Population Structure of *Epipactis palustris* at three sites in Oxfordshire

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Background

Marsh helleborine (*Epipactis palustris*) is an orchid species that is characteristic of fen and dune slack habitats. Published research indicates that the population of this species has declined, yet research using the online databases of the University of Oxford and the Botanical Society of the British Isles indicates that little detailed research into the population structure and habitat requirements of this species have been carried out, at least in the UK.

This paper reports research on three Oxfordshire sites, the Lye Valley, Parsonage Moor and Dry Sandford Pit.

Introduction

In the vicinity of Oxford, *Epipactis palustris* is found at three sites. Two of the sites, namely Lye Valley and Parsonage Moor SSSIs consist of ancient fenland habitat, whereas the third site, Dry Sandford Pit, was a working quarry as recently as the late 1950s and early 1960s (Porter 2002). The study therefore provides the opportunity to study the population structure of *Epipactis palustris* at ancient fenland habitats and a very recently created habitat and to see what differences there are between them, and particularly how *Epipactis palustris* responds to competition from other species.

The purposes of this study are as follows:

A: Distribution

1. To discuss the geographic distribution of Marsh Helleborine, nationally and internationally;
2. To discover details of the local population

B: Dispersion

To determine whether it is spaced, clumped or randomly distributed and to determine variations in this between the different sites.

C: Density

To determine the density of the local population as indicated by the number of flower spikes and to determine variations in this between the sites.

Also, to investigate the reasons why there are differences between the sites: is this due to physical factors, competition from other species, how Marsh Helleborine reproduces or is the management of the sites impacting on the population?

Characteristics and Distribution of species

Marsh Helleborine belongs to the genus *Epipactis*, a group of orchids that are largely confined to Europe and Asia. A and S Harrap state that, "the number of species is uncertain with the most conservative authors listing around 11 species in Europe, whereas Delforge (2005) details 59. There are eight species in the British Isles, including two endemics." Recent research has demonstrated that several have close relationships with fungi, which make a significant contribution to their nutrition. British helleborines are often divided into those which are cross pollinated and those which are self pollinated, with four in each category. *Epipactis palustris* is stated to be one of the cross pollinated species (Harrap and Harrap 2005).

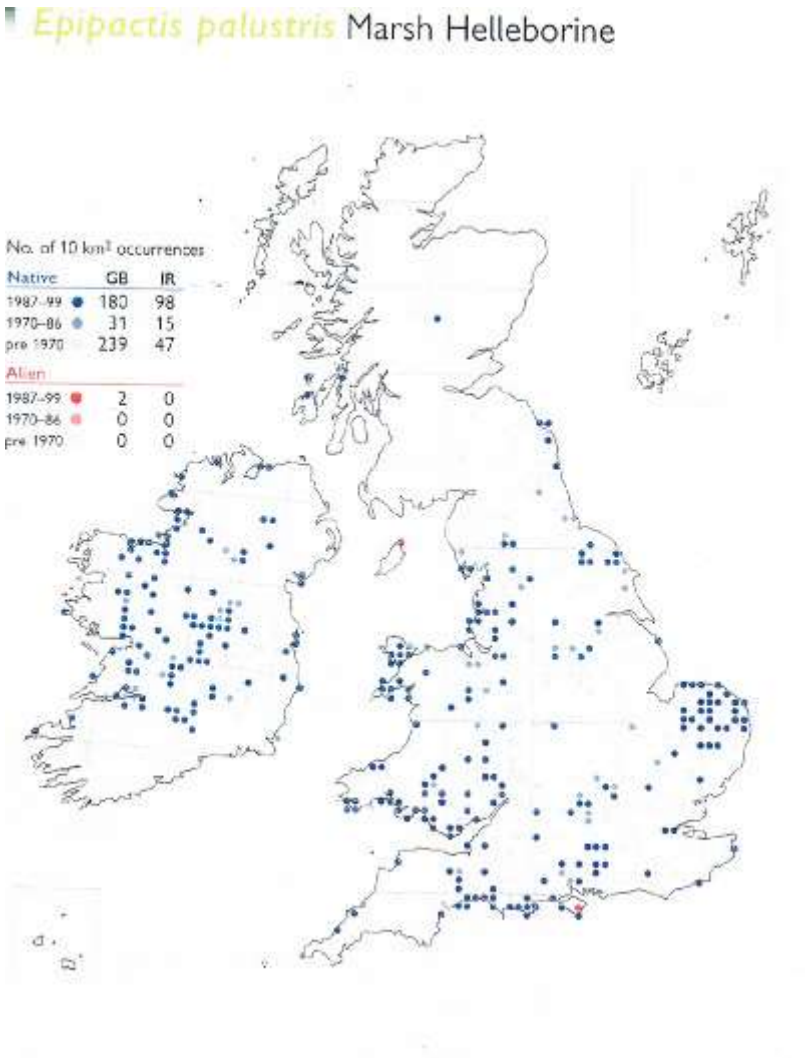

Figure 1. Distribution of *Epipactis palustris* in the British Isles (Preston *et al*, 2002)

Epipactis palustris has a widespread global distribution, being found in Europe from southern Scandinavia to the Mediterranean, including North Africa, and eastwards across Asia to Lake Baikal in eastern Siberia and Japan. It has been recorded at altitudes of up to 1600 m in the Alps but is absent from the Mediterranean lowlands. (Clapham 1962, Harrap and Harrap 2005 and Foley 2005). In England, Wales and Ireland it is widespread but often very local, being absent from large areas, including much of Kent, Sussex, Surrey, Wiltshire, Devon, Cornwall, the Midlands, Durham, Cumbria and mid Wales. Its strongholds are in Norfolk and Hampshire. It is very rare in Scotland, only being found at a handful of sites in Perthshire, Argyll and the islands of Islay and Colonsay (Harrap and Harrap 2005). The current and recorded historic distribution of the species is shown in Figure 1. There has been an extensive decline in the distribution of the species (change – 0.39) with Preston *et al* recording that most losses occurred before 1930, with the species being vulnerable to changes in water level and nutrient enrichment (Preston 2002). An example of this process occurring is at the rich fen at Newton Reigny Moss near Penrith in Cumbria, where a deep ditch was dug in the 1940s, lowering the water table, and thus leading to the encroachment

of willow carr and the disappearance of *Epipactis palustris* (Radcliffe 2002). More recently, the enrichment of groundwater by fertiliser run off has caused suitable fens to become overgrown with more vigorous vegetation leading to the loss of *Epipactis palustris* (Harrap and Harrap 2005). Table 1 indicates the decline of the species as recorded by A and S Harrap.

