

# *The Hardy Orchid Society* *Newsletter*



**No. 19 January 2001**

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Colour Insert between Pages 14 and 15

**Cover illustration: *Pogonia ophioglossoides* by Carol Dash**

## **From the Newsletter Editor**

This edition of the Newsletter includes our first experiment with colour printing. Let us know what you think.

## **HOS Autumn Meeting**

### **Orchid Conservation; What Determines Orchid Distribution and Abundance?**

**Helen S Scott**

After a very general introduction to the aims of this research back in 1998, I was delighted to re-attend and present the results of my three-year PhD. The work aimed to investigate several aspects of wild orchid conservation, from habitat management through to the complex relationship between orchid and mycorrhizal fungus.

Study species *Anacamptis pyramidalis* and *Gymnadenia conopsea* were chosen for their relative abundance and to provide a basis on which to address key orchidology questions. It is hoped that this grounding will provide us with the methodology and experience to approach the conservation of rarer orchid species.

Research centred around Porton Down SSSI in Wiltshire, which is owned and managed by the Ministry of Defence. Three other sites within a ten-mile radius of Porton (Cockey Down South; privately owned, Cockey Down North; Wiltshire Wildlife Trust and Pepperbox Hill; National Trust) were also chosen to provide comparable data on past and present habitat management strategies (Table 1) and population demographics.

Firstly, we addressed the question of what factors determine the performance of individual plants. Plots were constructed at each site in replicate and measurements of spike height, inflorescence height and the number of flowers per inflorescence were recorded, along with whether individuals were flowering, vegetative, absent or 'new' each year during the flowering season (early June to July). The term 'new' referred to plants that were not recorded within the population after year one. Unfortunately, the length of this study did not permit us to determine whether recruitment was occurring, as new individuals may also be re-emerging from a period of dormancy.

We also surveyed the vegetation within the plots and the effect of grazing intensity on plant performance, together with rainfall patterns for each year.



Figure 1. *Gymnadenia conopsea*

*Anacamptis pyramidalis*

Results highlighted a number of contributory factors. For both orchid species the proportion of flowering plants was very high for each year (as much as 90% of the total population). Life-stage transitions between flowering, vegetative and absence were also very low (approx. 5%) with flowering occurring in the same individuals throughout the three year survey. There was also very little evidence of new plants at all the study sites. This was particularly so on Pepperbox Hill, where the population density was very low. By determining the National Vegetation Classification code for each site by surveying quadrat plant species, Pepperbox Hill also differed from all the other sites – CG3 rather than CG2.

An increase in sward height coincided with an increase in spike height and the number of flowers per inflorescence in both species. Intense year-round pony grazing at Cockey Down South clearly had an effect on the *G. conopsea* population at the site, as spikes were considerably smaller than on the winter grazed neighbouring site of Cockey Down North.

Rainfall data, provided by the Environmental Change Network at Porton Down, was analysed against the performance of each orchid species with total rainfall for each year divided into winter (October – April) and summer (May – September). Whilst this kind of analysis should idealistically be performed on data collected from long term studies, the performance of *G. conopsea* in this study strongly correlated to the amount of winter rainfall prior to the flowering season – performance was reduced (smaller spikes with less flowers) after high winter rainfall. In comparison, *Anacamptis pyramidalis* showed no correlation to rainfall

Site	History of Land Use	Grazing Regime		
		1997	1998	1999
Porton Down	Last recorded arable in 1885. Grassland for 50-100 years.	Naturally grazed by rabbits.	Naturally grazed by rabbits.	Naturally grazed by rabbits. <i>Cornus sanguinea</i> cropping.
Cockey Down North	Unploughed for at least 100 years.	Grazed from Dec – Apr by young cattle.	Grazed from Dec – Apr by young cattle.	Grazed from Dec – May by young cattle.
Cockey Down South	Unploughed for at least 100 years.	Year round horse grazing.	Year round horse grazing.	Year round horse grazing.
Pepperbox Hill	Unploughed for at least 100 years.	Winter grazed by ponies.	No managed grazing.	No managed grazing.

Table 1. The history of each study site with past and present management strategies.

patterns, suggesting that either the data was insufficient, or that other factors were affecting their performance.

Secondly, we investigated whether plant density was related to soil nutrient composition. Soil samples were taken from each replicate plot at each study site and tested for a number of significant soil nutrients. Whilst the majority of nutrients varied little between sites, the form in which nitrogen was made available to plants did appear to be a critical factor in determining the distribution of a species. Sites populated by *A. pyramidalis* had soils containing high levels of ammonium nitrogen ( $\text{NH}_4^+$  -ions) whereas *G. conopsea* populated sites with comparatively lower levels of  $\text{NH}_4^+$  -ions and higher levels of nitrate nitrogen ( $\text{NO}_3^-$  -ions). The ‘patchiness’ of these particular nutrients may also account for the clumped distribution of both species at each site and nutrient flux can also disrupt the relationship between orchid and mycorrhizal fungus (Dijk *et al.*, 1997).

We went some way into investigating the microhabitat requirements for successful seed germination with an experiment designed to manipulate the indigenous fungi

with the fungicide benomyl. Using the seed packet method developed by Rasmussen and Whigham (1993), seeds were retrieved from the soil every month for analysis after treatment with either water or a suspension of benomyl in water (control of no treatment). Results indicated that both germination and infection were increased with both treatments. Although we are uncertain whether the beneficial effects of benomyl are due to fungal manipulation or fungicide composition, the fungicide did increase infection and germination beyond the addition of water. However, the largest contributing factor to successful seed germination was in fact the increase in soil moisture due to watering during the initial stages of the experiment.

Despite this apparent trigger, the majority of seeds reached only the very early stages of germination. This suggests that whilst soil moisture may be the trigger for germination, other factors such as temperature, light and depth may be playing a role in the further development of seeds beyond this point.

A percentage of seeds also showed levels of germination without the presence of mycorrhizal fungi, suggesting that these initial stages are not necessarily dependent on fungal infection. This prompted the question, is the relationship between orchid and fungus continuous throughout its life cycle and is there any specificity between orchid species and the fungi they associate with?

These questions were answered with the use of molecular analysis. Fungi were isolated from the seed and root tissues of both orchid species and pure cultured in the laboratory. DNA from each of these isolates was chemically extracted, chopped up and amplified to give large quantities of a specific section. Run on a gel, this gives a series of bands which migrate according to size, allowing us to differentiate between genetically distinct but morphologically similar fungi.

