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ORIGINAL PAPER

Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators

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Abstract Alpine flowers face multiple challenges in terms of abiotic and biotic factors, some of which may result in selection for certain colours at increasing altitude, in particular the changing pollinator species composition, which tends to move from bee-dominated at lower elevations to fly-dominated in high-alpine regions. To evaluate whether growing at altitude—and the associated change in the dominant pollinator groups present—has an effect on the colour of flowers, we analysed data collected from the Dovrefjell National Park in Norway. Unlike previous studies, however, we considered the flower colours according to ecologically relevant models of bee and fly colour vision and also their physical spectral properties independently of any colour vision system, rather than merely looking at human colour categories. The shift from bee to fly pollination with elevation might, according to the pollination syndrome hypothesis, lead to the prediction that flower colours should shift from more bee-blue and UV-blue flowers (blue/violet to humans, i.e. colours traditionally associated with large bee pollinators) at low elevations to more bee-blue-green and green (yellow and white to humans—colours often linked to fly pollination) flowers at higher altitude. However, although there was a slight increase in bee-blue-green flowers and a decrease in bee-blue flowers with increasing elevation, there were no statistically significant effects of altitude on flower colour as seen either by bees or by flies. Although flower colour is known to be constrained by evolutionary history, in this sample we also did not find evidence that phylogeny and elevation interact to determine flower colours in alpine areas.

Keywords Flower colour · Pollinator diversity · Insect vision · Alpine flowers · Pollination

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Introduction

Plants growing in mountainous regions are faced with a range of challenges. As well as having to contend, potentially, with high winds, desiccation and extremes of cold, they also face increased ultraviolet exposure and pollinator limitation when the temperatures and winds grow too extreme for pollinating insects to fly (Totland et al. 2000). Many strategies employed for dealing with such habitats have already been investigated in depth (Totland et al. 2000), but what still warrants further investigation is how flowers at high altitude might exhibit specific adaptations in terms of their pigmentation.

Why might some colours in high-alpine habitats be more beneficial than others? Flower colour is under selection by pollinators (Kevan and Baker 1983; Rodriguez-Girones and Santamaria 2004; Tastard et al. 2008; Waser 1983; Whibley et al. 2006). There are indeed several studies associating shifts in flower colours with shifts in pollinator type (Altshuler 2003; Bradshaw and Schemske 2003). In alpine areas the numbers of pollinators present will

decrease overall with increasing altitude, and will change in composition; some insect groups are less able to function at very high elevations than others (Kearns 1992; Totland 1992). Therefore, different pollinator guilds dominate at different elevations and therefore the selective forces on flower traits might be expected to differ. The pollination syndrome hypothesis, which has been used as the basis for studies of pollination systems for many years (Faegri and van der Pijl 1978), postulates a strong association between different pollinator guilds and particular suites of floral characteristics, in particular aspects of morphology and colour (e.g. the zygomorphic, closed and blue/purple "bee flowers"; large, white "moth flowers" with long corolla tubes). Based on this framework, a changing pollinator composition at different elevations may be expected to lead to different colours prevailing at different altitudes depending on the dominant pollinator types and the colours that appeal to them.

For example, the ability of flies to forage at higher elevations than bees (Kearns 1992; Lázaro et al. 2008; Totland 1993) might perhaps lead us to expect that the flower colours traditionally thought to be associated with fly pollination (appearing white and yellow to humans) would be more abundant at high altitudes. Flowers appearing white (and also pink) to humans are mostly blue–green for bees and other trichromatic insects (Kevan et al. 1996), whereas yellow flowers can be either green or UV-green, depending on their UV reflectance, to such insects (Chittka et al. 1994).

Bees appear to be especially dominant at low to medium elevations (i.e. below the treeline, in sub-alpine habitats) (Lázaro et al. 2008), their large body size allowing foraging in the relative cold, but the high energetic requirement demanded by maintaining their flight muscles at a high enough temperature perhaps restricting their activities at very high elevations (Arroyo et al. 1982). Bees of many species have an innate preference for UV-blue and blue flowers (Giurfa et al. 1995; Raine et al. 2006), leading perhaps to an expectation based on the pollination syndrome hypothesis that where bees dominate as pollinators (e.g. below the treeline in mountainous regions), bee-blue and UV-blue flower species should be more numerous. However, this preference is modifiable by learning, as are the innate preferences in many other pollinating insects such as hoverflies and butterflies (Lunau and Maier 1995).

There have been previous attempts to document the effects of altitude on flower colours present (see Totland et al. (2000) for a summary)—Weevers (1952) observed that there were more blue flower species in upland areas than in lowland areas (both in Switzerland above 1,100 m and Java above 1,500 m), and Kevan (1972) and Savile (1972) observed that flowers in alpine areas and arctic regions (which are climatically similar to alpine areas) tended to consist of a higher proportion of white and yellow species. McCall and Primack (1992) observed that purple and yellow flowers were the most visited colours in lowland woodland, whilst yellow and white were the two most visited colours in alpine tundra, with blue–purple flowers being much less frequently visited. Some of these earlier studies, however, contain primarily observational recordings that are not well supported by statistical power. More importantly, perhaps with the exception of Kevan (1972), some of these studies have considered flower colour principally from the human perspective, without fully taking into account the more recent understanding of pollinator visual systems and how these differ from human eyes (Chittka and Kevan 2005; Chittka and Menzel 1992; Menzel and Shmida 1993).

These differences are fundamental: all insects so far extensively tested have UV receptors with a maximum sensitivity between around 330 and 375 nm (i.e. in the UV range where human eyes have no sensitivity)—this includes bees and other hymenopterans, lepidopterans, coleopterans, hemipterans, dipterans, etc. (Briscoe and Chittka 2001). Bees, the most important pollinators in Norway at all but the high-alpine elevations (Lázaro et al. 2008), also have blue and green receptors, but typically lack red receptors (Peitsch et al. 1992) (see Fig. 1a). Other insects, including many butterflies and flies, have rather different colour vision systems, in some cases more complex than those of bees or humans (Briscoe and Chittka 2001; Morante and Desplan 2008). Figure 1b shows the spectral sensitivities of the four photoreceptors contributing to colour vision in the blowfly, *Lucilia* sp.

We investigated whether the flower community growing at high altitude has a different pollinator-relevant colour composition to that of lower altitude areas, by using a data set collected along a transect in the Norwegian Dovrefjell mountains from 700 m to 1,600 m elevation. This is of especial interest in light of the recent study by Lázaro et al. (2008), in which plant communities at different elevations in southern Norway were surveyed for floral colour and morphology, and this was combined with visitation data. The study found evidence of association between traits (including colour) and pollinator, showing that flowers in alpine areas generally seem to be visited by pollinators that could be predicted according to the pollination syndrome hypothesis. Thus, it seems that the predominance of pollinator types (and subsequently the main foraging strategies in evidence) varies with elevation and could potentially have strong effects on which flower species are most abundant.