Table 1. Past and present occurrence of *Epipactis palustris* in Britain and Ireland (based on presence or absence in 10 km squares of the National Grid; data from the New Atlas) (Harrap and Harrap 2005)
*** current range as a percentage of the total number of 10 km squares**

	Britain	**Ireland**
Total historical range, 1500 - 1986	450	160
Current range	180 (6.3%)*	98 (9.7%)*
% lost 1500 -1969	53%	29.5%
% lost 1970 - 1986	7%	9.5%
% lost total	60%	39%

Locally, the species has a very restricted population in Berkshire and Oxfordshire. (Numbers in brackets in this paragraph are SP grid references to various accuracies.) Killick *et al* record that in Oxfordshire it is found at Bullingdon Bog (5404) (this is an alternative name for Lye Valley), Headington Wick (5408), Spartan Bog (6400), Otmoor (5612), Woodcote (6482). In tetrad 6292 it has been recorded at Berrick Salome gravel-pit and at Yoke Marsh. They also state that it is abundant near Cothill (Killick 1998). In Berkshire, Crawley records that it is "very rare and extinct in most of its former sites" (Crawley 2005). He states that the species is extinct in East Berkshire, while in western Berkshire it is mostly confined to areas in the north within the former county boundary of what is now Oxfordshire. These sites include a fen near Hatford (39), Frilford Heath golf course (442986), Hitchcopse pit (4599), Barrow Farm fen (4697), Cothill fen (4699), Abingdon (49), Foxcombe Hill and on the south side of Wytham (40). He then implies that it may be extinct at some of these sites by stating "Still in Cothill fen, in the wetland at the bottom of Dry Sandford Pit at Cothill, and on Frilford Heath golf course nearby" (Crawley 2005). Bowen indicates that the records at Foxcombe hill, Wytham and Abingdon are from the Flora of Berkshire, 1897 (Bowen 1968).

Ecological Range and Ecological Characteristics of *Epipactis palustris*

A and S Harrap state that *Epipactis palustris* "requires neutral to alkaline ground water and and relatively short, open vegetation to thrive," being most often found in dune slacks and spring fed fens. It "may also be found in meadows which are seasonally flooded with chalky water, but it cannot compete with tall vegetation and such habitats must be regularly mown or grazed for it to survive." Preston *et al* (2002) likewise states that "It prefers flushed or seasonally inundated areas where competition from other vegetation is reduced." Summerhayes (1951), however, takes a different view with regard to the ability of *Epipactis palustris* to survive competition, stating that, "Owing to the comparatively tall stems and the leaves being

borne some way above the base, the Marsh Helleborine is able to maintain itself in quite rank vegetation, such as the common reed." He states that in central Ireland it is a "characteristic member of the extensive limy marshes which are covered to a great extent with coarse vegetation of sedges and other similar plants."

Figure 2. *Epipactis palustris*

Occasionally it occurs in other habitats, such as wet, slumped, clay cliffs (as in Dorset and the Isle of Wight), gravel pits and fly ash pools. Surprisingly it is also occasionally found on chalk grassland, but this is usually where quarrying and excavations have left a compacted surface that is prone to becoming waterlogged (Harrap and Harrap 2005). One example of this is at Morgan Hill in the Marlborough Downs, where a colony has been recorded since 1937 and where 2,184 flowering spikes were recorded in 2000, with the majority being found on the north facing slopes and flat areas. Denning suggests that what may have happened here is that one plant became established shortly after quarrying ceased and the plant has increased in number vegetatively since that time (Denning 2000). Surprisingly, Foley states that populations have been known to occur on open chalk downland which, owing to the permeable nature of chalk, is often very dry. He also records that the species can be found in damp, upland, limestone grassland in South Cumbria and that one of the Scottish populations is found on an upland meadow overlying metamorphosed limestone (Foley 2005).

Rodwell *et al* (1991) list *Epipactis palustris* as being present in the following British Plant Communities in their volumes on *Mires and Heaths*, and *Aquatic communities, swamps and tall herb fens*. See table 2.

Table 2. National Vegetation Communities where Rodwell *et al* list *Epipactis palustris* as being present (excluding coastal communities)

M13	Black Bog-rush (*Schoenus nigricans*) – Blunt-flowered Rush (*Juncus subnodulous*) mire (Extreme rich fen)	This is one of the fenland plant communities found at Parsonage Moor and Lye Valley (Wheeler 2002 and Morris 2002).
M13c	Marsh Marigold - Fen Bedstraw (*Caltha palustris – Galium uliginosum*),	Morris (2002) states that this community has fair to good similarities with some of the fen communities found at Parsonage Moor.
M22	*Juncus subnodulosus – Cirsium palustre* (Fen meadow)	
M24	*Molinia caerulea – Cirsium dissectum* (Fen-meadow)	Morris states that the fen meadow at Parsonage Moor shows fair to good similarities with M24 Purple Moor-grass - Meadow Thistle (*Molinea caerulea – Cirsium dissectum*) and/or M24a Hemp Agrimony (*Eupatorium cannabinum*) sub community.).
S1	*Carex elata* (Sedge-swamp)	
S24	*Phragmites australis – Peucedanum palustre* (Tall–herb)	
S27	*Carex rostrata – Potentilla palustris* (Tall–herb fen)	

Fojt (1993) subdivides fen species into principal fen species and rare fen species and places *Epipactis palustris* among the rare fen species. This suggests that within fen vegetation communities *Epipactis palustris* usually has a fairly low frequency and abundance.

A survey of Dry Sandford Pit in 1990 by English Nature found that the site could not be classified using the National Vegetation Classification. However, 35 principal fen species were recorded at the site, indicating that the fen was then in a favourable condition.

Epipactis palustris reproduces both by seed, with an average of over 80% of flowers setting seed, and vegetatively. The aerial stems grow from a rhizome that grows horizontally near the surface of the soil, and it has been claimed that over 100 flower spikes may grow from the same plant (Harrap and Harrap 2005). Summerhayes (1951)and Bowmer (2008) state that this rhizome is only 2 -3 cms or so below the surface, chiefly due to the scarcity of oxygen in the very damp ground where it is found. Figure 3 shows the underground rhizomes.