The results not only showed overlap between our own field isolates and two isolates pure cultured by Kew for the symbiotic propagation of orchids, but it was also clear that whilst there was an overlap between the fungi associating with each species (not surprising as these species are often found cohabiting the same site) there was a significant difference between those associating with seed or root tissues. Consequently, a site suitable for seed germination may not be suitable for adult plant development and vice-versa, limiting population distribution and recruitment.

Overall, the results of this PhD have been an eye-opener on a number of issues. It is hoped (with funding!) that research will continue with the support of Imperial College London and Kew and extend to those species which are under threat of extinction, both in the UK and world-wide. We hope to establish a database of specific fungi and their orchid associates and to complete the investigative thread

by determining the effects of cultured field isolates on the germination and development of laboratory reared seedlings.

## **A Small Corner Of Canada**

### **Carol Dash**

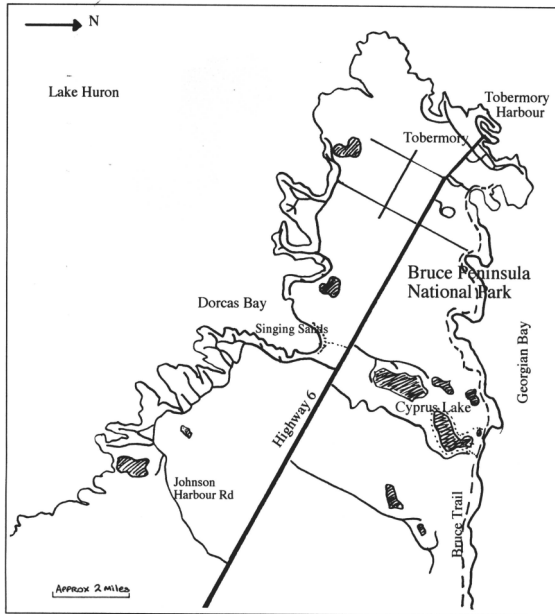
For most people Canada is a vision of spiky mountains. I have to say it was for me. So – when we decided to go in search of *Cypripediums* for our summer holiday it was a choice of Canada or NE USA and Canada won. However it was not to be as spiky as I had imagined. A friend and fellow orchid enthusiast living in Western Canada was e-mailed to make some suggestions and this, followed by more specific site information supplied by HOS fellow members (see acknowledgements) formed the basis for our trip.

So our visit was to be to South East Ontario, specifically to the Bruce Peninsula. Most of this area of Ontario is flat – very flat, and scenically not exciting. However there is a limestone escarpment stretching from Niagara in the South northwards to the Bruce Peninsula which juts out into Lake Huron. This rocky ridge is a World Biosphere Reserve and has a series of Conservation Areas and parks along its length. The long distance footpath called the Bruce Trail also follows this escarpment.

We flew into Toronto on 15<sup>th</sup> June 2000. The first night we collapsed in an airport hotel but then the next day picked up our campervan and after visiting a local supermarket (an experience in its own right) we set off northwest towards the Bruce. The campervan proved to be a brilliant way to explore and be self-contained as well as independent.

Interestingly the area of the Bruce Peninsula is at a latitude around that of Central France but obviously being within such a large landmass results in a Continental climate. The winters are very cold and the summers usually hot. This year the weather was cool and very wet during the first week of our visit but then warmer towards the end. As the Peninsula juts out into Lake Huron its climatic conditions are very much influenced by the proximity of the water. Indeed in appearance it was easy to imagine you were at the “sea side” with sandy beaches and waves lapping the shore. In winter icy cold winds blow onshore and freeze everything around the shore – hard to imagine when the sun is shining in the middle of June!

Our first base on the Peninsula was on the western side at the small coastal resort of Sauble Beach. Along this area of coastline are a series of shoreline fens, many of which are Nature Reserves. These are very rare fens with high alkalinity and little or no peat. They often have a covering of sedges, grasses and reeds with some shrub cover and a few white cedars. Most of the Peninsula is wetland or swamp.



THE UPPER BRUCE PENINSULA, ONTARIO.

Areas which are slightly more raised still have a very high water table underneath. Petrel Point is one such area. This is a 21 acre reserve with roughly half as shoreline fen and half as dense cedar swamp. It was easy to imagine that much of the coast had been like this before being cleared for development, indeed any undeveloped block of land seemed to be dense wooded swamp. *Cypripedium reginae* like the edges of these wooded swamps. When we arrived in the area around the 18<sup>th</sup> June they were just coming out and were truly spectacular.

Many plants stand 3 foot high with large globular flowers. From the road they were quite easy to spot because of the lightness of the green of the foliage and also the whiteness of the petals. In the fresh specimens the lip was bright, almost fuchsia pink!! Yet this was not what caught your eye first from the campervan. They were very fresh at this time with many flowers yet to come out. Although I would not say they were common they certainly were plentiful in this area. For me they were what I had gone to see! And I was not disappointed – actually very impressed. They were far more robust and spectacular than any I had previously seen in cultivation. Large clumps with perfect foliage and 20 or so flowers take some beating. As you can imagine much film was used! But remember swamp = mosquitoes! So it was not all fun and games. Some of the swamps looked very primaevial and it was easy to imagine dragonflies with 3 foot wingspans - certainly the mozzies were huge.

Typically the *Cypripedium reginae* were standing in water. They seemed to prefer being at the base of old or dead trees but always in water. Much of the undeveloped areas of vegetation as mentioned above were dense patches of scrub and woodland. These areas were impenetrable and it was therefore uncertain how many Cyps could be found within the woodland. However they appeared to prefer the looser woodland edge with dappled shade. We did find one area where trees had been fairly recently felled (last year?) and there were plants out in the open and it would be interesting to see how the population can cope with such exposure.



Possibly in the long term it may be beneficial if a more open shrubby woodland is allowed to regrow as long as undergrowth does not choke the Cyps.

The Peninsula is not only famous for its orchids but is recognised for the variety and rarity of its ferns. They were indeed many very beautiful ones but I am afraid we did not tackle identification other than the very obvious *Osmunda regalis* and *Onoclea sensibilis*. The latter was very common especially in amongst the *Cypripedium reginae*.