In our study we consider flower colours as seen by their pollinators, firstly using the well-studied model of bee colour vision, secondly using a model of fly colour vision, and also using the raw reflectance spectra of the flowers,

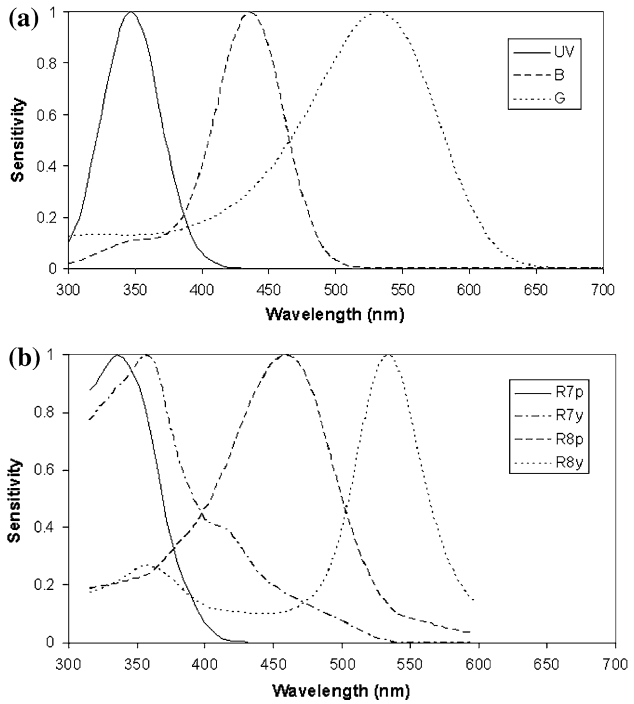

Fig. 1 Spectral sensitivities of the (**a**) bee photoreceptors and (**b**) fly photoreceptors that contribute to colour vision. Bee photoreceptors shown are UV-, blue- and green-sensitive for *Bombus terrestris* (Skorupski et al. 2007); fly receptors are for *Lucilia* sp. (Hardie and Kirschfeld 1983)

thereby considering their colours without bias towards any vision system. As bees and flies are the most important pollinators in most Norwegian alpine habitats (Lázaro et al. 2008), we considered how the colours might appear to these pollinators (using the bee colour hexagon model (Chittka 1992) and a model of the blowfly (*Lucilia* sp.) as described in Troje (1993)). Additionally, we analysed the raw spectral properties of the flowers. This encompasses wavelengths invisible to humans (<400 nm), but does not impose a particular visual system on to the results. To investigate whether the colours of flowers were constrained by their phylogeny, we tested whether phylogenetic distance correlates with differences in colours, and whether there is an interaction between elevation and phylogeny that affects flower colour. This is an important consideration because of the following possibility: if closely related flowers tend to have similar colours and also occur in similar elevation ranges, this could result in detection of an association between colour and elevation, but the cause would be phylogenetic constraints rather than selection for particular colours at particular altitudes. It is also possible that there may be no statistical association between elevation and colour, and equally no statistically detectable association between phylogeny and flower colour, but when the effects of phylogeny and elevation are combined there could be a tendency for certain related groups of flowers, when growing at particular elevations, to exhibit particular colours more often.

Materials and methods

Study sites and data collection

The study site was located in the Dovrefjell–Sunndalsfjella National Park (formerly Dovrefjell National Park) in Norway, near to Oppdal. Data were collected in June 1992 in the altitude range 700–1,600 m a.s.l. (sub-alpine to high-alpine), using Kongsvoll Biological Station (62° 18′N, 9° 36′ E, 900 m above sea level) as a basis. The entire altitude range from the valley floor to the Knutshø (also variously spelt Knutshøa or Knudshø) peaks (south peak altitude: 1,680 m; 62° 18′ N; 9° 41′ 0 E; north peak altitude: 1,684 m; 62° 18′ N; 9° 40′ E) east of the station was surveyed for 7 days, mostly using footpaths as trunk routes and thoroughly exploring the territory around. All flowering species found in this survey were noted, along with the elevation at which they were recorded. It is possible that some comparatively rare species might have escaped attention, but the vast majority of common species have been included (c.f. West and West (1910)), and also confirmed by local expert Simen Bretten (personal communication). Spectrophotometer readings from 300 nm to 700 nm (i.e. including the ultraviolet range) were taken of the flowers of all species present, using the methods described in Chittka and Kevan (2005) and Dyer and Chittka (2004). (All spectral reflectance curves are available from the Floral Reflectance Database http://www.reflectance.co.uk (Arnold et al. 2008).) A total of 74 species were sampled from this location and are listed in Appendix 1.

Effect of elevation on bee colour composition of the community

We divided the surveyed territory into three elevation ranges: lower altitudes (700–1,000 m), intermediate altitudes (1,000–1,300 m) and high altitudes (1,300–1,600 m), and recorded which species were found in each, and which spanned more than one range. At this location, the low altitude group corresponds to the vegetation of mountain meadows, stream beds, and some forests (mainly birch); the intermediate group covers the first zone above the tree line although scattered dwarf birch (*Betula nana*) and *Salix* trees still occur here (West and West 1910); 1,300 m is the lower boundary of permafrost in the Dovrefjell (Sollid et al. 2003); the high altitude vegetation is dominated by lichens and comprises flowering plants growing on rocky, unstable soils (West and West 1910). The range sampled

still extends into regions that can at times be too cold for many pollinator species to fly and thus the dominant types of pollinators will change significantly within the range sampled (Totland 1993; Totland et al. 2000), with an increase in muscoid fly species, a decrease in bee and beetle species and possibly an increase in butterfly species.

For bee pollinators, we categorised the flower species by colour, according to their loci in the bee colour hexagon (Chittka 1992; Gumbert et al. 1999). The colour hexagon is a graphical representation of the discriminability of different colours to a bee, based on the relative excitations of the three types of bee photoreceptor (UV, blue and green) elicited by the colours, and two unspecified colour opponency mechanisms. Previous studies have indicated that the division of the colour hexagon into six particular categories corresponds well to the actual distributions of flower colours present in nature (Chittka et al. 1994). Thus, we classified flowers as either bee-blue, blue–green, green, UV-green, UV or UV-blue (see Appendix 1), as shown in Fig. 2a.

Effect of elevation on fly colour composition of the community

We also looked at patterns in flower colour as seen by flies. Many dipteran species have five photoreceptor types, of which four are used for colour vision (Morante and Desplan 2008; Troje 1993). These are typically referred to as R7p (most sensitive in the UV), R7y (highest sensitivity to violet light), R8p (peak sensitivity to blue) and R8y (peak sensitivity to green). Two general types of ommatidia are present in fly eyes, containing either the two p-type ("pale") receptors or the y-type ("yellow") receptors—named according to how they appear in transmitted light (Troje 1993).

The model we used is that of Troje (1993), based on the blowfly *Lucilia* sp., in which spectral stimuli across quite wide ranges are not discriminated amongst, but are discriminated from stimuli in other spectral ranges, with category boundaries at 400 and 515 nm. The opponent system takes the difference in relative excitations between the two p-type receptors and the two y-type receptors and the receptor of each pair stimulated most strongly determines the colour the fly perceives. This results in four colour categories (p+ y+, p− y+, p− y− and p+ y−) which could be regarded as fly-UV, -blue, -yellow and fly-purple (purple referring in human vision to a colour where the shortest and longest wavelength receptors are stimulated most strongly in combination), with all stimuli within one category being chromatically indistinguishable to the fly. The fly colour loci are plotted in Fig. 2b, with the four quadrants labelled.