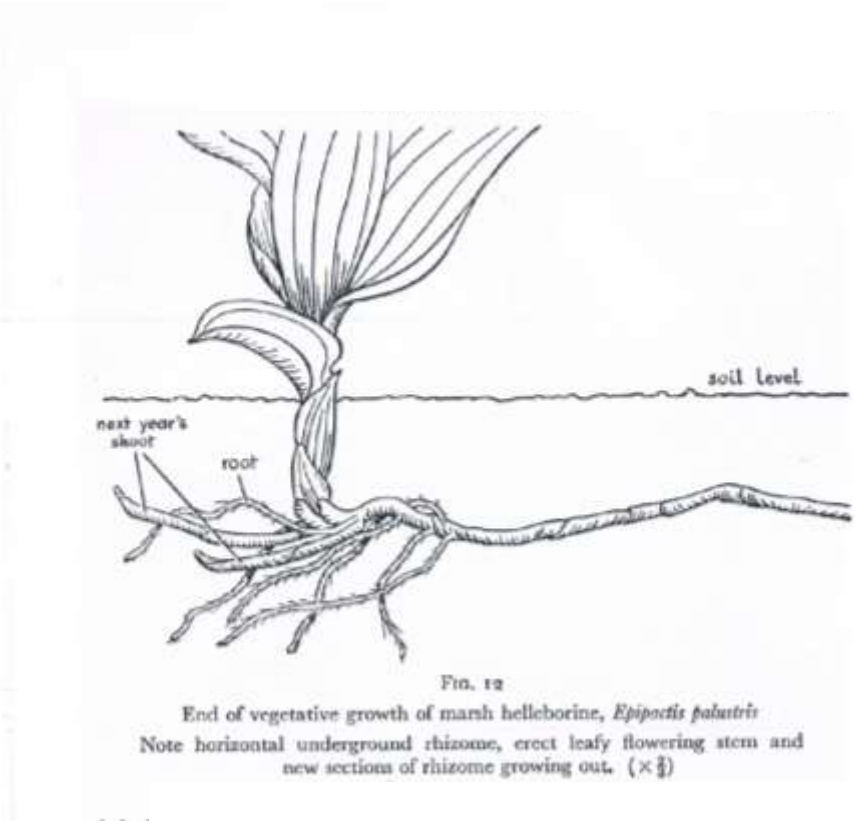

FIG. 12
End of vegetative growth of marsh helleborine, *Epipactis palustris*
Note horizontal underground rhizome, erect leafy flowering stem and new sections of rhizome growing out. (×⅔)

Figure 3. Underground rhizome of *Epipactis palustris* (Summerhayes 1951)

Roots are produced at many points along the rhizome and include both horizontal roots within the organic surface material and vertical roots that often grow deep into the mineral soil (Harrap and Harrap 2005). Summerhayes (1951) states that there are grounds for thinking that *Epipactis palustris* multiplies chiefly by the production of underground runners from the rhizome and that the function of the seeds is probably to enable the species to become established in new localities. It produces thousands of dust-like seeds which lack any endosperm and have a high wind dispersal potential. The seeds can therefore travel large distances of over 125 km. In this way it has been able to colonise former lignite mining areas in eastern Germany (Esfeld *et al* 2008). Due to its habit of spreading vegetatively it is very effective at colonising and is able to form fairly dense patches and can form huge colonies in dune slacks in Anglesey and the north of England (Foley 2005 and Bowmer 2008).

Characteristics of Dry Sandford Pit, Parsonage Moor, Cothill and Lye Valley

The three sites chosen for this study consist of two ancient fenland sites at Lye Valley and Parsonage Moor, Cothill, and Dry Sandford Pit, a former quarry (Porter 2002). Lye Valley is located within Oxford, close to the Slade and just to the south east of the Churchill hospital (Grid ref SP 547057). Dry Sandford Pit and Parsonage Moor are located to the south west of Oxford, near to the small village of Cothill. They are on opposite sides of the village, with Dry Sandford Pit (Grid ref SU 467995) being on the south eastern side and Parsonage Moor (Grid ref SU 461998) to the north west.

In contrast to Lye Valley and Parsonage Moor, which have deep deposits of peat, Dry Sandford Pit was a quarry until the 1950s and has developed tufa rich mud where springs emerge in the quarry floor (Porter 2002). The water from these springs flows through fen and ponds and then joins the Sandford Brook, which runs along the western boundary of the reserve. Porter (2002) describes this site as "really a primeval fen that in the coming centuries will begin to deposit peat and ultimately will resemble nearby Cothill. The nearest equivalent to Dry Sandford would have been the landscape emerging from the last ice age where ecological succession was literally starting from bare ground. At present the fen elements are represented by a few typical species such as swathes of Marsh Helleborine and a number of fen fly species. To the botanist the vegetation on Dry Sandford Pit defies classification." Changes to the characteristics of the site would appear to be already occurring. The Reserve Handbook published by the Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust in 1994 indicates extensive areas of open water (BBONT 1994), see Figure 7. However, apart from small depressions, most of this open water has now gone, replaced by reedbed and wet fenland habitat, with the only area of open water that still exists being at the southern end of the pit. A draft management plan produced in 2002 also states that "Reedmace, *Typha latifolia*, Birch, *Betula* spp, Goat Willow, *Salix caprea*, Osier, *S. viminalis*, and Elder, *Sambucus nigra*, continue to encroach into the fen" and that "the condition of the calcareous fen has decreased over time, as nutrients have built up and succession occurred. If this process is allowed to continue, such encroachment may result in the loss of many of the flora and fauna that are characteristic of such a habitat" (Dry Sandford Pit Management Plan 2002).

Parsonage Moor forms part of the Cothill Fen Site of Special Scientific Interest (SSSI), which "supports outstanding examples of nationally rare calcareous fen and moss-rich mire communities together with associated wetland habitats." The rich and nationally rare fen habitats "include a semi-floating Blunt-flowered Rush *Juncus subnodulosus* – Black Bog-rush *Schoenus nigricans* mire community" and "Purple Moor-grass *Molina caerulea* – Meadow Thistle *Cirsium dissectum* fen meadow," with the latter said to be the plant community supporting *Epipactis palustris* (SSSI citation). Originally the site consisted of a substantial depression consisting of four small valleys, but the present surface has become much more uniform owing to infilling by peat and highly calcareous sediment (Morris 1975). Although this is an ancient habitat, human activities such as peat cutting, burning, reed/litter cutting, scrub clearance and grazing have also been important in maintaining the fen habitats at Cothill, and Morris (2002) considers that without them "there seems no reason to doubt that the normal process of ecological succession would have resulted in the development of carr and wet woodland over the whole site."

There are two areas of open calcareous fen located at Lye Valley, which is a shallow valley drained by the Lye Brook. This study is only concerned with the northern area, due to the difficulty of obtaining permission for access to the southern area. The fen vegetation exists on waterlogged peat that is supplied predominantly from the lateral movement of water percolating through base-rich Corrallian Beds. Springs and seepage lines occurr where the calcareous grits and sands meet the impervious Oxford Clay. A base rich peat up to 1.5 m thick has been laid down along parts of the valley (Lye valley SSSI citation).

