain range that runs the entire length of the Italian peninsula. This area is well know for the outstanding beauty of the landscape and for is flowers. Teramo and L'Aquila are the nearest cities to the Gran Sasso, while Rome is 132 km by road. Parco Nazionale d'Abruzzo, Lazio e Molise (English: National Park of Abruzzo, Lazio and Molise, often abbreviated to Abruzzo National Park) is the first Italian national park and was founded in 1923. The majority of the park is located in the Abruzzo region though it is not constrained by regional boundaries and also includes territory in Lazio and Molise. The park headquarters are in Pescasseroli in the Province of L'Aquila. The park currently includes 506.82 km² (195.68 square miles).

We first based ourselves at the mountain village of Navelli and from here we visited nearby Capestrano where we found *Ophrys promontorii*, *Ophrys bertolonii* subsp. *bertolonii*, *Ophrys sphegodes* and hybrids between *O. bertolonii* and *O. promontorii* and between *O. sphegodes* and *O. promontorii*. From here we made our way north, exploring rich areas around Castel del Monte and Santo Stefano di Sessanio. Here, we encountered some fantastic meadows full of orchids and insects. There were lots of *Anacamptis morio*, *Orchis pauciflora*, *Orchis italica*, *Neotinia tridentata* and Man Orchids, plus very interesting butterflies including Italian Marbled White (*Melanargia argie*), Marocco Orange Tip (*Anthocharis belia*), Clouded Yellow (*Colias crocea*), Cleopatra (*Gonopteryx Cleopatra*), Red Underwing Skipper (*Spialia sertorius*), Queen of Spain Fritillary (*Issoria lathonia*) and Painted Lady (*Vanessa cardui*).

We explored most of the minor roads in the Campo Imperatore region which proved to be extremely rich in orchid species, including *Dactylorhiza sambucina* in its yellow and red forms and some fantastic specimens of Lady and Military Orchids, with lots of very robust hybrids between the two. At the summit of Campo Imperatore in the Gran Sasso Mountain we found some alpine flowers; Spring and Trumpet Gentians and near to the melting snow there was millions of Spring Crocus.

Figs 1 & 2: *Orchis xhybrida*, the hybrid between Lady and Military Orchids Figs 3 & 4: *Orchis pallens*

Photos by Gianpiero Ferrari



For the second part of our stay we moved our base south to the village of Villetta Barrea on the eastern side of the Parco Nationale D'Abruzzo. We took a circular route northwards towards Scanno and on to Anversa Degli Abruzzi before turning west to Pescina then south on the 83 back to base. This proved to be a wonderful and productive route with some very rich areas. The meadows around Monte Godi are well worth exploring, having a diverse and interesting array of orchids and other plants, with several hundred *Orchis pallens*, a few *Orchis mascula*, and interesting hybrids between the two. Just north of Scanno we located another orchid 'hotspot', with *Orchis provincialis, Orchis mascula* and *Orchis pauciflora*. Amongst the many specimens there were three albino plants of *Orchis pauciflora* and we also found a hybrid between *O. pauciflora* and *O. mascula*. This orchid is called *Orchis ×colemanii* and it is a very beautiful delicate pink colour. On the homeward section of this circular route is Gioia dei Marsi and the meadows in this area held vast quantities of White Helleborines, Late Spider Orchids, *Ophrys fusca* and other botanical high-lights.

South-east of Villetta Barrea is a wonderful, unspoilt area along the roadside (83) to Alfedena and here we found hundreds of Man Orchids, Adriatic Lizard Orchids (*Himantoglossum adriaticum*), Pyramidal Orchids and Late Spider Orchids. As well as the plants, there were huge numbers of butterflies, day-flying moths and other fantastic insects, including the very rare Black-veined Moth (*Siona lineata*), European Owl Moth (*Brahmaea europaea*), Adonis and Common Blue, Marsh, Knapweed, Spotted, Glanville and Pearl Bordered Fritillaries.



In the woodland area of the Camosciara we experienced a real highlight - a group of four Lady's Slipper Orchids in an idyllic setting and all in full flower. Nearby, there were Bird's Nest Orchids, Twayblades, Greenwinged Orchids and Sword-leaved Helleborines. We also walked along the valley Delle Rose and up to the snowline on Monte Camosciara in search of the endangered Abruzzo Chamois - we were rewarded with good sightings of this elusive mammal and found Spring Crocus, Primula auricula, Gentians and White Pasque Flowers.

Fig 5 (above) Hybrid between O. pauciflora and O. mascula (O ×colemanii)
Figs 6 & 7: Lady's Slipper Orchid (Cypripedium calceolus)
Fig 8: Adriatic Lizard Orchid, Himantoglossum adriaticum
Fig 9: Dactylorhiza sambucina in its yellow and red forms
Photos by Gianpiero Ferrari



A New Dactylorhiza Mutation Mark Smyth

In 2009 I obtained a small *Dactylorhiza* that was of interest because of its unusual foliage. It had creamy white edges to the leaves and a white tip which bled down the veins about a centimetre. The plant went dormant very quickly and was helped by the very warm weather. The trough I put it in is in full sun – when it shines – for many hours around midday.

In late winter 2010 I was very happy to see a small nose beginning to emerge from soil level. Once the bud began to open and become leaves I was reassured to see it had kept the cream tips. As the new leaves grew larger the variegation got better. By May it was obvious that it was going to flower. The bracts could be seen between the leaves.and were edged pink -I was expecting cream like the leaves. During June the spike grew and by the end of the month all the flowers were open and I tried some self pollinating.

In July I carefully lifted my plant and removed the new tuber which I planted in a different place. I couldn't let something happen to both plants! At the end of August I couldn't resist having a look at the original plant. It had produced a new but small tuber. Happy days and fingers crossed I should have three plants. Five of the flowers were fertilised and I collected the seed and spread it around the mother plant. I hope some come through and show the variegation!



Foliage and flowers of a variegated mutant of *Dactylorhiza* Photos by Mark Smyth



HERITAGE ORCHIDS

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Would you like to grow **Pleiones** like these? Then look no further. I have a fine assortment of Pleiones, both species and hybrids. Among them the beautiful **Pleione Tongariro** (left), which wins awards every year.

I also have a selection of Hardy Orchids and Cypripediums, all legally propagated from seed.

Please visit my website **www.heritageorchids.co.uk**. It contains a plant list, descriptions, detailed growing instructions and an order form.



Laneside Hardy Orchid Nursery



Visit our new web site <u>www.lanesidehardyorchids.com</u> for full details of plants available for sale on line, 2011 shows and events, cultural information and nursery opening.

A wide range of different hardy orchids are stocked, including pleiones for the first time.

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