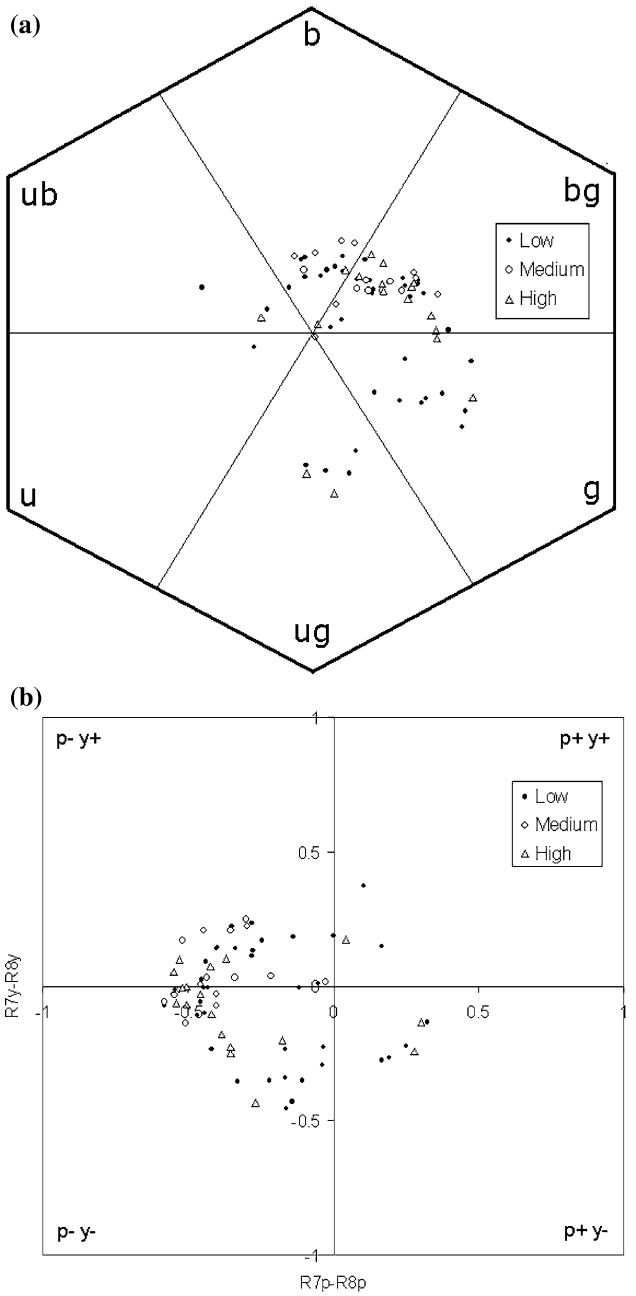

Fig. 2 **a** Bee colour hexagon of the flower species measured. The six segments correspond to the six bee colour categories used in this analysis (b = blue, bg = blue–green, g = green, ug = UV-green, u = UV and ub = UV-blue). Loci are calculated according to the relative stimulation of the three receptor types (UV, blue, green) elicited by the stimulus. "Low", "medium" and "high" refers to the highest elevation category in which the species was recorded. **b** Colours of flowers from the study site, according to how they would be perceived by the blowfly. Flower species are categorised according to the highest elevation range in which they were recorded. The model is used is that from Troje (1993) for *Lucilia* sp.; colours in the same quadrant of the graph are not discriminated by the fly, meaning that all flowers appear (clockwise, from top-left) fly-blue, UV, fly-purple or fly-green

We used Microsoft Excel with the Bootstrap add-in (available from http://www3.wabash.edu/econometrics/) to investigate whether there is an association between flower colour and elevation. We compared all the species that occurred in the same altitude group or combination of groups (e.g. low and medium) pairwise, counting the total number of times flower species occurring across the same ranges also shared the same colour, using either bee colours or fly colours in separate analyses. This yielded a measure, $N_{\cap}$, of the association between flower colour and altitude ranges of the flowers, derived from summing the counts of incidences in which species found across the same altitude groups share the same colours. For bee colours, $N_{\cap} = 251$ in our actual data, and for fly colours, $N_{\cap} = 343$.

We reassigned the flower colours across the sample 10,000 times, whilst keeping the altitude range over which each species is found constant, and recalculated $N_{\cap}$ with each trial, tracking how it varied and producing frequency distributions shown in Fig. 3. If particular colours are strongly dominant at some altitudes, $N_{\cap}$ will be disproportionately high, whilst if particular colours are found at unusually low frequency in a particular altitude range, $N_{\cap}$ will be low. Using the frequency distributions of $N_{\cap}$ obtained from our randomisations, we were able to ascertain whether the value corresponding to the original data differed significantly from expected, and thus, whether there was an association between elevation and colour. We will subsequently refer to this randomisation test as "Elevation versus Colour analysis".

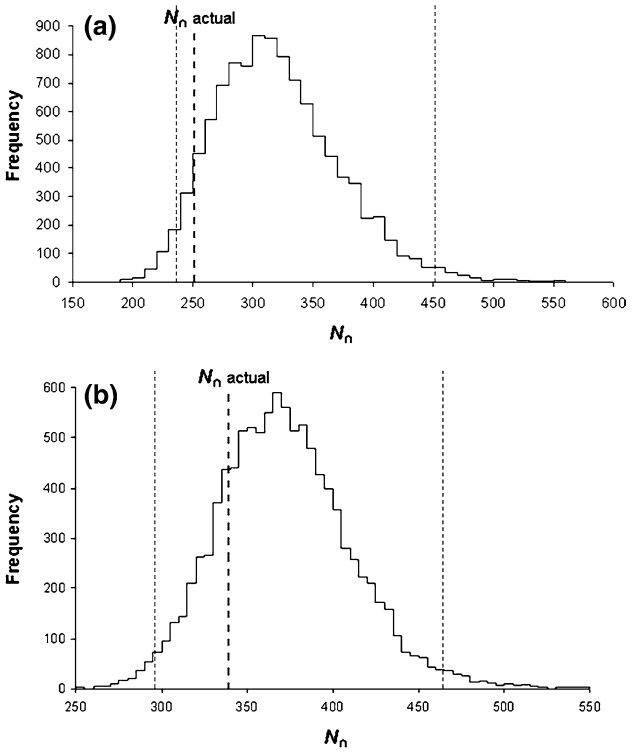

Fig. 3 Frequency distributions obtained for the value of $N_{\cap}$ when the flower species are categorised by (**a**) bee colour and (**b**) fly colour. $N_{\cap}$ is a measure of the number of times flower species that are present across the same altitude range share the same colour. If, at any elevation, one flower colour becomes disproportionately dominant or rare relative to chance, species occurring at that elevation will be either much more or much less likely to share the same colour, giving rise to an unusually high or low $N_{\cap}$ value. The thin dotted lines indicate the boundaries for the upper and lower 2.5% most extreme $N_{\cap}$ values

Distributions of spectral characteristics of flowers by elevation group

Since an alternative possibility to pollinator-mediated selection is that flowers' pigments are selected by abiotic factors, we also analysed the spectra independently of the consideration of any visual system. We simplified the spectra to the values obtained at 50 nm intervals over the range originally measured. This is justified because flowers typically have smooth reflectance functions, with only two or three strong changes in reflectance over a range from 300 nm to 700 nm (Chittka and Menzel 1992; Chittka et al. 1994). We performed a principal components analysis (PCA) on these data using SPSS for Windows. To test whether the coordinates fell into distinct clusters according to altitude range, we performed a MANOVA on the PCA scores, testing whether the groups of species from different elevations yield significantly different scores. This provides information on whether the reflectance spectra of flowers at the different elevations differ in terms of their physical properties, regardless of the visual system that perceives the flowers.

Effect of phylogeny on flower colour

It is possible that phylogeny is a stronger predictor or constraint of flower colour than any selective action of pollinators or abiotic factors within a habitat, as evidenced by the findings in Chittka (1997) that some plant families have flowers in only two or three bee colour groups, with fewer flowers of other colours. It may also be the case that plant clades are themselves distributed according to elevation.

We constructed a phylogenetic tree of the species studied, using published DNA sequence information of ribulose-1,5-bisphosphate carboxylase (*rbcL*) gene (Appendix 2) and ribosomal ITS1 (internal transcribed spacer 1) (Appendix 3) to resolve multiple species within genera. The *rbcL* gene has already been extensively used in phylogenetic studies as it is well conserved throughout the angiosperms (Chase et al. 1993). We used the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/) to search for *rbcL* sequences for the species present in the habitat. When a complete or

near-complete *rbcL* sequence was not available for a particular species we recorded, we substituted a sequence from a species of the same genus.

In some cases, no sequence was available for any species in the genus; in these cases we used a close relative from the same family. Thus, we used a *Dimorphotheca sinuata* sequence in the place of *Antennaria dioica* and a *Platanthera ciliaris* sequence in the place of the two *Dactylorhiza* species. This is justified by the studies of Kim and Jansen (1995) and Aceto et al. (1999), which place *Dimorphotheca* and *Antennaria*, and *Platanthera* and *Dactylorhiza* close together on phylogenetic trees. For three species (*Viscaria alpina*, *Tanacetum vulgare* and *Hieracium* sp.), we were unable to find any appropriate substitute sequences (sequences for other species from the same family were already included in the analysis, but we were unable to find species that were more closely related to the three above species than to the others in their families), and so these species remain unresolved in this study and were excluded from the subsequent statistical analysis.

