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Geographic variation in Sundew (*Drosera*) leaf colour: plant-plant interactions counteract expected effects of abiotic factors

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<https://doi.org/10.1111/jbi.13141>

PUBLISHER

© Wiley

VERSION

AM (Accepted Manuscript)

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REPOSITORY RECORD

Millett, Jonathan, G.W. Foot, Julia C. Thompson, and B.M. Svensson. 2017. "Geographic Variation in Sundew (*drosera*) Leaf Colour: Plant-plant Interactions Counteract Expected Effects of Abiotic Factors". Loughborough University. <https://hdl.handle.net/2134/28154>.

1 Article type: original article

2 **Geographic variation in Sundew (*Drosera*) leaf colour: plant-plant interactions**
3 **counteract expected effects of abiotic factors.**

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14 Running head: Geographic variation in *Drosera rotundifolia* leaf colour.

15 Number of words: 6586

16

Abstract

Aim

To identify geographical patterns in leaf colour of Roundleaved Sundew (*Drosera rotundifolia*) growing on ombrotrophic (rain fed) bogs across Europe and establish the controls over these patterns.

Location

North-west Europe.

Taxon

Angiosperms, *Drosera rotundifolia*

Methods

We measured leaf colour of *D. rotundifolia* plants growing on 24 ombrotrophic bogs across north-west Europe covering 26.4 degrees of longitude, and 21.1 degrees of latitude. We measured the height and cover of co-occurring vascular plant vegetation and the amount of incident light intercepted by the vegetation canopy. We determined the role of abiotic variables in controlling the patterns found. In a separate experimental study, we manipulated plant-plant interactions with *D. rotundifolia* by removing above-ground vascular plant vegetation and monitoring leaf colour over a single summer.

Results

Drosera rotundifolia leaf colour varied between bogs. Leaves were redder in northern latitudes and eastern longitudes, and in sites/plots with lower canopy influence, lower nutrient deposition, and a more continental climate. Canopy influence was greater on sites in southern latitudes, eastern longitudes, and with higher nutrient deposition, longer growing seasons and a more maritime climate. Nutrient deposition was higher at more southerly latitudes, eastern sites had a more continental climate, southern and western sites had warmer and longer growing seasons. In the *in-situ* experiment leaves became more red when canopy light transmission was increased by removing vegetation, but not when shade net was subsequently added to reduce light transmission.

Main conclusion

Geographic variation in *Drosera rotundifolia* leaf colour is strongly affected by its light environment, mediated by plant-plant interactions, but leaf colour is also affected by other abiotic factors. The relative importance of biotic and abiotic factors in determining geographic patterns in traits, and also species responses to environmental change, might depend on the growth form and competitive ability of a species.

53 **Keywords:** anthocyanin, *Drosera*, environmental gradients, leaf colour, light,
54 nutrients, plant-plant interactions, sundew, trait variation

55

56

57 Introduction

58 A major goal in ecology is to understand how biotic and abiotic processes influence
59 species distributions, and how individuals respond to these factors. Plant species
60 with broad geographic distributions experience a wide range of biotic and abiotic
61 conditions and plant traits can vary in response. Understanding the patterns and
62 causes of this within-species geographic variability in traits can, therefore, provide
63 insight into the links between plant function, biotic and abiotic interactions (Gaston *et al.*, 2008). It can also help us to understand the impacts on ecosystems of
64 anthropogenic drivers such as altered precipitation patterns and increased
65 temperature (Millien *et al.*, 2006; Zeuss *et al.*, 2014), and deposition of atmospheric
66 pollutants such as nitrogen and sulphur (Ellison & Gotelli, 2002; Millett *et al.*, 2015).
67 For example, phenotypic variability along latitudinal gradients reflects the
68 combination of adaptive and plastic effects, which can inform understanding of the
69 effects of warming on plant distributions (De Frenne *et al.*, 2013); latitudinal
70 gradients in nitrogen deposition have also been used to better understand species
71 responses to atmospheric nitrogen deposition (e.g., Millett *et al.*, 2012)

73 Biotic interactions vary geographically—it is predicted that the intensity of biotic
74 interactions will be greater closer to the equator (Schemske *et al.*, 2009)—and play
75 an important role in maintaining biodiversity (Brooker, 2006). Considerable
76 uncertainty remains, however, about the existence and shape of these geographical
77 patterns (Moles *et al.*, 2011; Ollerton *et al.*, 2011; Poore *et al.*, 2012; Dalrymple *et al.*, 2015), and many different patterns might actually exist. Geographic patterns of
78 plant-plant interactions might be particularly important because of their key role in
79 structuring plant communities. These interactions are predicted by the stress-
80 gradient hypothesis (SGH; Connell, 1983) to be more intense in more productive
81 environments. This has important implications for plant community responses to
82 global environmental change because many of the key drivers, such as
83 eutrophication and warming, will increase productivity in many systems (Brooker,
84 2006). Experimental evidence from altitudinal and latitudinal gradients supports the
85 SGH (Callaway *et al.*, 2002; Kikvidze *et al.*, 2011). These studies, however, are
86 limited in number and are manipulative so might suffer from artefacts of the
87 experimental approach. Another approach would be to investigate variability in plant
88 traits relating to plant-plant interactions along these productivity gradients, but this
89 relies on the identification of appropriate traits.

91 Carnivorous plants trap and digest animal prey, assimilating the nutrients into their
92 tissues. *Drosera rotundifolia* L. is a small carnivorous herb, which occupies
93 ombrotrophic (rain fed) bogs and catches prey with sticky mucilage secreted on the
94 end of leaf trichomes (tentacles). Like many carnivorous plants the leaves of *D.*
95 *rotundifolia* have a distinctive red colouration, due to the presence of anthocyanins
96 (Egan & der Kooy 2013). This red colour was thought to serve a prey attraction
97 function (Lloyd, 1942; Ichiishi *et al.*, 1999), but experimental studies have shown this
98 not to be the case (Foot *et al.*, 2014). Alternative hypotheses for the functional role of

leaf reddening are as a protection against excess light and UV-B radiation, herbivory defence or osmotic adjustment. Aboveground interactions with other plants might be crucial to the ecology of carnivorous plants (Ellison *et al.*, 2003) because the costs of carnivory mean that a net benefit should only occur in high-light environments (Givnish *et al.*, 1984). Aboveground interactions with shrubs might be particularly important for the ecology of *D. rotundifolia* because of its rosette form, with leaves close to the bog surface, meaning it is usually subordinate in the plant canopy.

We used both a correlative and experimental approach to explore in ombrotrophic (rain fed) bogs the relationship between latitude, vegetation structure and leaf colour of the carnivorous plant *Drosera rotundifolia*. The aim of the correlative study was to identify geographic patterns in *D. rotundifolia* leaf colour, vascular plant cover and canopy characteristics; the aim of the experimental study was to determine whether the red leaf pigmentation of *D. rotundifolia* leaves is light sensitive and influenced by aboveground plant-plant interactions. We hypothesised that 1. the red colour of *D. rotundifolia* leaves is light responsive; 2. the intensity of above-ground interactions between *D. rotundifolia* and other vegetation decreases towards the poles, due to decreased productivity; and 3. these changing interactions are indicated by a change in *D. rotundifolia* leaf colour, with leaves becoming redder due to increased light transmission through the vegetation canopy. An alternative hypothesis is that leaves will become less red towards the poles because, in the absence of plant-plant interactions, a strong latitudinal gradient of solar radiation (due to differences in the solar angle) reduces the requirement for photoprotective adaptation at higher latitudes. This alternative hypothesis has been demonstrated for photoprotective metabolites in other plant species (Willis *et al.*, 2011; Koski & Ashman, 2015).

