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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 **www.hardyorchidsociety.org.uk**, 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.

The Hardy Orchid Society Committee

Front Cover Photograph

Barry Tattersall's *Serapias* ×*godferyi* (*Serapias neglecta* × *cordigera*), winner of the 'Best in Show' Trophy in the 2012 HOS Plant Show.
Photo by Mike Gasson

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Editorial Note

This issue of *JHOS* draws on the experience of more of our members in providing articles from Tom Turner on UK orchid sites and David Pearce on orchid conservation. There are some interesting and previously unpublished results in the first part of a series from John Haggar and Svante Malmgren, as well as identification of the popular B1 fungus by Tony Heys. I have included photographs of an intriguing orchid found by Lorne Edwards in Rhodes. This possible hybrid between the Man Orchid (*Orchis anthropophora*) and the Dense-flowered Orchid (*Neotinea maculata*) has attracted interest after being posted on the internet. Claims of this hybrid have appeared rarely in the orchid literature and it features in the authorative book "*The Orchid Genera Anacamptis, Orchis, Neotinea*" by Kretzschmar *et al* (2007). There it is referred to as ×*Neotiaceras mattinatae* KOHLMÜLLER but listed as a doubtful hybrid, suggesting that the authenticity of earlier examples are questionable.

Photographic Competition at Kidlington, November 17th 2012

E-mail digital entries by 15th September 2011 to Anne Kitchen at knak@kenak.plus.com. Send notification of entries for print classes to Christine Hughes by 10th November 2011 at Linmoor cottage Highwood, Ringwood BH24 3LE or e-mail cchughes1@waitrose.com. For entrants who cannot come to the meeting Christine will accept postal entries by the same date, SAE if return of pictures is required. Full details with Rules & Classes are on the HOS website.

Chairman's Note

Celia Wright

Summer greetings! I hope that all your orchid plants have grown and flowered well this year, even if at unusual times as a result of the weather. UK flowering times in the wild were also a bit delayed early in the season but seem to have settled down as spring progressed. I've heard some excellent reports of HOS field trips, especially from some of our newer members when those with more experience have helped them to understand and enjoy the orchids they were seeing. Iain and I managed to get to the Derbyshire trip and had a thoroughly good time. Our highlight of 2012 for wild orchids has to be the masses of *Cypripedium calceolus* we saw at Kinnemulle, known as the Flowery Mountain, in Sweden at the end of May. The area is easily accessible and simply roped off with plants so close that you can touch them. In spite of this, the plants are never damaged, one of the local people explaining to us that respect for nature is universal and taught to all children in schools. I wish it were so everywhere.

The programmes and booking forms for the Northern and Southern Autumn Meetings are enclosed with this Journal. Both have good programmes that I hope include something for everyone. I'd like to remind anyone who wants Jean Claessens and Jack Kleynen to bring a pre-ordered copy of their book (The Flower of the European Orchid) to Kidlington that they should contact Jean and Jacques via their website - www.europeanorchids.com - explaining on the contact form notes that this is to be delivered and paid for at Kidlington. I will place an order for anyone who does not have internet access. Now is the time to be sorting through the season's photographs for your entries to the Photographic Competition at the November Kidlington meeting. As for the last two years, the winners in the projected images classes will go on as the HOS entries for the British Orchid Council Photographic Competition, so let's show the rest of the orchid world how to do it by winning at least one of the prizes again. I look forward to seeing many of you during the rest of the year and send my best wishes to you all.

Plant Show 2012

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

1st Barry Tattersall: *Anacamptis boryi* × *papilionacea* (Photo 2a), *Serapias bergonii* (Photo 2b), *Ophrys candica*(Photo 2c)
No further entries

Class 3 Three pots non-European hardy orchids, distinct varieties

1st Malcolm Brownsword: *Pleione* Shantung 'Ducat', *Pleione* 'Piton'(Photo 3a), *Pleione* Alishan 'Merlin' (Photo 3b)
No further entries

Class 5 One pot native British orchid

1st Barry Tattersall: *Anacamptis laxiflora* (Photo 5)
2nd Neil Evans: *Orchis simia*
3rd Malcolm Brownsword: *Anacamptis laxiflora*

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

1st Alan Blackman: *Serapias olbia* × *Anacamptis morio* (Photo 6)
2nd Andrew Bannister: *Serapias lingua* × *Anacamptis sancta*
No further entries

Class 7 One pot non-European hardy orchid
1st Andrew Bannister: *Satyrium corifolium* (Photo 7)
2nd Andrew Bannister: *Thelymitra macrophylla*
3rd Malcolm Brownsword: *Bletilla striata*

Class 9 One pot *Orchis*, *Anacamptis* or *Neotinea*
1st Richard Manuel: *Anacamptis papilionacea* (Photo 8)

Class 11 One pot *Serapias*
1st* Barry Tattersall: *Serapias* ×*godferyi* = *Serapias neglecta* × *cordigera* (Cover Photograph)
2nd Richard Manuel: *Serapias lingua*

Class 12 One pot *Cypripedium*
1st Barry Tattersall: *Cypripedium fasciolatum*
2nd John Haggar: *Cypripedium formosanum*

Class 13 One pot *Calanthe*
1st Malcolm Brownsword: *Calanthe Tranche hybrid* (Photo 13)
2nd Christine Hughes: *Calanthe tricarinata*

Class 14 One pot *Pleione*
1st Malcolm Brownsword: *Pleione formosana* 'Snowcap' (Photo 14)

(There were no entries in Classes 1, 4, 8, 10 and 15)

Winner of 'Best in Show' Trophy*
Barry Tattersall's *Serapias* ×*godferyi,* the winning entry in Class 11

Winner of RHS Banksian Medal
Barry Tattersall with 12 points

(Malcolm Brownsword 11 points; Richard Manuel 5 points, Andrew Bannister 5 points [3 points for 1st, 2 for 2nd, 1 for 3rd])

Thanks to Brian Walker for judging the Plant Show

The following pages feature some of the first placed winners in the 2012 Plant Show. A complete set of photographs of the first placed winning plants 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) that matches their order in the results list above.