At Petrel Point and similarly at Oliphant Fen, boardwalks have been constructed to enable you to walk across the fen. Whilst the Cyps tend to inhabit the woodland edge the more open exposed wetter fen areas hold a lot of interest also. Carnivorous plants are very spectacular in the area. In particular, *Sarracenia purpurea*, with its beautiful red-veined pitchers and tall red and yellow flowers. They were everywhere where the fen met the shoreline. They formed big clumps and obviously must be very hardy to survive in such exposed conditions on the lake shore. Other carnivorous plants included *Drosera linearis* and *D. rotundifolia*. Also *Pinguicula vulgaris* were common. Orchid-wise we found *Platanthera dilatata* just coming out, an impressive and robust white orchid.

Later in our holiday we returned to this area (23<sup>rd</sup> June) and the fens were bright with the Grass Pink *Calopogon tuberosus*. They were not easy to spot when not in flower but with the warmer weather they came out in profusion. Locals told us the display was not very good this year but we were impressed! They are a spectacular orchid with very slender foliage – very grass like - standing around 30 cm high with 2 or 3 flowers around 3 cm across and more buds to come. Mostly they were a bright cerise pink with the occasional pale flower too. In amongst the *Calopogons* were the much smaller *Pogonia ophioglossoides*. These were pale pink in colour and quite small plants – at most 10-12 cm tall. Typically they had one or two flowers per stem, the flowers reminding me of miniature pleiones. These two orchids together with the *Sarracenias* made a beautiful carpet on the lake shore. Often they were at their best where the ground had been “cleared” at the front of a holiday cottage and were able to grow without competition from grasses. Other shore plants included the pretty mauve *Lobelia kalmii* (Brook Lobelia) and the bright *Castilleja coccinea* (Indian Paintbrush).

Turning inland again the bright orange wood lilies (*Lilium philadelphicum*) were common at the road verge and woodland edge. So far we had only found the odd rather tattered specimen of *Cypripedium pubescens*. So we travelled further northwards. North of Petrel Point and Red Bay the limestone is raised as a pavement. This is exposed at the lake shore in this area. Here we found *C. pubescens* in profusion. Similarly inland, in areas where the limestone was exposed were large clumps and drifts of Cyps. We had never seen anything like it! And



*Calopogon tuberosus*

was a definite perfume. I think, at the risk of being controversial, that there is graduation between the two species and that certainly in our experience in this area they were not distinct species.

Our next base was Tobermory at the very tip of the Bruce Peninsula. Tobermory is a small town with a ferry to Manitoulin Island and also to the much smaller Flowerpot Island. The latter had been strongly recommended as a good place to see most of the orchid species of the region in a small area. We hung around several days hoping to go out but the combination of rough wet weather and a recent ferry tragedy meant the locals were not willing to set us down on the island. Despite this set back we did explore some nice sites and saw many – if not quite all - of the orchids in other areas.

Dorcas Bay nature reserve on the western side of the Peninsula was well worth a visit. *Cypripedium pubescens* were common. They were out perfectly but rain and dull weather made photography difficult. The *C. reginae* were more common just outside the reserve along the busy Highway 6. They were only just coming out but were noticeably rain damaged within a few days. This reserve includes sandy areas with woodland and clearings. A large but very localised colony of *C. arietinum* (Ram's

needless to say were very excited. Gradual progress northward revealed more and more and fresher clumps. Often they were growing in the more coniferous areas, again at the edge of the trees in preference. The ground was much drier than that favoured by the *C. reginae* but one got the feeling that the water table was still not far below. Many more were seen as we travelled north towards the tip of the Bruce Peninsula.

There was a wide variation in shape and yellowness of the lip and also in depth of colour and twistiness of the petals. There was also a variation in size of flower. However we only once came across one which we convinced ourselves was *C. parviflorum*. Its flowers were altogether neater and more compact and there



*Cypripedium reginae*

Head) was growing in one very sandy area. One flower remained photographable on the 19<sup>th</sup>, but most had been finished off by the rain. This small flower was nevertheless beautifully marked and was very similar in size and appearance to the Asian species *C. plectrochilum*. Other woodland plants were common on the reserve, including the beautiful *Anemone canadensis* (very garden-worthy) and the orange and yellow *Aquilegia canadensis*. *Trientalis borealis* (Starflower) was a pretty woodland floor plant along with *Cornus canadensis* with its creamy flowers. Other orchids included the green flowered *Piperia unilascensis* which was common at the woodland edge all over this region, and *Platanthera huronensis*, a chunky white orchid growing in the wet near the lake shore.



*Cypripedium arietinum*

The northeastern side of the Peninsula has a cliff-like coastline where the limestone pavement meets the water of Georgian Bay. Inland is Bruce Peninsula National Park, which includes Cyprus Lake Provincial Park. This is a large area of recreational trails, campground and lake. Camping pitches are made in the coniferous woodland and we camped several nights here enjoying the remoteness as well as the proximity of bird and animal life. Each pitch we used had at least one orchid beside it! Indeed it is not everyone who can open their door to look out at the *Cypripedium pubescens* in the morning! Equally stunning were the *Corallorhiza* – both *striata* and *maculata*. *C. striata* was very spectacular with evening sun through its striped translucent petals.

The ubiquitous *Epipactis helleborine* was very common wherever we went and certainly could tolerate a multitude of conditions. During our visit it was tightly in bud.

We meandered our way back down the Peninsula to revisit earlier sites. In general the *Cypripedium reginae* were past perfection – their pink pouches quickly fade after a few days sunshine and rain damage was also a problem. So we had caught them at their best on the way up. The revisit was well worthwhile however with new orchids coming out on the fens and shoreline. Our travels then took us south away from the Bruce. We were pleased to still find the odd patch of *C. reginae* by the road sides as we journeyed south to Lake Erie and along to Niagara.

Niagara was of course a huge contrast with the peace and remoteness of the Bruce. But it was spectacular and we were glad we made the journey to visit it. Despite the tourism we did not feel exploited and the sheer impressiveness of the falls was really an experience of a lifetime. Our holiday ended as it had begun – in Toronto.

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Reluctantly we gave back our van and spent 2 nights in a Toronto hotel, flying back on the 30th June. All that remained then was to get the films processed.....  
“How many did you take of that *reginae* ??”

### **Acknowledgements**

We are indebted to Darcy Gunnlaugson for recommending the area in the first place. Also very grateful to Mike Parsons and Graham Goodfellow for generous advice on sites and orchid lists.

Book-wise we used “Wild Orchids of the Northeastern United States, A Field Guide”, by Paul Martin Brown. ISBN 0-8014-8341-7. This is a very useable book with reasonable illustrations and although not directly relevant in terms of areas was nevertheless adequate.

Also there is “The Orchids of Bruce and Grey Counties, Ontario” published by Owen Sound Field Naturalists and available from several shops in Tobermory including the Tourist Office. ISBN 0-9680279-1-1.