Materials and Methods

Correlative study

For the correlative study 25 ombrotrophic bogs were chosen across western and northern Europe covering 26.4 degrees of longitude (-7.638 – 18.810 °E), and 21.1 degrees of latitude (47.239 – 68.356 °N) (site details are in Appendix S1 in Supporting Information, locations are in Fig. 1). To reduce variability due to habitat differences we only measured plants growing on *Sphagnum* spp. hummocks. At each site, hummocks (c. 4 m²) were selected on the intact ombrotrophic parts of the bog. The number of hummocks varied depending on the size of the bog and available time for sampling (median = 6, range 4 – 10). To reduce the possibility of seasonal differences, sampling took place in a four-week period from late July – early August; because of the work involved, we sampled bogs over three years (2011 – 2013) to keep sampling within this time window.

On each hummock we recorded the percent cover and height of vascular plants. Cover was based on the average of independent estimates of at least two people; height was based on the average of ten randomly located measurements. To

determine the impact of the vegetation canopy on the light environment at the bog surface we measured photosynthetically active radiation (PAR) above the plant canopy and on the bog surface at five points using a PAR quantum sensor (SKP 215, Skye Instruments, Powys, UK). PAR was always measured between 10:00 and 15:00. One fully formed leaf was removed from each of ten *D. rotundifolia* plants per plot and kept chilled at below 10°C but above 0°C before colour analysis. As soon as possible, and within 4 hours, we measured the spectral reflectance of the leaves within the wavelength range 400-800 nm using a VIS-NIR spectrometer (USB4000, Ocean Optics Inc.). The probe was held 15 mm from the leaf giving a measurement area of approximately 35 mm². *Drosera rotundifolia* leaf spectrometry was carried out for all sites where *D. rotundifolia* was present ($n = 24$) but vegetation characteristics only for a sub-set of sites ($n = 18$ for vegetation height, cover and light interception).

Canopy light transmission was calculated as PAR at the bog surface as a proportion of above-canopy PAR. This removed the impact of differences in incident light due to meteorological conditions or site differences. Canopy photographs were analysed using the software integrated into the CI-110 system. Sky and vegetation were differentiated in the photographs using a threshold determined manually, based on a visual assessment. From this the canopy transmission coefficient was calculated, this is the proportion of the photograph that is sky (rather than vegetation). We carried out the initial data analyses with and without these canopy photograph data. The results of both approaches were qualitatively identical; because of this, and because these canopy photography data were only available for a smaller sub-set of sites, we excluded these data from the analyses. At all sites, no shading of plots was present due to rocks or trees. Leaf reflectance data were used to calculate leaf colour using SPECTRASUITE 2.0 (Ocean Optics 2010). We determined the colour of the leaves using multiple colour measures: a^* (a measure of red – green colour) in CIE 1976 ($L^*a^*b^*$) colour space (CIELAB), ‘dominant wavelength’ (the dominant wavelength of the colour in CIE 1976 colourspace, removing differences in saturation or intensity), mean red (600-699 nm), green (500-599 nm) reflectance and the ratio of red:green reflectance. The choice of measures is non-exhaustive and was driven by a wish to define the redness or greenness of the leaves, balancing the number of measures used against simplicity. Further details are in appendix S2 in Supporting Information.

Experimental study

The experimental study was carried out in 2012 at Thorne Moor, part of the Humberhead Peatlands National Nature Reserve, in the UK (Lat: 53.6385°, Long: -0.9009°). We carried out this experiment within a single trench in the peat, c. 20 x 100 m. The trench was originally formed by hand cutting of the peat in the 19th century and has since revegetated with *Sphagnum* spp. (with a developing hummock hollow topography), small shrubs and *D. rotundifolia*, such that the surface of the *Sphagnum* is now level with the surface of surrounding uncut baulks. We chose five

plots, each on a different *Sphagnum* hummock. At each hummock, we established three 0.5 x 0.5 m sub-plots. Each sub-plot was randomly allocated to one of three treatments: 1. 'natural' – vegetation left intact; 2. 'removed' – all aboveground vegetation, except *D. rotundifolia*, removed; and 3. 'removed+shaded' – all aboveground vegetation, except *D. rotundifolia*, removed and a 0.5 x 0.5 m square wooden frame covered in neutral shade cloth (to give a reduction in light intensity of approximately 50%) placed over the plot at a height of approximately 20 cm. The removed+shaded treatment was included to determine the extent to which changes due to vegetation removal (*removed*) could be attributed to impacts of vegetation on the aboveground light environment.

We applied the treatments on 6th June 2012. The light environment on the *Sphagnum* surface was measured by measuring PAR above the plant canopy/shade frame (or at a height of c. 30 cm for *removed* plots) and at the *Sphagnum* surface. Canopy light transmission was calculated as for the correlative study. On 15th August one fully formed leaf was removed from each of ten *D. rotundifolia* plants in each sub-plot. Spectral reflectance of each leaf was determined using the approach detailed for the correlative study.

Environmental variables data sets

We compiled a set of abiotic variables for each site, chosen to reflect the potential to impact on vegetation or leaf colour characteristics. These variables covered meteorological, nutrient deposition and UV radiation data. We used UV-B dose as an indicator of likely radiative dose because in plants UV is responsible for approximately one third of photodamage (Takahashi & Badger, 2011). UV-B exposure data for each plot were obtained from glUV (Beckmann *et al.*, 2014). This dataset provides satellite-derived estimates of global surface UV-B dose at a 15 arc-minute resolution. We used the UVB5 layer, which provides the sum of monthly mean (2004-2013) UV-B during the highest quarter. Annual atmospheric N (total N, NO₃, NH₄ and the ratio of NO₃:NH₄) and total S deposition was modelled using a higher resolution national model for the UK (Smith *et al.*, 2000; NEG-TAP, 2001) and for non-UK sites using the lower resolution European Monitoring and Evaluation Program (EMEP)-based Integrated Deposition Model (IDEM) (Pieterse *et al.*, 2007). Meteorological data comprised the following variables: mean annual (July – June) temperature, minimum January and maximum July temperature, temperature variability (CV for temperature in Fahrenheit), growing degree days (base 5), and mean annual precipitation for the previous five years for each site (with a 0.25 degree latitude-longitude resolution). The variables were calculated using an interpolated data set from E-OBS (Haylock *et al.*, 2008) accessed through the KNMI climate explorer (KNMI, 2013). For these variables, the mean values for the five-years prior to plot measurements were used to represent the likely time-scale of impacts on *D. rotundifolia*, which has an average life span of six years, while balancing the likely larger influence of more recent abiotic conditions.

222 Data analysis

223 Data were analysed in IBM STATISTICS 22.0 (IBM Corp., 2013). Some data reduction
224 was carried out before analysis. Measurements within each plot were averaged to
225 give a mean per plot. These data were used to compare the relationship between
226 canopy characteristics and leaf colour. For all other analyses comparisons were
227 required at the site level ($n = 14 - 26$ sites), so were based on means at each site
228 (i.e. means of plot means, $n = 10$ plants per plot and $5 - 10$ plots per site). There
229 were correlations among abiotic variables, so we performed principal components
230 analysis (PCA) with Varimax rotation on these data. We also used PCA to reduce
231 the five leaf colour measurements to a single index of colour and to reduce the
232 canopy characteristics measures to a single index of canopy influence. These last
233 two PCAs were carried out separately for the site-level and plot-level data analysis.

