HOS *Platanthera* Spur-length Survey Concludes: Correlation Between Spur Length and Leaf Width is Weak

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Background

This highly collaborative "membership participation" project was conceived by us in order to extend the geographical coverage of our own efforts to obtain measurements in general, and spur lengths in particular, from across the geographical and habitat ranges of the two European butterfly-orchids; specifically, *Platanthera bifolia* (Lesser Butterfly-orchid) and *P. chlorantha* (Greater Butterfly-orchid). Our interest in these two species was driven by their remarkable genetic similarity, the limited but clear-cut morphological differences that distinguish them, and uncertainty regarding whether they hybridise in the British Isles. In particular, we wished to explore the presumed critical contribution of spur length towards ensuring different pollinators for the two species, as outlined in some classic studies of orchid–pollinator co-evolution (e.g. Nilsson 1983). We therefore provided detailed instructions to HOS members describing how best to measure spur length, aiming to maximise consistency among supposedly inexperienced analysts.

Early results and preliminary interpretations

By the close of the first (2007) field season, our combined database of spur lengths contained 120 datasets (49 for *P. bifolia*) totalling 1876 individual plants (625 for *P. bifolia*). Datasets generated by 19 HOS members ranged in sample size from a single plant to 118 plants, representing populations clustered in the Vercors, the Alps (both *P. bifolia* only), southern England, Cumbria, southern Scotland and northwestern Scotland. Duplicate measurements of several populations demonstrated that the data gathered were both accurate and reproducible.

The results were first presented to HOS members in *JHOS* (Bateman & Sexton 2008a) and then published as two peer-reviewed papers (Bateman & Sexton 2008b; Bateman *et al.* 2009). Data for both species contradicted spur lengths given in most floras and monographs, and identified convincing hybrids at a few localities. But most importantly, they challenged the widely held assumption that adaptation to accommodate the proboscis length of pollinating moths is the dominant factor controlling spur length. Instead, at any particular latitude, *P. bifolia* has spurs approximately two-thirds the length of those of *P. chlorantha*. Moreover, both species exhibited latitudinal gradients, spur length increasing by an average of 2.2% per 100 km from north to south (though results for *P. chlorantha* were weakened by the absence of data from southern Europe). We hypothesised that this gradation of spur size could simply reflect greater resourcing of plants in lower latitudes, perhaps permitted by greater photosynthetic activity generating much-needed energy for the plants.

A more focused survey

We wished to test the hypothesis that spur length is positively correlated with leaf width, which we selected as being the most readily measured proxy for leaf area. Thus, during the 2008 field season, surveyors were asked to measure not only spur length but also leaf width, and to count leaf number. In total, 14 teams of surveyors together generated usable data for 21 populations each of the two species, totalling 749 plants (402 for *P. bifolia*). The geographic coverage of the study improved considerably, not only finally encompassing much-needed populations of *P. chlorantha* from Continental Europe but also garnering a welcome additional set of populations from Poland (co-ordinated by Przemo Baraniecki).

The new results

As expected, most of the plants sampled possessed two expanded leaves. Approximately 1% of the individuals of each species produced a third expanded leaf but, surprisingly, the proportion of plants recorded as yielding only a single leaf was greater in *P. chlorantha* (6%) than in the typically smaller *P. bifolia* (1%).

The new spur-length data largely reinforced previous evidence of a significant positive correlation between spur length and decreasing latitude. The new data generally fitted well the regression lines generated from previous data. [Note added in proof: The two populations measured in western Ireland by RB in June 2009 also nicely fitted the previous regression line.] The exceptions were an unusually short-spurred population of *P. chlorantha* from Normandy and four populations of *P. bifolia* from Poland that had mean spur lengths more typical of populations from southern France than southern England (an equivalent latitude to Poland). When the new data only were analysed, the resulting plots of spur length against latitude for both species matched closely the results obtained in the previous (2007) survey; in both cases average spur length increased with decreasing latitude (Figs 1, 2).

However, plotting leaf width against latitude gave more equivocal results. In *P. bifolia* (Fig. 3), leaf width increased southward at an average rate similar to that of spur length, though the fit of the regression line to the data (r^2) was appreciably weaker. However, the regression line for leaf width in *P. chlorantha* (Fig. 4) even suggested that leaves are on average slightly smaller(!) further south, though the r^2 value was so low that the notional trend can be considered random.

