

# Is spur length of *Platanthera* species in the British Isles adaptively optimized or an evolutionary red herring?

R. M. BATEMAN

*Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS\**

and

R. SEXTON

*22 Alexander Drive, Bridge of Allan, Stirling FK9 4QB*

## ABSTRACT

A consistent methodology was used by the authors, and by several volunteers lacking experience of morphometrics, to measure the length of the nectariferous labellar spur in populations of two sister species within the orchid genus *Platanthera*, *P. bifolia* and *P. chlorantha*. Although they differ morphologically, primarily in details of the column and labellar spur, these two species appear molecularly identical. Much previous research using *Platanthera* as an evolutionary model system assumed that spur dimensions play a critical role in attracting specific pollinators and thus in determining fitness. The present analysis of data from 1876 individuals of 73 *Platanthera* populations from the British Isles, plus 10 populations from Continental Europe, suggests that spur length in both species follows a latitudinal cline, diminishing northward at an average rate of 2.2% per 100 km. More southerly populations are more likely to occur in shaded habitats, where their spurs (especially *P. bifolia*) are on average longer than in open habitats. The present data are insufficient to determine whether this trend is adaptive and pollinator-limited, as traditionally believed, or allometric and resource-limited, as suggested here. Most previous floras and monographs (a) offer ranges for spur length that are seriously erroneous, and (b) are surprisingly reluctant to admit the occurrence of hybrids between the two species, preferring to prioritise speculative assertions of strong fidelity between pollinator and orchid above field observations recording morphologies intermediate between the two parental species. Statistical comparisons of various kinds of duplicated datasets show that, given an explicit protocol, acceptably consistent measurements can be achieved among multiple, largely inexperienced, analysts. The main source of potential measurement error, premature measurement of flowers prior to full anthesis, is easily avoided. The success of this project should open the way for more ambitious initiatives that recruit substantial numbers of

inexperienced researchers into morphometric investigations of the British and Irish flora.

**KEYWORDS:** adaptation, allometry, citizen scientist, co-evolution, geographic cline, measuring error, morphometrics, nectar spur, orchid, *Platanthera chlorantha*, *Platanthera bifolia*, pollinator attraction.

## INTRODUCTION

### THE GENUS *PLATANTHERA* AS AN EVOLUTIONARY MODEL SYSTEM

The Butterfly Orchids (genus *Platanthera* Rich.) have benefited from recent molecular phylogenetic studies (based on the nuclear ribosomal ITS region: Hapeman & Inoue 1997; Bateman *et al.* 2003), which have resolved the genus into six monophyletic sections (including *Piperia*, which was previously widely recognized as a separate genus in North America: Bateman *et al.* 2003; Lauri 2007). Several species of *Platanthera* have increasingly been used as models of evolution in general and putative adaptation in particular. In North America, hybridization, allopolyploid speciation and facultative autogamy have been documented in the *P. dilatata*–*P. aquilonis* aggregate of Section *Limnorchis* (Wallace 2004, 2006), while in Section *Lacera* inbreeding depression and mutational processes were described in *P. leucophaea* (Wallace 2003; Holzinger & Wallace 2004) and pollination frequency, outcrossing-to-inbreeding ratio and selection for spur length were explored in *P. lacera* (Little *et al.* 2005).

The remaining evolutionary studies have focused on Section *Platanthera* – specifically, on the classic Eurasian pairing of *P. bifolia* and *P. chlorantha*. The former has been used to

\*e-mail: r.bateman@rbgkew.org.uk

investigate variable selection for male and female function (Maad 2000; Maad & Alexandersson 2004), while the latter has yielded details of nectar secretion and resorption (Stpiczynska 2003a, b). Perhaps the most interesting investigations have compared and contrasted aspects of the two species. A few studies have explored the potential of these species for non-adaptive macroevolution through saltation (a heritable, genetic or epigenetic modification that is expressed as a profound phenotypic change across a single generation and results in a potentially independent evolutionary lineage), notably via modification of the labellum to resemble the sepals (a transition termed pseudopeloria: Bateman 1985; Bateman & DiMichele 2002; Bateman & Rudall 2006b). However, most studies have focused on aspects of presumed adaptation. Nilsson (1978, 1983) used morphometric measurements from both herbarium collections and *in situ* populations in Scandinavia to quantify the features that encouraged placement of the pollinia on the tongues of pollinating moths in *P. bifolia* and the eyes of other, closely related moths in *P. chlorantha*. As predicted from morphological observations by Summerhayes (1951), optimization of morphological characters across ITS-based phylogenies suggested that the eye attachment of pollinia in *P. chlorantha* evolved from the more conventional proboscis attachment that characterizes *P. bifolia* (Hapeman & Inoue 1997; Bateman 2005). The two species differ in fragrance chemotypes (Tollsten & Berstrom 1993). Admixed populations of these two species containing phenotypic “intermediates” (i.e. presumed hybrids) have been studied in the Low Countries (Claessens & Kleynen 2006) and Scandinavia (Nilsson 1985), where the efficiency of pollinium import and export between the two species was estimated (Maad & Nilsson 2004). Thus far, DNA sequencing studies have revealed extraordinary similarity between the two taxa, despite the fact that they are morphologically distinct and so universally accepted as *bona fide* species (Bateman 2005; Bateman, James & Rudall in prep.).

Most of the evolutionary scenarios emerging from these studies paid particular attention to the key role of the morphology of the column (and, to a lesser degree, of the pollinia themselves) in placing the pollinia at appropriate locations on visiting Lepidoptera, and on the role of the nectar-secreting spur in initially

attracting those pollinators and persuading them to probe sufficiently deeply to acquire pollinia. Only a minority of terrestrial orchid species in the Northern Hemisphere offer their pollinators a genuine food reward (usually nectar); the remainder deceive insects into mistakenly believing that they will be rewarded with food or, less frequently, sex (e.g. van der Cingel 1995; Cozzolino & Widmer 2005). Although there have been repeated evolutionary transitions between the rewarding and non-rewarding conditions (Bateman *et al.* 2003; Cozzolino & Widmer 2005), the genus *Platanthera* appears to be uniformly rewarding (Hapeman & Inoue 1997), substantial quantities of nectar being secreted by labellar spurs (Stpiczynska 2003a, b) that differ considerably in mean length among the many species in the genus.

#### RATIONALE OF THE PRESENT STUDY

Not surprisingly, given their reputedly strong influence over pollination efficiency, the spurs of orchids in general (Darwin 1877; Rudall & Bateman 2002; Box *et al.* in press), and of *Platanthera* in particular (Nilsson 1983, 1985; Maad & Nilsson 2004; Little *et al.* 2005; Claessens & Kleynen 2006; J. Maad pers. comm. 2007), have long received considerable attention from evolutionary biologists.

The present authors independently established projects that involved extensively measuring the labellar spurs of the Greater Butterfly-orchid (*Platanthera chlorantha* (Custer) Rchb.) and Lesser Butterfly-orchid (*P. bifolia* (L.) Rich.) in the UK. Working with Paula Rudall, Bateman began his project in 2003 and focused on southern England (Bateman 2005). This study, which sought to better delimit the presumed boundary separating the two species, involved measuring 42 characters from each plant, which limited the number of individuals that could realistically be studied. In addition, many of the plants subjected to morphometric analysis were also subjected to sequencing of the nuclear ribosomal ITS region (Bateman, James & Rudall in prep.). Bateman subsequently added some data on spur lengths of *P. bifolia* from northwest Scotland.

Sexton's project began in 2005 and focused on south-central Scotland (Sexton & McQueen 2005). Having summarized prior evidence of considerable variation in the spur length of *P. chlorantha* across Europe, this project sought to uncover and explain local variation in spur

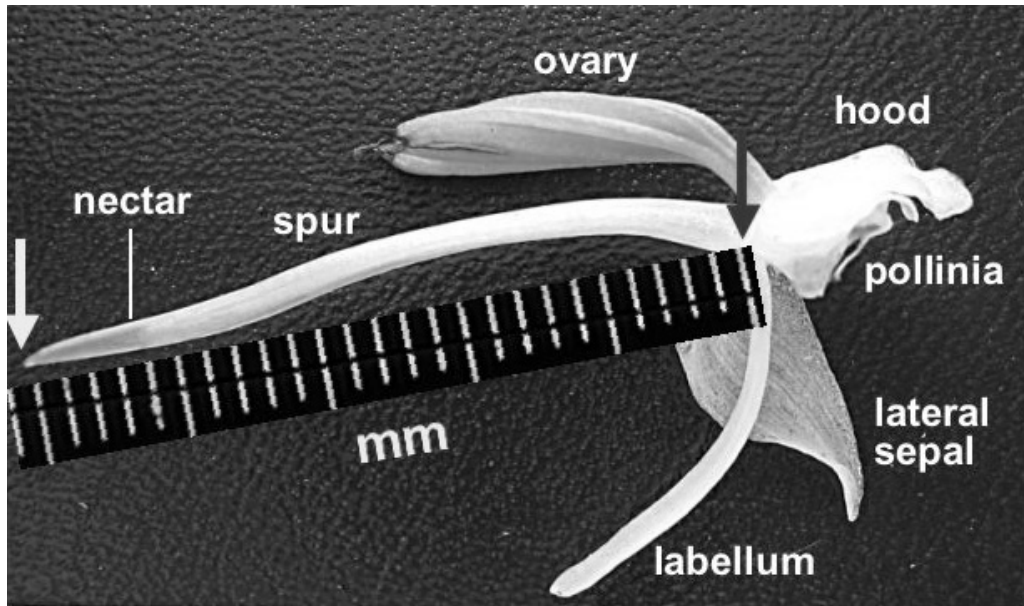


FIGURE 1. Lateral view of flower of *Platanthera chlorantha*, illustrating the method used to measure spur length in the present study (note that in the actual study the flowers remained attached to their parent plants). Photo: Roy Sexton.

lengths within the chosen study area. Other likely factors attracting pollinators to the flowers, including the volume of nectar and composition of scent, were considered. Success in attracting pollinators was judged indirectly, through frequency of fruit set and frequency of viable seed within individual fruit (Sexton & McQueen 2005).

It soon became evident that, given their contrasting geographical foci, our respective datasets on spur length would increase considerably in value if pooled. In addition, it was clearly highly desirable to extend the geographic coverage of the project to encompass the whole of the British Isles, and ideally to expand into mainland Europe. The most obvious way of achieving these more ambitious goals within a reasonable timescale was to involve substantial numbers of inexperienced researchers (often described as ‘interested amateurs’ or, more recently in science policy circles, “citizen scientists” – we recognize that neither term is especially appropriate). Therefore, in April 2007 we asked members of the UK’s Hardy Orchid Society to measure *Platanthera* populations in their local area and donate the resulting data to us for collation (Bateman & Sexton 2007).