As a general flower guide we used “Forest Plants of Central Ontario” by Chambers, Legay and Bentley. ISBN 1-55105-061-7.

Our campervan was rented through Canadream. [www.canadream.com](http://www.canadream.com)

All drawings by Carol Dash.

## **Photographic Competition 2000 - Results** **Tony Hughes - Show Secretary**

As usual, this year’s photographic competition generated a superb display at the Autumn Meeting at Wellesbourne and attracted a great deal of interest. Although there were significantly fewer entries and competitors than last year, the standard of photography was excellent and the range and quality of the flowers depicted were both extremely high. It is good to note that several of our “first time” competitors feature on the list of winners below. The globe-trotting of various members was most apparent, with several North American plants, a superb *Steveniella* from Turkey, and all sorts of goodies from various parts of Europe.

Our thanks go to Richard Laurence for agreeing not to compete but to exercise his considerable photographic skills as our judge, and then talking us through a show of all the transparencies entered.

For those of you who missed the show, all the pictures that won First Prizes are now displayed on our Internet website, and several of them are included in the central colour pages of this Newsletter.

Class 1: Landscape, 6x4in Prints (4 entries)

- |     |              |  |
|-----|--------------|--|
| 1st | Graham Giles | Hillside with <i>Barlia robertiana</i> |
| 2nd | Carol Dash   | <i>Pogonia ophioglossoides</i>         |

Class 2: Single Plant, 6x4in Prints (8 entries)

- |     |               |                                |
|-----|---------------|--------------------------------|
| 1st | Peter Sheasby | <i>Dactylorhiza purpurella</i> |
| 2nd | Tony Hughes   | <i>Orchis olbiensis</i>        |
| 3rd | Carol Dash    | <i>Cypripedium pubescens</i>   |

Class 3: Close-up, 6x4in Prints (12 entries)

- |     |               |                                 |
|-----|---------------|---------------------------------|
| 1st | Tony Hughes   | <i>Ophrys balearica</i>         |
| 2nd | Peter Sheasby | <i>Orchis papilionacea</i>      |
| 3rd | Bill Temple   | <i>Cypripedium fasciculatum</i> |

Class 4: Landscape, 10x7in Prints (3 entries)

- |     |                |   |
|-----|----------------|---|
| 1st | Allan Wild     | <i>Dactylorhiza fuchsii</i>                             |
| 2nd | Gwynne Johnson | <i>Epipactis palustris</i> & <i>Gymnadenia conopsea</i> |

Class 5: Single Plant, 10x7in Prints (6 entries)

- |     |               |                              |
|-----|---------------|------------------------------|
| 1st | Tony Hughes   | <i>Epipactis purpurata</i>   |
| 2nd | Peter Sheasby | <i>Gymnadenia conopsea</i>   |
| 3rd | Graham Giles  | <i>Cypripedium calceolus</i> |

Class 6: Close-up, 10x7in Prints (18 entries)

- |     |               |                               |
|-----|---------------|-------------------------------|
| 1st | Allan Wild    | <i>Dactylorhiza fuchsii</i>   |
| 2nd | Tony Hughes   | <i>Ophrys tenthredinifera</i> |
| 3rd | Peter Sheasby | <i>Orchis militaris</i>       |

Class 7: Landscape, 35mm Slides (9 entries)

- |     |                |  |
|-----|----------------|--|
| 1st | Kath Fairhurst | <i>Gymnadenia conopsea</i>             |
| 2nd | Colin Clay     | <i>Orchis italica</i>                  |
| 3rd | Graham Giles   | <i>Epipactis purpurata</i> (pink form) |

Class 8: Single Plant, 35mm Slides (11 entries)

- |     |               |                               |
|-----|---------------|-------------------------------|
| 1st | Graham Giles  | <i>Calypso bulbosa</i>        |
| 2nd | Nigel Johnson | <i>Epipogium aphyllum</i>     |
| 3rd | Harry Gill    | <i>Dactylorhiza sambucina</i> |

Class 9: Close-up, 35mm Slides (12 entries)

- |     |                   |                               |
|-----|-------------------|-------------------------------|
| 1st | Nigel Johnson     | <i>Epipogium aphyllum</i>     |
| 2nd | Gianpiero Ferrari | <i>Stenienella satyroides</i> |
| 3rd | Tony Hughes       | <i>Ophrys speculum</i>        |

## **Does DNA Reveal All About The Evolution Of Terrestrial Orchids?** **Report of a talk by Richard Bateman**

Prof. Richard Bateman gave an excellent talk, which was split into two parts separated by a short interval. The first part was on “Evolutionary relationships” and the second on “Speciation and conservation genetics”. This talk covered so much ground that it will be necessary to split the summary into more than one part in order to do the talk justice, and prevent my summary from taking over the whole Newsletter. Richard’s magnificent slides, which were enjoyed by those at the meeting, will inevitably be missing from this summary.

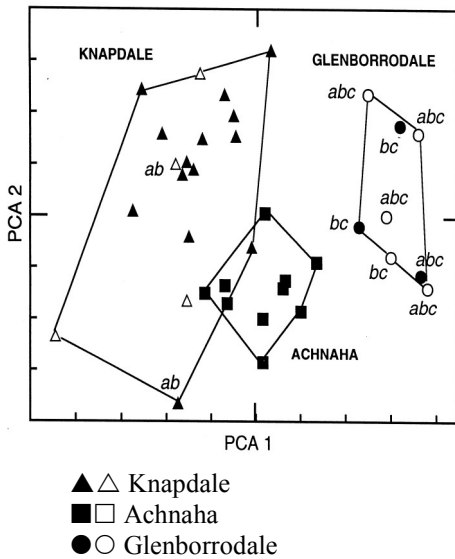
The first part of the talk was on DNA sequencing in Tribe Orchideae and was an update of the excellent talk which Richard gave us three and a half years ago. A brief summary of the original talk appeared in HOS Newsletter 5, July 1997. As a result of the interest generated by the talk and the brief summary, Tony Hughes wrote a more detailed summary, which appeared in HOS Newsletter 10 in October 1998. (*Copies of both issues are still available from me - Ed.*)

The second part of the talk dealt with classical methods and some conservation issues that have arisen in the UK, principally as a result of such methods being used for species identification. I have decided (with Richard’s consent) to cover the second talk first. This will allow members new to DNA sequencing to consult the old HOS Newsletter reports, or refer to the actual publications (the latter may be more challenging to non-biologists).