Appendix 2 lists the species originally recorded in the habitat, and also the data relating to the sequences used to resolve the relationships, including the species from which the *rbcL* sequences were obtained, the accession details of the samples and the relevant references.

We aligned the sequences using PAUP* (Swofford 2002), then constructed a tree using maximum parsimony. We used a heuristic search with the Tree Bisection Reconnection (TBR) swapping algorithm. We performed 1,000 replicates for stepwise addition, saving only the five best trees from each replicate. The best trees produced were used to create a strict consensus tree, with the two monocot genera (*Tofieldia* and *Dactylorhiza*/*Platanthera*) being used to root the tree following the Angiosperm Phylogeny Group (2003). Two genera (*Saxifraga* and *Silene*) contained three species that were all present at the study site. In order to resolve the relationships between the species within these genera, we used the ITS1 sequence information from the species in these genera.

Using MacClade (Maddison and Maddison 1992), we then manually substituted the original species names from the habitat in place of the species providing the *rbcL* sequence information. Because of the relatively small number of taxa in our sample, a few genera (specifically those genera in the Brassicaceae and Saxifragaceae) were misplaced according to the current phylogeny published by the Angiosperm Phylogeny Group. In these cases, we corrected the tree in MacClade according to the most recent information of the APG using http://www.mobot.org/MOBOT/APGroup/, moving taxa to their correct branches as given in this resource.

All major lineages contained at least two different bee colours, though the Ericaceae in this sample consisted only of bee-blue and bee-blue-green species. We used MacClade (Maddison and Maddison 1992) to test whether the distribution of colours with respect to the known phylogeny deviated significantly from random, and also whether species on the tree showed a pattern in their maximum elevations relative to their phylogeny. We tested for random versus non-random distribution of traits by shuffling the characters (colour or maximum elevation) 1,000 times and testing whether the tree lengths obtained differed significantly from the tree length obtained from the actual data. If the characters are clustered in the actual tree, this tree length will be significantly shorter than for the trees subsequently created with random reassignments of characters. This test will be referred to as "Elevation versus Phylogeny analysis".

To investigate whether the two variables, phylogeny and altitude, have a combined effect on the colours of flowers, we constructed a distance matrix based on phylogenetic distance between species on the tree. As the sequence data did not perfectly correlate with established trees, we did not use *rbcL* genetic distance in our measure as this would produce anomalous distances; instead we measured distance in terms of the number of nodes in the tree between pairs of species. We created two other similar distance matrices in SPSS, based firstly upon elevation ranges of the species, and secondly on colour distances derived from raw spectral reflectance data for the flower species.

If evolutionary history constrains flower colour, one would anticipate that the colour distance matrix would correlate significantly with the phylogenetic distance matrix. Furthermore, if there was a combined effect of phylogeny and elevation on flower colours, an aggregate distance matrix containing combined information on phylogenetic distance and dissimilarity of elevation range would be expected to correlate with a matrix of colour distances—i.e. the closer species are in evolutionary history and elevation range, the more similar their colours. We used the ade4 package in the R statistical package (R Development Core Team 2004) with 1,000 repeats to test whether this was the case.

Results

The colour composition of the flower populations in the different elevation groups is shown in Fig. 4; graphs show the bee colours of the flowers, as used in the analyses, and also the species as classified by human colours, for reference.

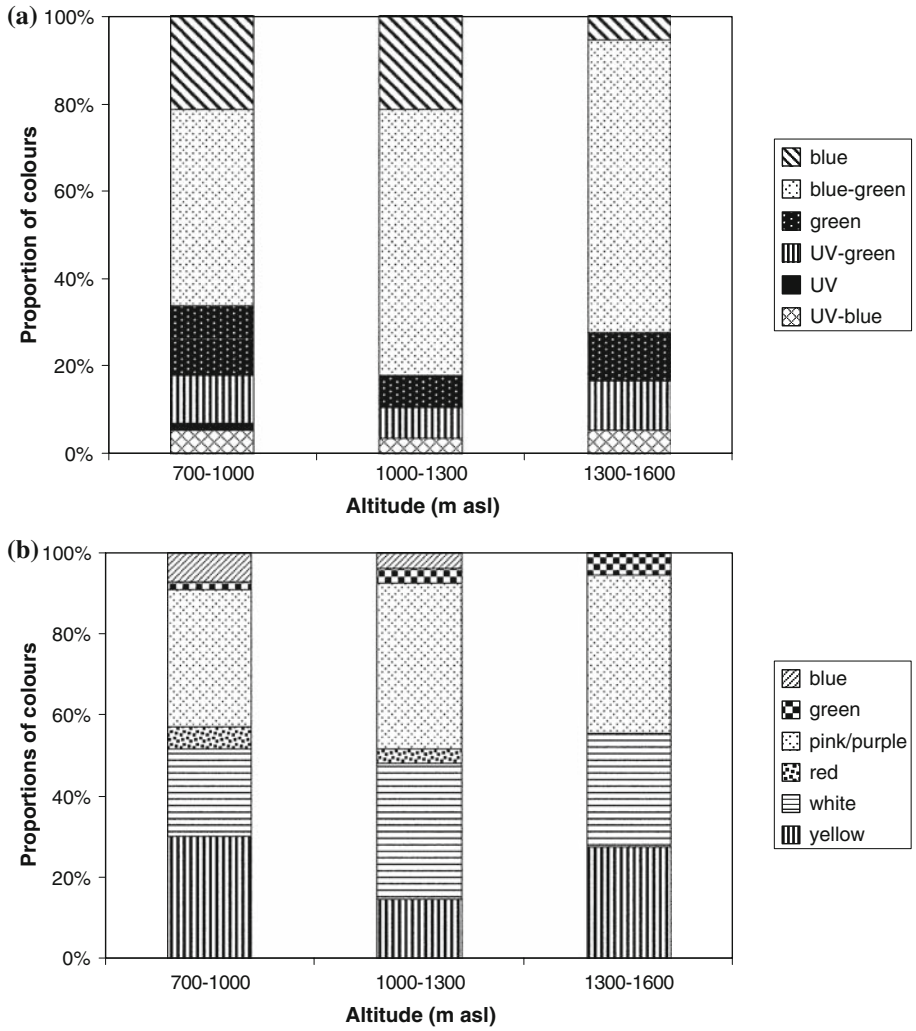

Fig. 4 Relative proportions of different flower colours present at the survey site, with increasing elevation. Flowers are classified on their appearance according to a) the bee visual system and b) the human visual system. (Number of species recorded at each elevation range: 700–1,000 m, 58; 1,000–1,300 m, 27; 1,300–1,600 m, 18.)

Effect of elevation on bee colour composition of the community

The commonest bee colour at all altitudes was blue–green (52% of flowers overall), and the proportion of blue–green flower species increased from low to high altitudes, from 45% in the low altitude group to 67% in the high altitude group. Whilst not significant (see below), this trend is in line with predictions based purely on the concept of pollination syndromes—at high altitudes where flies are the dominant pollinator type, flowers should, according to this hypothesis, be more likely to be human white, i.e. bee-blue-green, than at lower elevations. By contrast, the proportion of bee-blue flowers (usually blue or purple to humans) declined with increasing altitude; only 5% of the flower species recorded above 1,300 m were blue to a bee's eyes compared with 21% of species occurring at the lowest elevations below 1,000 m.

Figure 3a shows the frequency distribution of $N_{\cap}$ values obtained from the randomisation when species are classified by bee colour, and the actual value of $N_{\cap}$ obtained from the dataset. The analysis showed no significant tendency for species in the same altitude group to share the same colour more often than chance (Elevation versus Colour analysis, $N_{\cap} = 251$, $P = 0.144$), and this holds when species found at each elevation are considered individually (low elevation, $N_{\cap} = 221$, $P = 0.076$; medium, $N_{\cap} = 33$, $P = 0.608$; high, $N_{\cap} = 23$, $P = 0.457$). This indicates overall that no flower colour is more dominant than expected at any particular elevation; were a colour category to predominate more at any particular elevation or, equally, to decrease in importance, it would skew the value of $N_{\cap}$ and cause an unexpectedly high or low value to be obtained. The value of $N_{\cap}$ obtained for the actual dataset is somewhat lower than many of the random values obtained; although this is by no means significant, it suggests that, perhaps surprisingly, the aggregation of colours by elevation is somewhat less than chance would predict, i.e. the dominance of common colours in some altitude groups is (non-significantly) less than expected by chance.