234 To determine the relationships between variables we used either simple linear
235 regression (for single independent variables) or, where there were multiple
236 independent variables, stepwise ordinary least squares (OLS) regression with
237 forward and backward inclusion, an inclusion cut-off at $P = 0.05$ and an exclusion
238 cut-off at $P = 0.1$. We first determined any systematic geographic variability in the
239 data by using the PCA axes for the environmental, canopy and colour characteristics
240 as dependent variables and latitude and longitude as independent variables (sites
241 west of 0° were coded as negative). We also separately analysed geographical
242 variability in vegetation height, because plant height has previously been shown to
243 vary systematically with latitude (Moles *et al.*, 2009). To do this we used the 'curve
244 estimation' module in IBM Statistics. Appropriateness of the fitted model was
245 assessed by inspecting plots of residuals and the model with the highest r^2 value
246 was chosen.

247 Next, we used the three environmental PCA axes as independent variables and the
248 indices (i.e., PCA axis score) of vegetation influence and *D. rotundifolia* colour
249 characteristics as dependent variables to determine the relationship between the
250 abiotic environment and biotic variables. We also analysed the relationship between
251 UV-B and leaf colour separately because UV-B has been previously implicated in
252 differences in plant colour along latitudinal gradients (Koski & Ashman, 2015).

253 To determine the relationship between canopy characteristics and leaf colour, we
254 used a linear mixed model on plot-level data. We used a two-level model to account
255 for relationships between and within sites. We used plot as a random effect, nested
256 within site, and canopy influence as a fixed effect. We compared four models: 1. null
257 single-level model, 2. null multi-level model, 3. random intercepts model and 4.
258 random slopes and intercepts model. Differences in the appropriateness of each
259 model was tested with a 'log likelihood ratio test', comparing with the previous, less
260 complex model.

For the experimental study the impact of the three was tested by comparing differences in the ground-level light environment after treatments were established and the colour (CIE Lab a*) of *D. rotundifolia* leaves at the end of the experiment. We did this tested using a randomised block design ANOVA with hummocks as blocks and subplots as treatments within blocks. Differences between the three treatments were tested using *post-hoc* comparisons (Fisher's LSD).

Results

PCA results

For the bioclimatic variables, the first three PCA axes explained total of 85.5% of variation (29.5, 29.3 and 26.7% for axes 1, 2 & 3 respectively), and were successful in differentiating between the different groups of environmental variables (Table 1). Axis 1 reflected nutrient deposition differences among the sites, with high scores on this axis representing sites with a high N and S deposition load (higher total N, total S, NO₃ and NH₄ deposition). Axis 2 reflected differences among sites in summer meteorological conditions, sites with high scores on this axis had warmer summers and a longer growing season (higher mean summer temperature, high maximum July temperature and high GDD). Axis 3 reflected the continentality of the climate, sites with high scores on this axis had a more maritime, less continental climate (high January minimum temperature, mean annual temperature, and precipitation, and low monthly temperature variance).

We also used PCA to reduce multiple indices of leaf colour and multiple measures of canopy influence to single principle components. For canopy influence and leaf colour, only a single PCA axis was extracted. For canopy influence the first PCA axis explained 79.8% of between-site variability (Table 2). Sites with high scores on this axis had high vegetation cover, tall vegetation and high interception of incident PAR. The PCA axis for leaf colour explained 85% of the variability and differentiated on the basis of how red or green the leaves were (Table 2). Sites in which plants had redder leaves had high values on this axis, which indicated high CIE LAB a*, high values for the dominant wavelength reflected, red reflectance and the ratio of red:green reflectance, and low values for green reflectance.

Patterns

There are some clear geographic patterns in the three data components (leaf colour, vegetation influence, abiotic variables), and some clear patterns of variation between these different components (full analysis results are in Appendix S3 in Supporting Information). The multiple regression analysis showed that geographic variability in *D. rotundifolia* leaf colour was best explained by a linear regression model which included both latitude and longitude. This explained 65.7% of between-site variability in leaf colour (Table 3). Canopy influence and vegetation height varied with only latitude, which explained 81.6% and 84.7% of variability respectively. Abiotic PCA axis 1 (nutrient deposition) varied with only latitude; abiotic axis 2 (summer growing

conditions) varied with both latitude and longitude; abiotic axis 3 (continentality) varied with only longitude. (See appendix S3.)

When considering only abiotic variables, variability in canopy influence and vegetation height was best explained by a model which included abiotic PCA axes 3, 1 and 2 (continentality, nutrient deposition and summer climate respectively). This model explained 86.5% of the variability in canopy influence and 89.5% of the variability in vegetation height (Table 3). However, a model including only PCA axis 1 and 3 best explained variability in leaf colour, explaining 72.1% of variability in leaf colour. There was no significant relationship between summer temperature/growing degree days and leaf colour. (See appendix S3.)

Canopy influence (vegetation height, cover and light interception) was higher in more northerly and easterly sites (Fig. 2 a & b), though variation with longitude was not statistically significant. *Drosera rotundifolia* leaves were redder in more northerly and easterly sites (Fig. 2c & d). More easterly sites experienced a more continental climate (lower precipitation, lower winter temperature and higher climate variability (Fig. 3a) and higher summer temperatures/longer growing seasons (Fig. 3b). More northerly sites received lower levels of nutrient deposition (Fig. 3c) and lower summer temperatures/longer growing seasons (Fig. 3d).

Canopy influence was greater on sites with a more maritime climate than on sites with a more continental climate (Fig. 4a). *Drosera rotundifolia* on sites with a more continental climate had redder leaves than those with a more maritime climate (Fig. 4b). Sites with higher nutrient deposition had higher canopy influence than those with lower nutrient deposition (Fig. 4c). *Drosera rotundifolia* in these higher nutrient deposition sites had greener leaves than those in the lower nutrient deposition sites (Fig. 4d). Sites with high summer temperatures and longer growing seasons had greater canopy influence and taller vegetation (Fig. 4e).

For the relationship between canopy influence and leaf colour, the model which included both random intercept and slope was the highest ranked based on the -2log likelihood score (comparison is shown in appendix S4 in Supporting Information). This indicates differences between sites in both the relationship between canopy influence and leaf colour, and in the expected leaf-colour at average canopy influence (Fig. 5, appendix S5 in Supporting Information). For this model, overall there was a statistically significant relationship between canopy influence and leaf colour (LMM: d.f.=19.272, $t=-3.778$, $P=0.001$); leaves were less red when canopy influence was higher. The extent of this relationship varied between sites. A strong negative correlation existed between the intercept and slope of the fitted lines (Pearson's Correlation Coefficient: -0.85, $P<0.001$); this indicates that, for sites with a lower intercept (i.e. indicating greener leaves when canopy influence was at the mean of all plots), the slope of the relationship between canopy influence and leaf colour was less steep (i.e. leaf colour varied less with canopy influence).

We used UV-B radiation dose as an indicator of the potential for incident radiation to influence leaf colour. The above-canopy UV-B dose received by the sites was strongly dependent on latitude, decreasing towards the poles (linear regression: $F_{1,23} = 46.18$, $r^2 = 0.67$, $P < 0.001$), but the leaves of *D. rotundifolia* growing at sites which received a higher UV-B dose had greener leaves than those at sites with a lower UV-B dose (Linear regression: $F_{1,23} = 10.364$, $P = 0.004$, $r^2 = 0.311$). These were also the sites with higher canopy influence scores (Pearson correlation coefficient for UV-B vs canopy influence = 0.613, $P = 0.005$).