#### MATERIALS AND METHODS

We made considerable efforts to formulate clear instructions that contained sufficient detail to ensure consistency of measurement among different analysts (Bateman & Sexton 2007). Spur length is a more obviously self-defining parameter than, for example, spur width (see below). It is most readily measured by placing a 150 mm steel rule against the back of one of the lateral sepals (Fig. 1), with the flower still firmly attached to the inflorescence. As flower size has recently been shown to decrease considerably from the base to the apex of the inflorescences of several European orchid species (Bateman & Rudall 2006a), we specified that the measured flower should be chosen from the middle of the inflorescence and be fully open. We requested that analysts gather a sample of 20 individuals, though the small size of most *Platanthera* populations in the UK meant that some sample sizes were appreciably smaller (in partial compensation, other samples were substantially larger). Datasets were submitted electronically by most analysts, and were collated by Bateman in an Excel spreadsheet immediately after the 2007 flowering season had ended.

TABLE 1. SIGNIFICANCE TESTS OF COMPARISONS OF DUPLICATED ESTIMATES OF MEAN SPUR LENGTH OBTAINED FROM THE SAME POPULATION OF *PLATANATHERA*

Locality	Species	Analyst(s) and Year(s)	Difference between mean values (mm)
<i>(a) Same analyst, same year (4)</i>			
Wolstenbury	<i>chlorantha</i>	Stott/Pearce, 2007	0.79
Latterbarrow	<i>chlorantha</i>	Fenton, 2007	1.83
Levens Wood	<i>chlorantha</i>	Fenton, 2007	0.37
Quoigs Meadow	<i>chlorantha</i>	Sexton, 2007	0.42
<i>(b) Different analysts, same year (5)</i>			
Newtimber Hill	<i>chlorantha</i>	Stott vs Pearce, 2007	1.12
Latterbarrow	<i>chlorantha</i>	Gendle vs Fenton, 2007	0.48
A590/A591 junction	<i>chlorantha</i>	Gendle vs Fenton, 2007	2.33*
Waitby Greenriggs	<i>chlorantha</i>	Gendle vs Fenton, 2007	0.11
Kernsary	<i>bifolia</i>	Bateman vs D.+C. Hughes, 2007	0.17
<i>(c) Different analysts, different years (1)</i>			
Pewsey	<i>bifolia</i>	Bateman, 2004 vs A. Hughes, 2007	1.35
<i>(d) Same analyst, successive years (10)</i>			
Bomains Meadow	<i>chlorantha</i>	Sexton, 2005 vs 2006	0.68
Bomains Meadow	<i>chlorantha</i>	Sexton, 2006 vs 2007	0.91
Plean Park	<i>chlorantha</i>	Sexton, 2005 vs 2006	1.56
Plean Park	<i>chlorantha</i>	Sexton, 2006 vs 2007	1.06*
Kippen (A)	<i>chlorantha</i>	Sexton, 2006 vs 2007	2.26**
Kippen (B)	<i>chlorantha</i>	Sexton, 2006 vs 2007	1.65*
Wester Balgair	<i>chlorantha</i>	Sexton, 2006 vs 2007	1.34*
Braeleny Farm	<i>chlorantha</i>	Sexton, 2006 vs 2007	0.99
Quoigs Meadow	<i>chlorantha</i>	Sexton, 2006 vs 2007	0.49
Quoigs Meadow	<i>bifolia</i>	Sexton, 2006 vs 2007	2.21*
<i>(e) Same analyst, same year, different stages of anthesis (1)</i>			
Carnan	<i>bifolia</i>	Bateman, 2007	3.38**

Significance: \* =  $p < 0.05$ , \*\* =  $p < 0.01$

## RESULTS

The results of the study are summarized in Appendix 1 and Tables 1–2. By the close of the 2007 field season, our combined database of spur lengths contained 120 datasets (49 for *P. bifolia*) totalling 1876 individual plants (624 for *P. bifolia*); datasets ranged in sample size from a single plant to 118 plants (Appendix 1). Of these 120 datasets, 33 were generated by Bateman, 26 by Sexton and the remaining 61 by other recorders (notably two from southern England and five from Austria by Tony Hughes, four from southern England by Katherine Stott and David Pearce, 11 from Cumbria by Alan Gendle, a further four from Cumbria by James Fenton, seven from west-central Scotland by Sarah Longrigg, and 15

from Scotland by David and Christine Hughes). The net result was clustering of data-points in the Alps, southern England, Cumbria, southern Scotland and northeastern Scotland (interestingly, the middle three areas constitute the geographical “hot-spots” for *P. chlorantha* explicitly identified in the UK by Foley & Clarke [2005]).

Fifteen datasets consisted of only one or two plants, and so yielded data of very limited value. Each of the remaining 105 datasets yielded values for the mean and sample standard deviation, omitting a few rare plants wherein spur development was clearly seriously retarded. A further 21 datasets (four for *P. bifolia*) duplicated other datasets based on the same locality (Table 1). Most duplications of measurement were intentional,

TABLE 2. COMPARISON OF SPUR LENGTHS FROM THE LOWERMOST, MIDDLE AND UPPERMOST FLOWERS IN TEN PLANTS OF TWO POPULATIONS OF *PLATANThERA* FROM SOUTHWEST ENGLAND

Measurement	Locality					
	Bull's Cross			Pewsey		
	Mean	SD	CV	Mean	SD	CV
Species	<i>bifolia</i> + hybrids			<i>bifolia</i>		
Flowers per inflorescence	14.20	3.08	21.7	11.30	3.56	31.5
Spur lengths (mm)						
lowermost	23.50	1.73	7.4	18.80	1.75	9.3
middle	23.80	1.58	6.6	19.75	1.32	6.7
uppermost	22.67	2.19	9.7	18.75	3.60	19.2

None of the pairwise comparisons of positions on the inflorescence is statistically significant. (Data collected by Tony Hughes).

though three duplicated datasets from Cumbria, one from Wiltshire and one from northeast Scotland resulted from wholly accidental (but nonetheless welcome) convergences between two independent analysts (Gendle vs Fenton, Bateman vs A. Hughes, Bateman vs D. and C. Hughes, respectively). Five duplications were conducted by different analysts in the same year, one by different analysts in different years (Bateman vs A. Hughes), four by the same analyst in the same year, and ten by the same analyst (Sexton) in different years. Another kind of duplication study (Bateman) compared plants within the same population that had at least 50% open flowers with those where the lowermost flower was just beginning to open. The last category of duplication study (by A. Hughes) compared the spur lengths of flowers at the specified position in the middle of the inflorescence with the lowermost and uppermost flowers of the same inflorescence, focusing on two populations of *P. bifolia* from southwest England (Table 2).

These duplicate datasets, summarized in Table 1, allowed us to infer how much of the observed variation in spur length could be attributed to measuring error or non-genetic (i.e. environmental and/or developmental) factors rather than genetic factors. Subsequently excluding duplicated datasets (retaining the dataset from the site that was based on the largest sample size) left 83 datasets that could be statistically analyzed and so used to seek geographically related patterns in spur length variation for each species.

## DISCUSSION

### INTRAPOPULATION VARIATION AND POTENTIAL ANALYTICAL ERROR

Values for mean and sample standard deviations were used to calculate coefficients of variation for each usable dataset. Coefficients of variation ranged from 6 to 15% and were typically 8–12%; this moderate level of variation relative to other features of the plant suggests a considerable degree of functional latitude rather than the constraints inherent in strong selection pressure (cf. Bateman, James & Rudall in prep.). There were, however, two exceptions. The unusually low coefficient of variation (2%) for Murroch is probably an artefact of the unusually small sample size (Appendix 1). However, the exceptionally high coefficient (23%) for the Bix Bottom population of *P. bifolia* may be biologically meaningful, indicating that hybridization at the site is more extensive than was previously supposed, and that some of the plants designated “pure” by us on the basis of overall morphology may actually have experienced introgression (see below). Indeed, since this statement was first written, multivariate analysis required re-identification of one of the Bix plants originally assigned to *P. bifolia* as the hybrid between *P. bifolia* and *P. chlorantha*.

Comparison of mean values and sample standard deviations for different phases of measurement of the same population (Tables 1,

2) provided much useful information. Four populations were measured by the same analyst in the same year, the two phases of measurement typically occurring about a week apart. The mean values differed on average by 3.2% of the total spur length (range 1.3–6.3%), much of which can presumably be ascribed to sampling error. None of the disparities between these pairs of means was statistically significant (Table 1).

A further five populations were analyzed by different analysts in the same year; four pairs of measurements were taken within a week of each other but the fifth pair, at Kernsary, were separated by a remarkable three-week period. The mean values differed on average by only 0.4% more than when the same analyst collected both tranches of data (3.6%, range 0.6–7.8%), suggesting that our efforts to ensure consistency among analysts in the way that they measured spurs had been successful (but see below). Admittedly, one such population (A590/A591 junction near Kendal, Cumbria) showed a difference between the two population means that was marginally statistically significant, but contrasting subpopulations may have been sampled in this extensive, linear roadside population, which encompassed a range of habitats.

More interesting are ten comparisons made by Sexton in south-central Scotland, where in each case the same population was sampled (in most cases extensively) in successive years. These comparisons showed typical mean length differences of 5.1% (range 1.8–10.3%), and five of the ten comparisons were statistically significant, one strongly so (Table 1). In four of these five cases, spurs were on average shorter in 2007 than in 2006, suggesting that there is a significant environmental influence on mean spur length from year to year; presumably, this discrepancy primarily reflects local weather conditions. The single comparison of means determined by different analysts in different years (Pewsey) also fell naturally into this category; although strictly not statistically significant, the two means differed by 6.8%.

Another interesting experiment was conducted by Katherine Stott and David Pearce, who independently measured the same small number of plants at Newtimber Hill, Sussex. For most plants examined their respective estimates of spur length differed by at most only one unit of measurement (i.e. 0.5 mm), but in a few cases measurements differed by up to a worrying 3 mm (i.e. c. 10% of the

overall length of the spur in question). We initially suspected that, in these cases of divergence between analysts, different flowers had been measured on the same inflorescence. Our original emphasis on measuring flowers at consistent position in the middle of the inflorescence was prompted by the study of Bateman & Rudall (2006a), who demonstrated that spur and especially labellum dimensions decreased significantly from the base to the apex of the inflorescences of several species of European orchids. In contrast, these trends were barely evident in the single inflorescence of *Platanthera chlorantha* studied by them; indeed, spur length appeared to peak slightly in the middle of the inflorescence (Bateman & Rudall 2006a, fig. 9).