When mean values for spur length were plotted against leaf width, *P. bifolia* (Fig. 5) again showed a stronger positive correlation than *P. chlorantha* (Fig. 6), primarily reflecting the occurrence in the Alps of three populations that are both relatively long-spurred and large-leaved. But for both species the relationship was weaker than anticipated. This pattern was mirrored when spur length was plotted against leaf width for all measured individuals within each of the 42 populations measured. Only

eight populations (three of *P. bifolia*) showed statistically significant positive correlations (r^2 = 0.3–0.7), and these were more than offset by 13 populations (six of *P. bifolia*) that yielded flat or even in four cases negative graphs, which implied that in these populations larger plants generated smaller spurs!

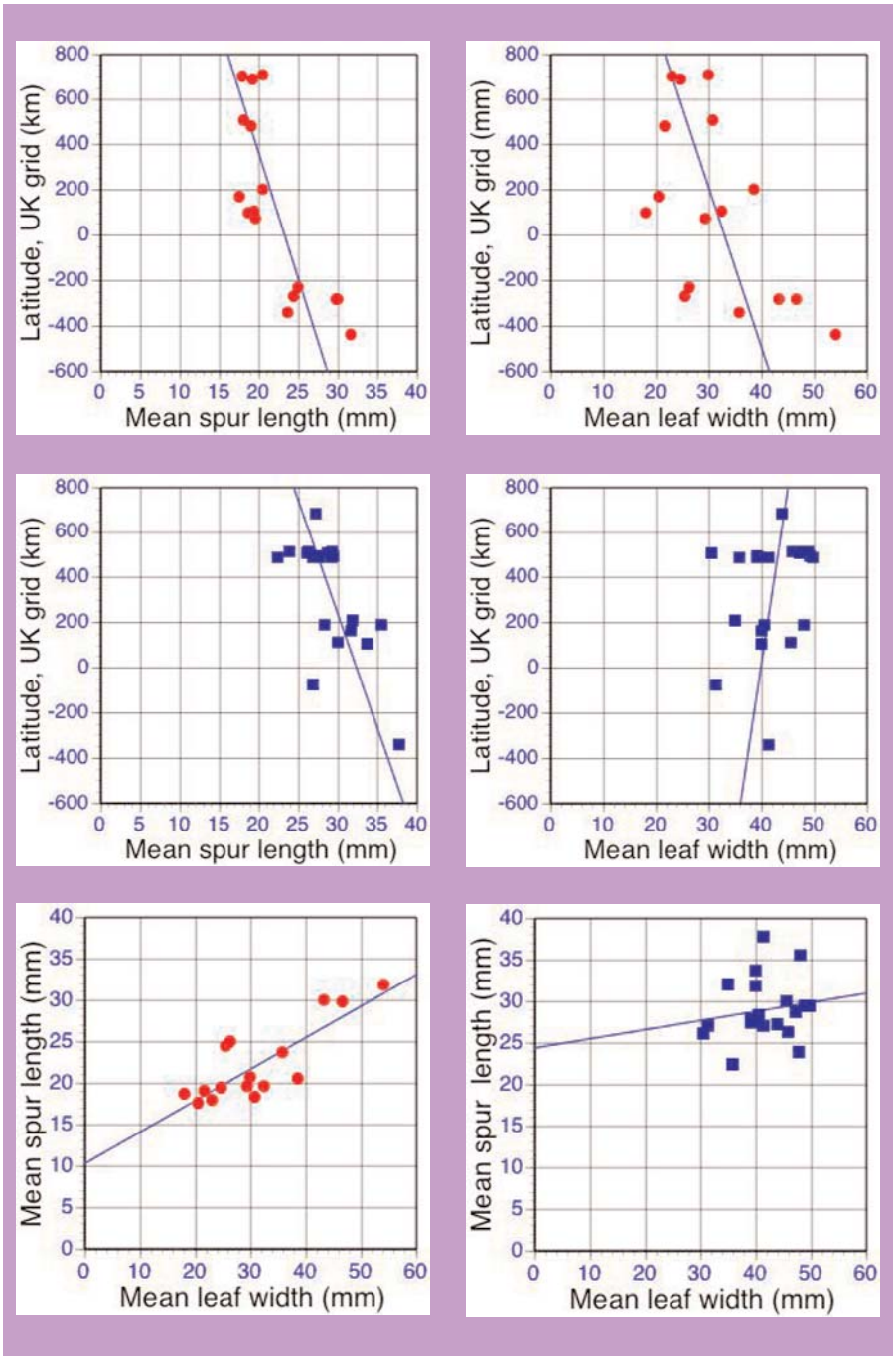
A more problematic interpretation

So the 2008 data show that the link between spur length and leaf size is weak at best, especially in *P. chlorantha*. Either levels of resourcing make a less important contribution to spur length than our original hypothesis suggested, or our method of assessing levels of resourcing has been not just simple but also simplistic. Both explanations are credible.

It could be argued that leaf width is an inadequate proxy for leaf area, which is more likely to determine photosynthetic ability. However, the majority of the field surveyors (including ourselves!) measured leaf length as well as width, permitting calculation of approximate leaf area. Leaf area gave equally poor fits when plotted against spur length. Also, one might predict that of two plants with equally sized leaves, the one that receives more incident light would generate more energy. However, as we already noted (Bateman & Sexton 2008a, b), at any particular latitude, populations growing in shaded habitats (especially those of *P. bifolia*) tend to have somewhat longer spurs than those growing in the open. In addition, summer day-length is actually greater at *higher* latitudes. These observations suggest that that the resourcing and vigour of the plants may be more strongly controlled by warmth and/or soil moisture than by light per se.