In our *in-situ* experimental study *D. rotundifolia* leaf colour changed as a result of interactions with co-occurring plants, due to impacts on the light environment. We doubled light transmission through the canopy (Fig. 6, ANOVA: $F_{2,8} = 48.94$, $P < 0.001$) by removing all aboveground vegetation. After ten weeks, the leaves of *D. rotundifolia* plants in these vegetation removal plots were redder than the leaves of plants in control plots (Fig. 6, ANOVA: $F_{2,8} = 11.93$, $P = 0.004$). We also controlled for possible impacts of plant removal independent of their shading effect by adding shade cloth to vegetation removal plots – intercepting approximately the same amount of light as the plant canopy. In these shaded plots, the change in *D. rotundifolia* leaf colour was not present (Fig. 6).

Discussion

We demonstrate systematic geographic variability in plant leaf colour for the first time. *Drosera rotundifolia* leaves were redder in more northerly and easterly sites. Our results provide evidence that this pattern may be controlled to some extent by geographic variability in plant-plant interactions, which are themselves influenced by variation in climate and nutrient deposition. While geographic variation in animal pigmentation has been well-studied (Gloger, 1883; Jablonski & Chaplin, 2000; Lai *et al.*, 2008; Alho *et al.*, 2010; Tuomaala *et al.*, 2012; Zeuss *et al.*, 2014), geographic variability in plant colour is poorly understood. Plants are, however, subject to different evolutionary and environmental constraints to animals (Bradshaw, 1972; Huey *et al.*, 2002). For example, the sessile nature of plants might result in more-pronounced stress resistance mechanisms (Huey *et al.*, 2002) such as pigmentation. Plants might also be influenced by biotic interactions to a greater extent. Moles *et al.* (2011) showed that plant height across ecosystems decreased as a function of latitude. We found the same pattern within a single ecosystem, ombrotrophic bogs. We also found that this, in association with an associated decrease in vegetation cover, reduced canopy light interception altering the light environment of *D. rotundifolia* plants.

The results of our experimental study show that *D. rotundifolia* leaf colour can be directly influenced by vegetation impacts on the light environment. Leaf colour was light sensitive, changing from green to red in response to short-term increases in light intensity due to a reduction in aboveground plant-plant interactions. Increased leaf redness of *D. rotundifolia* indicates higher concentrations of anthocyanins (Egan

& der Kooy 2013)—though this was not measured directly. These experimental results suggest that variability in the influence of aboveground vegetation is a plausible explanation for the geographic pattern of leaf colour. This is supported by the clear and strong relationship between canopy measurements and *D. rotundifolia* leaf colour in our correlative study. We suggest that these changes are at least partially responsible for the observed leaf colour changes, and as far as we are aware, this is the first direct, non-manipulative evidence of such a pattern.

The abiotic conditions for plant growth can vary significantly geographically. Within a species, adaptive and plastic responses of plants to these different and variable conditions result in geographic variability in traits. This phenotypic variability can itself have important impacts on ecological dynamics (Bolnick *et al.* 2011). Our data suggest that biotic interactions might also play a significant role in geographical patterns of plant traits, and can be more important than changes in abiotic conditions—reversing their impact even. Biotic interactions are thought to increase in importance towards the equator (Schemske *et al.*, 2009), and are considered to be one of the key drivers of latitudinal biodiversity gradients (Mittelbach *et al.*, 2007). There is, however, little evidence of broad geographical patterns in plant-plant interactions. Our results suggest that photosensitive plant secondary metabolites might provide a novel way to test for variability in aboveground plant-plant interactions.

Disentangling the correlative patterns in this study was challenging. Leaf-colour, canopy influence and abiotic variables all covaried. Leaf-colour varied with latitude and longitude; canopy influence only varied with latitude. Canopy influence varied in response to all three abiotic PCA axes; leaf colour did not respond to PCA axis 2, which related to growing season temperature. These results might indicate that canopy influence is not the only factor controlling geographic variation in leaf colour in our dataset. For example, the longitudinal gradient in leaf colour might be due to the corresponding impact of increasing continentality of the climate. Leaves were redder in sites which experienced larger extremes of temperature. Low temperatures have been previously suggested to influence anthocyanin synthesis (Close & Beadle 2003), and this could be the case here. The between-site differences in the relationship between canopy influence and leaf-colour support this. It is clear that further, experimental, studies are required to determine causation.

The adaptive function of *D. rotundifolia* leaf reddening was not tested, but has been implicated in photoprotective and stress response roles in other species (Mendez *et al.* 1999; Close & Beadle 2003; Boldt *et al.* 2014). While anthocyanin synthesis in response to light intensity does not directly demonstrate a photoprotective function *per se*, we consider it a plausible explanation. Were this the case, the latitudinal gradient in leaf colour is opposite to expected patterns—photoprotective pigments are predicted to increase towards the equator and higher altitudes, due to the increased intensity of solar radiation. This has been shown to be the case—UV absorbing pigments in *Argentina anserina* flowers (Koski & Ashman, 2015) and

concentrations of the UV-B absorbing compound *para*-coumaric acid in *Pinus sylvestris* (Willis *et al.*, 2011), for example, increase towards the equator. Conversely, Åkerström *et al.*, (2010), found that anthocyanin concentrations in the fruit of Bilberry (*Vaccinium myrtillus*) increased towards the pole in Europe. No clear mechanism for this unexpected pattern has yet been demonstrated, though decreased temperature at very high latitudes has been suggested as a possible mechanism. We also found that red colouration (and likely anthocyanin synthesis) increased towards the pole. The difference between these two groups of species is canopy position. *Argentina anserina* grows in relatively open habitats, *P. sylvestris* tends to be dominant or co-dominant in forest canopies; the *V. myrtillus* studied by Åkerström *et al.*, (2010) were growing under a forest canopy, and the *D. rotundifolia* in our study were growing under a shrub canopy. These observations and our study present a potential mechanism for this difference—plant-plant interactions. In plants, UV is responsible for approximately one third of photodamage (Takahashi & Badger, 2011), so incident UV dose would be expected to be positively correlated with photoprotective metabolites (Willis *et al.*, 2011; Koski & Ashman, 2015). We found the opposite. We therefore predict that, for plants that are subordinate in the vegetation canopy, geographic variability in plant-plant interactions, especially competition for light, are a more important determinant of the synthesis of photoprotective pigments than variability in above-canopy radiation dose. This is intuitive, but has not been previously suggested.

An alternative and intriguing hypothesis is that leaf redness in *Drosera rotundifolia* is an anti-herbivory adaptation—either providing crypsis, or as an aposematic warning. There is some evidence for this in other plant species (Manetas 2006). For *D. rotundifolia* in sites with more vegetation cover, the need to deter herbivores might be reduced because the plants will be ‘hidden’ by the vegetation canopy. In more open areas the plants might be more apparent and so more likely to be seen by herbivores. Foot *et al.* (2012) found that, in a study of prey capture by *D. rotundifolia*, fewer insects were attracted to red artificial leaves than to green artificial leaves. This might indicate that insects are deterred by red colouration. This hypothesis warrants further investigation in carnivorous plants.

In conclusion, understanding geographic variability in other traits of this widespread species and their underlying genetic basis would provide a useful model system for understanding how climate change might impact on species distributions. Biotic interactions and local adaptation are poorly represented in species distribution models (Gotelli & Stanton-Geddes, 2015); we suggest that plant-plant interactions with *D. rotundifolia* vary strongly with latitude and longitude so it might represent a species where current distribution models would be particularly unreliable. It is not unlikely that this is also true for other species. In addition, between-population variability in other traits such as nitrogen nutrition of *D. rotundifolia* has also been demonstrated (Millett *et al.*, 2012, 2015) and might warrant further investigation of the potential for local adaptation. Our data also raise a number of further questions.