Photos by Mike Gasson


3a


3b


6


14


2a


2b


2c


5


7


9


12


13

Reports From Early 2012 Field Trips

Reports from the first of the 2012 field trips to Purbeck and Derbyshire follow. The remainder of the season's field trips will be reported in the October *JHOS*.

Purbeck, 10th and 21th April led and reported by David Hughes

From late March reports came in on the Discussion Forum on the early flowering of *Ophrys sphegodes* on Purbeck. I did an early reconnaissance below Spyways Barn on 10th of April in the company of a number of very young future members of the HOS. "Sphegodes" were already in perfect condition so I offered the booked members an earlier meeting. Some accepted the offer on 21st April. The *O. sphegodes* were still in perfect condition and we were treated to the sight of a pod of dolphins off the Dancing Ledges. Above the ledges, a few diminutive multicoloured *Anacamptis morio* were just starting to flower. The rest of the team came on 28th April in the rain, and were rewarded, thanks to the cold spring, by continuing perfect "sphegodes". These were better maintained at the top of the escarpment and were mostly over nearer the sea. It was particularly enjoyable to have Ron Harrison, one of our older members, partaking in this strenuous field trip.

Derbyshire Dales, 20th May led and reported by Cathryn Frost

On an initially grey day, 15 members met at the Red Lion in Litton ready to visit the Early Purple Orchids in Cressbrook Dale. The timing for the orchids was perfect, with what seemed like thousands covering the hillside and flowering well. It was great to see the rarer white variety too. Trollius were also spotted just on the point of flowering. Other species in the Dale included Meadow Saxifrage and Mountain Pansy. After lunch, we travelled in a convoy of nine cars to Lathkill Dale (and didn't lose anyone!). The dew pond at the head of the Dale proved particularly interesting with smooth newts, dragonfly larvae, newt eggs and a couple of leeches fascinating the group. Lathkill Dale was covered in flowers and because the sun actually came out, so did the butterflies: Small Copper, Green-veined White, and Orange-tip. There were lots of Early Purple Orchids here amongst Cowslips, Wood Anemones, Mossy Saxifrage and Water Avens. It was interesting to see the Jacob's Ladder (red data book) which was about to flower. All in all, I believe a good day was had by all.

Fig. 1: White colour form of Early Purple Orchid Fig. 2: Early Purple Orchid
Fig. 3: Early Purple Orchids in Cressbrook Dale
Fig. 4: Purbeck coast Fig. 5 Resupinate form of Early Spider Orchid
Fig. 6: Early Spider Orchid
Photos by
Cathryn Frost (Figs. 1 & 3) and Malcolm Brownsword (Figs. 2, 4, 5 & 6)


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2


3


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Much ado about almost nothing? Part One

John Haggar & Svante Malmgren

A few years ago, a small colony of *Orchis purpurea* appeared at Hartslock, a site in Oxfordshire where previously plants of *Orchis simia* had been masters in their own field. Some years later, an increasing number of hybrids between *O. simia* and *O. purpurea* (*Orchis ×angusticruris*) appeared. Using DNA analysis, Bateman *et al*. (2008) showed that the *O. purpurea* was of non-British origin, most likely originating from an area in southern France. These authors discussed several theories regarding its arrival; were the plants intentionally planted by man, did they grow from seed deliberately scattered by man, or were they a natural arrival via wind-blown seed from France?

There has been much private and public discussion within the society regarding the increasing number of hybrid plants at the reserve and several concerns have been raised. Is it possible that the native *O. simia* plants could be "driven out of the market" by appearing less attractive to the pollinators than the hybrids? Could the native Monkey Orchid's British genome become "contaminated" with DNA from the French *O. purpurea*? (Bateman *et al*., 2008). Richard Bateman asks us to mention that personally he is comfortable with letting nature take its course and views this conundrum primarily as a fascinating detective story rather than a conservation calamity (R. Bateman pers. comm., 2012).

Incorporation of DNA from one species into another species by way of hybridisation is known as *introgression*. Not just a simple case of producing a primary hybrid, introgression refers to the gradual incorporation of foreign DNA into a species as a result of successive back-crossing of primary hybrids with one or both of the original parent species over multiple generations. For this to occur, it is of course a prerequisite that at least some fertile hybrids be produced from each generation. It is not necessarily evident in the morphology of the introgressed plant, but the foreign genes can be revealed by detailed DNA analysis. Introgression between the anthropomorphic *Orchis* species has been suggested as a possible explanation for morphological similarities between different genotypes of *O. simia*, *O. purpurea* and possibly also *O. militaris*, both in England and in some areas on the Continent, as a result of the DNA work of Bateman *et al* (2008).