Effect of elevation on fly colour composition of the community

The commonest fly colour categories were "fly-yellow" (50% of species) and "fly-blue" (38% of species); "fly-UV" and "fly-purple" categories contained only three and six species, respectively. Increasing elevation was associated with an increase in the proportion of fly-yellow flower species (51–73%) and a decrease in fly-blue and fly-purple flowers (from 36% to 20%, and 8 to 0% from the low elevation group to the high elevation group, respectively). However, these changes are not statistically significant: the randomisation analysis revealed no trend for flowers growing in the same altitude ranges to share the same fly colour (Elevation versus Colour analysis, $N_{\cap} = 343$, $P = 0.594$). This indicates that no colour of flower is more or less dominant than expected by chance at any elevation. The frequency distribution of $N_{\cap}$ values obtained in the randomisation is shown in Fig. 3b, including the value corresponding to the actual data.

Distributions of spectral characteristics of flowers by elevation group

The results of the principal components analysis are shown in Fig. 5. The distribution of colours from all three altitude groups appear to overlap heavily, and indeed the MANOVA reveals no difference between the groups of points (Wilks' lambda, $F = 0.247$, $P = 0.911$, $hdf = 4.0$, $edf = 140$), indicating that the distributions of reflectance spectra present in each elevation group are statistically indistinguishable, at least with the sample sizes available to us.

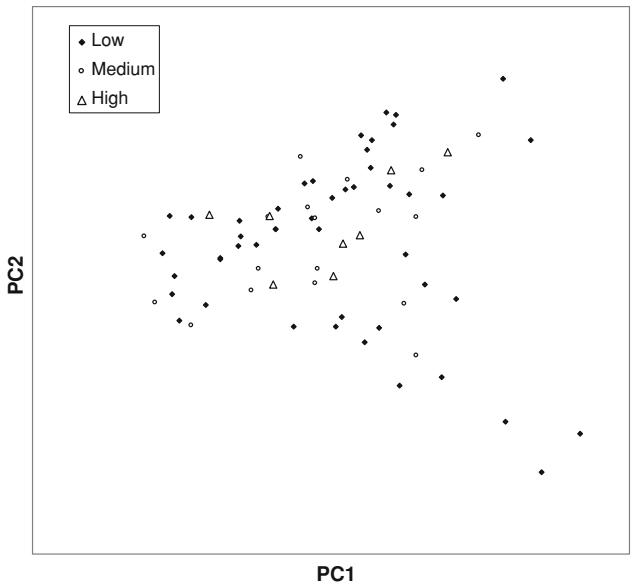

Fig. 5 Principal components analysis of spectral reflectance data. Here, flowers are classified as low, medium or high elevation based on the maximum elevation at which they were recorded (<1,000 m, <1,300 m and <1,600 m, respectively). Variation accounted for by principal component 1: 45.177%; principal component 2: 25.871%

Effect of phylogeny on flower colour

The phylogenetic tree of the flower species present at the study site is shown in Fig. 6, with the colours included for reference purposes. The tree length when maximum elevation was mapped on to the tree is significantly shorter than chance (Elevation versus Phylogeny analysis, $n = 71$, actual tree length = 24, $P = 0.029$), indicating that growing at high elevations is a trait that occurs non-randomly with respect to phylogeny. By contrast, in this analysis, the tree length for colour did not differ significantly from random (Elevation versus Phylogeny analysis, $n = 71$, actual tree length = 27, $P = 0.250$), giving no evidence in this particular analysis of a pattern of colour relative to phylogeny, perhaps because our sample included mostly distantly related species, where the phylogenetic signal is weak.

When we compared a matrix of phylogenetic distances with a matrix of colour distances based on raw flower spectra, there was also no significant correlation between the matrices (Mantel test, $r = 0.0169$, $n = 71$, $P = 0.174$). Although at certain scales there are inevitably constraints of evolutionary history on flower colour, the effect in this sample is not significant.

We also found no significant correlation between the matrix of colour distances and the aggregated matrix of phylogenetic distance and dissimilarity in altitude range when the colour distances were derived from raw spectra (Mantel test, $r = 0.0215$, $n = 71$, $P = 0.123$). This indicates that phylogenetic distance across the data set does not interact with altitude to affect flower colour.

Discussion

Previous authors have made observations about the colours of flowers in alpine areas, stating that the colour composition of high altitude and arctic communities differs from those at lower elevations (Kevan 1972; Savile 1972; Weevers 1952). In this study of flower colours along a single transect in the Norwegian alpine flora, we sought to test whether any of these observations can be supported statistically. Unlike the studies by Savile (1972) and Weevers (1952), we considered the flower colours as they would be seen by insect pollinators, as this better reflects the selective pressures on those flowers. We also analysed how spectral properties of flowers were distributed over a range of altitudes, making no a priori assumptions about the visual systems viewing any of the flower species.

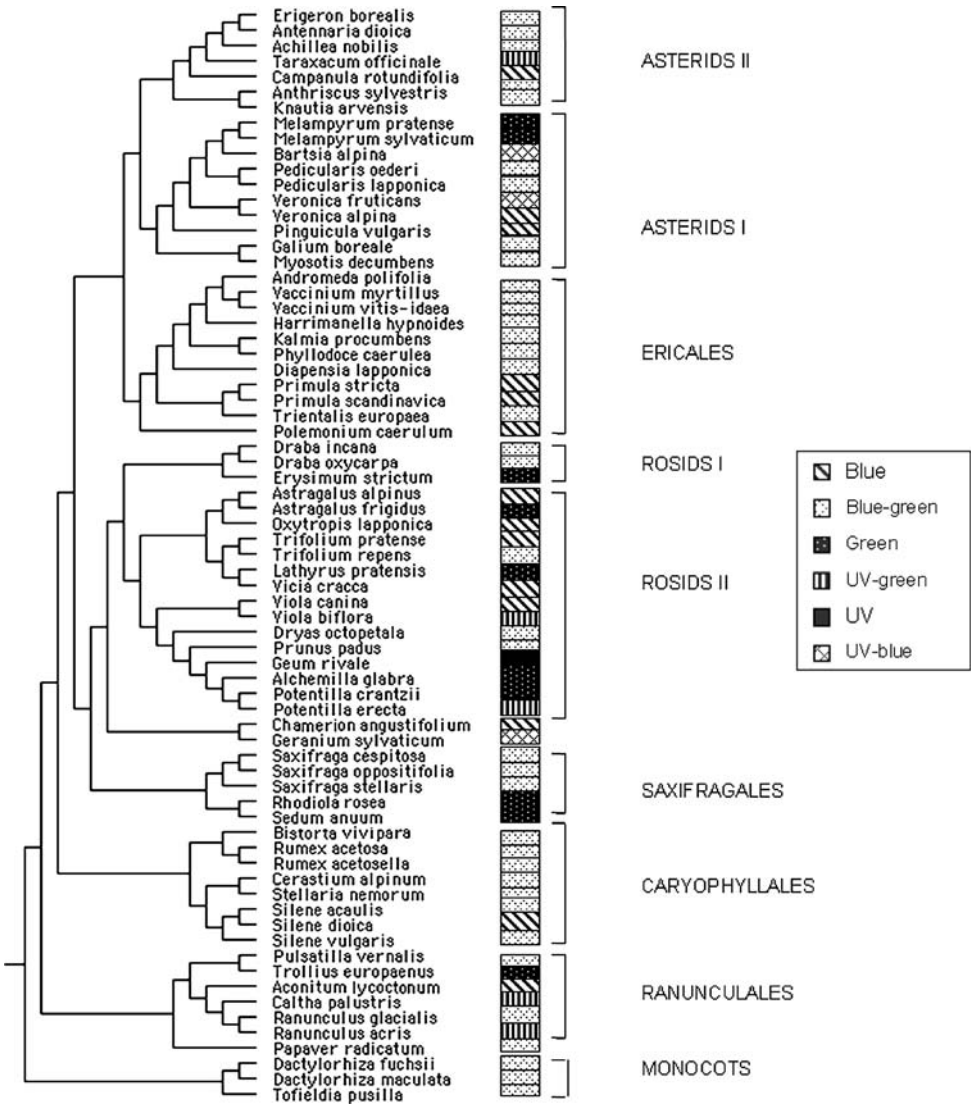

Fig. 6 Phylogenetic tree of the species recorded at the study site. We used published *rbcL* (the large subunit of the ribulose-1,5-bisphosphate carboxylase) gene sequences from the GenBank database to resolve relationships to the genus level, and ITS1 (ribosomal internal transcribed spacer 1) sequences to resolve species within genera where necessary