This pattern seemed most likely an artefact. However, data collected for the present study by A. Hughes show that this is in fact a genuine trend in *P. bifolia* that can be detected (albeit without statistical significance) at the population level. Table 2 shows maximum spur length in the centre of the inflorescence in both study populations, with lower coefficients of variation than are evident in either the lowermost or the uppermost flowers. We suspect that the unusually large, widely spaced flowers of these *Platanthera* species permit the development of a cylindrical rather than a conical inflorescence. Whatever the reason, there is evidently less risk of positional differences causing measuring error in *Platanthera* than in most other European orchids.

Our data suggest that the largest potential source of error is the stage of anthesis reached by the inflorescence. At the Carnan (Benbecula) population of *P. bifolia*, Bateman sampled 20 inflorescences where at least the lower half of the inflorescence bore fully open flowers, and a further 10 inflorescences where even the lowermost flowers were only just beginning to open or were about to open. We estimate that anthesis in the two cohorts of inflorescences only differed by less than a week, yet there was a statistically highly significant difference of 24% in their respective mean spur lengths (10.9 mm vs 14.2 mm: Table 1). This observation supports recent scanning electron microscope studies which suggested that spur elongation occurs surprisingly late in the development of the flowers of European orchids (Box *et al.* in press); it clearly demonstrates that the measured flower must be fully open before relevant and reliable spur dimensions can be obtained.

TABLE 3. RANGES OF SPUR LENGTHS GIVEN FOR *PLATANThERA* SPECIES IN FLORAS OF THE BRITISH ISLES AND CONTINENTAL EUROPE

Flora/monograph	Spur length (mm)	
	<i>P. bifolia</i>	<i>P. chlorantha</i>
<i>(a) British Isles</i>		
Godfery (1933)	15–25	25–30+
Clapham <i>et al.</i> (1962)	15–20	19–28
Sell & Murrell (1996)	15–20	19–28
Stace (1997)	15–20	19–28
Harrap & Harrap (2005)	13–23(–27)	19–35
<i>(b) Europe</i>		
Webb (1980)	25–30	18–27
Davies <i>et al.</i> (1983)	25–30	18–27
Baumann & Künkele (1988)	13–40	20–40 <sup>1</sup>
Buttler (1991)	12–23	18–40
	23–41 <sup>2</sup>	
Bournérias & Prat (2005)	20–30	20–45
Delforge (2006)	13–23	18–41
	20–41 <sup>2</sup>	

<sup>1</sup>Given erroneously in the original text as 2–4 mm.

<sup>2</sup>Both authors distinguished a short-spurred calcifugic ecotype in northwest Europe from a longer-spurred calcicolous ecotype occurring further south and east.

Lastly, conducting our own measurements led to a better appreciation of qualitative differences in spur morphology between the two species. In particular, Ettliger (1997) noted that the spur of *P. bifolia* appears to be a simple cylinder, whereas that of *P. chlorantha* is expanded towards the apex. We found spurs of both species to be bilaterally compressed (i.e. oval rather than circular in transverse section), but in *P. chlorantha* there was also a significant dorsiventral increase in wall thickness in the distal half to two-thirds of the spur. This often conferred on the spurs of *P. chlorantha* a gently sigmoid shape when viewed laterally (Fig. 1); these then require straightening prior to measurement.

#### PREVIOUS REPORTS OF SPUR LENGTH IN *P. CHLORANTHA* AND *P. BIFOLIA*

Appendix 1 and Figure 3 summarize our extensive data on *Platanthera* spur lengths for the British Isles and our more sporadic data obtained from populations occurring in Continental Europe. For comparison, in Table 3 we have abstracted ranges of spur lengths given for the two species in several widely-general florals and orchid monographs

covering (a) the British Isles and (b) part or all of Continental Europe.

Within the British Isles, Sell & Murrell (1996) and Stace (1997) followed the dimensions given for spurs of both species by Clapham *et al.* (1962), but their ranges are too narrow for *P. bifolia* and are skewed toward the lower end of the scale for *P. chlorantha*, failing to accommodate the long-spurred (mean >32 mm) populations that predominate in southern England (Fig. 3). Only the ranges given by Harrap & Harrap (2005) match our own observations for both *Platanthera* species, though we have not yet located any of the short-spurred (mean <23 mm) populations of *P. chlorantha* reported by all authors except Godfery (1933).

Moving on to mainland Europe, Webb (1980, echoed by Davies *et al.* 1983) gave lower and upper limits to *P. chlorantha* that were less than those for *P. bifolia*, apparently having given undue emphasis to the extraordinary reversal in this character reported along the Baltic coast by Nilsson (1978, 1985). The ranges fail utterly to accommodate British populations. The remaining authors (Baumann & Künkele 1988; Buttler 1991; Bournérias &

Prat 2005; Delforge 2006) succeeded in encompassing variation in both species, but only by offering ranges of spur length that are so broad that they lose any potential for being diagnostic of either species (and even then, Bournérias & Prat failed to accommodate the lower end of the spectrum of spur length recorded by us in both British and Continental *P. bifolia*).

Perhaps the most interesting decision was that taken by Buttler (1991) and Delforge (2006) to separate two putative ecotypes of *P. bifolia*, partly on the basis of ecological preference and partly on the basis of vegetative vigour and spur length. Delforge (2006) offered slightly more liberal spur-length ranges than Buttler (1991), allowing the northwestern, later flowering, calcifugic ecotype (subsp. *graciflora* Bisse, ? = var. *robusta* Seemen) to extend from 13–23 mm and the southern and eastern calcicolous ecotype to cover the range 20–41 mm. However, calcicolous populations from the UK approximate both the upper boundary of the smaller ecotype and the lower boundary of the larger ecotype (cf. Appendix 1 and Table 3). In Continental Europe, the four calcicolous populations of *P. bifolia* measured by Bateman & Rudall in southeastern France occupy the lower part of the range of the calcicolous ecotype, whereas the calcicolous population measured by Sexton in the Dolomitic Alps of northeastern Italy shows a typical “calcifugic” mean spur length of 17 mm. At best, the delimitation of these infraspecific taxa clearly requires further refinement.

The above discussion may appear to have become enmeshed in unnecessarily fine details. However, our aim is to address a broader underlying theme. Specifically, spur length is in fact diagnostic of the two northern European species of *Platanthera*, but only if the latitude of populations is taken into account (Fig. 3). When latitude is not taken into account, the majority of the published ranges of spur length either fail to accommodate a substantial proportion of *Platanthera* populations, if they are too narrow, or fail to suggest any diagnostic potential, if they are too broad. We suspect that similar patterns are commonly reflected in quantitative characters presented in diagnoses of herbarium-based floras. There is no substitute for access to geographically and ecologically extensive field data.

#### MUTATION

Visual inspection of the data matrix suggested that frequencies of both presumed hybridization and presumed mutation radically reducing spur length were surprisingly low.

With regard to putative mutation, of 1876 plants measured, only four plants (0.21%), apportioned equally between *P. chlorantha* and *P. bifolia*, yielded abnormally short spurs (defined as those less than 60% of the mean length for the population). This result contrasts with, for example, data for *Gymnadenia conopsea* and its close relatives, where the frequency of abbreviated spurs is substantially greater and has been inferred as a likely driver of speciation within the genus (Bateman 2005). Pseudopeloric individuals of *P. chlorantha*, possessing only simplified sepal-like labella bearing greatly reduced (essentially vestigial) spurs, have previously been reported from Keltneyburn, near Perth (averaging approximately 3% of the population: cf. McKean 1982; Bateman 1985) and Kennishead, Glasgow (averaging approximately 6% of the population: Dickson 1990). During the present study in 2007, Sexton identified 21 widely distributed pseudopeloric individuals out of 802 *P. chlorantha* flowering at Bomains Meadow, Falkirk (i.e. 2.6%).

Recent assertions that these pseudopeloric plants could constitute incipient species (Rudall & Bateman 2002; Bateman & Rudall 2006b) have arguably been weakened by Sexton’s observation that the Bomains mutants verge on being male-sterile. We suspect that they are epimutants, reflecting hypermethylation of the DNA bases rather than non-synonymous mutation of the bases themselves; a project is underway to test this hypothesis (Bateman, Rudall, Kidner & James, unpublished).

#### HYBRIDIZATION

Only five (0.27%) of the measured *platantheras* were accused by us of being hybrids between the two study species, identified primarily on the basis of their intermediate pollinaria positions. All five plants were reported by Bateman & Rudall: four from Bix Bottom, Oxfordshire (Fig. 2), plus one less certain identification from St Anne’s Chapel, Cornwall (regrettably, no data were obtainable from its gynostemium). Disappointingly, our published request for data from additional mixed



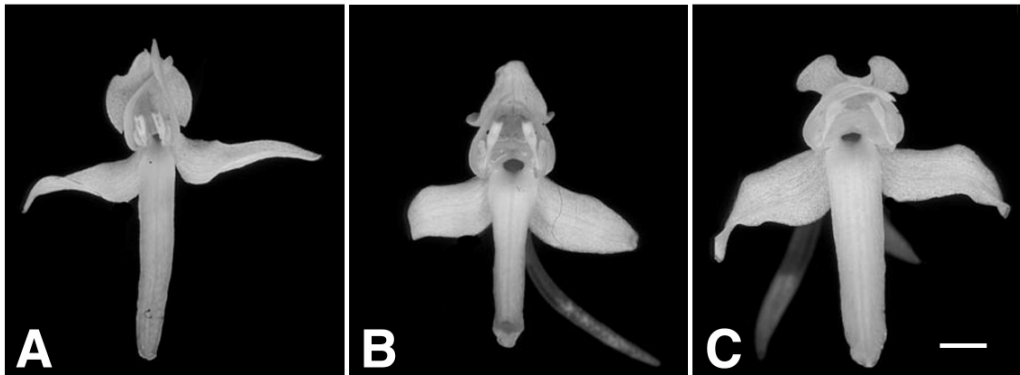


FIGURE 2. Flowers of *Platanthera bifolia* (A left), *P. chlorantha* (C right) and a putative hybrid (B centre) from the Warburg Reserve, Bix Bottom, Oxfordshire. Scale bar = 5 mm. Photos: Richard Bateman.

populations (Bateman & Sexton 2007) yielded only one new dataset (gathered near Perth, by Sexton), and no putative hybrids were reported from this locality. On the other hand, the Bull's Cross locality studied by Tony Hughes contained convincing hybrids, though they were not reliably separated from their co-occurring parents.