At this point, we will leave molecular analysis for a while and concentrate on a more pragmatic approach. Should we worry about the arrival of the French *O. purpurea*? Can we predict whether or not the hybrid plants will significantly "corrupt" the pre-existing British *O. simia*, and if so, how? Inter-specific hybridisation is a well known phenomenon in orchids. In general, the ability to form a hybrid is dependent on how closely the parent species are related, but there are remarkable exceptions where

Fig. 1: *Dactylorhiza* ×*grandis* in the wild
Fig. 2: A typical propagated specimen of *Dactylorhiza* ×*grandis*
Photos by John Haggar

even comparatively distantly related species can cross successfully to produce intergeneric hybrids. In nature, many potential possible hybrids never occur because they have different pollinators, flower at different times, grow in dissimilar habitats and/or at widely separated geographical sites, all examples of so-called *pre-zygotic isolation.* In some cases, cross pollination of different species will produce empty capsules containing no seed. In other cases, poorly viable seed that cannot grow to maturity is produced − the result of trying to unite poorly compatible genomes, "genetic mis-fitting", so-called *post-zygotic isolation*.

If seed does develop, a few tests to investigate potential viability have been described. The simplest example is to count the proportion of apparently normal embryos by examining the seed microscopically. Another method uses a chemical test, the TTC test (Scopece *et al*., 2007). In some hybrid seed capsules, however, seeds that look normal in all respects prove impossible to germinate. One example is *Orchis mascula* × *Anacamptis sancta*, where different results are obtained depending on which species is chosen as the pollen donor. If *A. sancta* is used as the mother plant, empty seed capsules form. When *O. mascula* is the mother plant, a large quantity of seed containing seemingly normal embryos develops, but not one of these seeds germinated in our tests. Different results according to which parent is the pollen donor and which the mother plant is called *asymmetric reproductive isolation*.
In our opinion, such tests of viability as described above, designed primarily for seed of non-hybrid origin, are inadequate for testing orchid hybrids. For the purposes of this paper our definition of "viable hybrid seed"

Fig. 3: *Dactylorhiza* ×*grandis* × *praetermissa*

Fig. 4: *Dactylorhiza* Patricia's Pride, a highly fertile hybrid

Photos by John Haggar

is that the hybrid seed must germinate and subsequently grow on sterile or symbiotic media using methods that we have previously used to germinate and grow successfully the seed of one or both parent plants. In addition, the hybrids so produced must be sufficiently vital to be able to survive and grow ex vitro on soil/compost.

In the laboratory and garden pre-zygotic isolation, at least, can be overcome and many hybrids can be created, giving insight and information that cannot be obtained from field studies. For example, early-flowering *Orchis* species can be hybridised with late flowering dactylorhizas, producing healthy primary hybrids. A most beautiful and garden-worthy orchid results from crossing the Madeiran *Dactylorhiza foliosa* with *Gymnadenia conopsea* from Sweden. Moreover, naturally occurring orchid hybrids can be reproduced in order to test viability and fertility and to supplement field observations. Many primary (F1) orchid hybrids are strong and vigorous, exhibiting "hybrid vigour". In many cases they are more robust and grow more strongly than either of their parents, and this appears to be the case with the Oxfordshire *Orchis* hybrids.

What happens next, though? Is it likely or even possible that these F1 hybrid plants will produce viable seed by self-pollination or by back-crossing with a parent thus "diluting" that original parent species via introgression or even creating a "new" species? To help answer these questions we are publishing data from our experimental work. Different orchid genera seem to behave differently with respect to hybrid fertility and their potential for further inter-crossing.

Prospective controlled studies provide a novel way of investigating the possibilities and probabilities of introgression in orchid hybridisation and give us an alternative method to DNA analysis, which by virtue of its very nature must be a retrospective technique. Indeed, our methods represent classic science, whereby hypotheses are tested by planned experiments.

SM has propagated *Gymnadenia conopsea* × *odoratissima* to flowering size as far as the F3 (third) generation. All the plants are morphologically very similar and seed production is perfect. These two species are clearly genetically very closely related, but it seems likely that they tend to remain largely separated in nature because of the pre-zygotic isolation consequent to their different spur lengths.

Dactylorhiza frequently produces F1 hybrids with a degree of fertility. Indeed, the seed of selfed F1 *Dactylorhiza* ×*grandis* (*D. praetermissa* × *fuchsii*) experimentally cultured by JH is 25% viable and this remains true of the F2 and F3 generations too. Back-crossing of *D.* ×*grandis* with *D. praetermissa* produces seed of extremely low fertility (less than 0.2% of the seeds germinate in symbiotic culture), but the few seeds of this back-crossed F2 hybrid that do germinate and grow normally appear to produce plants that are nearly 100% fertile when selfed or back-crossed again with true *D. praetermissa* to produce an F3 generation. In this way, a method whereby introgression of novel *fuchsii* genes into plants that broadly speaking could be classified as *D. praetermissa* can be demonstrated experimentally. This form of introgression, however, seems to rely strongly on at least one of the original parents being allotetraploid and the hybrid being crossed with a plant whose genome is already represented within that tetraploid parent. For example, *D.* ×*wintoni* (*D. praetermissa* × *incarnata*) behaves fairly similarly to *D.* ×*grandis* although a lower percentage of its seed germinates, but no similar fertility is seen when the hybrid *D.* Lindholm (*D. praetermissa* × *sambucina*) is selfed. This latter hybrid has proven 100% sterile to date despite some apparently normal seeds being seen microscopically.

Experimental evidence of introgression is much more difficult to find in purely diploid *Dactylorhiza* species. These F1 hybrids appear to be almost completely sterile. Despite attempts to produce an F2 generation from crosses such as *D. fuchsii* × *viridis* (*D.* ×*mixtum*), *D. fuchsii* × *sambucina* and *D. fuchsii* × *incarnata* (*D.* ×*kernerorum*), we have only ever managed to germinate a single seed, that being of the back-cross *D.* ×*kernerorum* × *fuchsii*, which has been grown to flowering size. This plant has not produced any fertile seed to date.