The extent to which geographic variability in leaf colour is a result of adaptive vs plastic variability cannot be determined, though we present evidence that leaf colour can clearly exhibit phenotypic plasticity. Furthermore, it is still not clear why *Drosera* are red, and more widely, what the adaptive function (if any) is of leaf/trap redness in carnivorous plants. The commonness of leaf redness in carnivorous plants, across unrelated organisms, might provide a useful system for studying the adaptive significance of leaf reddening in plants.

Acknowledgements

We thank the various landowners and statutory bodies for allowing access to sites for this study. JM was funded by the British Ecological Society, the Botanical Research Fund and Loughborough University, GF was funded by the Engineering and Physical Sciences Research Council, BS was funded by Uppsala University. We thank Fei Mengjie for help with colour analyses and Mark Szegner for help with drawing Fig. 1. The comments of three reviewers were invaluable and their input is gratefully acknowledged.

Appendices

Appendix S1 Characteristics of study sites.

Appendix S2 Leaf colour measurement methods

Appendix S3 Multiple regression results.

Appendix S4 Linear mixed model results.

Appendix S5 Data used in the study.

References

- Åkerström, A., Jaakola, L., Bång, U. & Jäderlund, A. (2010) Effects of latitude-related factors and geographical origin on anthocyanin concentrations in fruits of *Vaccinium myrtillus* L. (Bilberries). *Journal of Agricultural and Food Chemistry*, **58**, 11939–11945.
- Alho, J.S., Herczeg, G., Söderman, F., Laurila, A., Jönsson, K.I. & Merilä, J. (2010) Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? *BMC Evolutionary Biology*, **10**, 317.
- Beckmann, M., Václavík, T., Manceur, A.M., Šprtová, L., von Wehrden, H., Welk, E. & Cord, A.F. (2014) glUV: A global UV-B radiation dataset for macroecological studies. *Methods in Ecology and Evolution*, **5**, 372–383.
- Boldt, J.K., Meyer, M.H. & Erwin, J. E. (2014) Foliar Anthocyanins: A Horticultural Review. *Horticultural Reviews: Volume 42* (ed. by J. Janick), pp. 209-252. John Wiley & Sons, Inc., New Jersey. doi: 10.1002/9781118916827.ch04
- Bolnick, D.I., Amarasekare, P., Araújo, M.S, Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why

504 intraspecific trait variation matters in community ecology. *Trends in Ecology and*
505 *Evolution*, **26**, 183-192.

506 Bradshaw, A. (1972) Some evolutionary consequences of being a plant. *Evolutionary*
507 *Biology*, **5**, 25-47.

508 Brooker, R.W. (2006) Plant – plant interactions and environmental change. *New*
509 *Phytologist*, **171**, 271-284.

510 Callaway, R.M., Brooker, R.W., Choler, P., Kividze, Z., Lortie, C.J., Michalet, R.,
511 Paolini, L., Pugnaire, F.I., Newingham, B., Aschehoug, E.T., Armas, C.,
512 Kikodze, D. & Cook, B.J. (2002) Positive interactions among alpine plants
513 increases with stress. *Nature*, **417**, 844-848.

514 Close, D.C. & Beadle, C.L. (2003) The ecophysiology of foliar anthocyanin. *The*
515 *Botanical Review*, **69**, 149-161.

516 Connell, J. (1983) On the prevalence and relative importance of interspecific
517 competition: evidence from field experiments. *American Naturalist*, **122**, 661-
518 696.

519 Egan, P.A., & der Kooy, F. (2013) Phytochemistry of the carnivorous sundew genus
520 *Drosera* (Droseraceae)—future perspectives and ethnopharmacological
521 relevance. *Chemistry & Biodiversity*, **10**, 1774-1790.

522 Ellison, A.M., Gotelli, N.J., Brewer, J.S., Cochran-Stafira, D.L., Kneitel, J.M., Miller,
523 T.E., Worley, A.C. & Zamora, R. (2003) The evolutionary ecology of carnivorous
524 plants. *Advances in Ecological Research*, **33**, 1-74.

525 Ellison, A.M. & Gotelli, N.J. (2002) Nitrogen availability alters the expression of
526 carnivory in the northern pitcher plant, *Sarracenia purpurea*. *Proceedings of the*
527 *National Academy of Sciences*, **99**, 4409-4412.

528 Foot, G., Rice, S.P. & Millett, J. (2014) Red trap colour of the carnivorous plant
529 *Drosera rotundifolia* does not serve a prey attraction or camouflage function.
530 *Biology Letters*, **10**, 20131024.

531 De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrierie, O., Decocq,
532 G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R.,
533 Hermy, M., Lenoir, J., Plue, J., Coomes, D. a. & Verheyen, K. (2013) Latitudinal
534 gradients as natural laboratories to infer species' responses to temperature.
535 *Journal of Ecology*, **101**, 784-795.

536 Gaston, K.J., Chown, S.L. & Evans, K.L. (2008) Ecogeographical rules: elements of
537 a synthesis. *Journal of Biogeography*, **35**, 483-500.

538 Givnish, T.J., Burkhardt, E.L., Happel, R.E. & Weintraub, J.D. (1984) Carnivory in the
539 bromeliad *Brocchinia reducta*, with a cost/benefit model for the general
540 restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *The*
541 *American Naturalist*, **124**, 479-497.

542 Gloger, C. (1883) *Das Abändern der Vogel durch Einfluss des Klimas*, A. Schulz,
543 Breslau.

544 Gotelli, N.J. & Stanton-Geddes, J. (2015) Climate change, genetic markers and
545 species distribution modelling. *Journal of Biogeography*, **42**, 1577-1585.

546 Haylock, M., Hofstra, N., Klein Tank, A., Klok, E., Jones, P. & New, M. (2008) A
547 European daily high-resolution gridded dataset of surface temperature and
548 precipitation. *Journal of Geophysical Research*, **113**.

549 Huey, R.B., Carlson, M., Crozier, L., Frazier, M., Hamilton, H., Harley, C., Hoang, A.
550 & Kingsolver, J.G. (2002) Plants versus animals: do they deal with stress in
551 different ways? *Integrative and Comparative Biology*, **42**, 415–423.

552 IBM Corp. (2013) IBM SPSS Statistics for Windows.

553 Ichiishi, S., Nagamitsu, T., Kondo, Y., Iwashina, T., Kondo, K., Tagashira, N. &
554 Orikazu (1999) Effects of macro-components and sucrose in the medium on in
555 vitro red-colour pigmentation in *Dionea muscipula* Ellis and *Drosera spatulata*
556 Labill. *Plant Biotechnology*, **16**, 235–238.

557 Jablonski, N.G. & Chaplin, G. (2000) The evolution of human skin coloration. *Journal*
558 *of Human Evolution*, **39**, 57–106.

559 Kikvidze, Z., Michalet, R., Brooker, R.W., Cavieres, L.A., Lortie, C.J., Pugnaire, F.I. &
560 Callaway, R.M. (2011) Climatic drivers of plant–plant interactions and diversity
561 in alpine communities. *Alpine Botany*, **121**, 63–70.

562 KNMI (2013) KNMI Climate Explorer.

563 Koski, M.H. & Ashman, T.-L. (2015) Floral pigmentation patterns provide an example
564 of Gloger's rule in plants. *Nature Plants*, **1**, 14007.

565 Lai, Y.C., Shiroishi, T., Moriwaki, K., Motokawa, M. & Yu, H.T. (2008) Variation of
566 coat color in house mice throughout Asia. *Journal of Zoology*, **274**, 270–276.