Figure 4. Dry Sandford Pit (view from north end of site)

Figure 5. Parsonage Moor (middle of site)

Figure 6. Lye Valley (middle part of fen)

Methodology

The survey technique used was to take two transects across the habitat where the *Epipactis palustris* were located, with each quadrat 1 x 1 m in size and 10 m apart. At each of the three sites the fen community grades into other vegetation types often dominated by Common Reed (*Phragmites australis*) and tall herbs such as Hemp Agrimony (*Eupatorium cannabinum*) and the study aimed to establish the relationship between the population of *Epipactis palustris* and changes in vegetation community. At both Dry Sandford Pit and Lye Valley there are also changes in relief both within the fen and with adjoining habitats, which could also affect the population structure of *Epipactis palustris*. The transects were located to enable each of these factors to be assessed. Other purposes of survey were to determine the characteristics of dispersion and estimate the density of the species.

At both Dry Sandford Pit and Lye Valley the transects were taken across almost the entire fen area. At Dry Sandford Pit the long transect ended after 14 quadrats with a barbed wire fence. South of this area there is an area of open water and the landowner's evident wish for no public access beyond this point was respected. Quadrat 14 was taken immediately adjoining the fence and viewing the area south of the fence it was evident that while there were a few *Epipactis palustris* immediately adjoining the fence, the habitat then changed so that omitting the area would not have a significant effect on estimating the total *Epipactis palustris* population at this site. At Lye Valley the long transect also ended 45 m north of the southern boundary of the

fen habitat. The *Epipactis palustris* is concentrated in the middle part of the site. After taking four quadrats with no *Epipactis palustris* in an area of tall rush and reed, I then walked to the southern edge of the tall reed habitat where it changes to woodland to see if any more *Epipactis palustris* could be found, but none was seen. I am therefore confident that the two transects cover the whole population of *Epipactis palustris* at Lye Valley. At Parsonage Moor, the situation was somewhat different as the site is very large, being at least 300 m long and 200 m wide so a very large number of quadrats would be needed to cover the whole site, which would be beyond the scope of this study. Two transects through a range of fenland vegetation communities were therefore taken close to a minor watercourse in the middle of the site as shown on Figure 8. Walking was undertaken in other parts of the site to check that no large populations of *Epipactis palustris* had been missed, but none were found. The location of the transects at each site are shown in Figures 7, 8 and 9.

The surveys took place on the 8th, 9th and 11th July 2011 at the three sites.

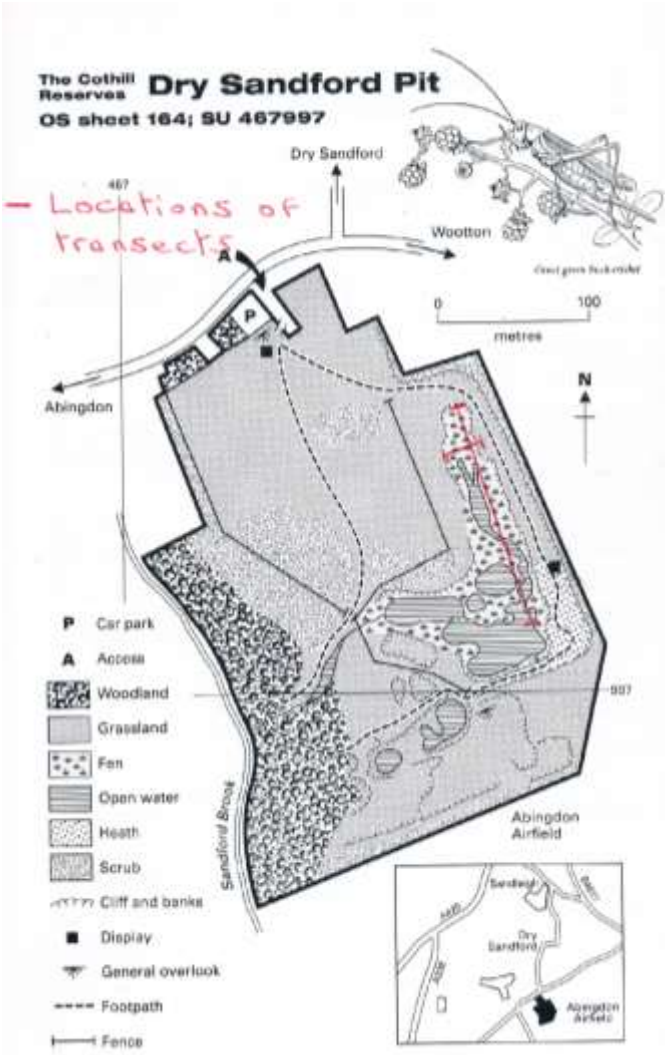

Figure 7. Dry Sandford Pit showing Transect Locations (BBOWT 1994)

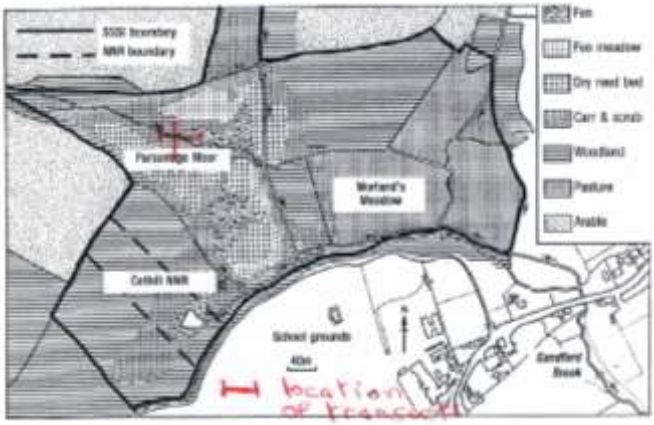

Figure 8. Parsonage Moor showing Transect Locations (Morris 2002)