The hybrids recorded from southern England by Bateman & Rudall (Fig. 2), Tony Hughes (Hughes 2007) and Sarah Whild (Whild & Lockton 2007) during the present survey have some significance, as most authors of floras have been uncharacteristically reluctant to accept the occurrence of hybridization between the two species of *Platanthera*, especially within the British Isles. For example, Godfery (1933) reported an occurrence of a putative hybrid from Sligachan, Skye, but then qualified this observation by noting that the "hybrid" had co-occurred with a pseudopeloric mutant (*sensu* Bateman 1985). This sceptical viewpoint was adopted by most subsequent authors, who argued that evidence of hybridization in British *platantheras* was inconclusive and that most if not all putative hybrids were actually aberrant individuals of one of the parents (cf. Hunt 1975; Sell & Murrell 1996; Stace 1997, in press; Foley & Clarke 2005; Harrap & Harrap 2005). Further questionable arguments frequently deployed against the existence of hybrids included supposed differences in habitat preference, peak flowering period and, most bizarrely, the presumed reduced fitness of the hybrids caused by suboptimal presentation of the viscidia to pollinators. (This is actually an argument against introgression, rather than against hybridization *per se*, since the key assumption is that primary hybrids could only act as pollen recipients, not as pollen donors

[Nilsson 1983]. However, recent field observations question this assumption, as moths have been photographed removing pollinia from hybrids: Claessens *et al.* in press.)

In adopting this sceptical viewpoint, these authors were echoing Charles Darwin, who took considerable exception to the decision of Bentham & Hooker (e.g. 1886) to treat the two British *platantheras* as mere varieties of a single species – a decision taken on the grounds that "intermediate" forms occurred between them (cf. Summerhayes 1951). Darwin was driven to uncharacteristically waspish exaggeration in his opposition, stating that "the two forms differ in a large number of characters, not to mention general aspect and the stations inhabited", and that "these two forms certainly differ from one another more than do most species belonging to the same genus". Having noted that loose waxy scales on the heads of visiting moths most likely confine placement of pollinia to the proboscis (*P. bifolia*) or eyes (*P. chlorantha*), Darwin (1877, pp. 73–4), apparently prompted in part by Muller (1865), concluded that there is no "doubt that the Larger and Lesser Butterfly Orchids are distinct species, masked by close external similarity" (thereby somewhat undermining his own earlier statement regarding their appreciable morphological distinctness). Given the continuing uncertainties over the nature of morphological intermediates between these species, the surprising failure thus far of molecular systematists to identify genetic markers that reliably separate the two species is particularly unfortunate – indeed, it once again raises questions regarding the now generally accepted status of these taxa as separate species (Bateman 2005; Bateman *et al.* in prep.).

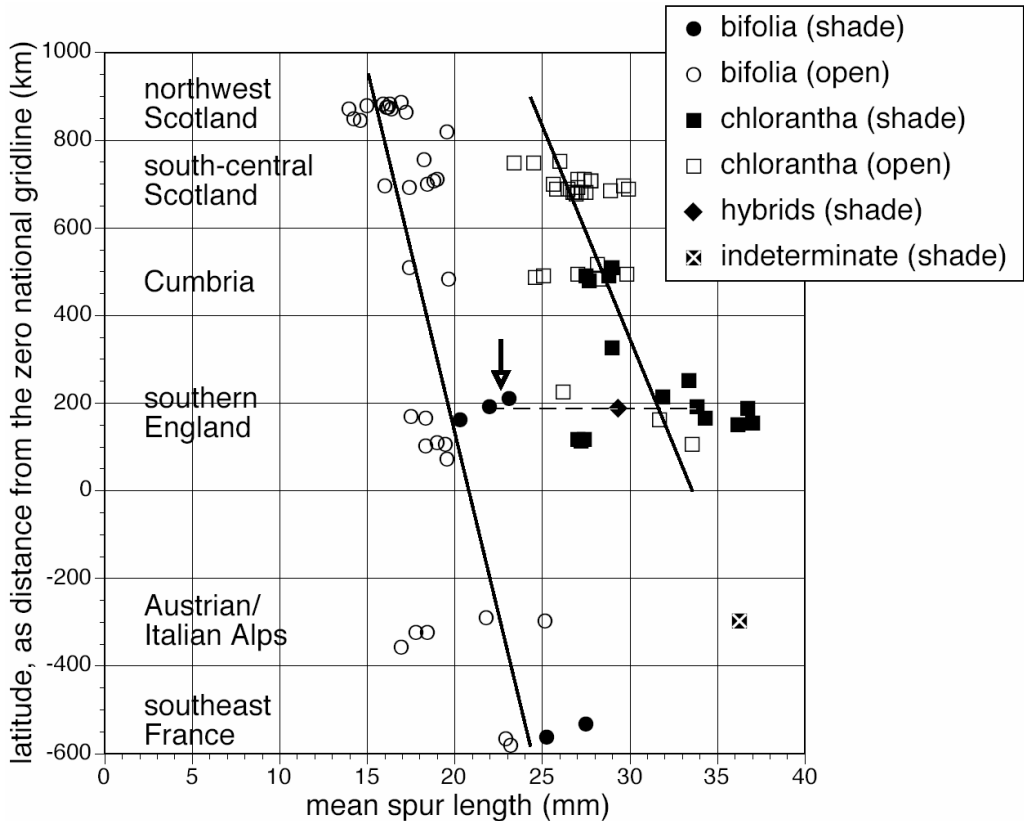


FIGURE 3. Mean spur length (mm) regressed against latitude for populations of *Platanthera bifolia* (left,  $n = 38$ ,  $r^2 = 0.56$ ) and *P. chlorantha* (right,  $n = 43$ ,  $r^2 = 0.42$ ). Shade and open habitats are also distinguished. The dashed line connects mean values for both parents and the putative hybrids at the Bix Bottom locality; the two juxtposed arrowed populations of *P. bifolia* show evidence of introgression. The Alpine population of three flowering plants labelled “indeterminate” was reported as *P. bifolia* but has a mean spur length more characteristic of *P. chlorantha*; this, together with the hybrid group, was omitted from the regression analysis.

Returning to the present study, the putative hybrids from Bix had spur lengths intermediate between those of the parental species co-existing at the two relevant localities (Appendix 1, Fig. 3). Indeed, we suspect that hybridization in the Bix and Bull’s Cross populations may be more extensive than we originally believed, since spur-length measurements for supposedly pure plants of *P. bifolia* in these two populations are the longest of any in southern England (Fig. 3), suggesting the genetic influence of co-occurring *P. chlorantha*. Similar observations of spur intermediacy in hybrids were made on larger numbers of putative hybrids of *P. chlorantha* × *bifolia* (= *P. × hybrida* Bruegger) along the Baltic coast of Sweden by Nilsson (1985), though here *P. bifolia* typically has spurs that are substantially longer than those of *P.*

*chlorantha* – an extraordinary character reversal relative to the more typical condition (Bateman 2005). Indeed, intermediacy of spur dimensions has characterized all of the many quantitative case-studies of hybridization among spurred European orchids known to us (reviewed by Bateman & Haggard in prep.). This reliable intermediacy renders extraordinary the report of extensive hybridization in two mixed populations of *Platanthera* in the Netherlands, where the putative hybrids show longer average spur lengths (32 mm) than co-existing populations of both *P. chlorantha* (26 mm) and *P. bifolia* (23 mm) (Claessens & Kleynen 2006; Claessens *et al.* in press). It seems likely that the evolutionary processes operating in these ambiguous Dutch populations are more complex than simple introgression.

## GEOGRAPHICAL AND ECOLOGICAL TRENDS

Prior to the present survey, we wrote that “as expected, spurs of *P. chlorantha* were typically considerably longer than those of *P. bifolia* (averaging 34 mm vs 19 mm) ... Data from Scotland (very limited in the case of *P. bifolia*) suggest that *P. bifolia* retains its English dimensions there, whereas the spurs of *P. chlorantha* are significantly shorter” (Bateman & Sexton 2007, p. 61). The addition of substantial datasets from southeast France, the Alps, Cumbria and Scotland requires substantial revision of that preliminary viewpoint. Regression of mean spur length against latitude (Fig. 3) revealed strong correlation coefficients, especially for *P. bifolia* ( $r^2 = 0.56$ , vs 0.42 for *P. chlorantha*), and suggested similar trends in both species; each shows a reduction in mean spur length of approximately 2.2% per 100 km of increasing latitude. Figure 3 also demonstrates that spur length distinguishes the two species only if latitude is taken into account, as populations of *P. bifolia* from the Vercors in southeast France show similar mean spur lengths to populations of *P. chlorantha* occurring approximately 1300 km to the north in southern Scotland (c. 26 mm). Those occurring a little further north of the Vercors in the Alps also fit into the general trend, though the spread of populations appears relatively wide (even discounting the exceptional mean length of 35 mm obtained in Pettneu, western Austria, which is rendered suspect by both the unusually small sample size of three plants and uncertain species assignment).

The obvious temptation is to interpret these latitudinal trends as reflecting adaptation of the spur to proboscis length in the respective pollinators of these two orchid species. However, data gathered by Bateman and co-workers from all major features of the plants of both species suggest that, at least in southern England, most features of *P. bifolia* are on average only two-thirds of the dimensions of equivalent features in *P. chlorantha* (Bateman 2005; Bateman *et al.* in prep.). A similar ratio was evident among floral dimensions recorded in south-central Scotland by Sexton (unpublished). If this ratio is repeated across most of the (largely coincident) ranges of the two species, the latitudinal gradient in spur length observed in this study (Fig. 3) could simply represent an allometric ratio across most organs of the plant that is merely reflected in spur length. In this case, the overall size

differences could, for example, ultimately reflect the greater relative degree of insolation impacting on the (usually paired) leaves of plants at lower latitudes (though note that, during the summer months when *Platanthera* leaves are fully expanded, day length at least is longer in higher latitudes). Spur length would then be inferred to be under at most relatively weak and/or sporadic selection pressure (a conclusion reached, on the basis of more sophisticated data, for the North American *P. lacera* by Little *et al.* 2005).

Furthermore, re-examination of the present data (Fig. 3) suggests that application of more complex algorithms for fitting curves to the spur-length data might ultimately achieve a more exact fit. For example, the spur-length data for *P. bifolia* could legitimately be interpreted as showing a constant size of approximately 18 mm for most of the latitudinal range, deviating only at the two extreme ends of the latitudinal gradient (i.e. upwards in southeast France and downwards in northwest Scotland). Similarly, data for *P. chlorantha* could be considered to be constant for most of its latitudinal range, plateauing at approximately 28 mm (a similar figure also characterizes this species in the Low Countries: Claessens & Kleynen 2006), but to have deviated upward to values of 32–37 mm over much of southern England (a mean spur length of 32 mm was also reported for a co-latitudinal population of *P. chlorantha* from Poland by Stpiczynska 2003a). Positive deviations in the spur length of *P. bifolia* (mean values 27–34 mm) characterize much of Scandinavia, falling to 18–21 mm in the birch forests of the north (Nilsson 1983, 1985; Tollsten & Berstrom 1993; Maad 2000). Moreover, on the Baltic island of Oland there has been divergence between short-spurred populations of *P. bifolia* in grassland (means 19–23 mm) and those occupying deciduous woodland (means 28–40 mm) (Nilsson 1983; J. Maad pers. comm. 2007).

