

University of Groningen

## Competition for pollinators and intra-communal spectral dissimilarity of flowers

van der Kooi, C. J.; Pen, I.; Staal, M.; Stavenga, D. G.; Elzenga, J. T. M.

*Published in:*  
 Plant Biology

*DOI:*  
[10.1111/plb.12328](https://doi.org/10.1111/plb.12328)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
 Publisher's PDF, also known as Version of record

*Publication date:*  
 2016

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van der Kooi, C. J., Pen, I., Staal, M., Stavenga, D. G., & Elzenga, J. T. M. (2016). Competition for pollinators and intra-communal spectral dissimilarity of flowers. *Plant Biology*, 18(1), 56-62.  
<https://doi.org/10.1111/plb.12328>

### Copyright

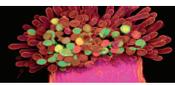
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*



## RESEARCH PAPER

# Competition for pollinators and intra-communal spectral dissimilarity of flowers

C. J. van der Kooi<sup>1,2</sup>, I. Pen<sup>3</sup>, M. Staal<sup>1</sup>, D. G. Stavenga<sup>2</sup> & J. T. M. Elzenga<sup>1</sup>

<sup>1</sup> Plant Physiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

<sup>2</sup> Computational Physics, Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands

<sup>3</sup> Theoretical Biology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

## Keywords

Biotic interactions; flower colouration; plant–pollinator signalling; principal components analysis; reproductive character displacement.

## Correspondence

J. T. M. Elzenga, Plant Physiology, Centre for Ecological and Evolutionary Studies, University of Groningen, Nijenborgh 7, NL-9747 AG Groningen, The Netherlands.

E-mail: j.t.m.elzenga@rug.nl

## Editor

A. Dafni

Received: 21 December 2014; Accepted: 3 March 2015

doi:10.1111/plb.12328

## ABSTRACT

**Competition for pollinators occurs when, in a community of flowering plants, several simultaneously flowering plant species depend on the same pollinator. Competition for pollinators increases interspecific pollen transfer rates, thereby reducing the number of viable offspring. In order to decrease interspecific pollen transfer, plant species can distinguish themselves from competitors by having a divergent phenotype. Floral colour is an important signalling cue to attract potential pollinators and thus a major aspect of the flower phenotype. In this study, we analysed the amount of spectral dissimilarity of flowers among pollinator-competing plants in a Dutch nature reserve. We expected pollinator-competing plants to exhibit more spectral dissimilarity than non-competing plants. Using flower visitation data of 2 years, we determined the amount of competition for pollinators by different plant species. Plant species that were visited by the same pollinator were considered specialist and competing for that pollinator, whereas plant species visited by a broad array of pollinators were considered non-competing generalists. We used principal components analysis to quantify floral reflectance, and found evidence for enhanced spectral dissimilarity among plant species within specialist pollinator guilds (*i.e.* groups of plant species competing for the same pollinator). This is the first study that examined intra-communal dissimilarity in floral reflectance with a focus on the pollination system.**

## INTRODUCTION

Community structures are shaped by many types of biotic interactions, *e.g.* plant–herbivore, plant–pathogen and plant–pollinator interactions (Gumbert *et al.* 1999; Sargent & Ackerly 2008; McEwen & Vamosi 2010). Plant–pollinator interactions are essential for outcrossing in many flowering plant species in order to set seed. Simultaneously, flowering plant species that strongly depend on the same shared pollinator are likely to experience competition for that pollinator. Neighbouring plant species that compete for pollinators will not only suffer from a reduction in number of pollinator visits, but will also enhance interspecific pollen transfer, which often reduces the amount of viable offspring (reviewed in Ashman & Arceo-Gómez 2013). Competition for pollinators might therefore influence the community assembly.

Flowering plants can avoid interspecific pollen transfer by changing their flowering period, but if this period is relatively fixed (*e.g.* by environmental factors, such as light, water and nutrient availability; see Pleasants 1980), interspecific pollen transfer can be prevented through phenotypic differentiation from simultaneously flowering competitors (Heinrich 1975; Caruso 2001). The latter process is often referred to as character displacement (Wilson & Brown 1953; Heithaus 1974; Pleasants 1980; Armbruster *et al.* 1994; Muchhala & Potts 2007). Character displacement is a change in appearance, effectively

leading to a greater distinction between plants that flower simultaneously and share pollinators. In order to achieve character displacement, various floral features can be changed, *e.g.* corolla length (Armbruster *et al.* 1994; Eaton *et al.* 2012), corolla width (Caruso 2000), inflorescence height (Waddington 1979), nectar production (Eaton *et al.* 2012) and pollen placement on the pollinator (Brown & Kodric-Brown 1979; Armbruster *et al.* 1994; Muchhala & Potts 2007). In addition to these morphological traits, floral colour is an important feature for pollinator attraction (*e.g.* Kevan & Baker 1983; Barth 1991; Schiestl & Johnson 2013; Shrestha *et al.* 2013; Renoult *et al.* 2014).