We have focused on the visual systems of bees and flies because these are the two dominant pollinator types in Norwegian alpine habitats. However, other pollinators are, of course, present in plant communities. The recent study by Lázaro et al. (2008) noted that butterflies in the Norwegian mountains are of relatively minor importance when compared to the above groups (constituting just 2% of the flower-visiting insects at the lowest altitude study site, increasing to 7.9% at the highest altitudes). Butterflies' innately preferred colours can vary vastly depending on species and individual so it is difficult to generalise (Lunau and Maier 1995; Neumayer and Spaethe 2007); diurnal lepidopterans also have very variable numbers of photoreceptor types (Briscoe 2000; Briscoe and Bernard 2005; Sison-Mangus et al. 2006). For these reasons, we have chosen not to include a quantitative analysis of flower colours as seen by butterflies here.

The diversity of butterfly visual systems, species-to-species differences in colour preferences and the comparatively small contribution they make to the total pollinator visitation relative to that of bees and flies means that attempting to model the flowers according to their visual system was not feasible. Equally, there is insufficient information about beetle colour vision to be able to make predictions about how they view flowers, and this group makes a very small contribution to pollination in Norwegian alpine habitats (Lázaro et al. 2008).

The study by Lázaro et al. (2008) found an association between pollinator type (e.g. bees, flies, butterflies, etc.) and flower colour and other aspects of morphology in Norwegian habitats at various altitudes. Given this, and the fact that the pollinator community changes in composition at different elevations, a possible prediction arising from this is that as different pollinator types change in

importance at different elevations, and each group is associated with particular colours of flower, then the colours of flower species present should also vary in accordance with these preferences. Although visit frequency taken alone does not perfectly assess an insect's contribution to pollination of a particular plant, visit frequency is one measure of total interaction (Vázquez et al. 2005) and therefore a colour that is associated with more visits from a pollinator is probably also receiving more benefit from that pollinator than a flower of another colour visited less frequently. This could apply regardless of whether the colour association is based on innate preferences (Lunau and Maier 1995) or the result of pollinators learning which flowers are most suitable for them (Raine et al. 2006), given flower morphology and rewards.

However, our analysis provides no evidence for altitude variation in the distribution of flower colours, either as perceived by bees or by flies. Indeed, even when considering the flower colours without any model of insect perception, no differences between the altitude groups emerged; the PCA indicates that the distributions of spectral reflectance properties in the three altitude categories are statistically indistinguishable. The lack of association between elevation and colour is unlikely to be a result of insufficient data: all species found along our transect during a full week's careful survey were recorded, and the length of the transect spanned sufficient distance that there were substantial changes in the habitat type, from woodland and stream beds to unstable, rocky high-alpine substrates. Although the transect began at around 700 m, and did not extend to such low elevations as in Lázaro et al. (2008), the change in habitat types suggests a significant change in pollinator composition, such that a change in flower colour composition of the communities could be anticipated.

It is known from other studies that evolutionary history constrains flowers' colours (Kalisz and Kramer 2008; Menzel and Shmida 1993), since not all families have the biochemical pathways to produce particular pigments. Some plant lineages only contain particular floral pigments, and therefore flowers in those groups can only assume a limited range of colours. However, most plant families are ultimately capable of producing a variety of colours (Chittka 1997), and in our study there is no evidence of a combined effect of phylogeny and elevation predicting flower colour similarities according to relatedness and shared elevation range.

There are a number of reasons why such a lack of change in the proportions of flower colours with changing elevation, even when the effect of phylogeny is factored into the analysis, may be observed. The first is the phenomenal learning ability of insect pollinators. Even though certain pollinator types have specific innate preferences for colours of flowers (Giurfa et al. 1995; Lunau et al. 1996; Raine et al. 2006), many are able to learn to overcome these preferences easily if a flower is sufficiently rewarding (Menzel 1985). Therefore, simply because a flower's colour matches the innate preference of a dominant pollinator, this may not necessarily constitute a fundamental selective advantage for the flowers, since the vast majority of pollinator visits will be by experienced individuals (Raine and Chittka 2007). The pollination market hypothesis, in fact, advocates that a range of distinct and discriminable colours in a habitat would be most advantageous to plants (Friedman and Shmida 1995; Gumbert et al. 1999). Even if the diversity of pollinators decreases with elevation, rather than appealing to the innate preferences of those remaining pollinator types, a plant species may benefit more from displaying floral signals that are distinctive and recognisable (Chittka et al. 1999).

There can also be selection for certain pigments for reasons other than pollinator preference, particularly because of their protective ability on the plant. Examples include protecting against desiccation, cold, drought, herbivory or UV damage (Ben-Tal and King 1997; Chalker-Scott 1999; Chittka et al. 2001; Fineblum and Rausher 1997; Mori et al. 2005; Warren and Mackenzie 2001), and to other challenges such as herbivory (Johnson et al. 2008), all factors which are likely to differ in importance at different elevations. Similarly, the increase in ultraviolet at high elevations can be damaging to some plant cells, and it has been found that floral pigments such as anthocyanins (usually conferring a blue or red colouration) may also confer protection against UV damage (Mori et al. 2005). It is therefore conceivable that in some cases the pigments favoured by physical factors conflict with those that pollinators may favour, resulting in an overall observation that colour frequencies at different altitudes do not differ, in spite of various selection pressures favouring particular colours in particular circumstances. Based on current knowledge of the protective effects of anthocyanin pigments, one might expect that the flowers of plants subjected more often to extreme environmental conditions such as high altitude would bear such pigments in increased quantities. However, at high altitudes, selection according to the concept of fly pollination syndrome might dictate that flowers would be principally pollinated by flies and therefore "should" be white or yellow (Lázaro et al. 2008). This could result in a trade-off situation in which flowers must compromise between the colours that appeal to pollinators' innate preferences, and those which serve other protective functions. Analogous trade-offs in which traits or behaviours are beneficial in some contexts and disadvantageous in others are relatively abundant in nature, for example when zooplankton face trade-offs between risks of UV damage and risk of predation when migrating

between depths in lakes or developing protective pigments (Boeing et al. 2004; Hansson 2000).

Overall, our study demonstrates that there is no simple pattern to the colours of flowers in mountainous areas as elevation increases, whether flowers are considered according to either of two insect colour vision models or based only on their reflectance spectra, and that the pollinator types present cannot account for the lack of differences if considered purely within the context of the pollination syndrome concept.

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Appendix

See Tables 1, 2 and 3.

Table 1 List of plant species analysed, including colour information (as seen by bees, and also humans, after categorising the flower colour into one of six human colours) and elevation of collection (in m a.s.l.)