Fig. 5: Plants of *Dactylorhiza* ×*wintoni* bearing seed capsules
Fig. 6: Propagated specimens of *Dactylorhiza* ×*wintoni*
Fig. 7: *Dactyorhiza foliosa* × *Gymnadenia conopsea*
Fig. 8: *Dactylorhiza* ×*kernerorum* × *fuchsii*
Photos by John Haggar (Figs 5, 6 & 8) and Svante Malmgren (Fig. 7)


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One real exception to the general infertility of F1 diploid *Dactylorhiza* hybrids is the hybrid *Dactylorhiza* Patricia's Pride (*D. incarnata* × *aristata*), which has proven to be highly fertile when selfed up to the F3 generation at least. Although significant genetic differences between Western European *D. incarnata* and *D. aristata* have been found (R. Bateman, pers. comm., 2006), the distribution of the former species across Northern Eurasia is almost contiguous with that of the latter in the Far East, where the two species are geographically separated by no more than a couple of mountain ranges. Evidently, the differences in the two species' genomes offer little hindrance to effective recombination when the two are crossed, indicating that the two species may be more closely related than the genetic data would suggest. Similar fertility might be expected from hybrids of other isolated species that are known to be closely related, like *D. incarnata* × *umbrosa*, for example. We would maintain that such exceptions involving normally pre-zygotically isolated but closely related species are more the fault of nomenclature and classification than of our conclusions being flawed. For example, different orchids that hybridise freely over multiple generations could be regarded as belonging to the same species. Alternatively, it could be argued that the binomial system of naming plants (genus plus species) is too insensitive to express adequately differing degrees of relatedness and thus the likelihood of a hybrid being fertile.

With the above proviso, it thus appears that introgression between co-existing *Dactylorhiza* species is strongly dependent on one or another of the parent species being tetraploid (usually allotetraploid). The progeny are always polyploid and this should be looked upon as a special case. It is doubtful that introgression occurs naturally other than exceptionally rarely between genetically well separated diploid *Dactylorhiza* species without a novel tetraploidy event being involved. In Part 2 of this three-part series, we will discuss hybrids between species of the genus *Ophrys*, *Anacamptis* and *Orchis*, and return to the question of the Hartslock *Orchis* hybrids.

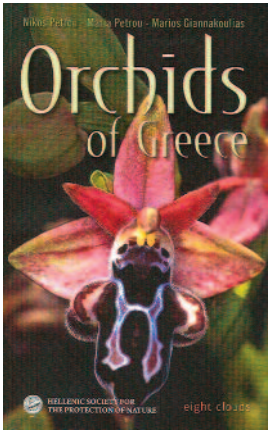
References

Bateman, R.M., Smith, R.J. & Fay, M. (2008) Morphometric and population genetic analysis elucidate the origin, evolutionary significance and conservation implications of *Orchis* ×*angusticruris* (*O. purpurea* × *O. simia*), a hybrid orchid new to Britain. *Botanical Journal of the Linnean Society* 157: 687-711.

Scopece, G., Musaccio, A., Widmer, A. & Cozzolino, S. (2007) Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* 61: 2623-2642.

Book Review: End of a Greek Odyssey

Richard Bateman



Orchids of Greece by Nikos Petrou, Maria Petrou and Marios Giannakoulias (2011), Koan, Athens. Softback. ISBN 978-960-789595-0. 320 pp. Price variable; typically £70.00+

There can be very few HOS members who have not yet experienced the many pleasures of orchid hunting in parts of Greece. Inevitably, this geographically fragmented country, especially rich in attractively diverse yet tractable islands, has lent itself to equally fragmented orchidological treatments. Beginning with treatments of the delightful but declining gem that is Crete, we have been treated to several regional guides – early examples basic, some later examples impressive – but nothing that ties together all the riches of this remarkable country. That omission has now been rectified with aplomb. *Orchids of Greece* qualifies as a pocket guide on size (a little taller than A5), though its surprising density – a consequence of the high quality paper permitting colour throughout – might cause the bearer to lean slightly in the direction of the relevant coat pocket.

The book's vital statistics are helpfully summarised on the back cover: 888 colour photos illustrate 212 putative species and subspecies. At its core is a 250-page systematic treatment of these taxa, equally divided between *Ophrys* and the remaining genera. Little space is wasted. A well-informed page of text introduces each genus. Treatments of most species and subspecies are also confined to a single page, two thirds of which is occupied by three high-quality colour images – one distant, two close-ups, almost all the work of Nikos Petrou – and the final third by a compact description that explicitly highlights key identification features, but largely eschews quantification. Distributional data appear rudimentary in comparison with those available for countries further west, though of course this incomplete knowledge offers the welcome prospect of making further exciting discoveries in the field.

The taxonomy used is a remarkable hybrid. The DNA-circumscribed monophyletic genera prescribed by *Genera Orchidacearum* are happily deployed, the authors choking only on the still relatively unpopular (but undoubtedly correct) inclusion of *Coeloglossum* within *Dactylorhiza*. Species circumscriptions follow the data-free nomenclatural compendium of Kreutz (2004) but with Kretzschmar et al.'s (2007) monograph of the former genus *Orchis* grafted on, both in turn superimposed with a few autonomous taxonomic decisions made by the authors to reflect local condi-

tions. Marios Giannakoulias indulges in an informed but ultimately unresolved debate regarding classifications and species concepts, eventually offering a pragmatic 'Goldilocks' justification of the decisions taken – the preferred classifications are neither too lumping nor too splitting.