This observation suggests that, beyond latitude, the other extrinsic factors that could potentially have influenced spur length are those relating to habitat preference, particularly soils (pH and moisture content) and degree of shade experienced by the orchids. Our present data for soils are inadequate, though there exists much anecdotal evidence that *P. bifolia* is more tolerant than *P. chlorantha* of wet acidic soils. In contrast, we were able to amalgamate the habitat descriptions listed in Appendix 1 into two broad categories of

exposure to light: shaded (woodland and scrub) and open (grassland, heathland and moorland).

Among our sampled populations, shaded populations do not extend northward beyond southern England for *P. bifolia* and beyond northern England for *P. chlorantha*. In the case of *P. bifolia*, within specific latitudinal zones, all populations occurring in shade have longer spurs than those occurring in the open (all occur on the positive side of the regression line in Fig. 3). The pattern is less clear for *P. chlorantha*, for which in addition we presently lack data from Continental Europe. In southern England, shaded populations appear to span a wider range of spur lengths than exposed populations (only shaded populations span the upper end of the range of means, at 34–37 mm). However, no significant difference between shaded and open populations was observed in northern England, and no shaded populations were recorded in Scotland. Nonetheless, potential correlations between average spur length and environmental parameters evidently merit more detailed scrutiny.

Lastly, we note that although some of our study populations of both *Platanthera* species encompassed several hundred flowering plants, the majority were small, many typically yielding less than ten flowering plants in any one year. Where such small effective population sizes pertain, deviation from previous distributions of spur length can be readily achieved via genetic drift, whereas the potential for long-term directional or disruptive selection is greatly reduced (e.g. Tremblay *et al.* 2005). Genetic drift merits consideration in any study of spur-length variation in *Platanthera*, though its random effects might have been expected to obscure the apparent correlation between habitat preference and mean spur length.

#### POLLINATOR SPECIFICITY REVISITED

As noted earlier, past assertions of strong selection pressure on spur length have focused on morphological and ecological data for the two *Platanthera* species in Scandinavia (Nilsson 1978, 1983, 1985; Maad 2000; Maad & Nilsson 2004), where mean spur length of *P. bifolia* in particular appears to show greater regional variation than is evident in our measurements taken elsewhere in western Europe. The Swedish data suggest that strong pollinator specificity among different species of sphingid moth provides a meaningful level

of reproductive isolation separating the two species and different geographic/ habitat races within *P. bifolia*; indeed, the observed mechanism has become a model system of plant–pollinator co-evolution (e.g. Hapeman & Inoue 1997).

The classical model states that *P. bifolia* places its pollinaria anywhere along the length of the moth's proboscis, whereas *P. chlorantha* requires the moth to ram its head into the column in order to attach the viscidia to the surface of its eyes. This contrast in the mechanism of pollinium placement would suggest that spur length should be more critical to the reproductive success of *P. chlorantha* than to that of *P. bifolia*. Setting aside sites that yielded very small sample sizes and those showing evidence of introgression, coefficients of variation for spur length within our study populations range from 5–18% for *P. bifolia* (typically approximately 11%) and 6–14% for *P. chlorantha* (typically approximately 9%; Appendix 1). Thus, if there is a contrast in selective pressure on spur length between the two species it does not appear to be a strong one.

Darwin (1877) and subsequent commentators (e.g. Summerhayes 1951; Nilsson 1983) argued adamantly that only long-tongued moths effect pollination in European *Platanthera*. However, Darwin also persistently rejected observations of other natural historians, notably Müller (1865), that many European orchids did not actually reward their pollinators, whereas subsequent studies have proven Müller right; many orchid species succeed by deceiving rather than rewarding pollinators (e.g. van der Cingel 1995; Neiland & Wilcock 1998; Cozzolino & Widmer 2005). Many authors have noted that species offering genuine rewards to pollinators generally achieve substantially higher frequencies of pollination than do co-existing food-deceptive species (Neiland & Wilcock 1998; Cozzolino & Widmer 2005). However, fewer authors have noted that this comparatively successful pollination rate has not translated into greater numbers of rewarding than non-rewarding species or to greater average size or number of populations per rewarding species. And even fewer authors have noted that there is no obligation on pollinators to accept any reward offered. Surely, an insect that is demonstrably willing to be duped into pollinating an unrewarding orchid will not balk at the prospect of

pollinating a species that offers a reward that the insect cannot satisfactorily exploit?

Having thus hypothesized that at least some occasional pollinators of *Platanthera* would show behaviour patterns that are unlikely to be influenced by spur length, we were briefly excited when our 2007 spur-length survey prompted Tony Hughes (2007) to capture daytime images in Gloucestershire of a distinctive *Oedermera* beetle transporting pollinia across a putatively hybrid *Platanthera* inflorescence at Bull's Cross. Unfortunately, more careful scrutiny suggests that the pollinia in question were obtained from adjacent plants of *Neottia (Listera) ovata*. Feasibility studies using moth traps at *P. chlorantha* sites in south-central Scotland showed that moths with pollinia attached to their eyes were caught fairly readily (Sexton & McQueen 2005). Moreover, John Knowler (pers. comm. 2006) reported that catches included the Beautiful Golden Y (*Autographa pulchrina*), Silver Y (*A. gamma*) and Gold Spangle (*A. bractea*); all three moths are prominent pollinators of *P. chlorantha* in Sweden (Nilsson 1978).

Nonetheless, recent combined population genetic and morphological studies of gene flow in other groups of European orchids also counsel caution when assuming strong pollinator specificity. Even in the notionally most pollinator-specific genus, *Ophrys*, natural hybrids occur among all major species groups, demonstrating both their interfertility and the fact that pollinators frequently transfer pollinia between species (cf. Paulus & Gack 1990; Devey *et al.* in press; S. Cozzolino pers. comm. 2007). In addition, the ability to identify pollinia through DNA sequencing (e.g. Soliva & Widmer 2003) means that we can assess pollinator error rates, which even in *Ophrys* are proving to be relatively high. Also, artificial crosses among *Ophrys* species have demonstrated fertility in F1s, F2s and backcrossed progeny (S. Malmgren pers. comm. 2006; S. Cozzolino pers. comm. 2007). Current (albeit limited) evidence suggests that *P. bifolia* and *P. chlorantha* may similarly introgress whenever they co-exist in substantial numbers in the same or immediately adjacent habitats.

For the present, the jury is out regarding the degree of reproductive isolation enjoyed by the two European *Platanthera* species. Clearly, it is desirable to gather data on the range and frequency of success of pollinators from other regions of Europe to test the long-standing hypotheses of strong specificity.

## CONCLUSIONS

We have confidently identified latitudinal and habitat influences on mean spur length in *Platanthera bifolia* and *P. chlorantha*. Once these factors have been compensated for, *P. chlorantha* maintained spurs approximately 50% longer than those of *P. bifolia* throughout our latitudinal transect, though previous studies (Nilsson 1983, 1985; Maad & Nilsson 2004; Boberg *et al.* 2007; J. Maad pers. comm. 2007) suggest that this apparently linear relationship breaks down in parts of Scandinavia. We recognize that detection of further patterns of regional highs and lows would once again encourage consideration of potential local adaptation of spur length to optimally fit the morphology of co-evolving pollinating moths, and that our observations do not necessarily reject previous selectionist interpretations. However, we do suggest that, in future, potential non-adaptive causes of variation in spur length, relating to allometric relationships with other structures of the plant and/or to vigour of growth influenced by habitat and latitude, are considered alongside more conventional co-evolutionary explanations.

The new data have allowed us to formulate more precise questions regarding the evolution of spur length in the study species, even if they have not provided unequivocal answers. Adding further spur-length data, preferably targeted to fill current geographical lacunae in our trans-European sampling, should help to clarify patterns of variation. However, the above hypothesis of a non-adaptive allometric relationship between spur length and other parts of the plant can only be tested effectively by expanding the study to encompass measurements of other characters from the same plants that are presently yielding spur-length data. Top priority for such supplementary characters would be metric measurements of the column/pollinia, labellum and (typically paired) expanded leaves. Data on the ontogeny of the spurs of *Platanthera*, and on the underlying developmental genetic mechanisms, would also be welcome. Similarly, data on pollinator specificity are needed across the full geographical ranges of both species before meaningful interpretations of the possible relationship between adaptation and speciation can be made.

More generally, we hope that we have successfully demonstrated that field-oriented projects recruiting inexperienced researchers

can be successfully extended beyond the conventional limit of simply recording the presence of a particular species, into the realm of quantitatively measuring features of potentially high biological significance. Returning to butterfly-orchids, we note that it is B.S.B.I.-inspired field surveys that identified *Platanthera bifolia* as one of the most rapidly declining species of flowering plants in the British Isles (Preston *et al.* 2002; Braithwaite *et al.* 2005), and led to a subsequent resurvey of its localities in Scotland that relied largely on “amateur” botanists (Farrell 2006; Lavery 2007). It would therefore be appropriate if inexperienced researchers also contributed further scientific data with the potential to explain those declines. Such an explanation should be facilitated by the co-occurrence of a very closely related species, *P. chlorantha*, that does not appear to be experiencing a rapid decline and therefore constitutes an ideal comparative yardstick. Comparison of rare and/or rapidly declining species with closely related species that are common and/or rapidly expanding is presently an under-exploited approach to exploring the British and Irish flora.

## ACKNOWLEDGMENTS

We warmly thank the following members of the Hardy Orchid Society for donating data to the project (number of datasets is given in parentheses): Peter Daltry (1), Ian Denholm (2), Jonathan Fenton (5), Alan Gendle (11), Lois and Nigel Harbron (2), Tony Hughes (7), Christine and David Hughes (15), David Johnston (2), Harold and Jane Lambert (1), Sarah Longrigg (7), Kathy Stott and Dave Pearce (5), Jonathan Tyler (1) and Iain Wright (1). Several of these contributors showed considerable inventiveness in designing their own supplementary analytical approaches. Salvatore Cozzolino, Jean Claessens and Barbara Gravendeel kindly shared their observations on orchid pollination. Richard Bateman thanks the Botanical Research Fund and Botanical Society of the British Isles for partially supporting his fieldwork through small grants, and is grateful to Johanne Maad, Paula Rudall and an anonymous referee for critically appraising the manuscript.

