Hand pollination to increase seed-set of red helleborine *Cephalanthera rubra* in the Chiltern Hills, Buckinghamshire, England

Newman R.D., Showler A.J.[1], Harvey M.C.[2] & Showler D.A.[3]
[1]*12 Wedgwood Drive, Hughenden Valley, High Wycombe, Buckinghamshire HP14 4PA, UK;* [2]*Buckinghamshire and Milton Keynes Environmental Records Centre, Museum Resource Centre, Tring Road, Halton, Aylesbury, HP22 5PN, UK* (Email mcharvey@buckscc.gov.uk); [3]*CEEC, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK* (Email d.showler@uea.ac.uk)

SUMMARY

In 2007 and in previous years, as part of ongoing attempts to improve red helleborine *Cephalanthera rubra* seed-set, hand pollination of florets has been undertaken at a small colony of this species in Buckinghamshire, southern England. Natural pollination rarely occurs (one mature pod recorded in 10 years) at this site. In 2007, hand pollination resulted in the production of four seed pods, of which one withered and died. Upon ripening, the three remaining pods were removed for attempted micropropagation of the seeds. Ongoing conservation management has probably benefited the solitary bee *Chelostoma campanularum* which now appears fairly plentiful at the site, but despite the presence of this red helleborine flower visitor, natural pollination remains virtually unrecorded at this locality; field observations suggest that *C.campanularum* is in fact probably not large enough to act as an effective red helleborine pollinator as it can slip in and out of the flowers without removing the pollinia, unlike it larger relative *C.fuliginosum*, absent from the UK but which is a known pollinator of red helleborine in continental Europe.

BACKGROUND

In the British Isles, the red helleborine *Cephalanthera rubra* is now known only from single localities in the counties of Buckinghamshire, Gloucestershire and Hampshire, southern England. In the UK this orchid is classified as Critically Endangered and is a fully protected species (Cheffings & Farrell 2005). It occurs sporadically throughout much of Europe, where it is considered vulnerable. In England it typically inhabits deciduous woodlands dominated by beech *Fagus sylvatica*, on free-draining slopes with calcareous soils and a short, patchy ground flora (Wigginton 1999). Declines in its UK range have been attributed to habitat loss and lack of woodland management, neglect leading to problems of excessive shading, and poor seed-set due presumably, to a lack of suitable pollination insects, but also perhaps due to the purported intrinsic partial fertility of the species.

On continental Europe flower visitors are mostly bees, especially males of the solitary bees *Chelostoma fuliginosum* and *C.campanularum* (Megachilidae), and pollination is reported as being regularly effected by males of *C.fuliginosum* (Nilsson 1983); this species however, does not occur in Britain. *C.campanularum* does on the other hand occur, but *C.campanularum* males are smaller in size and have been observed to slip out of flowers with ease without removing the pollinia (Evans 1931). Recently, Mike Edwards and George Else have also studied the insects visiting red helleborine, and have likewise questioned whether *C.campanularum*, although a regular *flower visitor*, is in fact large enough to act as an effective red helleborine pollinator (pers. comm. 2006).

Due to its critically endangered status, it was decided in 2007 to re-establish the UK Red Helleborine Group in order to share ideas and develop plans for the conservation of the species. Local wardens from Gloucestershire, Hampshire and Buckinghamshire were joined by specialists from the Royal Botanic Gardens, Kew (London) and Natural England, visiting each of the extant sites and the Kew laboratories. This provided opportunities to consider management options and to learn more about the ecology of the species. It was decided that hand pollination attempts (ongoing for several years) should be continued in an attempt to increase seed-set for propagation purposes in light of very low natural pollination success at these three remaining small colonies.

In the case study presented here, hand pollination attempts, observations of insects visiting red helleborine flowers, and ongoing management to encourage possible pollinators at the Buckinghamshire locality are described.

ACTION

Study site: The hand pollination of red helleborine flowers and observations of insect flower visitors was undertaken at the BBOWT (Berks, Bucks and Oxfordshire Wildlife Trust) reserve that supports the single extant Buckinghamshire red helleborine population situated in the Chiltern Hills, southern England. This locality supports a small colony of red helleborines consisting of up to 10 plants. In a good year, as in 2007, several plants will flower.