### **Speciation and conservation genetics**

Most orchid books when describing a particular orchid species begin by giving physical measurements of the plants, usually such measurements as leaf size, height of flower spike, number of flowers per spike, position of sepals and appearance and size of the labellum. Richard said that he has spent 20 years measuring 52 parameters in UK *Dactylorhiza* populations in order to discover whether physical measurements can be relied upon in order to identify species. He then used a slide showing the physical characteristics of *Dactylorhiza praetermissa* (the Southern Marsh Orchid) as listed in a book, and then in the next illustration, showed that none of these covered more than 80% of the plants that he had studied and measured. He also showed a bar chart of the number of species of *Dactylorhiza* believed to exist by different well-publicised authors, the range being from 5 to over 70 species. Although we already knew of the problems of identifying *Dactylorhiza* species, this huge range from ‘authorities’ on orchids illustrates the full magnitude of the problem of *Dactylorhiza* identification. There then followed a series of multivariate ordinations (loosely called scatter diagrams) where a number of physically measured parameters are plotted for different species and you look to see if there is an overlap. Most of these showed significant or

considerable overlap and illustrated the difficulty in trying to decide what species you are looking at by reference to the measurements



The solid symbols indicate plants with leaf markings, and the outline symbols indicate plants without leaf markings.

This illustration was kindly provided by Prof. Richard Bateman and is from his forthcoming article in the Journal of European Orchids.

Figure 1. Simultaneous principle components ordination of morphometrics and allozymic data for three populations of *Dactylorhiza traunsteineri* s.l. in western Scotland, with superimposed allozymes genotypes for the *6-pgd* locus. Four *6-pgd* genotypes (*ab*, *ac*, *bc* & *abc*) are present; individuals are genotype *ac*

unless otherwise shown.

The above illustration is the result of the comparison of populations from one site believed to contain only *D. lapponica* (Achnaha) and two sites believed to contain both *D. lapponica* and *D. traunsteineri*. A total of 20 plants from each population was measured morphometrically, using the characters and methods of Bateman & Denholm (1985), and single leaves were also removed from each plant for allozymes analysis. Only the *6-pgd* allozymes system provided useful variation. This was then incorporated with the 52 morphometric characters in a simultaneous ordination analysis to produce the above illustration.

Richard showed a diagram of the cell structure of orchids and indicated the three different possible sources of DNA that can be used for sequencing – nuclear, mitochondrial and plastid. The nuclear DNA is inherited 50:50 from each parent, the mitochondrial DNA from the mother only and the plastid DNA from the mother only. Plastids come in a variety of forms (e.g. chloroplasts, which carry out photosynthesis and chromoplasts, which produce colours in the flowers). These additional sources of DNA can be used to gain extra information. They have even pinpointed which species was father and which was mother, and from which part of the distribution range the mother of the plant originated.

The most taxonomically troublesome *Dactylorhizas* are the many rather similar allotetraploids, which appear to reflect multiple origins by hybridisation between the diploids *D. fuchsii* and the *D. incarnata* aggregate. Recent studies of allozymes (the protein products of DNA) indicate up to four independent origins of allotetraploids: the *D. purpurella* group, *D. traunsteineri* group, *D. praetermissa* – *D. majalis ssp.* group, and *D. elata*. Moreover the *D. purpurella* group may have originated in northern Britain, presumably following the retreat of the last glaciers about 10,000 years ago. In contrast *D. maculata*, and (surprisingly) *D. occidentalis*, appear to be autotetraploids resulting from chromosome doubling without hybridisation. Unpublished DNA fingerprinting research by M. Hedrén and colleagues suggests an even more complex pattern of evolution, with some allotetraploids groups originating multiple times.

Similarly allozymes were used to establish that *Dactylorhiza cruenta*, which has only two sites in the UK, is of Scandinavian origin. The interesting thing about *D. cruenta* is that at present it is not being specially conserved, in spite of its rarity in the UK. On the other hand *D. ebudensis* (syn. *D. majalis ssp. scotica*) and *D. lapponica* are being actively conserved, the latter as Schedule 8 “species” even though they are actually varieties of *D. traunsteineri*. These two varieties were afforded status as species on the basis of their appearance. They appear different if one focuses on morphologically extreme individuals and fails to take account of environmental differences between sites. In contrast *D. occidentalis* and *D. maculata*, which look very similar, and are both autotetraploids, are distinct species.

Although there will always be a need for careful observation in the field, the work by Richard Bateman and colleagues has shown that claiming/assigning new species as a result of limited morphological observation alone is now difficult to justify. Reluctantly, I must admit that I personally don't think that this will prevent dubious claims for new species being made in the immediate future, but hopefully it will in the longer term as results of DNA sequencing become more widely known and recognised.

#### References.

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RM Bateman. & I Denholm (1985): The diploid marsh-orchids. – *Watsonia*, **15**: 321-355.

Richard Bateman's talk was reported by Bill Temple. Bill is happy to answer queries on this summary at [bill@wtemple.f9.co.uk](mailto:bill@wtemple.f9.co.uk).

## **Sterilisation Of Orchid Seed**

**Ted Weeks**

I have recently had requests, from new members requiring seed, about information regarding the sterilisation, sowing and germination of orchid seed, and what equipment to use. Over the past six years I have had success using the following method and, hopefully, this may be of some use to new members wishing to “have a go” themselves.

For sterilising I use a specialised seed disinfection and filtration phial with a filter inside and a rubber bung at each end. The sterilising solution is made up of ordinary household bleach, the most inexpensive “own” brand from Asda or any other supermarket. This is diluted 1ml bleach to 9ml distilled water, for fresh seed, and 1.5ml bleach to 8.5ml distilled water for older, darker seed. This quantity is sufficient for one phial. Fresh seed is sterilized for approximately 20 minutes and older seed can take up to 1.5 hours to reach the required stage for sowing.

The phial is first thoroughly washed out and a rubber bung placed in the filter end. The seeds are gently tapped from a small, folded square of paper into the phial, making sure none stick to the inside edge. Trapped seed, between the top bung and inside edge of the phial remain unsterilised and can lead to contaminated flasks. The phial is filled to within 15mm of the top, with the bleach solution, and one drop of washing up liquid (original) is added, making sure again none touches the inside edge of the glass. As an extra precaution I dip the other bung in any remaining bleach solution before I put it into the open end of the phial.