An efficient way of character displacement is to increase floral colour contrast (*i.e.* to increase dissimilar spectral properties) between simultaneously flowering plant species (Levin 1985; Dyer & Chittka 2004; McEwen & Vamosi 2010; de Jager *et al.* 2011; Muchhala *et al.* 2014). In community-wide studies, reproductive character displacement by means of divergent floral reflectance has been relatively little studied (but see McEwen & Vamosi 2010; de Jager *et al.* 2011; Eaton *et al.* 2012; Muchhala *et al.* 2014). An elegant study on a subalpine plant community from McEwen & Vamosi (2010) reported significant spectral differences among co-flowering plant species. Presumably this represents an example of character displacement, but unfortunately actual flower visitations by pollinators in this community were not recorded (as is often

the case in studies on character displacement; see Waser 1983).

Plant–pollinator interactions can be either specialised, *i.e.* when a plant species is pollinated by a single pollinator species, or generalised, *i.e.* when a species is serviced by a broad range of pollinators (reviewed in Johnson & Steiner 2000; Ollerton *et al.* 2007). Compared to generalists, specialist plants, which depend strongly on a specific pollinator, are subjected to increased selection pressure for strong floral signalling (Heithaus 1974). Consequently, spectral dissimilarity is expected to be higher between specialists than between generalists (Waser & Ollerton 2006).

In this study we determined the spectral dissimilarity of flowers among specialists relative to generalists. We used detailed insect visitation data collected in the Dutch nature reserve Drentsche Aa (Hoffmann 2005). We grouped plant species into either the generalist or a specialist guild based on the width of the range of pollinator species that visits them. We expected that in plant species that heavily depended on only a few pollinator species, competition for pollination would be stronger than between generalist species, and that therefore flowers of specialist plant species would exhibit stronger spectral differences. Using principal components analysis (PCA), we found that the spectral dissimilarities of flowers within each specialist guild were indeed higher than those within the generalist guild. This is the first study that documents how character displacement by means of spectral characteristics of flowers might be related to the specialisation of plant species to pollinators.

## MATERIAL AND METHODS

### Flower visitation observations

Flower and inflorescence visitation data were obtained from a comprehensive database collected in 2000 and 2001 in the Drentsche Aa (Hoffmann 2005). This nature reserve is characterised by homogeneous, open grasslands that are separated by rows of trees. The Drentsche Aa is rich in flowering plants, including several rare plant species such as orchids (Grootjans *et al.* 2002). Observations of insects on flowering plants were done from May until October in 2000 and from May until August in 2001, respectively. In total, the database comprises observations of over 38,000 individual insects visiting more than 1 million flowers of 88 different plant species (for details, see Hoffmann 2005). We pooled insect visitation data per plant species. To correct for rare flower morphs and species with reproductive systems that do not rely on pollinators *per se* (*i.e.* self-fertilisation or apomixis), we included only plant species with a minimum of 20 individual insect visits (following Hoffmann 2005). In addition, we combined various Diptera species to the genus level since pollination is often achieved by groups of pollinator species (reviewed in Fenster *et al.* 2004). We pooled only species with similar ecologies and avoided pooling of species with different spectral sensitivities (following Vogt 1989).

To determine the importance of pollinators per plant species rather than per individual plant, we calculated the relative visitor numbers for all plant species. In the database, the vast majority of insects visiting flowers were identified to the species level, but for some visiting insects the species or genus could

not be determined (Hoffmann 2005). Therefore, to avoid a bias in our analysis, we excluded plant species with more than 10% unknown visitors. The observations on *Bombus terrestris* include the less abundant *B. lucorum* because these bumblebee species cannot be distinguished in the field (following Hoffmann 2005). Due to the large number of observations in the database it was impossible to determine whether all flower visitors in our study area are indeed pollinators. However, the effects of *e.g.* occasional visitation or pollen theft by insects will be negligible given the large number of visits recorded in the database. Most importantly, all insect species or genera that we considered as ‘specialist pollinators’ were documented elsewhere to indeed be plant pollinators (Barth 1991; D’Arcy-Burt & Blackshaw 1991; Goldblatt *et al.* 2005; Clement *et al.* 2007; Ssymank *et al.* 2008; Garibaldi *et al.* 2013). We therefore considered all flower-visiting insects as pollinators.