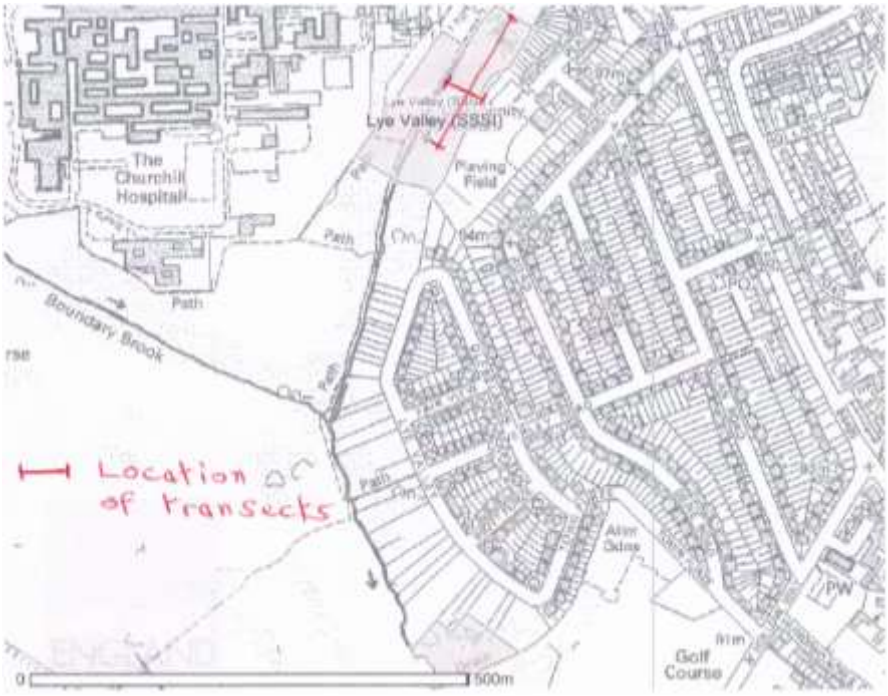

Figure 9. Lye Valley showing Transect Locations (SSSI citation)

The information recorded is provided in Table 3 below.

Table 3. Information recorded in surveys at Dry Sandford Pit, Parsonage Moor and Lye Valley

1.	**Location of quadrat**	The plot number column indicates the location of each quadrat in relation to the transects plotted on the site location plans.
2.	**Number of flower spikes and their height**	As these were clearly visible they could be counted and measured accurately.
3.	**Plants without flower spikes**	Many quadrats had substantial numbers of *Epipactis palustris* which did not have flower spikes. However, as these were often hidden within the other vegetation, the numbers counted may be less accurate than the counts of flower spikes.
4.	**Other key components of vegetation in quadrat**	This was to indicate the main vegetative components, not to provide a full species list.
5.	**Vegetation height**	Recorded with a tape measure. As the vegetation of the various plants

		within each quadrat often varied considerably, it only provides an approximate estimate.
6.	**Percentage of vegetation cover of ground**	This was a visual estimate
7.	**pH level**	Most of these were recorded with a pH Gardman Soil pH testing kit, which involves the use of pH test solution. Some of the samples were also taken with a 'rapitest probe,' which involves putting a probe stick in the ground. Often both techniques were used in the quadrats. The reason for using the probe is that the test instructions for the chemical test state that the soil is to be left to dry before testing. As the samples were saturated it was thought that inaccurate results might be obtained. However, for the last three samples at Dry Sandford Pit plus all those taken at Parsonage Moor and Lye Valley this problem was overcome by taking soil samples in the field and then drying them out before taking the chemical pH test
8.	**Light Level (Lux)**	This was taken using a light meter and recording the Lux levels. The results do not give a very good picture of variations in light levels as on the first two days there was a mixture of cloud and sunshine and the recorded light level was greatly influenced by the weather at the time. The final survey on the 11.7.11 was in the evening which meant that the light levels recorded were low despite often being in open locations.
9.	**Soil moisture**	This estimate was derived from taking a sample of soil and squeezing it between fingers. If water came out it was described as saturated.
10.	**Height of ground in relation to surroundings**	This was a visual estimate. Dry Sandford Pit is a relatively flat area at the bottom of a former quarry, with sloping sides at the northern and eastern edge, with the later rising about 2 m above the fen to a footpath that runs along the eastern edge. There is also a low sandstone ridge within the fen which is about 70 cm above the surrounding fen vegetation, where quadrat 17 at Dry Sandford Pit was taken.

Results

Spatial distribution

A useful method of classifying spatial distributions uses the following three types:

- random
- clumped
- uniform

In a random distribution the distribution of each plant is independent of the others, so there would be no fixed pattern to the distribution. In a uniform distribution each plant would be a set distance from the others, so there would be a fixed pattern. In a clumped distribution the plants would be clumped together in certain parts of the site while in other parts there would be none.

The variance (s^2) to mean ratio has been used to measure population dispersion. The interpretation of s^2/mean values is as follows:

s^2/mean > 1.0	clumped distribution
s^2/mean = 1.0	random distribution
s^2/mean < 1.0	uniform distribution

Tables 4 and 5 indicate the results obtained for this test for the three sites, with Table 4 relating to the flower stalks and Table 5 to all *Epipactis palustris* plants including those with no flower stalks. From this it can be seen that the distribution is clumped at all three sites but that this is most marked at Dry Sandford Pit and least at Parsonage Moor. In order to calculate whether the distribution is significantly different from a random distribution (i.e. s^2/Mean = 1) a test statistic (t) is calculated, where:

$$t = \frac{|(s^2/\text{mean}) - 1.0|}{\sqrt{2(n-1)}}$$

Where n = sample size (number of quadrats sampled). Under this test an absolute value of t > 1.96 indicates a distribution that differs from random with a certainty of 95%. The value of t is given in the fourth column of tables 4 and 5. From this it will be seen that the distribution of *Epipactis palustris* is statistically significantly different from random at all three sites, but again this is most pronounced at Dry Sandford Pit and least at Parsonage Moor. One of the reasons why plants often have a clumped distribution is because they spread by rhizomes. Given that this is a characteristic of *Epipactis palustris*, it is not surprising that it has a clumped distribution. Other reasons can be due to variations in soil chemistry, moisture content or the characteristics of the plant community or vegetation height. The possible influence of these factors is discussed further below (Lock 2011).

Table 4. Population Dispersion of *Epipactis palustris* Flower Stalks using variance (s^2) to mean ratio

Sample Details	s^2/Mean Ratio	Indicated dispersion	t	Significant
Site 1: Dry Sandford Pit (19 quadrats sampled)	18.4	Clumped Distribution	52.3	Yes
Site 2: Parsonage Moor, Cothill. (10 quadrats sampled)	2.0	Clumped Distribution	2.1	Yes
Site 3: Lye Valley, Oxford. (17 quadrats sampled)	8.5	Clumped Distribution	21.2	Yes

Table 5. Population Dispersion of all *Epipactis palustris* including Flower Stalks and non flowering plants using variance (s^2) to mean ratio

Sample Details	s^2/Mean Ratio	Indicated dispersion	t	Significant
Site 1: Dry Sandford Pit (19 quadrats sampled)	27.2	Clumped Distribution	78.6	Yes
Site 2: Parsonage Moor, Cothill. (10 quadrats sampled)	3.0	Clumped Distribution	4.2	Yes
Site 3: Lye Valley, Oxford. (17 quadrats sampled)	24.9	Clumped Distribution	67.7	Yes

Estimate of total Population Size

The total population size is estimated by first calculating the area of the site in m^2 and then using the formula:

$$N = (A/a) * n$$

Where N = the estimated total population size
A = the total study area (in m^2)
a = the area of one quadrat (in m^2)
n = the average number of *Epipactis palustris* per quadrat

The total areas surveyed were rectangular, so the study area was calculated by squaring the total distance covered by the two transects. The results of applying this equation are given below, with Table 6 only relating to flower spikes, while Table 7 includes non-flowering plants.