I then agitate or shake the phial, at intervals, for 20 minutes or longer, until the seeds reach the stage at which I like to sow them. I like the embryo to be opaque and creamy, resembling a tiny pearl. Some older seed can take a while to reach this stage, but, if the embryos are fertile, they will reach it. I check at intervals during sterilisation, under the microscope, but these stages can be checked simply using a watchmaker's eyeglass.

Upon reaching the required stage, the seed has to be washed free of the sterilising

solution, using sterilised, distilled water. This is done in a glass tank, which has been adapted to create a sterile environment in which to work. Wearing disposable gloves, the bottom bung is removed over a small glass container; the top bung is then taken out, allowing the bleach solution to drain into the container, leaving the seed in the phial. The bottom bung is then replaced and the phial is filled again, to within 15mm of the top, with sterile water. The top bung is inserted and the phial is gently shaken, for just a few seconds. This operation is repeated three times, the seed is then ready to sow. I usually sterilise enough seed to sow about three flasks to make sure, in case of contamination, I still have sterile seed sowings.

This sterilisation method is considered by some to be slow, but I prefer this method to the packet method, although with the latter a few packets can be sterilised at one time. I like to check the progress of the seed and using the packet method the seed is not visible. This method has worked well and I have had success with *Orchis*, *Ophrys*, *Dactylorhiza* and a few other species.

The phial can be obtained from Ratcliffe Orchid Supplies, e-mail [ratcliffe@zoo.co.uk](mailto:ratcliffe@zoo.co.uk).

## **Early Spring in the Canadian Rockies**

### **Mike and Veronica Baker**

It is always a delight to find orchids, especially in situations where one is not expecting to do so. Starting at the end of June 1999, we spent three weeks in the Canadian Rockies with the objective of finding alpiners. Life being what it is, spring in 1999 was somewhat late, so our three weeks was in fact spent in early spring conditions. One beneficial effect of this was that we spent time at the start of the holiday at lower altitudes, and saw a range of orchid species, some in large numbers.

After the flight to Calgary, we spent the first three days in Kananaskis Country, a splendid area located between Banff and Calgary. The area consists mainly of Alberta Provincial Parks, and being less well known than the National Parks of the province, is comparatively and delightfully less crowded. The whole of Kananaskis Country is well served by a splendid through-road over Highwood Pass, with a good supporting road system. The tree line is about 7500 ft, so with Highwood Pass at 7,000 ft alpiners should have been reasonably accessible. However, in our first trip to a Visitors' Centre we talked to a ranger who was a botanist, and established that alpiners were out of the question because of snow: however she did draw our attention to a couple of locations for *Cypripedium pubescens* and *C. passerinum*. We had noted these species in the flower books, but in our ignorance had not really considered finding them – after all the UK flower books show *C.*

*calceolus* and *Epipogium aphyllum* without offering the reader much hope of seeing either. Our walk to the first location, in the North Barrier Lake area, produced a few *Cypripedium pubescens* and a single *C. passerinum*, along with several *Coeloglossum viride*. The second location which we visited that afternoon in Bow Valley Provincial Park produced both *Cypripedia* literally in hundreds, along with a few *Amerorchis rotundifolia*; the sight of so many *C. pubescens* in such a small area, and in good condition, was breathtaking, and we could hardly believe our luck. In addition there was a large number of other wildflower species, including *Lilium philadelphicum*. What a marvellous start to a holiday!

Our second day in Kananaskis Country we spent in Peter Lougheed Provincial Park, rather higher and nearer the snowline, where we found rafts of *Erythronium grandiflorum* in the snowmelt areas, and also *Calypso bulbosa*. The latter were in groups of up to 30 flowers in their traditional conifer woodland setting: magnificent. Our third day added *Platanthera obtusata* to the tally.

After this splendid and unexpected start, we reverted to our original plan and attended the North American Rock Garden Society annual conference in Banff. The three day meeting consisted of a number of excellent talks, with two full days of field trips. The first field trip to which we were allocated was Grassi lakes Trail, back in Kananaskis Country: in steady and continuous rain, we saw numerous flowers of the same *Cypripedia* and *Amerorchis rotundifolia* again, together with *Platanthera dilatata*, and a large number of alpinines including *Pinguicula vulgaris*, *Androsace chamejasme* and *Linnaea borealis*. Because of the amount of snow, the organisers had to re-arrange the second day's field trips, and again we saw orchids in the rain, with only one new species, *Listera caurina*.

After Banff, we stayed for a couple of days in Radium Hot Springs to walk in Kootenay National Park. Here a day's walking with one of the local NARGS members – again in the rain – produced *Corallorhiza striata* and a single specimen each of *Cypripedium montanum* and *Spiranthes romanzoffiana*, along with a few *C. pubescens* and *C. passerinum*, and, noteworthy among the non-orchids, *Moneses uniflora*.

Our next three days were spent in Golden (BC), with the objective of walking in Yoho National Park: however the continual heavy rain and low cloud, coupled with Wimbledon on the television, led to enforced hibernation relieved by occasional dashes to the off-licence. However, our subsequent journey from Golden to Jasper along the Icefields Parkway was a sheer delight as there had been a fall of fresh snow and all was white and sparkling; stops produced wildflowers, but no orchids. In Jasper the weather was much kinder, and we made relatively snow-free trips to Maligne Lake and to Whispers, the latter at 7600 ft providing some good alpinines. However, Cavell Meadows, which should have produced good

alpines, was still under snow: a long plod through the snow was scenically rewarding, but botanically not so. We saw few orchids in this phase of the holiday – the occasional *Cypripedium pubescens* and *Calypso bulbosa*, but no exciting displays. A number of black bears forage in the outskirts of Jasper, and we were lucky enough to see one of these from a safe distance: a gentle lope is surprisingly quick, and their height when they stand on back legs impressive.

Our final day was spent back in Kananaskis Country above Highwood Pass, where the snow had finally melted, the sun was shining and the alpines were just beginning to show themselves: a highlight was *Saxifraga oppositifolia*. To cap all this, on the way back from Highwood Pass, we spent some fifteen minutes watching a pair of young grizzlies grazing by the side of the road – from the safety of the car!

And so to Calgary and the journey home.

### **Crustacare Nursery** **A visit to the Belgian Orchid Nursery**

With well-known orchid companies like Sander and Linden, with world-renowned hybridisers like Lambeau, Peeters and Vuylsteke, Belgium surely has played an important role in Orchid history. This international role was entirely lost after World War II. Currently only a handful of orchid retailers remain, most of whom all resell the same mericloned *Phalaenopsis*, *Cymbidiums*, *Dendrobiums* and *Cambria* that are produced by the thousands in Holland. Only very few, who really deserve to be called orchid growers, offer non pot plant orchids and do their own sowing and hybridising.