Unfortunately, we orchidologists are such ornery critters that few of us will find the resulting species 'just right' (cf. Bateman, 2009; Davies, 2012). This taxonomic porridge boiled over (at least, from my pot) when I encountered the argument that plant descriptions from Delforge (2006) can routinely be combined with formal names sanctioned by Kreutz (2004). It is not only ranks that differ between classifications but also circumscriptions; for example, I now circumscribe all four of the British and Irish tetraploid *Dactylorhiza* species differently from the way I did 30 years ago, having used fresh sources of data to reassign several infraspecific taxa to different species (admittedly, a practice viewed by some as rearranging the deckchairs on the Titanic). The more pragmatic worldview of Petrou *et al*. is epitomised by the statement that, among Greek Platantheras, "species identification is child's play" – a statement that is true only if one accepts the traditional species at face value. Personally, after pursuing a decade of research on the genus, I'm still operating at the kindergarten level.

Grazing through the taxonomic descriptions reveals a significant number of rare species, divided between supposed endemics and less contentious species occurring at the margins of their ranges. Useful notes draw attention to a few of the many ambiguities, such as the questionable presence in Greece of *bona fide Himantoglossum hircinum* and the precise nature of the *quadripunctata*-like *Orchis sezikiana*. Other points of interest can be extracted from the illustrations; for example, I suspect that the majority of the six illustrated plants assigned to the reliably diploid *Dactylorhiza incarnata* are actually tetraploids, though I could easily be wrong – and if by chance I am right, I have no idea whether appropriate names are already available. Much remains to be learned about the Greek orchid flora.

The systematic treatment is preceded by short but excellent summaries of Greek geography and habitats, orchid morphology and biology (revealing a welcome scepticism regarding pollinator specificity), and the multitude of threats that challenge the future well-being of this diverse flora. These sections are supported toward the end of the book by an idiosyncratic but nonetheless useful glossary, a selective bibliography that suggests familiarity with a reasonable cross-section of recent scientific literature, and a comprehensive taxonomic index. Particularly welcome are 'cigarette card' plates of 69 hybrids and 49 floral "abnormalities". Only rarely does the wealth of information provided lapse into outright error (anyone who could conclusively demonstrate that the sole Greek population of *Ophrys bertolonii* originated through "wind-borne spores [sic] from Italy" would be guaranteed membership of the Royal Society).

The distinctly informal tone of the text is set by its short prologue and epilogue, wherein Maria Petrou vividly describes the highs and lows of obsessively seeking, and determinedly identifying, Greek orchids. I was particularly struck by the excitement evident in the text when the authors graduated from the infuriating myriad of lowland *Ophrys* to the more taxonomically diverse orchids of mountains and wetlands – many of which are sufficiently familiar to western European orchidologists that, foolishly, we have not bothered to pursue them in Greece. This book has shaken me out of that particular complacency.

It took Odysseus 20 years to triumph in, and then return home from, the Trojan war. The present authors required the same period of time to complete this labour of love. They have thereby performed a great service for all European orchid enthusiasts, producing not only the first rigorous orchid flora of Greece but also one that is modern and user-friendly in concept, engaging in execution, and published in English. I can only hope it will soon be made more readily available, as at present it is difficult to find – even on the Web, where it is seriously over-priced for a medium-sized softback, however useful its contents.

References

Bateman, R.M. 2009. What's in a name? *JHOS* 6: 53–63, 88–99.

Davies, P.H. 2012. What's in a face? *JHOS* 9: 51–58.

Delforge, P. 2006. *Orchids of Europe, North Africa and the Middle East* (3rd edn). A. & C. Black, London.

Kretzschmar, H., Eccarius, W. & Dietrich, H. 2006. *The orchid genera* Anacamptis*,* Orchis *and* Neotinea. EchinoMedia, Bürgel, Germany.

Kreutz, C.A.J. 2004. *Kompendium der Europäischen Orchideen.* Published by the author, Landgraaf, Netherlands.

The B1 Fungus is a *Ceratobasidium*

Tony Heys

B1 Fungus

The B1 fungus strain is well known to amateur growers of hardy orchids from seed in the U.K. It forms a robust and vigorous growth on oat based agar medium. The growth is typically pale or colourless with some white "hairy" patches of aerial hyphae, and concentric ring patterns can often be seen. A distinguished member of our Hardy Orchid Society, Jim Hill, is the one who first isolated it, and I have taken much advice from him in writing this article. Jim extracted B1 from the root of a *Dactylorhiza fuchsii* growing on a disused allotment at Troopers Hill in East Bristol. He has described the method used for isolation in a previous issue of this journal (Hill, 1997). A question arose at the April 2012 HOS meeting as to whether the B1 fungus had been identified. We can now give an answer to that question.

B1 is known to be capable of supporting the germination and early growth of most if not all species of *Dactylorhiza*, some species of *Serapias* and *Anacamptis* (e.g. *A. morio*, *A. laxiflora, S. lingua*), *Gymnadenia conopsea* and probably others. A strong orchid mycorrhiza is established such that mature flowering orchid plants can be reliably obtained, typically within 2 to 5 years of sowing seed on B1 culture. John Haggar kindly gave me a sample of B1 in Spring 2007, and I have been using it every year since then, occasionally re-isolating it from healthy protocorms. There has been no noticeable loss of germinating ability, and I have been able to produce more than 1500 plants in that time. B1 can thus be regarded as a very reliable symbiotic fungus for growing orchids. The growth habit and seed germination profile have led to speculation that the fungus may be *Ceratobasidium cornigerum*.