567 Lloyd, F.E. (1942) *The Carnivorous Plants*, The Chronica Botanica Company.

568 Mendez, M., Jones, D.G. & Manetas, Y. (1999) Enhanced UV-B radiation under field
569 conditions increases anthocyanin and reduces the risk of photoinhibition but
570 does not affect growth in the carnivorous plant *Pinguicula vulgaris*. *New*
571 *Phytologist*, **144**, 275–282.

572 Manetas, Y. (2006) Why some leaves are anthocyanic and why most anthocyanic
573 leaves are red? *Flora*, **201**, 163–177.

574 Millett, J., Foot, G.W. & Svensson, B.M. (2015) Nitrogen deposition and prey
575 nitrogen uptake control the nutrition of the carnivorous plant *Drosera*
576 *rotundifolia*. *Science of The Total Environment*, **512–513**, 631–636.

577 Millett, J., Svensson, B.M., Newton, J. & Rydin, H. (2012) Reliance on prey-derived
578 nitrogen by the carnivorous plant *Drosera rotundifolia* decreases with increasing
579 nitrogen deposition. *The New phytologist*, **195**, 182–8.

580 Millien, V., Kathleen Lyons, S., Olson, L., Smith, F. a., Wilson, A.B. & Yom-Tov, Y.
581 (2006) Ecotypic variation in the context of global climate change: revisiting the
582 rules. *Ecology Letters*, **9**, 853–869.

583 Mittelbach, G.G., Schemske, D.W., Cornell, H. V, Allen, A.P., Brown, J.M., Bush,
584 M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H. a, McCain, C.M.,
585 McCune, A.R., McDade, L. a, McPeck, M. a, Near, T.J., Price, T.D., Ricklefs,
586 R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M. & Turelli, M. (2007) Evolution
587 and the latitudinal diversity gradient: speciation, extinction and biogeography.

Ecology Letters, **10**, 315–31.

Moles, A. T., Warton, D. I., Warman, L., Swenson, N. G., Laffan, S. W., Zanne, A. E., Pitman, A., Hemmings, F. A. and Leishman, M. R. (2009), Global patterns in plant height. *Journal of Ecology*, **97**, 923–932. doi:10.1111/j.1365-2745.2009.01526.x

NEGTA (2001) *Regional estimation of pollutant gas dry deposition in the UK: model description, sensitivity analyses and outputs*, Edinburgh.

Ocean Optics (2009). Spectrasuite spectrometer operating software. *Ocean Optics Inc., USA*.

Pieterse, G., Bleeker, A., Vermeulen, A.T., Wu, Y. & Erismn, J.W. (2007) High resolution modelling of atmosphere-canopy exchange of acidifying and eutrophying components and carbon dioxide for European forests. *Tellus*, **59B**, 412–424.

Schemske, D.W., Mittelbach, G.G., Cornell, H. V., Sobel, J.M. & Roy, K. (2009) Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, **40**, 245–269.

Smith, R.I., Fowler, D., Sutton, M.A., Flechard, C. & Coyle, M. (2000) Regional estimation of pollutant gas dry deposition in the UK: model description, sensitivity analyses and outputs. *Atmospheric Environment*, **34**, 3757–3777.

Takahashi, S. & Badger, M.R. (2011) Photoprotection in plants: a new light on photosystem II damage. *Trends in Plant Science*, **16**, 53–60.

Tuomaala, M., Kaitala, A. & Rutowski, R.L. (2012) Females show greater changes in wing colour with latitude than males in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae). *Biological Journal of the Linnean Society*, **107**, 899–909.

Willis, K.J., Feurdean, A., Birks, H.J.B., Bjune, A.E., Breman, E., Broekman, R., Grytnes, J.-A., New, M., Singarayer, J.S. & Rozema, J. (2011) Quantification of UV-B flux through time using UV-B-absorbing compounds contained in fossil Pinus sporopollenin. *New Phytologist*, **192**, 553–560.

Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann, C. F., Forchhammer, M. C., Grytnes, J.-A., Guisan, A., Heikkinen, R. K., Høye, T. T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.-C., Normand, S., Öckinger, E., Schmidt, N. M., Termansen, M., Timmermann, A., Wardle, D. A., Aastrup, P. & Svenning, J.-C. (2013), The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological Reviews*, **88**, 15–30. doi:10.1111/j.1469-185X.2012.00235.x

Zeuss, D., Brandl, R., Brändle, M., Rahbek, C. & Brunzel, S. (2014) Global warming favours light-coloured insects in Europe. *Nature Communications*, **5**, 3874.

Data accessibility

Data are available in supplementary material.

Biosketch

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631 Sciences, in the Department of Geography in Loughborough University. His work is
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637 Editor: W. Daniel Kissling

638

Tables

Table 1 Results of principle components analysis of between site variability in 13 environmental variables for sites across north-western Europe. Presented are the loadings for each variable on the first three axes.

Variable	Axis 1	Axis 2	Axis 3
Total N deposition	0.895	0.235	0.321
Total S deposition	0.836	0.431	0.084
NO ₃ deposition	0.951	0.052	0.237
NH ₄ deposition	0.762	0.376	0.365
July maximum temperature	0.162	0.908	-0.142
Growing Degree Days (base 5)	0.388	0.813	0.400
Mean summer temperature	0.292	0.912	0.127
NH ₄ :NO ₃	-0.158	0.542	0.502
UV-B	0.368	0.630	0.132
January minimum temperature	0.349	0.242	0.887
Variance in monthly temperature	-0.349	-0.207	-0.888
Annual precipitation	0.139	-0.216	0.773
Mean annual temperature	0.403	0.523	0.727
Cumulative proportion of variance explained	0.295	0.593	0.855

Table 2 Results of principle components analysis of between site variability in three measures of vegetation influence, and *Drosera rotundifolia* leaf colour characteristics, for plants growing on bogs in north-western Europe. Presented are the loadings for each variable on the first axes.

Vegetation influence		Leaf colour	
Variable	Axis 1	Variable	Axis 1
Vegetation cover	0.870	CIE LAB a*	0.996
Vegetation height	0.949	Dominant wavelength	0.991
% light transmitted through canopy	-0.960	Red:green	0.974
		Red	0.774
		Green	-0.853
% of variance explained			
	85.97		85.01

Table 3 Results of stepwise regression for relationship between *Drosera rotundifolia* leaf colour, vegetation characteristics and environmental variation with geographic location, and between leaf colour and vegetation characteristics with environmental variation, for sites across north-western Europe.

Dependent variable	Stepwise selected independent variables	r^2	Adjusted r^2	F	P
Geographic variables					
Leaf colour	Latitude, longitude	0.686	0.657	23.976	<0.001
Canopy influence	Latitude	0.816	0.806	75.583	<0.001
Vegetation height	Latitude	0.847	0.838	94.346	<0.001
Abiotic PCA axis 1	Latitude	0.291	0.262	9.873	0.004
Abiotic PCA axis 2	Latitude, Longitude	0.551	0.512	14.126	<0.001
Abiotic PCA axis 3	Longitude	0.749	0.738	71.472	<0.001
Abiotic variables					
Leaf colour	PCA Axis 1 & 3	0.744	0.721	32.008	< 0.001
Canopy influence	PCA axis 3, 1, 2	0.865	0.838	32.116	< 0.001
Vegetation Height	PCA axis 3, 1, 2	0.895	0.874	42.486	<0.001

Figure Legends

Figure 1

Location of study sites (site details in Table S1). The colour of the markers is indicative of the average colour of the leaves of *Drosera rotundifolia* growing on *Sphagnum* hummocks. The white marker at site UK10 is because no *D. rotundifolia* were present. The markers for SW1, 2 & 3 are offset to make them visible.