One of these white ravens is the company Crustacare. In fact, it is, as far as we know, the only Belgian firm that specialises in hardy orchids. The owner, Jan Moors began to study local orchids at a young age and once bitten by the orchid virus, he never got rid of it. The inheritance of the entire outstanding hardy orchid collection of his good friend the late Marcel Clapdorp, one of the pioneers in Belgium in the field of hardy orchids, made him decide to start, together with friends, to propagate hardy orchids commercially. Having a degree in microbiology, he is very aware of the role of micro organic elements and their relation to different soil types and soil additions.

Professionally, Jan has a lobster import business. With his skills and experience in this field, he developed large-scale lobster holding systems that are being installed all over Europe.

### **Into the garden**

When Jan acquired his house, the back of the garden was a flat apple orchard. Realising that the top part of the garden was too fertile to grow orchids in, he had the upper 30cm of soil mechanically removed and put up as small mounds on the borders of his garden. It is unnecessary to explain that his family and friends all declared him mad, destroying such a beautiful flat garden only to end up with a moon landscape.

However, Jan covered the bare soil with 1 cm of peat in which he mixed orchid seeds. The result now is astonishing. The seeds germinated, flowered and set seeds that in their turn germinated and flowered. In the lowest part of the garden, some underground wells provide enough water for a 20 square metre pond. The garden is covered with hundreds of *Dactylorhiza praetermissa*, *D. majalis*, *D. maculata* and *D. fuchsii* and their hybrids, especially the area where the excess water of the pond flows to a small river on the boundary. Also commonly present in these wetter soils are *Orchis morio* and *Epipactis palustris*. On the dryer slopes grow *Orchis militaris*, *O. purpurea*, *Anacamptis pyramidalis*, *Gymnadenia conopsea*, *Platanthera chlorantha* and *Epipactis atrorubens*. In a few adapted corners one can find *Cypripedium calceolus*, *C. reginae* and *Pogonia ophioglossoides*. As well as these orchids other beauties like *Narcissus*, *Sarracenia* and rarities in the Belgian flora like *Parnassia*, *Fritillaria meleagris* and *Leucojum aestivum* are growing abundantly. To top it all, the pond houses, besides the water and the common frog, three different and very rare species of salamander.

In the part of the garden nearest to the house, Jan Moors grows several fruit trees and bushes. More than 20 old varieties of pears, 20 different plum trees, several peaches, apricots and raisins together with the best varieties of blackcurrants and raspberries which abundantly produce delicious fruit every year. It is also in this part that both orchid greenhouses are located. Under several large pine trees, is a more shady space accommodating *Dactylorhiza fuchsii*, *D. sambucina*, *Orchis mascula*, *O. simia*, *Aceras anthropophorum* and other woodland orchids. Nursery beds to hold seedlings and small plants of *Epipactis (gigantea, palustris, Renate and Sabine)*, *Dactylorhiza (maculata, alpestris)*, *Orchis morio* and *Cypripedium (calceolus and reginae)* can be found at different places in the garden, depending which growing conditions (humidity, light level, temperature) are optimal for the plants they house.

### **The greenhouses**

The oldest greenhouse is a 6 by 8-metre aluminium framed glass house built against the western wall of a little shed. It houses mainly a huge collection of *Disa* whose more than 500 adult plants must make a fabulous display in late spring. Most of the plants are seedlings produced in co-operation with the company orchidaceae.org. It contains also the mother stock, the award quality plants

imported from South Africa as well as selected plants from his own breeding. The clever automated watering system ensures phenomenal growing results. Also in this greenhouse is a wonderful private collection of near-hardy Chinese *Cymbidium* species. *Cymbidium kanran*, *C. goeringii*, *C. ensifolium* and *C. sinense* can be found in different flowering forms and leaf designs.

In the spring, after spending the winter in a 12 m<sup>3</sup> refrigerator, the *Cypripedium* mother plants, (of which *Cypripedium kentuckiense* ‘Judith’ PC/RHS with its 80 cm height and two flowers per stem is rightly Jan’s pride) are placed under the benches that run along the entire south side. (See centre colour pages for photo. Ed.) No worry for overheating here however, as the large pine tree deprives this whole side from direct sunlight. After flowering, most of the *Cypripedium* pots are relocated in the shadiest places of the garden, next to the wettest part in order to keep the plants as cool as possible in summer. The benches themselves are used to the last square centimetre to hold the styrofoam boxes with young *Cypripedium* seedlings, who, like their mother plants, spend the winter in the refrigerator.

Finally there is one bench with European orchids. It contains some of Europe’s rarest species like *Orchis coriophora*, *O. spitzelii*, *O. patens* and *Ophrys* species, all clearly labelled according to the classification of Delforge. (See centre colour photos for *Orchis patens*. Ed.) Thus you can find, besides the ‘normal’ *Ophrys lutea* the lesser-known varieties *phryganae* and *melena*. As you probably would have guessed, the space under this bench is not wasted either: it is stuffed with some 15 different near hardy *Calanthe* species. In the winter, an electric heater keeps the temperature just above freezing. Once the outside temperature rises above 10 degrees, all windows and doors are opened to have the plants enjoy the smallest breeze and to keep the temperature as low as possible.

The second greenhouse is a 4 by 8 tunnel construction. Unheated, it houses besides a small *Bletilla* and *Pleione* collection the seedlings and sellable stock of European orchids.

### **An ongoing story**

Crustacare co-operates intensively with the company orchidaceae.org. In fact they do all Jan’s sowing work. They also sell the flasked seedlings that are sold by Crustacare. However, besides *Disa*, orchidaceae.org focuses itself on warmer growing orchids, thus leaving the commercialisation of ex-vitro seedlings and mature plants of (near) hardy orchids to Jan Moors’ company.

Crustacare concentrates its sowing on three groups of plants. First there are the *Cypripediums*. More than 20 species and several colour and/or regional varieties can be found in the private collection. Almost all of them are currently being propagated. Beside species, more than 40 hybrids (of which more than 10 with *C.*

*reginae* as one of the parents) have been sown this year alone. These hybrids, that will be available in a few years, include crosses with *chenii*, *flavum*, *farreri*, *kentuckiense*, *macranthos*, *margaritaceum* and *lichiangense*.