DNA Identification

In July 2011 I approached Professor David Read and Dr Martin Bidartondo to have the B1 strain identified by DNA analysis. Martin analysed the nuclear ribosomal internal transcribed spacer region (nrITS) of the fungal genome, giving a DNA sequence and the following description: "A type of *Ceratobasidium* (a Rhizoctonia-forming fungus), similar on the National Center for Biotechnology Information (NCBI) database to other fungi detected in orchid roots. Either an undescribed species or a described species not yet represented in the database. It does not exactly match *C. cornigerum*". David added that species concepts are difficult to apply here and that changes to just one or two bases in a sequence seem to be able to change fundamental properties like infectivity for orchid roots.

Martin then referred me to Professor Marc Cubeta at North Carolina State University in the U.S.A., who has an extensive curated database of similar fungi. Marc replied: "B1 belongs to the genus *Ceratobasidium*. Its closest relatives in our database are two strains of anastomosis group C (AG-C) isolated from sugar beet, NCBI no. AB290021 with 94% shared identity, and CBS 148.54 from an unknown plant host with 93% shared identity. In NCBI the closest relatives appear to be in the range of 93-94% shared identity and are usually endomycorrhizal isolates associated with orchids. According to Sharon *et al*. (2008) the percent nrITS identity within the AG-C group varies from 87-98%, so it is likely that the B1 strain belongs to AG-C. Furthermore AG-C strains are usually associated with mycorrhizal behaviour (Sneh *et al.,* 1991)".

To summarise, B1 is a species of *Ceratobasidium* belonging to anastomosis group C and is closely related to other fungi in this group that form mycorrhizae with orchids. Since species are challenging to define for these fungi, the genus *Ceratobasidium* has previously been sub-classified into anastomosis groups (AG). This system is based on the ability of hyphae of related isolates of the same species to recognise and fuse or anastomose with each other. In practice this is done by pairing two iso-

lates on a glass slide and observing the interaction zone. Those that exhibit hyphal fusion are considered to be the same species and belong to the same AG. Prof Cubeta's laboratory has done much research in this area and at least 21 Ceratobasidium AG groups are recognised internationally, designated from AG-A to AG-U (Gonzalez et al 2001). Their research has found that analysis of ribosomal DNA largely accords with the AG classifications.

***Ceratobasidium* and Orchids**

Ceratobasidium is a genus of fungi in the Phylum *Basidiomycota*. These fungi occur worldwide and their mode of nutrition is principally saprotrophic, feeding on dead plant material either close to the soil surface or on decaying wood or bark. However they have other strings to their bow, being commercially important pathogens in living crop plants. Amongst many others they cause sharp eyespot disease of cereals, turfgrass diseases, black rot of coffee, and aggregate sheath spot disease of rice. Bearing in mind the origin of B1 from an allotment, it is interesting to note that many of the *Ceratobasidium* isolates in Prof Cubeta's paper originated from crops – sweet potato, rice, peanut, cucumber etc as well as grasses and cereals. Some are known to

Left: *Dactylorhiza fuchsii* protocorms growing on agar medium with visible areas of B1 fungal growth
Right: *Anacamptis morio* flowering only 20 months after sowing seed with B1 fungus. Flowering from the third season of growth is more usual, though.
Photos by Tony Heys

cause "damping off" disease of young plant seedlings grown in humid, crowded conditions, which can also include orchid seedlings.

Of course, the third, and most interesting lifestyle to us is that some can establish stable mycorrhizae with orchids. *Ceratobasidium* species have often been isolated from the roots of naturally growing orchids and may well play a continuing role in the plant's nutrition. It is uncertain whether this is a mutualism because it is not known what the fungus is deriving nutritionally from the relationship. However, the plant may be providing the fungus with a more favourable environment to live in. The *in vitro* properties of the B1 fungus in supporting germinating orchid seeds suggest that it or similar species of *Ceratobasidium* may well play a key role in successful germination of orchids in the wild.

Where does *Ceratobasidium* fit into the classification of known orchid mycorrhizal fungi? *Ceratobasidium* species do produce sexual fruiting structures, although these are not borne in very obvious or showy mushrooms or toadstools. Rather they are sometimes visible on dead wood or bark as white or grey, spreading encrustations. This is a "resupinate" or "corticoid" fruiting habit and the spore producing part of the fruiting body appears as if it has been painted on the substrate with its web-like appearance. The fruiting bodies produce sexual spores (basidiospores), and this is known as the teleomorph form of a fungus. The various species classified within *Ceratobasidium* are thus teleomorph forms.

On the other hand, many of the fungi that are important mycorrhizal partners of orchids have been described only by their asexual form, classified under Rhizoctonia. The asexual mycelium of a fungus that does not produce or has not been observed to produce sexual spores is called the anamorph form. Rhizoctonia is a form genus, i.e. a type of morphologically defined "umbrella" group that is not a true evolutionary one, and includes fungi that are not closely related to each other. Within it are the groups *Ceratorhiza*, *Moniliopsis*, and *Epulorhiza* that contain most of the important mycorrhizal partners of orchids. These are the anamorph equivalents of the teleomorph genera *Ceratobasidium*, *Thanatephorus*, and *Tulasnella*. In practice, the connections between anamorph and teleomorph genera are difficult to define, and the anamorph names are not widely used by the scientific community. Two examples of species other than *Ceratobasidium* associated with supporting orchid germination are *Tulasnella calospora* and *Thanatephorus cucumeris*.

In conclusion, it is hoped this identification of B1 will help to throw a little more light on the nature of the fungi that hardy orchids so depend on in nature. Surely an area where there is still much to be learned?

Acknowledgements

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References

Hill, J. (1997) Symbiotic culture of hardy orchid seedlings. *The Hardy Orchid Society Newsletter* 5: 14-16.

Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., and Sneh, B. (2008) Classification of *Rhizoctonia* species using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience* 49 (2): 93-114.

Sneh, B., Burpee, L., and Ogoshi, A. (1991) Identification of Rhizoctonia Species. *American Phytopathological Society*, St Paul, USA.

Gonzalez, D., Carling, D., Kuninaga, S., Vilgalys, R., and Cubeta, M. (2001) Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with Rhizoctonia anamorphs. *Mycologia* 93 (6): 1138-1150.

Orchid Conservation on Wolstonbury Hill

David Pearce

Situated seven miles north of Brighton, Wolstonbury Hill is a commanding northerly outpost on the South Downs. Mainly owned by the National Trust, and within the South Downs National Park, the hill features mixed farming comprising arable and grazed pasture. The summit of Wolstonbury reaches an altitude a little over 200m and offers an impressive 360° panoramic view ranging from the English Channel in the south to the Sussex Weald in the north. Such is this vantage point that the multiple earthworks found at the summit are indicative of the protection offered to early settlers from the Bronze Age and later to the Romans.

Some five years ago a small group of volunteers 'Friends of Wolstonbury Hill' was formed with support from the National Trust to assist with conservation work. The working group gives two days per month and undertakes a wide range of activities to assist with the management of this chalkland hill. One of the principle interests for the group has been conservation of native orchids.

Across the hill, and according to season, some twelve species of orchid can be found. A list of those seen in the last couple of years is given below. Of particular interest to the conservation group is an area of approximately 1.5ha on the northwest facing escarpment affectionately referred to as the 'orchid bank'. This has received many hours of work where the removal of low bush and brambles has been achieved; it is now mowed regularly in early autumn. It is hoped that this area will eventually return to general grassland so that it can be grazed by sheep.

To the south and upper part of the orchid bank resides a small stand of silver birch and mature beech trees, which offer shade and a varied habitat to a range of woodland flora. In regard to the orchids, this has produced improved populations of *Orchis mascula*, *Platanthera chlorantha*, *Cephalanthera damasonium*, *Ophrys insectifera* and the ubiquitous *Neottia ovata*. By way of example, the Greater Butterfly Orchid has increased its flowering spike count in recent years from around 20 per year to well over 300. It is this population that has provided plant morphology data to Professor Richard Bateman and his research team for their work investigating the synergistic evolutionary relationship between plant and pollinator.

The Fly Orchid is another success story. Where this plant was showing two or three flowering spikes each year, it has now increased to an encouraging population in excess of twenty with a widening distribution across the orchid bank.

The orchid bank in Spring (top)and winter (bottom)
Photos by David Pearce

Although Friends of Wolstonbury Hill is a small group of enthusiasts it has been remarkably successful in improving the habitat for native orchids and hopes to continue this work with the agreement of the National Trust. Native orchids found on Wolstonbury Hill are *Orchis mascula*, *Neottia ovata*, *Dactylorhiza fuchsii*, *Platanthera chlorantha*, *Cephalanthera damasonium*, *Ophrys insectifera*, *Ophrys apifera*, *Orchis anthropophora*, *Gymnadenia conopsea*, *Dactylorhiza viridis*, *Anacamptis pyramidalis* and *Spiranthes spiralis*.

On and around Box Hill

Tom Turner

Box Hill is a famous open space, now owned by the National Trust. The main tourist area overlooks the Mole Gap, a break in the chalk of the North Downs near Dorking, but the estate is very extensive and even a short walk from the lookout point leaves most visitors behind and one is free to run, walk, picnic, fly kites or, in our case, look for orchids. I have seen 15 of the 16 species that are commonly to be found in and around the hill, a remarkable number for one area, and this article includes my reflections on finding them. I'm using local names around the area that are given on the National Trust leaflet and elsewhere, and reviewing the species in the approximate order in which they flower on the hill.

First to flower are the Early Purple Orchids. I know of only one location and that is about 400m north of the lookout point. Since the introduction of cattle grazing, their numbers have increased slowly but still only two or three dozen flower. In 2011 there were none, perhaps because the cattle were left too long in the area, or because of the very dry spring. Perhaps a dozen flowered this year. They are out in the open, and a good proportion (about a quarter) are pink.

Vying for second place are the White Helleborines and Bird's Nest Orchids. The former are particularly numerous at Juniper Bottom, but they are quite widespread in many of the woods. Bird's Nest Orchids occur on the wooded slopes beside the Zigzag Road, but are often in splendid numbers in the woodland above the Headley Road. A path that cuts across White Hill is a good place to look. In some years small areas are carpeted with them, providing a splendid sight.

Man Orchids are remarkably widespread, and one can come across an isolated specimen almost anywhere. A few years ago over 100 flowered on Duke's Meadow, but

Fig. 1: Early Purple near the tourist centre
Fig. 2: Early Purple overlooking Dorking
Fig. 3: Close up of Bird's Nest Orchids
Fig.4: White Helleborine
Photos by Tom Turner