Figure 2

Geographic variation in vascular plant canopy influence and *Drosera rotundifolia* leaf colour, for plants growing across north-western Europe. Presented are the mean site values (circles) for variation with longitude (a and b) or latitude (c and d) in the axis 1 scores from a principle components analysis (PCA) of measures of canopy influence (a and b) and *D. rotundifolia* leaf colour (c and d), with the fitted regression line (where statistically significant). The simple linear regression model is shown in b; c and d are partial regression plots where the axes and regression line are adjusted (corrected) to remove variability due to the other variables included in the full multiple regression model, to allow the bivariate relationship to be visualised. Individual leaf colour and vegetation influence measures are shown in table 2.

Figure 3

Geographic variation in the abiotic environment on bogs in north-weestern Europe. Presented are the mean site values (circles) for variation with longitude (a and b) or latitude (c and d) in the first three axes from a principle components analysis (PCA) of environmental variables, with the fitted regression line. The simple linear regression model is shown in a and c; b and d are partial regression plots where the axes and regression line are adjusted (corrected) to remove variability due to the other variables included in the full multiple regression model, to allow the bivariate relationship to be visualised. Individual environmental measures are shown in table 1.

Figure 4

Relationship between the abiotic environment on bogs in north-western Europe and vascular plant canopy influence and *Drosera rotundifolia* leaf colour. Presented are the mean site values (circles) for variation in the axis 1 scores from a principle components analysis (PCA) of measures of canopy influence (a, c, and e) or leaf colour (b and d), and variation in the first three axes from a principle components analysis (PCA) of environmental variables. All graphs are partial regression plots, where the axes and regression line are adjusted (corrected) to remove variability due to the other variables included in the full multiple regression model, to allow the bivariate relationship to be visualised. Individual environmental, leaf colour and vegetation influence measures are shown in tables 1 and 2.

Figure 5

The relationship between canopy influence and *Drosera rotundifolia* leaf colour on bogs across north-western Europe. Presented are the values (circles) for the axis 1 scores from a principle components analysis (PCA) of measures of canopy influence and leaf colour for individual plots within bogs. The lines represent the fitted regression line for the plots on each bog, based on a multi-level linear mixed model analysis. The equation for each line is presented in appendix S7, site codes are explained in appendix S1. Individual leaf colour and vegetation influence measures are shown in table 2.

Figure 6

The impact of vegetation removal and vegetation removal plus shade addition treatments on canopy light transmission and *Drosera rotundifolia* leaf colour at the Humberhead Peatlands. Presented are the mean ($\pm$ se) for measurements of three different treatments: no vegetation removal ('*natural*'), all above-ground vegetation removed ('*removed*'), all above-ground vegetation removed and shade cloth added ('*removed+shaded*'). Treatments that are significantly different from each other ($P < 0.05$, based on Fisher's LSD) are indicated by different letters. Leaf/trap colour is presented in CIE 1976 ($L^*a^*b^*$) colour space (CIE LAB), where a^* represents a green – red axis of colour. Positive values represent red colours while negative values represent green colours.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

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SUPPORTING INFORMATION

**Geographic variation in Sundew (*Drosera*) leaf colour: plant-plant interactions
counteract expected effects of abiotic factors.**

Jonathan Millett, George W Foot, Juila C Thompson, Brita Svensson

Appendix S1 Characteristics of study sites.

Table S1.1 Characteristics of study sites.

Site	Name	Longitude (°E)	Latitude (°N)	Measurement date	Total atmospheric S deposition (mg m ⁻² yr ⁻¹)	Total atmospheric NO ₃ deposition (mg m ⁻² yr ⁻¹)	Total atmospheric NH ₄ deposition (mg m ⁻² yr ⁻¹)	Total atmospheric N deposition (mg m ⁻² yr ⁻¹)	Mean annual precipitation (mm) *	Mean annual temp. (°C)*
SW1	Abisko 1	18.810	68.355	20.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW2	Abisko 2	18.797	68.355	23.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW3	Abisko 3	18.778	68.341	25.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW4	Lappmyran	19.583	64.164	17.08.2013	0.114	0.138	0.082	0.220	634	2.37
NO1	Skjetnmyra	9.962	63.319	20/21.08.2011	0.069	0.092	0.090	0.182	1135	6.07
NO2	Lysklett-Myran	10.493	63.315	17/18.08.2011	0.069	0.092	0.090	0.182	1065	5.16
SW5	Sundsvall	17.156	62.326	19.08.2013	0.137	0.163	0.101	0.264	659	4.00
SW6	Åkerlänna Römossen	17.354	60.016	21.08.2013	0.183	0.262	0.174	0.436	630	5.54
NO3	Bervamyra	6.748	58.519	23/24.08.2011	0.239	0.300	0.191	0.492	2269	5.59
UK1	Laxford Bridge	5.017	58.371	06.08.2013	0.121	0.302	0.334	0.637	1118	8.15
UK2	Moidach More	-3.614	57.457	9/10.08.2012	0.153	0.407	0.352	0.759	905	7.13
SW7	Saxnäs Mosse	13.508	56.904	23.08.2013	0.522	0.631	0.555	1.186	878	6.40
UK3	Black Loch Moss	3.835	55.904	03.08.2013	0.381	0.630	0.823	1.453	1291	8.37
UK4	Dogden Moss	2.504	55.740	01.08.2013	0.228	0.534	0.702	1.237	1039	8.11
UK5	Butterburn Flow	-2.507	55.072	23/24.08.2012	0.258	1.078	0.919	1.997	1474	7.45
UK6	Bowness Common	-3.265	54.923	31.07.2013	0.373	0.678	0.498	1.176	1158	8.49
UK7	Caresgowan Moss	4.455	54.899	08.08.2013	0.316	0.642	0.830	1.472	1418	8.8
UK8	Meathop Moss	2.856	54.228	30.07.2013	0.428	0.729	0.901	1.629	1093	9.65
UK9	Malham Tarn	-2.175	54.097	29.07.2013	0.502	1.319	1.094	2.413	1310	7.25
UK10	Ringinglow Bog	-1.597	53.348	20/21.08.2012	0.943	1.493	1.271	2.764	956	8.86
IRE	Clara Bog	-7.638	53.322	09.08.2013	0.207	0.157	0.716	0.873	975	9.23
NL	t Groot Veen	6.448	52.834	20/21.07.2011	0.619	0.611	1.635	2.246	876	10.02
CZ1	Chiadla	15.249	50.680	7/8.08.2011	0.762	0.667	0.671	1.338	749	8.82
CZ2	Mrtvy' luh	13.878	48.870	10/11.08.2011	0.442	0.617	0.681	1.298	963	6.65
CH	Etang de la Gruere	7.043	47.239	26/28.07.2011	0.381	0.545	0.806	1.351	1105	8.3

*Mean annual (JUL-JUN) precipitation and temperature for the 5 years before sampling

Appendix S2 Leaf colour measurement methods

Leaf reflectance was measured using an Ocean Optics USB4000 VIS-NIR miniature spectrometer. This spectrometer measures reflectance in the range 350-1000 nm in steps of 25 nm. Because plant pigments degrade over time, we measured colour in the field. We therefore used the sun as the light source and adjusted using a Kodak white balance card (90% reflectance). We held the sensor 2 cm from the leaf surface, data were captured using the Ocean optics SpectraSuite software. One single measure of reflectance was used for each leaf based on the mean of 20 measurements. Data were smoothed using a boxcar width of 20 data points. Once captured we calculated colour measures using the SpectraSuite software assuming a D75 illuminant and a 2 degree observer. We calculated the location of the colour of the leaf in CIE 1976 ($L^*a^*b^*$) colour space (CIELAB). This standard colour space includes all colours perceivable to humans. Each colour is defined by its position in the colour space based on the 'Lightness' (L^*) of a colour, its position between red and green (a^* , negative = green, positive = red) and its position between blue and yellow (b^* , negative-blue, positive = yellow). Thus CIELAB enables assessment of colour difference and how red or green an object is by the value of the a^* axis. We also used the Dominant Wavelength from the SpectraSuite analysis. This is the dominant wavelength of the colour in CIE 1976 colourspace, removing differences in saturation or intensity. It is calculated by We used the reflectance spectrum to calculate mean red reflectance (average reflectance in the range: 600-699 nm) and mean green reflectance (average reflectance in the range 500-599 nm). We then used these values to calculate the ratio of red:green reflectance.