Family	Species name	Bee colour	Human colour	Fly colour	Elevation
Campanulaceae	*Campanula rotundifolia*	Blue	Blue	p− y+ ("blue")	700–800
Caryophyllaceae	*Silene dioica*	Blue	Pink/purple	p− y+ ("blue")	700–1,200
Caryophyllaceae	*Viscaria alpina*	Blue	Pink/purple	p− y+ ("blue")	1,050–1,180
Fabaceae	*Astragalus alpinus*	Blue	Pink/purple	p− y+ ("blue")	700–1,500
Fabaceae	*Oxytropis lapponica*	Blue	Pink/purple	p− y+ ("blue")	700–900
Fabaceae	*Trifolium pratense*	Blue	Pink/purple	p− y+ ("blue")	700–900
Fabaceae	*Vicia cracca*	Blue	Pink/purple	p− y+ ("blue")	700–800
Lentibulariaceae	*Pinguicula vulgaris*	Blue	Pink/purple	p− y+ ("blue")	700–1,300
Onagraceae	*Chamerion angustifolium*	Blue	Pink/purple	p− y+ ("blue")	700–900
Plantaginaceae	*Veronica alpina*	Blue	Blue	p− y+ ("blue")	1,100
Polemoniaceae	*Polemonium caerulum*	Blue	Pink/purple	p− y+ ("blue")	700–1,000
Primulaceae	*Primula stricta*	Blue	Pink/purple	p− y+ ("blue")	700–900
Primulaceae	*Primula scandinavica*	Blue	Pink/purple	p− y+ ("blue")	1,050–1,150
Ranunculaceae	*Aconitum lycoctonum* subsp. septentrionale	Blue	Pink/purple	p− y+ ("blue")	700–900
Violaceae	*Viola canina*	Blue	Blue	p− y+ ("blue")	700–900
Apiaceae	*Anthriscus sylvestris*	Blue–green	White	p− y− ("yellow")	700–920
Asteraceae	*Achillea nobilis*	Blue–green	White	p− y− ("yellow")	700–900
Asteraceae	*Antennaria dioica*	Blue–green	Pink/purple	p− y− ("yellow")	920–1,200
Asteraceae	*Erigeron borealis*	Blue–green	White	p− y− ("yellow")	1,400–1,500
Boraginaceae	*Myosotis decumbens*	Blue–green	Blue	p− y+ ("blue")	800–1,000
Brassicaceae	*Draba incana*	Blue–green	White	p− y− ("yellow")	700–1,500
Brassicaceae	*Draba oxycarpa*	Blue–green	Yellow	p− y− ("yellow")	1,600
Caryophyllaceae	*Cerastium alpinum*	Blue–green	White	p− y+ ("blue")	700–1,100
Caryophyllaceae	*Silene acaulis*	Blue–green	Pink/purple	p− y+ ("blue")	1,000–1,600
Caryophyllaceae	*Silene vulgaris*	Blue–green	White	p− y− ("yellow")	700–900
Caryophyllaceae	*Stellaria nemorum*	Blue–green	White	p− y− ("yellow")	700–900
Crassulaceae	*Sedum annuum*	Blue–green	Red	p− y− ("yellow")	800–900
Diapensiaceae	*Diapensia lapponica*	Blue–green	White	p− y− ("yellow")	1,040–1,100
Dipsacaceae	*Knautia arvensis*	Blue–green	Pink/purple	p− y+ ("blue")	700–900
Ericaceae	*Andromeda polifolia*	Blue–green	White	p− y− ("yellow")	1,040–1,260
Ericaceae	*Harrimanella hypnoides*	Blue–green	White	p− y− ("yellow")	1,040–1,160
Ericaceae	*Kalmia procumbens*	Blue–green	Pink/purple	p− y+ ("blue")	1,040–1,160
Ericaceae	*Phyllodoce caerulea*	Blue–green	Pink/purple	p− y+ ("blue")	700–1,500
Ericaceae	*Vaccinium vitis-idaea*	Blue–green	Pink/purple	p− y− ("yellow")	700–900

Table 1 continued

Family	Species name	Bee colour	Human colour	Fly colour	Elevation
Ericaceae	*Vaccinium myrtillus*	Blue–green	Red	p− y+ (“blue”)	1,000–1,100
Fabaceae	*Trifolium repens*	Blue–green	White	p− y− (“yellow”)	700–900
Orchidaceae	*Dactylorhiza maculata*	Blue–green	Pink/purple	p− y+ (“blue”)	700
Orchidaceae	*Dactylorhiza fuchsii*	Blue–green	Pink/purple	p− y+ (“blue”)	700
Orobanchaceae	*Pedicularis lapponica*	Blue–green	Yellow	p− y− (“yellow”)	700–800
Orobanchaceae	*Pedicularis oederi*	Blue–green	Yellow	p− y− (“yellow”)	920–1,600
Papaveraceae	*Papaver radicatum*	Blue–green	Yellow	p− y− (“yellow”)	900
Polygonaceae	*Bistorta vivipara*	Blue–green	White	p− y− (“yellow”)	960–1,100
Polygonaceae	*Rumex acetosa*	Blue–green	Red	p− y− (“yellow”)	700–800
Polygonaceae	*Rumex acetosella*	Blue–green	Red	p− y+ (“blue”)	700–900
Primulaceae	*Trientalis europaea*	Blue–green	White	p− y− (“yellow”)	900–1,200
Ranunculaceae	*Pulsatilla vernalis*	Blue–green	Pink/purple	p− y+ (“blue”)	1,000–1,200
Ranunculaceae	*Ranunculus glacialis*	Blue–green	Pink/purple	p− y− (“yellow”)	1,600
Rosaceae	*Dryas octopetala*	Blue–green	White	p− y− (“yellow”)	900–1,500
Rosaceae	*Prunus padus*	Blue–green	White	p− y− (“yellow”)	700–800
Rubiaceae	*Galium boreale*	Blue–green	White	p− y− (“yellow”)	700–900
Saxifragaceae	*Saxifraga stellaris*	Blue–green	Pink/purple	p− y− (“yellow”)	1,400–1,500
Saxifragaceae	*Saxifraga oppositifolia*	Blue–green	Pink/purple	p− y+ (“blue”)	1,400–1,500
Saxifragaceae	*Saxifraga cespitosa*	Blue–green	White	p− y− (“yellow”)	1,100–1,600
Tofieldiaceae	*Tofieldia pusilla*	Blue–green	White	p− y− (“yellow”)	1,400
Asteraceae	*Tanacetum vulgare*	Green	Yellow	p− y− (“yellow”)	800
Brassicaceae	*Erysinum strictum*	Green	Yellow	p− y− (“yellow”)	700–900
Crassulaceae	*Rhodiola rosea*	Green	Green	p− y− (“yellow”)	1,160–1,600
Fabaceae	*Astragalus frigidus*	Green	Yellow	p− y− (“yellow”)	700–1,000
Fabaceae	*Lathyrus pratensis*	Green	Yellow	p− y− (“yellow”)	700–800
Orobanchaceae	*Melampyrum sylvaticum*	Green	Yellow	p− y− (“yellow”)	700–960
Orobanchaceae	*Melampyrum pratense*	Green	Yellow	p− y− (“yellow”)	700–800
Ranunculaceae	*Trollius europaenus*	Green	Yellow	p− y− (“yellow”)	900
Rosaceae	*Alchemilla glabra*	Green	Green	p− y− (“yellow”)	700–1,000
Rosaceae	*Potentilla crantzii*	Green	Yellow	p− y− (“yellow”)	900–1,600
Rosaceae	*Geum rivale*	UV	Pink/purple	p+ y+ (“UV”)	700–1,000
Geraniaceae	*Geranium sylvaticum*	UV-blue	Pink/purple	p+ y+ (“UV”)	700–1,000
Orobanchaceae	*Bartsia alpina*	UV-blue	Pink/purple	p+ y+ (“UV”)	700–1,500
Plantaginaceae	*Veronica fruticans*	UV-blue	Blue	p− y+ (“blue”)	700–900
Asteraceae	*Hieracium spec.*	UV-green	Yellow	p+ y− (“purple”)	900
Asteraceae	*Taraxacum officinale*	UV-green	Yellow	p+ y− (“purple”)	700–1,000
Ranunculaceae	*Caltha palustris*	UV-green	Yellow	p+ y− (“purple”)	900–1,000
Ranunculaceae	*Ranunculus acris*	UV-green	Yellow	p+ y− (“purple”)	700–1,500
Rosaceae	*Potentilla erecta*	UV-green	Yellow	p+ y− (“purple”)	700–900
Violaceae	*Viola biflora*	UV-green	Yellow	p+ y− (“purple”)	800–1,500