Red helleborines in 2007: In 2007, red helleborine plants first appeared around 10 May and a total of eight plants grew in the usual location with three developing flowers. These had between 10 and 14 florets on each stem or 'flower spike', the tallest of which reached 50 cm (about the maximum height recorded at this site).

Hand pollination: Attempts at hand pollination were deemed appropriate due to the apparent lack of natural insect pollinators, and consequent very low natural pollination and seed-set; over the last 10 years only one seed pod developed in response to natural pollination. Hand pollination is undertaken as florets open and the pollinia mature, usually over several days at the end of June. The procedure that was used in 2007 had been successfully trialled before: when the horse-shoe shaped pollinia is mature (about when a floret is fully open) using a small 'cocktail-type' stick with a sharpened end, the pollinia is gently lifted off, transferred to another floret and rubbed against the stigmatic surface (Fig. 1). When possible (dependent upon how many plants are flowering in that year) transfer is usually made to a floret on another flower spike. In 2007, six florets were pollinated in this way.

Maturing pods resulting from hand pollination are gathered when still green (at around 50 or so days after pollination) as micropropogation of other *Cephalanthera* species suggests that higher germination success is achieved from less mature seeds (e.g. Yamazaki & Miyoshi 2006). Pods are harvested by simply cutting the pedicel with a pair of sharp scissors. Each pod is placed in a small glass tube plugged with cotton wool (to allow it to 'breathe' and prevent build-up of humidity which may cause it to rot) and sent on day of collection to the Royal Botanic Gardens, Kew (London).

Weather: The relatively mild and sunny winter of 2006/7 was followed by the warmest, driest and sunniest April since 1914, with temperatures 5º C above normal. From May onwards, southeast England had the wettest and dullest early summer recorded, with exceptionally high rainfall very much above the average through to August. How or if this affected maturation of pollinia, hand-pollination success or subsequent seed pod, development is not known.

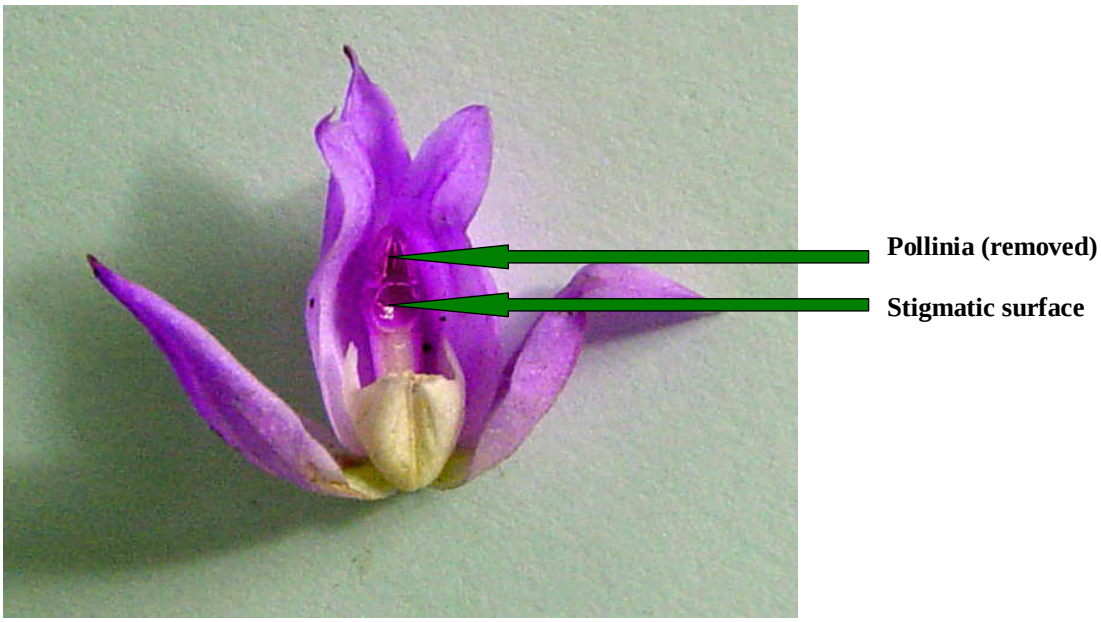

Figure 1. Single red helleborine floret showing position of pollinia and stigmatic surface (Photo: Roger Newman)