Appendix S3 Results of stepwise multiple regression.

Table S3.2 Results of stepwise multiple regression analysis of geographical variation in a. *Drosera rotundifolia* leaf colour, b. canopy influence of vascular plant vegetation in which the *D. rotundifolia* are growing, and c. the height of vascular plant vegetation in which the *D. rotundifolia* are growing.

a. *Drosera rotundifolia* Leaf colour.

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β <i>P</i>
Constant	-7.759***	1.365		-6.151***	1.371	
Latitude	0.135***	0.023	0.768	0.102***	0.025	0.578
Longitude				0.043*	0.017	0.363
R ²		0.590			0.686	
F for change in R ²		33.098***			6.680*	

* $P < 0.005$, *** $P < 0.001$

b. Canopy influence

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	β
Constant	10.814***	1.176	
Latitude	-0.174***	0.020	-0.904
Longitude†	-0.146		
R ²		0.816	
F for change in R ²		75.583***	

†excluded from model, *** $P < 0.001$

c. Vegetation height

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	β
Constant	91.779***	7.922	
Latitude	-1.308***	0.135	-0.921
Longitude†	0.004		
R ²	0.847		
F for change in R ²	94.346***		

*** $P < 0.001$

Table S3.3 Results of stepwise multiple regression analysis of geographic variation in principle component axis scores from principle components analysis of abiotic conditions at each site. a. Axis 1, which reflects nutrient deposition differences among the sites, with high scores on this axis representing sites with a high N and S deposition load (higher total N, total S, NO₃ and NH₄ deposition); b. axis 2 which reflects differences among sites in summer meteorological conditions, sites with high scores on this axis had warmer summers and a longer growing season (higher mean summer temperature, high maximum July temperature and high GDD); c. axis 3 which reflects the continentality of the climate, sites with high scores on this axis had a more maritime, less continental climate (high January minimum temperature, mean annual temperature, and precipitation, and low monthly temperature variance).

a. Abiotic PCA axis 1

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	5.41	1.730	
Latitude	-0.094**	0.030	-0.540
longitude			
R ²		0.291	
F for change in R ²		9.873**	

***P*<0.001

b. Abiotic PCA axis 2

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	5.760	1.682		8.300	1.596	
Latitude	-0.100**	0.029	-0.575	-0.152***	0.029	-0.874
longitude				0.065**	0.019	0.557
R ²		0.330			0.551	
F for change in R ²		11.839**			11.321**	

P*<0.001, *P*<0.001

c. Abiotic PCA axis 3

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	0.716	0.131	
Latitude	-0.100***	0.012	-0.865
longitude			
R ²		0.749	
F for change in R ²		71.472***	

****P*<0.001

Table S3.4 Results of stepwise multiple regression analysis of relationship between environmental variation and a. *Drosera rotundifolia* leaf colour, b. canopy influence of vascular plant vegetation in which the *D. rotundifolia* are growing, and c. the height of vascular plant vegetation in which the *D. rotundifolia* are growing. Axis 1, 2 and 3 are principle component axis scores from principle components analysis of abiotic conditions at each site. Axis 1 reflects nutrient deposition differences among the sites, with high scores on this axis representing sites with a high N and S deposition load (higher total N, total S, NO₃ and NH₄ deposition); axis 2 reflects differences among sites in summer meteorological conditions, sites with high scores on this axis had warmer summers and a longer growing season (higher mean summer temperature, high maximum July temperature and high GDD); axis 3 reflects the continentality of the climate, sites with high scores on this axis had a more maritime, less continental climate (high January minimum temperature, mean annual temperature, and precipitation, and low monthly temperature variance).

a. *Drosera rotundifolia* leaf colour

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	-0.036	0.157		-0.030	0.109	
Axis 1	-0.809***	0.189	-0.666	-0.792***	0.131	-0.653
Axis 2†						
Axis 3				-0.549***	0.108	-0.548
R ²		0.444			0.744	
F for change in R ²		18.352***			25.843***	

†excluded from model, ****P*<0.001

b. Canopy influence

Variable	Model 1			Model 2			Model 3		
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	0.010	0.161		-0.006	0.106		0.061	0.096	
Axis 1				0.490***	0.103	0.524	0.296*	0.118	0.252
Axis 2				0.620***	0.099	0.685	0.552***	0.089	0.591
Axis 3	0.664***	0.150	0.732				0.567***	0.089	0.626
R ²		0.536			0.809			0.865	
F for change in R ²		19.676***			22.810***			6.278*	

****P*<0.001

56 c. Vegetation height

Variable	Model 1			Model 2			Model 3		
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	15.189***	1.071		15.097***	0.830		15.784***	0.628	
Axis 1	5.286***	0.997	0.789	5.036***	0.775	0.752	4.493***	0.580	0.671
Axis 2				2.811**	0.799	0.407	3.446***	0.603	0.499
Axis 3							3.017**	0.771	0.348
R ²		0.623			0.787			0.895	
F for change in R ²		28.080***			12.368**			15.300**	

57 ***P*<0.01, ****P*<0.001

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Appendix S4 Linear mixed model results

Table S4.5 Characteristics of Linear Mixed Models for the relationship between vascular vegetation canopy influence and *Drosera rotundifolia* leaf colour. Presented are characteristics for four models in ranked order based on -2log likelihood.

Model ranking	Model	Included parameters	-2log likelihood	-2log likelihood (difference from next ranked model)	P-vale for comparison with next ranked model
1	Random slopes and intercepts	Site, intercept, slope	192.647	0	<0.05
2	Random intercepts	Site, intercept.	201.407	8.76	<0.001
3	Null multi-level	Site	361.475	160.068	<0.001
4	Null single-level	None	560.897	229.422	<0.001

Table S4.6 Equations of fitted regression lines for the relationship between valcular plant canopy influence (x) and *Droserea rotundifolia* leaf colour (y) for each of 16 site around Europe. Fitted lines are derived from a multi-level Linear Mixed Model analysis. Site codes are in table S1.

site	Fitted regression equation ⁷¹
UK10	$y = 0.1394x - 0.7313$
UK5	$y = -0.122x - 1.1802$
UK1	$y = -0.1331x - 0.4048$
UK9	$y = -0.1852x - 0.5511$
UK2	$y = -0.189x - 0.4951$
UK7	$y = -0.2123x - 0.1967$
UK8	$y = -0.2733x - 0.8668$
IRE	$y = -0.357x + 0.3118$
SW3	$y = -0.3591x + 0.1357$
SW1	$y = -0.4062x + 0.2608$
UK4	$y = -0.4265x + 0.052$
SW6	$y = -0.4598x + 0.4499$
SW2	$y = -0.4755x + 0.6513$
SW5	$y = -0.4889x + 0.7929$
SW4	$y = -0.4955x + 0.4686$
SW7	$y = -0.5921x + 0.7443$