The second group Crustacare focuses on is the European orchids. The year 2000 was a real *Dactylorhiza* year with more than 10 species (*alpestris*, *cruenta*, *foliosa*, *fuchsii*, *incarnata*, *maculata*, *majalis*, *praetermissa*, *purpurella*, *sambucina*, *traunsteineri*) and several hybrids sown. But also *Orchis* (*coriophora*, *fragrans*, *italica*, *militaris*, *morio*, *papilionacea*, *patens*, *provincialis*, *purpurea* and *spitzelii*) and *Ophrys* (*apifera*, *bombyliflora*, *garganica*, *holoserica*, *incubaceae*, *insectifera*, *lutea*, *mammosa*, *regis-ferdinandii*, *saratoi*, *sphogodes*, *tenthredinifera*) are expected in the years to come. And then we have not yet mentioned *Barlia robertiana*, *Anacamptis pyramidalis* and *Spiranthes spiralis*.

The last, but definitely not the smallest of Jan's interests are the *Disa*. They are not only numerically the largest genus in the heated greenhouse, this year alone more than 100 hybrids were made in cooperation with orchidaceae.org, most of them being unregistered as yet.

### **How to reach Crustacare**

Due to the irregular hours Jan Moors has to spend for his lobster importing business, visitors can only be received by appointment.

Jan can be contacted at the following address: Crustacare, Eshagenstraat 25, B 1840 Londerzeel, Belgium, by phone (+32) 52 303906 or by fax (+32) 52 311051. He can also be contacted by e-mail at [keepalive@crustacare.be](mailto:keepalive@crustacare.be). His website URL is [www.crustacare.be](http://www.crustacare.be).

### **Note from Ed.**

For the second of our occasional articles on nurseries specialising in hardy orchids, Peter Peeters of Crustacare has provided this fascinating account. Although he was very willing, we couldn't ask Trevor Marks, our usual roving reporter to travel all the way to Belgium. Members who attended the May 2000 meeting at Pershore will recall that Crustacare had plants on display as well as for sale.

(Peter has explained that "white raven" is a common Belgian expression meaning exceptional).

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## Researchers Ask (One)

**Dr. Richard Gulliver**

Dr Richard Gulliver is researching Irish Ladies Tresses (*Spiranthes romanzoffiana*) and would like to make contact with anybody who grows them. He is observing conditions of growth, tolerance to grazing, leaf number and other characteristics in the wild population. He is interested in growers' observations of their plants in cultivation, such as identification of pollinators and to make comparisons. Their origin is also of interest, if it is known, particularly from Europe. Has anyone attempted to pollinate them?

Richard can be contacted on 01496 302432, rlg2@tutor.open.ac.uk Carraig Mhor, Imeravale, Port Ellen, Isle of Isla, Argyll PA42 7AL.

He is an independent botanist and keeps records of the Island flora for a number of the Scottish Islands. The work on Irish Ladies Tresses is a project for the Scottish Natural Heritage (equivalent to English Heritage).

## Researchers Ask (Two)

**Prof. Richard Bateman**

Richard Bateman would especially welcome specimens of the following Orchid taxa for sequencing purposes. Depending upon flower size, half a dozen or so individual flowers of each would be required. He can supply bags of silica gel in order to transport the samples; in order to avoid DNA transfer, it would be best if samples could be taken without being handled e.g. by cutting with clean scissors straight into the bag of silica gel.

### Orchidinea

*Amitostigma* (Asian); *Aceratorchis tschiliensis* (Asian); *Aorchis* (Asian); *Barlia metlecicsiana*; *Chondradenia* (Asian); *Dactylorhiza osmanica*; *Dactylorhiza cilicica*; *Himantoglossum affine*; *Himantoglossum formosum*; *Nigritella* (any uncommon species); *Ophrys arachnitiformis*; *Ophrys argolica*; *Platanthera oligantha*; *Platanthera micrantha/azorica*; *Ponerorchis* (Asian); *Chusua* (Asian); *Gymnadenia/Pseudorchis frivaldii*; *Piperia maritima/elegans* (W USA); *Symphiosephalum* (Asian).

### Habenariinae

*Brachycorythis* (mostly African); *Habenaria* (mostly African); *Herminium* (any uncommon species) (mostly Asian); *Neobolusia* (African); *Pecteilis* (African); *Peristylus* (Asian); *Schizochilus* (African).



Neottideae

*Cephalanthera* (any uncommon species); *Epipactis* (any uncommon species);  
*Limodorum trautmanianum*.

Richard can be contacted as follows – Prof. R M Bateman, Department of Botany,  
Natural History Museum, Cromwell Road, London SW7 5BD.

### **Seed and Fungus Bank Officer Asks Ted Weeks**

It is the aim of the Seed & Fungus Bank to allow interested members of the society to access seed and fungus, which will enable them to sow seed, using various growing mediums and methods. For myself, it is an interesting and satisfying pastime. Of course for the Seed & Fungus Bank to remain viable, members with flowering plants who set seed, need to send any spare seed they may have to the Seed Bank. To members who donated seed this year and who I haven't thanked personally, can I say a big thank you. An updated seed list is now available, please send s.a.e to Ted Weeks, 74 Over Lane, Almondsbury, Bristol, BS32 4BT.

### **Publicity Officer Asks Simon Tarrant**

At a recent meeting the HOS Committee discussed the need for a publicity strategy for the Society. Three key objectives were recognised :

1. To increase membership.
2. To improve the status of the Society among the scientific, research, conservation and horticultural communities.
3. To promote awareness of hardy orchids.

Methods of achieving these aims were explored and are being acted upon. A new membership form has been produced, and was distributed to participants at the British Orchid Council Congress at Leeds in September, and we are looking to produce posters for distribution to other societies and shows.

If you are able to help with the design or production of posters the Publicity Officer would love to hear from you. If you want a supply of membership forms to distribute wherever, please let me know. Address inside front cover, or e-mail me at [s.tarrant@virgin.net](mailto:s.tarrant@virgin.net).

## And Finally.... Orchid thieves caught in the act!

Seriously though, members of the HOS participated in a White Helleborine (*Cephalanthera damasonium*) rescue which was organised by Bill Temple in co-operation with the Berks Bucks and Oxon Wildlife Trust.



## And a view from Ted Unsworth....



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Copies are £2.50 each or £8 for four, including p&p.

BUT copies of issues 8 to 14 inclusive are still available to members at the sale price of £1 each.

Contact the Newsletter Editor by post or by e-mail at [m.tarrant@virgin.net](mailto:m.tarrant@virgin.net)



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