## REFERENCES

- BATEMAN, R. M. (1985). *Peloria* and pseudopeloria in British orchids. *Watsonia* **15**: 357–359.
- BATEMAN, R. M. (2005). Circumscribing and interpreting closely related orchid species: *Platanthera*, *Dactylorhiza* and the crucial role of mutation. *Journal of the Hardy Orchid Society* **2**(4): 104–111.
- BATEMAN, R. M. & DIMICHELE, W. A. (2002). Generating and filtering major phenotypic novelties: neoGoldschmidtian saltation revisited. In Q. C. B. CRONK, R. M. BATEMAN & J. A. HAWKINS (eds.), *Developmental genetics and plant evolution*, pp. 109–159. Taylor & Francis, London.
- BATEMAN, R. M. & HAGGAR, J. (in prep.). Infrageneric and intergeneric hybridization of *Dactylorhiza* Necker ex Nevski in the British Isles. *Botanical Journal of the Linnean Society*.
- BATEMAN, R. M., HOLLINGSWORTH, P. M., PRESTON, J., LUO, Y.-B., PRIDGEON, A. M. & CHASE, M. W. (2003). Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *The Botanical Journal of Linnean Society* **142**: 1–40.
- BATEMAN, R. M., JAMES, K. E. & RUDALL, P. J. (in prep.). Morphological versus molecular divergence between two closely related species of *Platanthera* (Orchidaceae) in southern England: recent evolution with a strong allometric overprint. *Annals of Botany*.
- BATEMAN, R. M. & RUDALL, P. J. (2006a). Evolutionary and morphometric implications of morphological variation among flowers within an inflorescence: a case-study using European orchids. *Annals of Botany* **98**: 975–993.
- BATEMAN, R. M. & RUDALL, P. J. (2006b). The Good, the Bad, and the Ugly: using naturally occurring terata to distinguish the possible from the impossible in orchid floral evolution. *Aliso* **22**: 481–496.
- BATEMAN, R. M. & SEXTON, R. (2007). Survey of the spurs of European butterfly-orchids. *Journal of the Hardy Orchid Society* **4**(2): 60–63.
- BAUMANN, H. & KÜNKELE, S. (1988). *Die wildwachsenden Orchideen Europas*. Kosmos, Stuttgart.
- BENTHAM, G. & HOOKER, J. D. (1886). *Handbook of the British flora* (5th edn). Reeve, London.
- BOBERG, E., ALEXANDERSSON, R., JONSSON, M., MAAD, J., AGREN, J. & NILSSON, L. A. (2007). Pollinator shifts and evolution of spur length in the moth-pollinated orchid *Platanthera bifolia*. *Abstracts of the Sixth Biennial Conference of the Systematics Association (Edinburgh)*: 69.
- BOX, M. S., BATEMAN, R. M., GLOVER, B. J. & RUDALL, P. J. (in press). Floral ontogenetic evidence of repeated speciation via paedomorphosis in subtribe Orchidinae (Orchidaceae). *Botanical Journal of the Linnean Society*.

- BOURNÉRIAS, M. & PRAT, D., eds. (2005). *Les Orchidées de France, Belgique et Luxembourg* (2nd edn). Mezé.
- BRAITHWAITE, M. E., ELLIS, R. W. & PRESTON, C. D. (2006). *Change in the British flora 1987–2004*. Botanical Society of the British Isles, London.
- BRZSOKO, E. (2003). The dynamics of the island population of *Platanthera bifolia* in the Bierbrza National Park (NE Poland). *Annali Botanici Fennici* **40**: 243–253.
- BUTTLER, K.-P. (1991). *Field guide to orchids of Britain and Europe*. Crowood, Swindon, Wilts.
- CLAESSENS, J. & KLEYNEN, J. (2006). Annehmungen zur Hybridbildung bei *Platanthera bifolia* und *P. chlorantha*. *Journal Europäischer Orchideen* **38**: 3–28.
- CLAESSENS, J., GRAVENDEEL, B. & KLEYNEN, J. (in press). *Cucullia umbratica* L. als Bestäuber von *Platanthera × hybrida* Bruegg. in Süd-Limburg (Niederlands). *Journal Europäischer Orchideen*.
- CLAPHAM, A. R., TUTIN, T. G. & WARBURG, E. F. (1962). *Flora of the British Isles* (2nd edn). Oxford University Press, Oxford.
- COZZOLINO, S. & WIDMER, A. (2005). Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology and Evolution* **20**: 487–494.
- DARWIN, C. (1877). *The various contrivances by which orchids are fertilised by insects* (2nd edn). Murray, London.
- DAVIES, P., DAVIES, J. & HUXLEY, A. (1983). *Wild orchids of Britain and Europe*. Chatto & Windus, London.
- DELFORGE, P. (2006). *Orchids of Europe, North Africa and the Middle East*. A. & C. Black, London.
- DEVÉY, D. S., BATEMAN, R. M., FAY, M. F. & HAWKINS, J. A. (in press). Friends or relatives? Phylogenetics and species delimitation in the controversial European orchid genus *Ophrys*. *Annals of Botany*.
- DICKSON, J. H. (1990). Aberrant Greater Butterfly Orchids (*Platanthera chlorantha*) in the Glasgow area. *Glasgow Naturalist* **21**: 599–602.
- ETTLINGER, M. T. (1997). *Notes on British and Irish orchids*. Published by the author, Dorking, Surrey.
- FARRELL, L. (2006). Lesser Butterfly Orchid – *Platanthera bifolia*. SNH-BSBI-Plantlife survey leaflet, pp. 1–4.
- FOLEY, M. J. Y. & CLARKE, S. (2005). *Orchids of the British Isles*. Griffin Press, Cheltenham, Gloucs.
- GODFREY, M. J. (1933). *Monograph and iconograph of native British Orchidaceae*. Cambridge.
- HAPEMAN, J. R. & INOUE, K. (1997). Plant–pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). In T. J. GIVNISH & K. J. SYTSMAN (eds), *Molecular evolution and adaptive radiation*, pp. 433–454. Cambridge University Press, Cambridge.
- HARRAP, A. & HARRAP, S. (2005). *Orchids of Britain and Ireland*. A & C Black, London.
- HOLZINGER, K. E. & WALLACE, L. E. (2004). Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). *Molecular Ecology* **13**: 887–894.
- HUGHES, A. (2007). Beetlemania. *Journal of the Hardy Orchid Society* **4**(3): 88–90.
- HUNT, P. F. (1975). *Platanthera* Rich. In C. A. STACE (ed.), *Hybridization and the flora of the British Isles*, p. 488. Academic Press, London.
- LAURI, R. (2007). The systematic and phylogenetic study of the subgenus *Piperia* (Orchidaceae) and closely related *Platanthera*. *Botanical Society of America abstracts* (Chicago), p. 241.
- LAVERY, L. (2007). Species Action Framework report on Lesser Butterfly Orchid (*Platanthera bifolia*) [in Scotland]. Unpublished report to Scottish Natural Heritage. 30 pp.
- LITTLE, K. J., DIERINGER, G. & ROMANO, M. (2005). Pollination ecology, genetic diversity and selection on nectar spur length in *Platanthera lacera* (Orchidaceae). *Plant Species Biology* **20**: 183–190.
- MAAD, J. (2000). Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. *Evolution* **54**: 112–123.
- MAAD, J. & ALEXANDERSSON, R. (2004). Variable selection in *Platanthera bifolia* (Orchidaceae): phenotypic selection differed between sex functions in a drought year. *Journal of Evolutionary Biology* **17**: 642–650.
- MAAD, J. & NILSSON, L. A. (2004). On the mechanism of floral shifts in speciation: gained pollination efficiency from tongue- to eye-attachment of pollinia in *Platanthera* (Orchidaceae). *Biological Journal of the Linnean Society* **83**: 481–495.
- MCKEAN, D. R. (1982). *× Pseudanthera breadalbanensis* McKean: a new intergeneric hybrid from Scotland. *Watsonia* **14**: 129–131.
- MÜLLER, H. (1865). Beobachtungen an westfälischen orchideen. *Verhandlungen des Naturalische Vereins für Provinz Rheinlandee Westfälischen Jahrg.* **25**(3): 36–38.
- NEILAND, M. R. M. & WILCOCK, C. C. (1998). Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* **85**: 1567–1581.
- NILSSON, L. A. (1978). Pollination ecology and adaptation in *Platanthera chlorantha* (Orchidaceae). *Botanisk Notiser* **131**: 35–51.
- NILSSON, L. A. (1983). Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich. and *P. chlorantha* (Custer) Reichb. (Orchidaceae). *Botanical Journal of the Linnean Society* **87**: 325–350.

- NILSSON, L. A. (1985). Characteristics and distribution of intermediates between *Platanthera bifolia* and *P. chlorantha* (Orchidaceae) in the Nordic countries. *Nordic Journal of Botany* **5**: 407–419.
- PAULUS, H. F. & GACK, C. (1990). Pollinators as prepollinating isolating factors: evolution and speciation in *Ophrys* (Orchidaceae). *Israel Journal of Botany* **39**: 43–97.
- PRESTON, C. D., PEARMAN, D. A. & DINES, T. D. (2002). *New atlas of the British and Irish flora*. Oxford University Press, Oxford.
- RUDALL, P. J. & BATEMAN, R. M. (2002). Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. *Biological Reviews* **77**: 403–441.
- SELL, P. D. & MURRELL, G. (1996). *Flora of Great Britain and Ireland. 5. Butomaceae–Orchidaceae*. Cambridge University Press, Cambridge.
- SEXTON, R. & MCQUEEN, A. E. D. (2005). Greater Butterfly-orchids: *Platanthera chlorantha* (Custer) Reichenbach. *Forth Naturalist and Historian* **27**: 77–90.
- SOLIVA, M. & WIDMER, A. (2003). Gene flow across species boundaries in sympatric, sexually deceptive *Ophrys* (Orchidaceae) species. *Evolution* **57**: 2252–2261.
- STACE, C. A. (1997). *New Flora of the British Isles* (2nd edn). Cambridge University Press, Cambridge.
- STACE, C. A. (in press). *Platanthera* Rich. In C. A. Stace (ed.), *Hybridization and the flora of the British Isles* (2nd edn). Botanical Society of the British Isles, London.
- STPICZYNSKA, M. (2003a). Floral longevity and nectar secretion of *Platanthera chlorantha* (Custer) Rchb. (Orchidaceae). *Annals of Botany* **92**: 191–197.
- STPICZYNSKA, M. (2003b). Nectar resorption in the spur of *Platanthera chlorantha* Custer (Rchb.) Orchidaceae: structural and microautoradiographic study. *Plant Systematics and Evolution* **238**: 119–126.
- SUMMERHAYES, V. S. (1951). *Wild orchids of Britain* (1st edn). Collins, London.
- TOLLSTEN, L. & BERSTROM, L. (1993). Fragrance chemotypes of *Platanthera* (Orchidaceae): the result of adaptation to pollinating moths. *Nordic Journal of Botany* **13**: 607–613.
- TREMBLAY, R., ACKERMAN, J. D., ZIMMERMAN, J. K. & CALVO, R. N. (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* **84**: 1–54.
- VAN DER CINGEL, N. A. (1995). *An atlas of orchid pollination: European orchids*. Balkema, Rotterdam.
- WALLACE, L. E. (2003). Molecular evidence for allopolyploid speciation and recurrent origins in *Platanthera huronensis* (Orchidaceae). *International Journal of Plant Sciences* **164**: 907–916.
- WALLACE, L. E. (2004). A comparison of genetic variation and structure in the allopolyploid *Platanthera huronensis* and its diploid progenitors, *Platanthera aquilonis* and *Platanthera dilatata* (Orchidaceae). *Canadian Journal of Botany* **82**: 244–252.
- WALLACE, L. E. (2006). Spatial genetic structure and frequency of interspecific hybridization in *Platanthera aquilonis* and *P. dilatata* (Orchidaceae) occurring in sympatry. *American Journal of Botany* **93**: 1001–1009.
- WEBB, D. A. (1980). *Platanthera* L.C.M. Rich. In T. G. TUTIN, V. H. HEYWOOD, N. A. BURGESS, D. M. MOORE, D. H. VALENTINE, S. M. WALTERS & D. A. WEBB (eds.), *Flora Europaea* 5, pp. 331–332. Cambridge University Press, Cambridge.
- WHILD, S. & LOCKTON, A. (2007). New records. *Shropshire Botanical Society Newsletter (Autumn 2007)*: 4–5 + cover.