However, the fact that southerly populations of *P. chlorantha* do not have larger leaves than northerly populations suggests that the plants do not increase in average vigour toward the equator, and that additional explanations should be sought for the southward increases in mean spur lengths. Studies of spur growth cause us to remain sceptical that spur length can be precisely adaptively optimised. For example, in 2007, R. Bateman compared spur lengths on late-stage buds and recently opened flowers in a population of *P. bifolia* on Benbecula, Outer Hebrides, and found that average spur length increased by 24% between the two developmental stages. During the 2008 survey, two teams (G. Goodfellow plus A. Skinner and R. Bateman plus P. Rudall) measured the large population of *P. bifolia* at Strawberry Banks in the

Figures showing the relationship between spur length, leaf width and/or latitude in a N–S transect of *P. bifolia* (red circles, 16 populations only, as Polish data were omitted) and *P. chlorantha* (blue squares, 20 populations). **(1)** Mean spur length versus latitude, *P. bifolia*. **(2)** Mean leaf width versus latitude, *P. bifolia*. **(3)** Mean spur length versus latitude, *P. chlorantha*. **(4)** Mean leaf width versus latitude, *P. chlorantha*. **(5)** Mean leaf width versus mean spur length, *P. bifolia*. **(6)** Mean leaf width versus mean spur length, *P. chlorantha*.


Latitude, UK grid (km)
Mean spur length (mm)
Latitude, UK grid (mm)
Mean leaf width (mm)
Latitude, UK grid (km)
Mean spur length (mm)
Latitude, UK grid (km)
Mean leaf width (mm)
Mean spur length (mm)
Mean leaf width (mm)
Mean spur length (mm)
Mean leaf width (mm)

Cotswolds. The latter team measured the population a month later than the former, and consequently found the average spur length to be 15% greater. Clearly, the length of spur encountered by a visiting insect is significantly influenced by whether the long-lived *Platanthera* flower is fresh, mature or nearing senescence.

In conclusion, it was always likely that this morphometric survey would raise as many questions as it answered; this is the usual outcome of any scientific endeavour, and it in no way detracts from the many insights gained from this highly successful project.

In drawing this project to a close we would like to end by thanking the following HOS members for their invaluable contributions to the 2008 survey: R. Bateman & P. Rudall (70 plants), L. Dudek (40), A. Gendle (117), G. Goodfellow & A. Skinner (175), W. Hanak (9), L. & N. Harbron (70), D. & C. Hughes (43), N. Johnson & R. Webb (41), L. Krajowski (32), P. Cieslak (15), K. Stott, D. Pearce & N. Henderson (83), M. Scelina (10), R. Sexton (38), and S. & M. Tarrant (6),

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