Table 6. Estimate of total population size of *Epipactis palustris* flower stalks at the three surveyed sites

Sample Details	Total study area (m^2)	Quadrat Size (m^2)	Average numbers of *Epipactis palustris* per quadrat	Estimated Population
Site 1: Dry Sandford Pit	6,336	1	11.42	72,357
Site 2: Parsonage Moor, Cothill.	2,520	1	0.18	454
Site 3: Lye Valley, Oxford.	1,872	1	1.35	2,527

Table 6 indicates that the number of flowering spikes at Dry Sandford Pit is large. This is consistent with information from Debbie Lewis, BBOWT Reserves Ecology Manager, that there are tens of thousands at the reserve. However, she also reported that the total number of plants at Parsonage Moor is about 5, so the figure of 454 is almost certainly an exaggeration (Lewis 2011). The plants cannot therefore be spread around the study area, which they would have to be to make the equation effective. At Lye Valley the total number of flowering spikes is estimated at 2,527, which is very much less than at Dry Sandford Pit, but more than at Parsonage Moor.

Table 7. Estimate of total population size of *Epipactis palustris* including non flowering plants at the three surveyed sites

Sample Details	Total study area (m^2)	Quadrat Size (m^2)	Average numbers of *Epipactis palustris* per quadrat	Estimated Population
Site 1: Dry Sandford Pit	6,336	1	26.47	167,714
Site 2: Parsonage Moor, Cothill.	2,520	1	0.27	680
Site 3: Lye Valley, Oxford.	1,872	1	9.24	17,297

When non flowering plants are taken into account there is only a limited increase in the estimated population at Parsonage Moor, but very substantial increases at Dry Sandford Pit and Lye Valley owing to the large number of non flowering plants at these locations.

One interesting question is how these populations have changed over time. In this respect there is some information from Parsonage Moor, where Morris (2002) undertook vegetation surveys in the early 1970s. He used a 20 m grid over the whole site. "Quantitative data were obtained by means of presence/absence observations in 40 randomly placed 0.25 m^2 quadrats in each grid square, from which % frequency values were calculated." The results therefore indicate frequency rather than the number of plants. He recorded *Epipactis palustris* in 11 of the 124 20 m grid squares that he used and the frequencies he obtained are given in Table 8 below.

Table 8. Grid squares at Parsonage Moor where Peter Morris recorded *Epipactis palustris* and the frequency score obtained (Morris 1975)

Grid square	Species frequency % for grid numbers
13	2.5
14	5.0
24	2.5
25	10.0
26	5.0
30	7.5
37	10.0
38	2.5
43	7.5
67	2.5
110	2.5

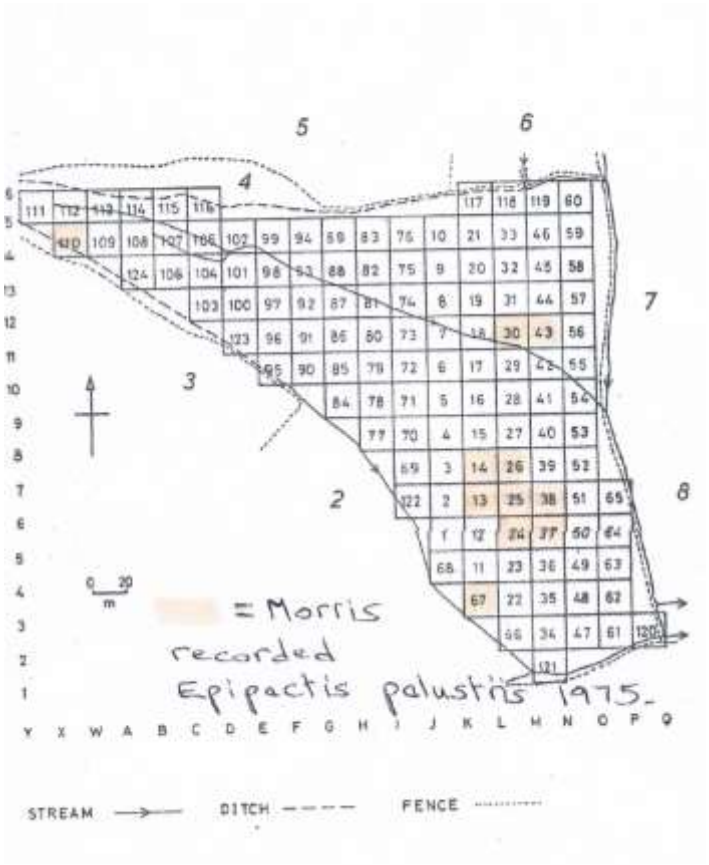

Figure 10: Morris's Grid Squares at Parsonage Moor showing locations where *Epipactis palustris* was recorded (Morris 1975)

Figure 10 gives the grid layout and shows which parts of the site Morris recorded *Epipactis palustris* in. While this indicates that the plant then had a restricted distribution on the site and low frequency, it was present on various parts of the site. This would suggests that if Lewis is correct that there are now only five plants on the whole site, the number of *Epipactis palustris* at this site has declined over the last forty years.

The results for other criteria recorded and their relationship to *Epipactis palustris* population structures are set out below:

Criteria monitored at the three survey sites and their relationship to the population structure of *Epipactis palustris*

Height of *Epipactis palustris*

There was a marked difference in the length of Epipactis palustris flower stalks at the 3 sites. At Dry Sandford Pit the height ranged from 13 to 75 cm, with the mean height being 36.4 cm. At Parsonage Moor the height of the two specimens recorded was 25 cm and 33 cm, giving an average of 29 cm, while at Lye Valley the height varied from 12 cm to 23 cm, giving a mean of 18.3 cm. The mean height at Lye Valley was therefore only half that at Dry Sandford Pit, although the number of plants measured was also much smaller. However, even at Dry Sandford Pit not all of the flower stalks were tall; there was a substantial range, with the smallest recorded being only 13 cm, similar to the smallest at Lye valley, but the largest flower stalk was 75 cm, 5.8 times as big. These height ranges are within the normal range. A and S Harrap (2005) state that the height is "usually 20 - 45 cm, occasionally up to 82 cm. Dwarf forms, only 10 cm high but with normal sized flowers, have been recorded, especially in dry habitats." Foley (2005) states that plants found in disused chalk pits or excavations "are often of smaller stature"; Summerhayes (1951) states that "very dwarf plants of *E. palustris* occur in a disused chalk pit near Greenhithe, Kent" and states that on the whole "sand dune individuals are shorter and stockier, and bear fewer flowers than those growing in other places, a difference which is related to the more exposed position of the plants." The current survey was not able to examine sufficient variables to be able to determine causality for the variations in flower stalk height., Curiously, however, the tallest specimens (45 – 75 cm) were found in quadrat 17 at Dry Sandford Pit, which is on the low sandstone ridge, which did not have typical *Epipactis palustris* habitat. The quadrat is described as consisting of "bare rock, grass, bramble, ash sapling, part overshadowed by birch sapling, horsetail. Other woody species." The quadrat is 70 cm above the adjoining fen and contained mineral soil rather than organic matter and the soil was moist rather than saturated as in most of the other quadrats. A number of other plots at Dry Sandford Pit included specimens that exceeded 40 cm (quadrats 4, 5, 7, 9, 12), and these too usually contained fairly rank vegetation including woody species, sedge and reed. Whether the greater height was due to competition or variations in nutrients or fungi it is not possible to determine from this study.