(Accepted November 2007)



APPENDIX 1. DETAILS OF LOCALITIES AND SPUR MEASUREMENTS ENCOMPASSED BY THE PRESENT STUDY OF BRITISH AND IRISH *PLATANThERA*, LISTED ACCORDING TO INCREASING LATITUDE

Species	Recorder(s), Date(s)	Locality	Habitat	Shaded	N	Mean	SSD	CV (%)
<b>ALPS</b>								
bifolia	R Bateman/P Rudall, 2007	S BARNAVE, S Die, SE of Vercors, SE France	coarse grassland/ scrub	N	10	23.2	3.79	16.3
bifolia	R Bateman/P Rudall, 2007	Robinia site, S CHAMALAC, SE of Vercors, SE France	coarse grassland	N	20	22.98	2.75	12
bifolia	R Bateman/P Rudall, 2007	TRESANNE Road, NW Clelles, SE of Vercors, SE France	open scrub	Y	20	25.25	2.97	11.8
bifolia	R Bateman/P Rudall, 2007	Croix des Toutes AURES, NE Royans, NW Vercors, France	mixed woodland	Y	11	27.55	2.07	7.5
bifolia	R Sexton, 2006	CORVARA, SE Brixen, Dolomites, Italy	calcareous flush	N	48	17	2.44	14.4
bifolia	A Hughes, 2007	Main road, OBERGURGL, NW Bozen, W Austria	open rocky slope	N	5	17.8	1.64	9.2
bifolia	A Hughes, 2007	Road W from VENT toward Roifen, NW Bozen, W Austria	grassland	N	6	18.5	3.39	18.3
indeterminate	A Hughes, 2007	PETTNEU, 10 km E St Anton, E Bludenz, W Austria	dense woodland	Y	3	36.33	4.62	12.7
bifolia	A Hughes, 2007	MOOS, NW outskirts of St Anton, E Bludenz, W Austria	grassland (hay meadow)	N	5 <sup>3</sup>	25.2	1.3	5.2
bifolia	A Hughes, 2007	OBERLECH, 13 km NW St Anton, E Bludenz, W Austria	grassland (hay meadow)	N	5 <sup>3</sup>	21.8	1.64	7.5
<b>UK</b>								
bifolia	R Bateman/P Rudall, 2003	Sylvia's Meadow, ST ANNE'S Chapel, Tavistock, Cornwall	coarse grassland	N	8	19.63	1.41	7.2
chlorantha <sup>1</sup>	R Bateman/P Rudall, 2003	Sylvia's Meadow, ST ANNE'S Chapel, Tavistock, Cornwall	coarse grassland	N	1	26	NA	NA
hybrids <sup>1</sup>	R Bateman/P Rudall, 2003	Sylvia's Meadow, ST ANNE'S Chapel, Tavistock, Cornwall	coarse grassland	N	1	23	NA	NA
bifolia	R Bateman/P Rudall, 2003	Elkham's Grave, MEAD END, Rhinefield, S Hants	wet heath/bog	N	8	18.38	1.51	8.2
chlorantha	R Bateman/P Rudall, 2004	BADBURY Rings, Shapwick, NW Wimborne, Dorset	coarse grassland	N	9	33.56	3.57	10.6
bifolia	R Bateman/P Rudall, 2003	PIG BUSH, Denny Lodge, S Hants	heath	N	10	19.5	2.07	10.6
bifolia	R Bateman/P Rudall, 2003	STEPHILL Bottom, Denny Lodge, S Hants	heath	N	4	19	1.83	9.6
chlorantha	K Stott/D Pearce, 2007	NEWTIMBER Hill, Sussex	woodland	Y	4 <sup>3</sup>	27.2	1.78	6.5
chlorantha	K Stott/D Pearce, 2007	WOLSTENBURY Hill, Sussex	woodland	Y	37	27.46	3.24	11.8

<sup>1</sup>not plotted, as sample size only one or two plants; <sup>2</sup> not plotted, as these datasets duplicate other larger datasets; <sup>3</sup> mean excluded one plant with an exceptionally short spur.

Appendix 1 continued on next page

## APPENDIX 1 CONT....

Species	Recorder(s), Date(s)	Locality	Habitat	Shaded	N	Mean	SSD	CV (%)
<b>UK</b>								
chlorantha <sup>2</sup>	K Stott/D Pearce, 2007	WOLSTENBURY Hill, Sussex	woodland	Y	12	26.58	3.6	13.5
chlorantha <sup>1</sup>	K Stott/D Pearce, 2007	CLAYTON Hill, Sussex	woodland	Y	5	27.1	3.15	11.6
chlorantha <sup>1</sup>	R Bateman/P Rudall, 2004	Broad Down, WYE Downs, SE Wye, E Kent	coarse grassland	N	1	28	NA	NA
chlorantha <sup>1</sup>	R Bateman/P Rudall, 2004	PARK GATE Down, Elham, E Kent	grassland	N	1	28	NA	NA
chlorantha	R Bateman/P Rudall, 2003/4	YOCKLETT'S Bank, Waltham, E Kent	open woodland	Y	5	36.2	3.03	8.4
chlorantha	D Johnson, 2007	BONSAI BANK, SE Canterbury, E Kent	chalk scrub	Y	20	37	2.55	6.9
chlorantha <sup>1</sup>	R Bateman/P Rudall, 2003	SHEEPLEAS Wood, West Horsley, Surrey	coarse grassland/ scrub	N	1	36	NA	NA
bifolia <sup>1</sup>	R Bateman/P Rudall, 2004	Great CHEVERELL Hill, SW Lavington, Wilts	coarse grassland	N	1	17	NA	NA
bifolia <sup>1</sup>	R Bateman/P Rudall, 2004	Coppice NW SHELDWICH, S Faversham, E Kent	woodland	Y	1	23	NA	NA
chlorantha	R Bateman/P Rudall, 2004	Traffic island, LONGREACH Wood, Stockbury, W Kent	coarse grassland/ scrub	N	10	31.7	4.47	14.1
bifolia	R Bateman/P Rudall, 2003/4	STOCKBURY Hill Wood, Elham, W Kent	open woodland	Y	3	20.33	1.53	7.5
bifolia	R Bateman/P Rudall, 2004	Walkers Hill, PEWSEY Downs, N Alton Barnes, Wilts	grassland	N	5	18.4	1.95	10.6
bifolia <sup>2</sup>	A Hughes, 2007	Walkers Hill, PEWSEY Downs, N Alton Barnes, Wilts	grassland	N	10	19.75	1.32	6.7
chlorantha	D Johnson, 2007	CULVERSTONE Woods, Meopham, NW Kent	woodland	Y	20	34.3	2.3	6.7
bifolia	R Bateman/P Rudall, 2004	MORGAN'S HILL, Calstove Wellington, SE Calne, Wilts	grassland	N	10	17.5	1.58	9
chlorantha	R Bateman/P Rudall, 2003/4	HOMEFIELD Wood, Medmenham, Bucks	woodland/scrub	Y	8	36.75	3.2	8.7
bifolia	R Bateman/P Rudall, 2003/4	Warburg Reserve LNR, BIX Bottom, Oxon	woodland/scrub/ grassland	Y	4	22	4.24	19.3
chlorantha	R Bateman/P Rudall, 2003/4	Warburg Reserve LNR, BIX Bottom, Oxon	woodland/scrub/ grassland	Y	11	33.91	3.42	10.1
hybrids	R Bateman/P Rudall, 2003/4	Warburg Reserve LNR, BIX Bottom, Oxon	woodland/scrub/ grassland	Y	3	29.33	2.08	7.1
chlorantha <sup>1</sup>	P Daltry, 2007	Near Great MISSENDEN, Bucks	?woodland	?Y	2	29.25	NA	NA
bifolia	A Hughes, 2007	BULL'S CROSS, B4070, NE Stroud, Gloucs	woodland	Y	5	23.1	1.52	6.6
hybrids? <sup>2</sup>	A Hughes, 2007	BULL'S CROSS, B4070, NE Stroud, Gloucs	scrub	Y	5	24.5	1.46	6
chlorantha <sup>1</sup>	R Bateman, 2004	DANCERS END LNR, Aston Clinton, Bucks	scrub/open woodland	Y	1	34	NA	NA
chlorantha	R Bateman/P Rudall, 2004	ASTON CLINTON Ragpits LNR, Bucks	scrub/coarse grassland	Y	10	31.9	2.23	7