Observations of flower visitors: On 1 July 2006 two of the authors of this present paper (Martin Harvey and Roger Newman) spent approx. 1½ hours observing insects visiting red helleborine flowers at the Chiltern Hills locality (Harvey 2006). The insects most frequently seen were male *Chelostoma campanularum* bees which were mostly observed flying rapidly around the flowers and then moving off, but every so often one entering a flower and emerging a few seconds later. A single female was also seen to enter a flower. This individual was bright yellow on the underside of the abdomen as a result of carrying a full pollen load presumably collected from nearby nettle-leaved bellflowers *Campanula trachelium* (*C.campanularum* is obligately associated with bellflowers *Campanula* spp.) although it is possible that red helleborine pollen could also have been present.

Several other species of insects (bees and flies) were also seen to land on red helleborine flowers:

- a small solitary bee, possibly a species of *Lasioglossum* (specimen not caught for species confirmation);
- small empid flies (Diptera: Empipidae) landing on the flowers, usually on the outside of the petals. Most did not enter the flowers; these tiny (2-3 mm) flies are assumed to be too small to be effective pollinators. The single specimen collected was a female *Rhamphomyia longipes*;
- hoverflies (Diptera: Syrphidae): a female *Platycheirus albimanus* was twice seen to land on the lower lip of a flower and use her mouthparts on the petal, but did not fully enter the flower. A smaller hoverfly (not collected for identification) was seen around the flowers but did not enter them;
- an anthomyiid fly (Diptera: Anthomyiidae) landed on a flower but did not appear to be using it as anything other than a perch.

CONSEQUENCES

Hand pollination success: In 2007, hand pollination resulted in four seed pods, of which one withered and died before reaching maturity. Figure 2 shows two of the three apparently healthy seed pods on one of the red helleborine plants (Plant D) that flowered in 2007, about 5 weeks after pollination. Upon ripening but whilst still green, on 12 and 20 August these pods (two

at 47 and one at 53 days after pollination) were carefully removed and sent to the Royal Botanic Gardens (Kew) for attempted micropropagation (see below).

Over the previous 10 years, hand pollination has been attempted in five seasons (in some years no flowers were produced thus hand pollination was not possible) in addition to 2007, with around 50% of hand-pollinated flowers resulting in seed pods. Only one seed pod has been produced as a result of natural pollination over this 10-year period.

In past years some flowering and non-flowering spikes (and associated seed pods) have been lost due to grazing by muntjac *Muntiacus reevesi*, even though plants were partially protected by wire mesh cages. This situation has now been rectified with the installation of a wire mesh fence. No loss or damage to pods via insect herbivory has been noted, although damage to plants by slugs has been apparent in some years. Steps are being taken to prevent this, including the placement of copper rings around plants, to protect them from slug herbivory (Newman & Showler 2007).

Figure 2. Two seed pods on Plant D, lower (D5) at 36 days and upper (D8) at 34 days after pollination, 1 August 2007 (Photo: Roger Newman)

Micropropagation: Attempts are currently ongoing at Kew to germinate and grow red helleborine seeds at the micropropagation unit. As yet propagation has proved unsuccessful. Even if successful germination is achieved, it is thought that seedlings may take several years from germination until the first leaves are produced and over 10 years until they first flower (anon 2007).

Site management: The evidence from the short visit on 1 July 2006 confirms the observations of Nilsson (1983) that *Chelostoma* bees, especially males, are regular visitors to red helleborine flowers, and suggests that conditions at the site (in 2006 at least) are favourable for *C.campanularum*. Current management aims to enhance conditions for this bee, although observations suggest that it appears doubtful that *C.campanularum* is in fact capable of pollinating red helleborine (Evans 1931, Edwards & Else pers.comm. 2006). Recent management work has opened up the tree canopy with a resultant increase in the number of nettle-leaved bellflower plants. Numerous *C.campanularum* were observed visiting their flowers during the July 2006 visit and although it was impossible to accurately estimate the numbers of these small, fast-flying bees seen, it seemed likely that they were in hundreds rather than tens.