***Epipactis palustris* without flower spikes**

More plants were recorded without flower spikes than with them. At Dry Sandford Pit the numbers were 217 flower spikes to 286 non flowering plants; and at Lye valley 23 flower spikes to 134 non flowering plants. Only 43.1% of plants therefore had flower spikes at Dry Sandford Pit and 14.6% at Lye Valley. There is a possibility of bias in recording as the quadrats had taller woody vegetation at Dry Sandford Pit which may mean that some non flowering plants were missed. It would seem that large numbers of non flowering plants is not unusual, as Clark (1968) records finding two colonies of 40 and 60 flowering spikes at Magilligan, Co Londonderry "which had ten times as many non flowering plants in each group," and another colony in a valley further west with 50 flower spikes "with a remarkable number of young non-flowering plants." At Parsonage Moor only two flowering spikes and one non flowering plant were recorded. Further studies would be required to establish whether the non flowering plants are young plants, plants growing in less suitable conditions, or parts of larger plants which do have flowering spikes or a combination of these.

Other key components of the vegetation

No detailed record of all the species present in each quadrat was made, but the key components of the vegetation were recorded. One key difference between the sites is that many of the quadrats at Dry Sandford Pit contained saplings of woody species, such as birch, willow, alder, ash and guelder rose, while these species were almost totally absent from the fen at Lye Valley. Often the quadrats with woody species also contained *Epipactis palustris* e.g. quadrats 2, 5, 6, 7, 11, 13, 14, and 17. However, given that there are no records of *Epipactis palustris* in woodland, it would seem likely that over time they would disappear if the saplings were allowed to grow to maturity. The reason for this difference is unknown and could be due to differences in management or ecological differences e.g. differences in nutrient status between the two sites. Another difference between Dry Sandford Pit and Lye Valley and also Parsonage Moor is that at Dry Sandford Pit, *Epipactis palustris* was sometimes growing in large numbers in moderately dense reedbed, whereas at the other 2 sites

Epipactis palustris was entirely absent from tall reedbed, even where it was relatively open such as quadrats 13 and 14 at Lye Valley. However, in more dense reedbed at Dry Sandford Pit there were no *Epipactis palustris* e.g. quadrat 8 while two other reedbed quadrats, 9 and 10, had none or a small number of flower spikes, with most of the plants being non flowering.

Vegetation height

As many of the quadrats contained reed, or rushes, the vegetation height within quadrats varied considerably, and even where most of the vegetation was short there were often some components that were tall, though they were at low density. For this reason it is difficult to draw any firm conclusions from this data, though much of the fen at Lye Valley and parts of the surveyed area at Parsonage Moor is characterised by relatively short vegetation, and would therefore seem to provide ideal conditions for *Epipactis palustris*, which is said to require relatively short, open vegetation to thrive (Harrap and Harrap 2005), but the population was more restricted than at Dry Sandford Pit, where the vegetation height was generally greater.

% vegetation cover

For many of the quadrats this was 100%. Often when it was less the remaining space was occupied by water e.g. quadrats 6 and 8 at Dry Sandford Pit, and quadrat 9 at Cothill. Although growing in saturated ground and close to surface water *Epipactis palustris* was almost totally absent from standing water, with just one *Epipactis palustris* growing in it in quadrat 6 at Dry Sandford Pit.

pH Level

The pH levels recorded were generally high. Indeed at Parsonage Moor all of the chemical tests gave a reading of 7.5, while at Lye Valley they ranged from 7 – 7.5. At Dry Sandford Pit the figures recorded were more variable and ranged from 6 – 7.5. However, a number of the records here were only obtained from the 'rapidtest' probe or from wet samples of soil. When dry soil samples were used for the last 4 quadrats (16, 17, 18 and 19) the chemical test gave 7.5 at this site also. The figures for Lye Valley and Parsonage Moor can be corroborated by other studies. Lamberth records pH for the Lye Valley fen ranging from 7.0 – 7.8 (Lamberth 2007). Similarly at Parsonage Moor, Morris gives figures ranging from 6.9 to 7.8. (Morris 1975). At Dry Sandford Pit a mature Scots Pine was noted growing from the low Sandstone ridge, indicating that locally more acid conditions may be present.

Light levels

The levels recorded cannot be relied upon to give an accurate indication of variations in light across the sites due to variations in the weather and time of day when the readings were taken. However, the areas where *Epipactis palustris* was growing at Parsonage Moor and Lye Valley were very open, and this was also true of some of the quadrats at Dry Sandford Pit. However, there were some areas of moderately dense reed at this site, where light levels must be lower but which still had good populations of *Epipactis palustris*.

Soil Moisture

In most of the quadrats at the three sites, including those where *Epipactis palustris* was found, the ground was saturated. At Dry Sandford Pit one quadrat was taken 2 m above the fen, where the soil was relatively dry. No *Epipactis palustris* was recorded here, nor were any others seen away from the fen area on a perambulation around the site. However, within the fen there are areas that are slightly raised above their surroundings, often containing blocks of sandstone. These include quadrats 12, 13 and 17. In these the soil was moist at the surface but not saturated. These quadrats did contain *Epipactis palustris*, with the most notable being quadrat 17 which was 70 cm above the surrounding fen and contained the tallest flower spikes recorded, which were up to 75 cm tall. Most authors consider that *Epipactis palustris* needs wet conditions and its name *palustris*, means "of swampy ground". A and S Harrap (2005) state that "seasonally inundated areas are often favoured." However, Summerhayes (1951) refers to it growing in and around chalk pits near the top of the Downs near Calne in Wiltshire where the "ground is not very damp, the other plants being normally inhabitants of moist or dryish localities." Thus while *Epipactis palustris* clearly usually grows in saturated soil, it appears that it can also sometimes grow in less moist conditions.