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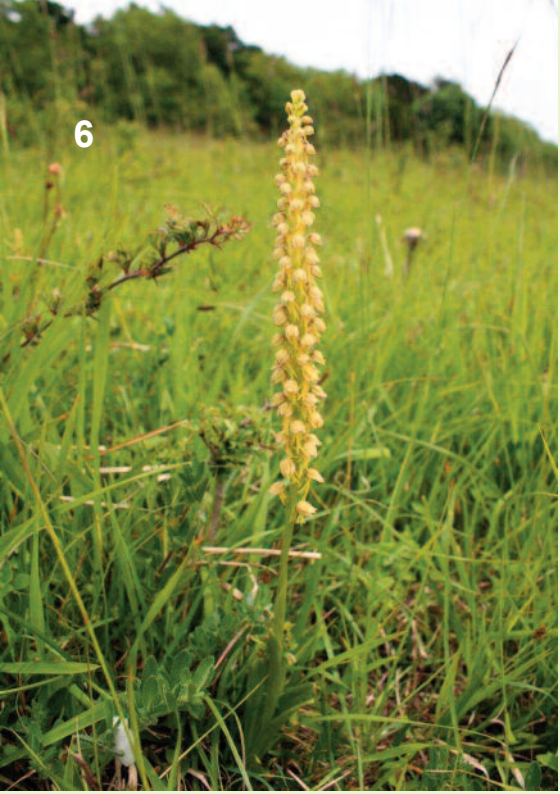
only in tens since then. That's where I first saw them a few years earlier, wandering along beside a bank, and there they were! Quite unexpected, and all the better for that. The finest examples can be found beside the A24 near Riker's Café; look in light scrub under hawthorn bushes and you could be rewarded by finding dozens, some up to 40 cm tall.

Greater Butterfly Orchids are only found near Juniper Bottom, and in small numbers. The location was, until recently, becoming overgrown by scrub, and only a few hung on between the bushes and the electric fence at the boundary. (Yes, it is electrified, no need to test it, I've done that for you!) Yet apparently when there was little scrub the flowering spikes were bitten off. I was told that rabbits were responsible but suspect deer, and recent articles in *JHOS* have added to my suspicion. In the autumn all the scrub in this area was cleared away and the ground is open. I had noticed that there were leaf rosettes under the scrub, which is positive news, and I shall ask permission to place hawthorn twigs around some of the flower spikes.

Fig. 5 (above): Man Orchids beside the A24
Fig. 6: Man Orchid on Duke's Meadow
Fig. 7: Greater Butterfly Orchid protected by the fence
Fig.8: A Fly Orchid being pollinated in Brockham Lime Works
Fig. 9: Common Spotted Orchids are always a welcome sight
Photos by Tom Turner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I've never seen a Fly orchid on the hill proper, though they are reported to flower in some woods. They are, however, present in nearby Brockham Lime Works, particularly where scrub has been cleared. Man Orchids can be found here too, with a good range of colours. There are also banks of Common Twayblades. Mention of Common Twayblades brings us to other widespread species: Common Spotted Orchid, Fragrant Orchid, Bee Orchid and, a little later, Pyramidal Orchid. My favourite in this list is the Common Spotted Orchid. I wonder how many miles we would travel to see it, and how the variation of its flowers would be admired if there were only four sites in the country where it occurred! So let's appreciate a pretty plant when we see it and be glad it's available for us all to admire.

Musk Orchids flower on the slopes overlooking the Zigzag Road. Their numbers vary greatly from a handful to perhaps a hundred. They are somewhat smaller than I've seen elsewhere, but seem to have a stronger scent. The same location hosts Frog Orchids, or so I'm told, as this is the species I've not found − yet!

Fig. 10 (above): An Autumn scene on Box Hill
Fig. 11: Common Twayblades are too often overlooked
Fig. 12: Autumn Lady's Tresses are very widespread
Fig.13: Broad Leaved Helleborines always deserve a closer look
Fig. 14: Broad Leaved Helleborines are widespread
Photos by Tom Turner


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Fig. 15: Violet Helleborines beside a tourist path
Photo by Tom Turner

There is a fair chance of finding a Broad Leaved Helleborine anywhere in the woods, but good numbers are beside the paths above the Headley Road. It was there that I once saw a wasp leave a flower, do a loop-the-loop and crash into the ground. Strange that alcohol should have similar effects on such different life forms. Violet Helleborines can be found growing on the clay-with-flints at the top of the hill, as the book says they do! One splendid plant, with several stems, is beside the main path up the hill, and is almost entirely ignored. Others grow in the hedge between the road and the car park. For some years one flowered in the open, right beside the 'car park' notice. It's fortunate that they are attractive only to the connoisseur.

So finally we look out for Autumn Lady's Tresses. Almost any grassland slope can be infested with these splendid little flowers. I was on Juniper Top looking at the eyebright, when I failed to focus on them. Then the penny dropped: I'd inadvertently come across 'ALTs', that I'd never seen before. And the only 1st that I've got in the photo competition was of this species. Any time you're not too far away, try to get to Box Hill. If you'd like to email me at tomturner@ntlworld.com I could give you more specific help in locating the species.

Possible Hybrid in Rhodes

Lorne Edwards

Our last full day on Rhodes and we were checking some of the orchids that had just come out and some others that would just be a "trip tick". We drove up to Profitis Ilias, one of the highest mountain sites, but unfortunately heavy rain fell for over an hour. When it eased we walked up the hillside to look for *Neotinea lactea* and *Anacamptis morio* ssp.*picta*, *Orchis provincialis* and a few *Orchis anthropophora*. *Neotinea maculata* is common all over the Profitis Ilias area, but when taking a record shot of Man orchid I noticed this odd looking Dense-flowered Orchid so took a record shot of that too. When I got home and was working through my photos, I noticed the difference and realised that it was possibly a hybrid. A rather odd possibility until, on checking Delforge, I realised that he has the two species listed as *Orchis intacta* and *Orchis anthropophora*.

Man Orchid *Orchis anthropophora* (left) and Dense-flowered Orchid *Neotinea maculata* (right) together with the possible hybrid between them (centre)
Photos by Lorne Edwards