About 5 m south of the exclosure there is a standing dead ash *Fraxinus excelsior* tree trunk (c.3 m tall and 110 cm dbh) in partial sunlight. This has many hundreds of small holes (c. 2 mm diameter) made by wood-boring beetles (Figs. 3 and 4), which were providing nest-sites for the bees. *C.campanularum* uses particles of soil to seal the entrance to its nest holes, and bees were seen apparently collecting soil from bare ground within the helleborine exclosure for this purpose. The slope thus provides, within a fairly small space, good quantities of nectar-plants for the bees, and plentiful nesting sites. The aim is to maintain the population of these bees through appropriate habitat management, e.g. by continuing to create new openings in the tree canopy where bellflowers as well as other nectar plants (and hopefully helleborines) can grow, while retaining dead wood, especially standing dead wood. Such standing dead timber will also provide habitat for other saproxylic invertebrates.

In the winter of 2007 an approximately 12 x 12 m area directly below the present red helleborine

exclosure was cleared of bramble *Rubus fruticosus* and ash seedlings and the ground lightly scarified by hand-raking to remove the cut material and accumulated leaf litter.

Figure 3. Standing dead ash *Fraxinus excelsior* trunk – valuable habitat for solitary bees and wasps (Photo: © Martin C. Harvey/BMERC)

Figure 4. Small boreholes in a dead ash *Fraxinus excelsior* tree used as nest locations by *Chelostoma campanularum* (Photo: © Martin C. Harvey/BMERC)

Long-term conservation: There has recently been extra attention given to conservation of red helleborine in the UK. As well as ongoing habitat management, seed has been collected over recent years: some has been banked at the Millenium Seed Bank at Wakehurst (part of Kew Gardens) in Sussex, southern England; some has been used for DNA analysis of the UK populations; and some has been used for propagation purposes. Botanists at Kew and Natural England are continuing to research improved methods of germination and propagation (as yet unsuccessful) with a view to producing plants that may be used for reintroduction purposes.

REFERENCES

Anon (2007) Royal Botanic Gardens, Kew: plant profiles – orchids: *Cephalanthera rubra* http://www.kew.org/plants/orchids/red_helleborine.html

Cheffings C. & Farrell L. (eds.) (2005) *The vascular plant Red Data List for Great Britain*. Joint Nature Conservation Committee, UK. http://www.jncc.gov.uk/page-3354

Evans G.H. (1931) *Proc. Royal Entomological Society London*, A6, 59 cited in: Nilsson L.A. (1983) Mimesis of bellflower (*Campanula*) by the red helleborine orchid *Cephalanthera rubra*. *Nature*, **305**, 799-800.

Harvey M.C. (2006) Potential insect pollinators of red helleborine *Cephalanthera rubra*, at Windsor Hill, Buckinghamshire, 2006. Unpublished report.

Newman R.D. (2007) Red helleborine (*Cephalanthera rubra*) at Windsor Hill, 2007. Unpublished report.

Newman R.D. & Showler A.J. (2007) The use of copper rings to reduce losses of red helleborine *Cephalanthera rubra* to slug and snail herbivory in the Chiltern Hills, Buckinghamshire, England. *Conservation Evidence*, **4**, 66-68. http://www.conservationevidence.com/Attachments/PDF908.pdf

Nilsson L.A. (1983) Mimesis of bellflower (*Campanula*) by the red helleborine orchid *Cephalanthera rubra*. *Nature*, **305**, 799-800.

Wigginton M.J. (1999) *British Red Data Books 1; Vascular Plants. 3rd Edition*. JNCC, Peterborough, UK.

Yamazaki J. & Miyoshi K. (2006) *In vitro* asymbiotic germination of immature seed and formation of protocorm by *Cephalanthera falcata* (Orchidaceae). *Annals of Botany*, **98**, 1197-1206. (a summary of this paper can be viewed at: http://www.conservationevidence.com/ViewEntry.asp?ID=959)