Height of ground

In most of the locations where *Epipactis palustris* was recorded the ground was low at the bottom of the site, apart from the low sandstone areas noted above. At Lye Valley the fen is located in a valley and gradually slopes down from north to south, with *Epipactis palustris* being found in the middle section. However, despite the slope, no difference in the moisture of the underlying peat was found in the transect taken down this slope.

Discussion

The *Epipactis palustris* population at the three sites surveyed is clumped. This is not surprising given that most authors state that the species is able to spread vegetatively using rhizomes. What is surprising is the varying population sizes between the three sites, given their history. Dry Sandford Pit consisted of fields until quarrying commenced about 1927 which continued until the late 1950s (Draft Management Plan 2002 and Porter 2002). *Epipactis palustris* can therefore only have been present at the site for a relatively short period of time, but now numbers tens of thousands. By contrast both Parsonage Moor and Lye Valley are ancient fenland sites where *Epipactis palustris* could have been present for thousands of years. Yet surprisingly only two flower stalks and one non flowering plant were recorded at Parsonage Moor. This is even more surprising given the habit of *Epipactis palustris* of spreading by underground rhizomes, as it would be expected that a least one substantial clump would be present. The absence of the species over most of the site, even in those areas of fenland habitat where one might expect it, would suggest that something is unfavourable for it.

One possible factor is that Morris states that comparison of Parsonage Moor with other wetland sites show it to be one of the most eutrophic (Morris 1975). Generally, at the spring-fed fens where *Epipactis palustris* is found, the groundwater is nutrient poor (Harrap and Harrap 2005). Esfeld *et al* (2008) report that in Saxony–Anhalt, *Epipactis palustris* has disappeared from 90% of its natural habitats due to the effects of eutrophication and land use changes.

Another unknown factor is its relationship to fungi at the sites. Older studies such as Summerhayes (1951) suggested that in contrast to the other species of *Epipactis*, *Epipactis palustris* "maintains an almost independent existence, and relies very little on its fungal partner for food." However, A and S Harrap (2005) state that "recent isotope studies have shown that Marsh Helleborine may acquire around 30% of its nitrogen from its fungal partner but it does not appear to receive any carbohydrates via that route." The part played by fungi at the three sites is unknown.

Another factor that may influence the amount of *Epipactis palustris* at the three sites is succession. At Dry Sandford Pit the site would have been clear of vegetation after quarrying, providing ample scope for colonisation in suitable conditions, whereas at the other two sites it has had to compete in an established vegetation community. It is apparent from Dry Sandford Pit that the species has the ability to rapidly colonise sites in a relatively short period of time. This is also confirmed by the research of Esfeld *et al* (2008) in eastern Germany, who researched *Epipactis palustris* populations at five former lignite mining areas within the Geiseltal coal district, where mining had ceased at least 25 years ago. They found the populations varied between 5,000 and 1,000,000 individuals at the former mining sites, whereas the population at seven natural sites varied between 150 and 1,500 individuals. It would seem therefore that in the absence of competition the species is able to act as a pioneer species. Interestingly, using DNA and microRNA primed genomic fingerprinting, they found high within-population variability and no evidence for genetic bottlenecks causing reduction in genetic diversity in the mining populations. They explain the rapid colonisation of these sites as being due to "independent and/or successive colonisation events from different populations, high rates of immigration, rapid population growth after founding and effective seed dispersal." While acknowledging that clonal growth

is possible, they state that "this kind of reproduction does not play a major role after colonisation, neither in the primary nor in the secondary habitats." This contradicts other authors such as Summerhayes (1951) who considered that *Epipactis palustris* multiplies chiefly by the production of underground runners from the rhizome.

While generally it would seem that established fen habitats may support relatively small populations of *Epipactis palustris*, a site visit to the southern part of Lye Valley SSSI in 2007 indicated that it supports a much more dense population than the northern area, so there are clearly differences between sites in this respect.

Given the relatively isolated nature of Dry Sandford Pit there is also the question of where the population originally came from. Clearly Parsonage Moor is one likely source, but given the ability of the plant's seeds to travel long distances, more distant sources could also be involved. There is considerable disagreement among researchers about reproduction among *Epipactis palustris* with one school of thought maintaining that self fertilisation is regular and most important, while the other school maintains that cross fertilisation is predominant (Talalaj and Brzosko 2008). Research by Nilsson at Oland in Sweden and Brantjes at Haren in the Netherlands indicates that a broad spectrum of insects feed on the nectar and that solitary wasps, hoverflies, honey bees and ants pollinate the flowers, with the latter being responsible for both self and cross pollination (Nilsson and Brantjes). Certainly in this respect both Dry Sandford Pit and Parsonage Moor are well located as they are nationally important for their invertebrate populations (SSSI citations).

Conclusion

This study has indicated that the population structure of *Epipactis palustris* at the three sites studied is clumped. It has also confirmed that around Oxford the species is rare and is only found in wet calcareous fen type habitats, such as the three surveyed. However, it has also indicated some unexpected characteristics, such as the very small population at the exceptionally important ancient fen habitat at Parsonage Moor, where the species seems to be struggling to survive, while a large population has succeeded in establishing itself as a pioneer plant at Dry Sandford Pit, where quarrying only ceased about 50 years ago. Also unexpected is the presence of *Epipactis palustris* growing in moderately dense reedbed at Dry Sandford Pit, though this may only be a temporary feature in the process of succession, and further development of the reedbed may result in the disappearance of the species. The species appeared to be able to grow in a range of vegetation communities at Dry Sandford Pit, whereas it seemed only able to grow in the most open fen areas at Parsonage Moor and Lye Valley. The reasons for these differences were beyond the scope of this study which could only look at a few very basic factors.

Indeed one of the chief conclusions of this project is just how little we know about *Epipactis palustris*. Sometimes it is found on sites which seem to provide unsuitable habitat such relatively dry habitats on the Wiltshire Downs; or in rank vegetation such as the limey marshes in central Ireland. However, there are more fundamental gaps in our knowledge. As noted above, there is not agreement even about whether it is self or cross pollinated. Even the widespread assumption that the species frequently spreads by rhizomes is not accepted by Elsfeld *et al* (2008). According to the Draft Management Plan for Dry Sandford Pit (2002) even the preferred water level for *Epipactis palustris* is "unknown". A and S Harrap (2005) also state that "The

subterranean seedling stage has never been observed in the wild and there is no information on the early development of this species." These are major gaps in knowledge, surprising in group of plants which has been very well studied. This emphasises the importance of continuing research if we are going to be able to ensure that the requirements of this relatively rare and declining species can be met at those sites where it is present, such as those surveyed in this study.

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