SPUR LENGTH IN *PLATANThERA*

Species	Recorder(s), Date(s)	Locality	Habitat	Shaded	N	Mean	SSD	CV (%)
<b>UK</b>								
chloranthes	R Bateman/H+J Lambert, 2004	Ty-Commins, LLANBEDR, Crickhowell, Powys	coarse grassland	N	10	26.2	3.05	11.6
chloranthes	R Bateman/J Tyler, 2004	Lineage Wood, E Bridge Street, W LAVENHAM, W Suffolk	woodland	Y	9	33.44	4.45	13.3
chloranthes	I Wright, 2004	LLYNCHLYS Common, Oswestry, Shrops	open woodland	Y	26	29.02	2.69	9.3
chloranthes	A Gendle, 2007	ARNSIDE Cemetery	woodland	Y	4	27.75	2.47	8.9
chloranthes <sup>1</sup>	A Gendle, 2007	MEATHROP Quarry	coarse grassland	N	1	28	NA	NA
chloranthes <sup>1</sup>	A Gendle, 2007	SANDSIDE Quarry	coarse grassland	N	2	29.75	NA	NA
bifolia	A Gendle, 2007	OUTLY Moss	valley mire	N	20	19.65	2.27	11.6
chloranthes	J Fenton, 2007	LATTERBARROW LNR, SW Kendal, SE Cumbria	grassland	N	20	28.23	2.73	9.7
chloranthes <sup>2</sup>	A Gendle, 2007	LATTERBARROW LNR, SW Kendal, SE Cumbria	grassland	N	12	28.71	2.61	9.1
chloranthes	A Gendle, 2007	Canny Hill, NEWBY Bridge	grassland	N	16	24.63	1.55	6.3
chloranthes	J Fenton, 2007	LEVENS Wood, SW Kendal, SE Cumbria	?woodland	Y?	17	28.85	2.07	7.2
chloranthes	A Gendle, 2007	Piers Gill, DENTDALE	hay meadow	N	12	25.13	3.45	13.7
chloranthes	J Fenton, 2007	A590/A591 junction, SW Kendal, SE Cumbria	woodland	Y	21	27.5	2.47	9
chloranthes <sup>2</sup>	A Gendle, 2007	A590/A591 junction, SW Kendal, SE Cumbria	woodland	Y	20	29.83	2.15	7.2
chloranthes	A Gendle, 2007	A6/A591 junction, S Kendal, SE Cumbria	coarse grassland	N	19 <sup>3</sup>	29.87	2.61	8.7
chloranthes	A Gendle, 2007	FIRBANK churchyard	coarse grassland	N	6	27.08	1.59	5.9
chloranthes	L+N Harbron, 2007	SMARTDALE NNR, W Kirkby Stephen, SE Cumbria	woodland	Y	67	29.04	2.85	9.8
bifolia	A Gendle, 2007	WAITBY Greenriggs, W Kirkby Stephen, SE Cumbria	grassland	N	20	17.45	2.23	12.8
bifolia <sup>2</sup>	J Fenton, 2007	WAITBY Greenriggs, W Kirkby Stephen, SE Cumbria	(grassland)	N	19	17.34	1.22	7
chloranthes	S Longrigg, 2007	MILGAVIE, East Dumbarntonshire	grassland (meadow)	N	6	27	2.3	8.5
chloranthes	S Longrigg, 2007	CARDROSS, W. Dumbarntonshire	coarse grassland	N	11	26.73	2.61	9.8
chloranthes	S Longrigg, 2007	MURROCH, Dumbarnton, E. Dumbarntonshire	grassland (meadow)	N	4	27.25	0.5	1.8
chloranthes	S Longrigg, 2007	RENTON, East Dumbarntonshire	coarse grassland (waste)	N	29	27.52	2.23	8.1
chloranthes	R Sexton, 2005	BOMAINS Meadow LNR, Bo'ness, Falkirk, ?Fife	coarse grassland	N	55	26.83	2.23	8.3
chloranthes <sup>2</sup>	R Sexton, 2006	BOMAINS Meadow LNR, Bo'ness, Falkirk, ?Fife	coarse grassland	N	19	26.15	2.49	9.5
chloranthes <sup>2</sup>	R Sexton, 2007	BOMAINS Meadow LNR, Bo'ness, Falkirk, ?Fife	coarse grassland	N	24	27.06	2.16	8

<sup>1</sup> not plotted. as sample size only one or two plants; <sup>2</sup> not plotted, as these datasets duplicate other larger datasets; <sup>3</sup> mean excluded one plant with an exceptionally short spur.

## APPENDIX 1 CONT.....

Species	Recorder(s), Date(s)	Locality	Habitat	Shaded	N	Mean	SSD	CV (%)
<b>UK</b>								
chlorantha	S Longrigg, 2007	BALLOCH, East Dumbartonshire	coarse grassland	N	20	28.9	2.51	8.7
chlorantha	S Longrigg, 2007	PIRNIEHALL, Croftamie, Stirlingshire	coarse grassland	N	20	29.98	1.94	6.5
chlorantha	R Sexton, 2007	PLEAN Country PARK	grassland (meadow)	N	75	25.88	1.66	6.4
chlorantha <sup>2</sup>	R Sexton, 2005	PLEAN Country PARK	grassland (meadow)	N	28	26.94	2.3	8.5
chlorantha <sup>2</sup>	R Sexton, 2006	PLEAN Country PARK	grassland (meadow)	N	6	28.5	2.73	9.6
chlorantha	R Sexton, 2007	PLEAN Primary SCHOOL	grassland (meadow)	N	49	26.53	1.57	5.9
bifolia	R Sexton, 2007	Wester BALGAIR, Stirling	wet meadow	N	22	17.41	1.47	8.4
bifolia <sup>2</sup>	R Sexton, 2006	Wester BALGAIR, Stirling	wet meadow	N	15	18.75	1.73	9.2
chlorantha	R Sexton, 2007	Glinns Road, KIPPEN (B), Stirling	coarse grassland	N	25	27.06	2.12	7.8
chlorantha <sup>2</sup>	R Sexton, 2006	Glinns Road, KIPPEN (B), Stirling	coarse grassland	N	6	28.71	1.38	4.8
bifolia	S Longrigg, 2007	Glen LUSS, Argyll & Bute	wet meadow	N	20	16.05	1.64	10.2
chlorantha	R Sexton, 2006	KIPPEN (A) Horse Field, Stirling	coarse grassland	N	118	29.65	2.53	8.5
chlorantha <sup>2</sup>	R Sexton, 2007	KIPPEN (A) Horse Field, Stirling	coarse grassland	N	24	27.36	2.07	7.6
chlorantha	R Sexton, 2005	Keith Hills, KINROSS	grassland (meadow)	N	47	25.69	2.27	8.8
bifolia	R Sexton, 2006	BALLANGREW Meadow, Stirling	wet meadow	N	5	18.5	1.63	8.8
chlorantha	R Sexton, 2006	ASHFIELD, Dunblane, Perth	grassland (meadow)	N	8	27.78	2.17	7.8
chlorantha	R Sexton, 2007	QUOIGS Meadow, Perth	coarse grassland	N	24	27.8	2.18	7.8
chlorantha <sup>2</sup>	R Sexton, 2006	QUOIGS Meadow, Perth	coarse grassland	N	10	27.31	2.11	7.7
bifolia	R Sexton, 2007	QUOIGS Meadow, Perth	coarse grassland	N	36	18.86	2.36	12.5
bifolia <sup>2</sup>	R Sexton, 2007	QUOIGS Meadow, Perth	coarse grassland	N	24	18.48	2.49	13.5
bifolia <sup>2</sup>	R Sexton, 2006	QUOIGS Meadow, Perth	coarse grassland	N	12	20.6	1.86	9
chlorantha	R Sexton, 2005	FLEECEFAULDS Meadow, Ceres, Fife	grassland (meadow)	N	27	27.09	2.89	10.7
bifolia	R Sexton, 2006	CALLANDER Golf Course, Stirling	coarse grassland	N	8	19	2.1	11.1
chlorantha	R Sexton, 2007	BRAELENY Farm, Callander, W Stirling	coarse grassland	N	99	26.47	2.21	8.4
chlorantha <sup>2</sup>	R Sexton, 2006	BRAELENY Farm, Callander, W Stirling	coarse grassland	N	6	27.46	3.45	12.6

SPUR LENGTH IN *PLATANThERA*

21

Species	Recorder(s), Date(s)	Locality	Habitat	Shaded	N	Mean	SSD	CV (%)
<b>UK</b>								
chlorantha	D+C Hughes, 2007	Roadside, LOCHALINE, Morvern, Westernness	grassland	N	18	23.44	2.45	10.5
chlorantha <sup>1</sup>	D+C Hughes, 2007	Lochside, LOCH ALNE, Lochaline, Morvern, Westernness	moorland	N	1	23	NA	NA
chlorantha	D+C Hughes, 2007	Roadside, ARDTORNISH, Morvern, Westernness	grassland verge	N	8	24.5	3.51	14.3
chlorantha	I Denholm, 2006	KELTNEYBURN LNR, Fortingall, Perth	coarse grassland	N	20	26	2.29	8.8
bifolia <sup>1</sup>	D+C Hughes, 2007	Mountainside, Beinn na h-UAMHA, Morvern, Westernness	moorland	N	2	15	NA	NA
bifolia	D+C Hughes, 2007	Woodside, FORFAR, Angus	marshy meadow	N	22	18.32	2.53	13.8
chlorantha <sup>1</sup>	D+C Hughes, 2007	DRIMNIN, Morvern, Westernness	damp meadow	N	2	24.5	NA	NA
bifolia	I Denholm, 2006	GLENCAIRN, S Boat of Garten, Abernethy, Inverness	coarse grassland	N	20	19.63	1.88	9.6
bifolia	R Bateman/I Denholm, 2007	Loch a Mhuilinn, S APPLECROSS, W Ross	calcareous flush/ heath	N	20	14.6	1.35	9.2
bifolia	R Bateman/I Denholm, 2007	CARNAN, N Benbecula, Outer Hebrides	wet heath/bog	N	20	14.23	2.05	14.4
bifolia	D+C Hughes, 2007	Roadside, KINLOCHEWE, Wester Ross	grassland	N	7	17.29	2.69	15.6
bifolia	D+C Hughes, 2007	Roadside E TALLADALE, S Loch Maree, Wester Ross	grassland	N	12	14	2.09	14.9
bifolia	D+C Hughes, 2007	Loch na h-ORDNACHE, S Flowerdale, Wester Ross	moorland	N	12	16.42	2.71	16.5
bifolia	D+C Hughes, 2007	E DUBH LOCH, S Flowerdale, Wester Ross	moorland	N	20	16.2	2.42	14.9
bifolia	D+C Hughes, 2007	E Kerrysdale, FLOWERDALE, Wester Ross	moorland	N	7	16.14	0.9	5.6
bifolia	R Bateman/I Denholm, 2007	NE Lochdrum Farm, LOCHDROMA, Braemore, W Ross	calcareous flush/ heath	N	7	15	1.91	12.7
bifolia	D+C Hughes, 2007	S Kernsary, NW Loch MAREE, Wester Ross	grassy bog	N	13	16.31	1.8	11
bifolia	R Bateman/I Denholm, 2007	N bank Loch KERNSARY, E Poolewe, NE Gairloch, W Ross	calcareous flush/ heath	N	28	15.93	1.91	12
bifolia <sup>2</sup>	D+C Hughes, 2007	N bank Loch KERNSARY, E Poolewe, NE Gairloch, W Ross	calcareous flush/ heath	N	10	16.1	2.92	15.29
chlorantha <sup>1</sup>	D+C Hughes, 2007	Roadside N INVEREWE, Wester Ross	grassland	N	1	22	NA	NA
bifolia	D+C Hughes, 2007	MIDTOWN, NW Poolewe, Wester Ross	meadow	N	3	17	1	5.9

<sup>1</sup> not plotted, as sample size only one or two plants; <sup>2</sup> not plotted, as these datasets duplicate other larger datasets; <sup>3</sup> mean excluded one plant with an exceptionally short spur.