Species names are as in *Norsk Flora* (Lid and Lid 2005)

Table 2 Details of the *rbcL* sequences used to build the phylogenetic tree, including accession and citation details for the species providing rbcL sequences

Species measured	Species sequence used	Family	Accession	Citation
Achillea nobilis	*Achillea millefolium*	Asteraceae	EU384938	Panero and Funk (2008)
Aconitum septentrionale	*Aconitum racemulosum*	Ranunculaceae	AY954488	Wang et al. (2005)
Alchemilla glabra	*Alchemilla mollis*	Rosaceae	AMU06792	Soltis et al. (1993)
Andromeda polifolia	*Andromeda polifolia*	Ericaceae	AF124572	Kron et al. (1999)
Antennaria dioica	*Dimorphotheca sinuata*	Asteraceae	EU384966	Panero and Funk (2008)
Anthriscus sylvestris	*Anthriscus aemula*	Apiaceae	D44554	Kondo et al. (1996)
Astragalus alpinus/frigidus	*Astragalus membranaceus*	Fabaceae	EF685978	Guo et al. (2007)
Bartsia alpina	*Bartsia alpina*	Orobanchaceae	AF190903	Olmstead et al. (1992)
Bistorta vivipara (syn. *Polygonum viviparum*)	*Polygonum cuspidatum*	Polygonaceae	AB019031	Inamura et al. (1998)
Caltha palustris	*Caltha palustris*	Ranunculaceae	AY395532	Silvertown et al. (2006)
Campanula rotundifolia	*Campanula trachelium*	Campanulaceae	DQ356118	Antonelli (2008)
Harimanella hypnoides (syn. *Cassiope hypnoides*)	*Cassiope mertensiana*	Ericaceae	L12603	Kron and Chase (1993)
Cerastium alpinum	*Cerastium glomeratum*	Caryophyllaceae	M83542	Manhart et al. (1991)
Chamerion angustifolium (syn. *Epilobium angustifolium*)	*Epilobium rigidum*	Onagraceae	AF495763	Levin et al. (2003)
Dactylorhiza maculata/fuschii	*Platanthera ciliaris*	Orchidaceae	AF074215	Cameron et al. (1999)
Diapensia lapponica	*Diapensia lapponica*	Diapensiaceae	L12612	Kron and Chase (1993)
Draba incarna/oxycarpa	*Draba nemorosa*	Brassicaceae	NC_009272	Hosouchi et al. (2007)
Dryas octopetala	*Dryas drummondii*	Rosaceae	U59818	Swensen (1996)
Erigeron borealis	*Erigeron tenuis*	Asteraceae	EU384973	Panero and Funk (2008)
Erysimum hieracifolium	*Erysimum capitatum*	Brassicaceae	AY167980	Cummings et al. (2003)
Galium boreale	*Galium mollugo*	Rubiaceae	AY395538	Silvertown et al. (2006)
Geranium sylvaticum	*Geranium albanum*	Geraniaceae	DQ452884	Fiz et al. (2008)
Geum rivale	*Geum macrophyllum*	Rosaceae	U06806	Soltis et al. (1993)
Hieracium spec.	N/A			
Knautia arvensis	*Knautia intermedia*	Dipsacaceae	Y10698	Backlund and Bremer (1997)
Lathyrus pratensis	*Lathyrus pratensis*	Fabaceae	AY395544	Silvertown et al. (2006)
Kalmia procumbens	*Kalmia procumbens*	Ericaceae	U49288	Kron and King (1996)
Melampyrum sylvaticum/pratensis	*Melampyrum sylvaticum*	Orobanchaceae	AM503854	Li et al. (2008)
Myosotis decumbens	*Myosotis discolor*	Boraginaceae	AY395552	Silvertown et al. (2006)
Oxytropis lapponica	*Oxytropis anertii*	Fabaceae	EF685981	Guo et al. (2007)
Papaver radicatum	*Papaver* sp. *Goldblatt 12541*	Papaveraceae	AM235045	Forest et al. (2007)
Pedicularis oederi/lapponica	*Pedicularis coronata*	Orobanchaceae	AF206803	Soltis et al. (1999)
Phyllodoce caerulea	*Phyllodoce caerulea*	Ericaceae	AF419829	Kron (2001)
Pinguicula vulgaris	*Pinguicula caerulea*	Lentibulariaceae	L01942	Albert et al. (1992)
Polemonium caerulum	*Polemonium reptans*	Polemoniaceae	L11687	Olmstead et al. (1992)
Potentilla erecta/crantzii	*Potentilla fruticosa*	Rosaceae	U06818	Soltis et al. (1993)
Primula stricta/scandinavica	*Primula stricta*	Primulaceae	AF394975	Trift et al. (2002)
Prunus padus	*Prunus padus*	Rosaceae	AF411485	Jung et al. (2001)
Pulsatilla vernalis	*Pulsatilla cernua*	Ranunculaceae	AY954492	Wang et al. (2005)
Ranunculus acris/glacialis	*Ranunculus acris*	Ranunculaceae	AY395557	Silvertown et al. (2006)
Rumex acetosa and acetosella	*Rumex acetosella*	Polygonaceae	D86290	Yasui and Ohnishi (1996)
Saxifraga stellaris/ oppositifolia/cespitosa	*Saxifraga stellaris*	Saxifragaceae	AF374732	Soltis et al. (2001)
Sedum anuum and *Rhodiola rosea* (syn. *Sedum rosea*)	*Sedum rubrotinctum*	Crassulaceae	L01956	Albert et al. (1992)
Silene acaulis/dioica/vulgaris	*Silene dioica*	Caryophyllaceae	EF646928	Muir and Filatov (2007)

Table 2 continued

Species measured	Species sequence used	Family	Accession	Citation
Stellaria nemorum	*Stellaria media*	Caryophyllaceae	AF206823	Soltis et al. (1999)
Tanacetum vulgare	N/A			
Taraxacum officinale	*Taraxacum officinale*	Asteraceae	AY395562	Silvertown et al. (2006)
Tofieldia pusilla	*Tofieldia pusilla*	Tofieldiaceae	AJ286562	Bremer (2000)
Trientalis europaea	*Trientalis europaea*	Primulaceae	U96655	Anderberg et al. (1998)
Trifolium pratense/repens	*Trifolium pratense*	Fabaceae	AY395564	Silvertown et al. (2006)
Trollius europaenus	*Trollius laxus*	Ranunculaceae	AY954486	Wang et al. (2005)
Vaccinium vitis-idaea/myrtillus	*Vaccinium vitis-idaea*	Ericaceae	AF419837	Kron (2001)
Veronica alpinus/fruticans	*Veronica anagallis-aquatica*	Plantaginaceae	AY034021	Wagstaff et al. (2002)
Vicia cracca	*Vicia cracca*	Fabaceae	AY395566	Silvertown et al. (2006)
Viola biflora/canina	*Viola philippica*	Violaceae	AB354436	Tokuoka (2008)
Viscaria alpina	N/A			

"N/A" indicates that a suitable sequence from a species in the same genus or a closely related genus was not available

Table 3 Details of the ITS sequences used to discriminate species within genera

Species	Accession	Citation
Saxifraga cespitosa	AF087604	Conti et al. (1999)
Saxifraga oppositifolia	AF087592	Conti et al. (1999)
Saxifraga stellaris	AF374827	Soltis et al. (2001)
Silene acaulis	U30949	Desfeux et al. (1996)
Silene dioica	U32568	Desfeux et al. (1996)
Silene vulgaris	U30969	Desfeux et al. (1996)

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