### Specialists and generalists

The degree of specialisation of a plant species to a pollinator strongly depends on its habitat (reviewed in Richardson *et al.* 2000; Vázquez & Aizen 2006). Preliminary analysis of the relative visitor numbers in our study area showed a distinct separation of generalists from specialists at *ca.* 40% visits (Figure S1). This visit level has no fundamental justification but can be substantiated. A lower threshold yielded plant species that were simultaneously ‘specialist’ for multiple insect guilds. For example, some plant species were specialist for two insect guilds, each guild accounting for 30% of the relative visitation number. Using the 40% criterion, the remaining visitations (maximum 60%) always consisted of many different, non-related insect species. We thus considered plant species visited at least 40% by one insect genus as specialist for that genus (for further details regarding this criterion, see the Discussion). Two pollinator guilds comprised only one plant species (of which one was specialist for the honeybee *Apis mellifera*). We excluded these guilds because it is impossible to compare plant species within a guild with only one plant species. After applying our criteria to the plant species in the Drentsche Aa dataset, 39 plant species remained, which were subsequently assigned to either the generalist guild or to one of the six specialist guilds. The remaining 39 plants had relatively long flowering periods, and based on the insect observations, we found that the overlap of the flowering time for plants within the same guild was at least 1 month.

### Flower species and reflectance measurements

Flower samples were either collected locally from meadows around Groningen, the Netherlands, or grown from seed (obtained from Cruydt-Hoeck, Nijbeerkoop, the Netherlands). Reflectance spectra of the flowers were measured with a bifurcated fibre-optic probe (Avantes FCR-7UV200; Avantes, Eerbeek, the Netherlands) using an AvaSpec 2048-2 CCD detector array spectrometer. The light source was a halogen-deuterium lamp (AvaLight-D(H)-S); a white diffuse tile (WS-2; Avantes) was used as a reference. We measured several reflectance spectra from the dominant coloured petal areas (following McEwen & Vamosi 2010). In these areas, the shape of the spectra was virtually constant and only the amplitude varied slightly. We obtained reflectance spectra of additional flowering plant

species from the online floral database [www.reflectance.co.uk](http://www.reflectance.co.uk) (Arnold *et al.* 2010).

To correct for brightness, we subtracted, for each species, the mean percentage reflectance, and thus specifically compared spectral quality differences (Cuthill *et al.* 1999; McEwen & Vamossi 2010). We analysed the wavelength range from 300 to 600 nm (which includes the ultraviolet light wavelength range and excludes the red wavelength range) as insects that are sensitive in the red part of the spectrum (*e.g.* beetles and butterflies; Briscoe & Chittka 2001) only very rarely visited our plant species (Table S1).

Ideally, floral reflectance is analysed by incorporating pollinator spectral sensitivity (Peitsch *et al.* 1992; Lunau *et al.* 2011; Dyer *et al.* 2012; Shrestha *et al.* 2013; Burd *et al.* 2014). However, for many important flower-visiting insects in our study area (notably Diptera), we currently have insufficient reliable information to model colour vision for these species (Lunau 2014). We therefore strictly aim to describe the spectral properties of co-occurring plants, rather than floral colour as perceived by pollinators.

### Statistical analyses

Statistical analyses were conducted using R Statistical Software (R Core Team 2012). We calculated the spectral differences using two complementary methods. The spectral differences were calculated based on the raw spectra and by transforming the spectra using PCA. PCA allowed us to easily visualise the spectral differences, whereas calculations based on the raw spectra provided more statistical power. The PCA was performed using the 39 standardised reflectance spectra with bins of 1 nm. The principal components 1 (PC1) and 2 (PC2) together largely explained the variance (see below), in accordance with similar studies (*e.g.* Cuthill *et al.* 1999; Grill & Rush 2000; Renoult *et al.* 2013; Sun *et al.* 2014). To visualise the differences in spectra between guilds, we constructed a PCA scatterplot comprising all plant species using the PC1 and PC2 values of each plant species as *x*- and *y*-coordinates, respectively. To quantify the spectral contrast, we calculated the Euclidean pair-wise distances between the plant species within each guild, resulting in a mean pair-wise distance (MPD) value per guild. To account for the total variance, and not only the 92% explained by PC1 and PC2, the MPDs were calculated using all 39 principal components. The MPD was also determined directly from the raw spectra. For all flowers within a

guild we calculated the absolute difference between the reflectance values for each nanometre, and then we averaged the obtained values over the examined wavelength range (300–600 nm). The guild's MPD was then calculated by averaging the obtained spectral differences.

The MPDs derived from both the PCA and the raw spectra were used in two different randomisation tests. We calculated the difference between the average MPD of the specialist guilds and the MPD of the generalist guild, denoted by  $\Delta$ MPD. Based on our hypothesis, we expected  $\Delta$ MPD to be positive (*i.e.* more spectral dissimilarity exists between the flowers that belong to a group of specialist plants and the flowers of generalist plants) and therefore we performed a one-tailed test. To test its significance, we generated 1,000,000 'random' plant communities by randomly assigning the 39 plant species to one of the six specialist guilds or the generalist guild, keeping the sample size for each guild identical to that in the original plant community (see Table 1). For each of the random communities we then calculated and stored  $\Delta$ MPD, thus generating a null distribution of  $\Delta$ MPD values. Finally, we tested if the observed spectral dissimilarity was larger than that expected by chance, by inspecting the quantile of the observed  $\Delta$ MPD value in the null distribution (Vázquez & Aizen 2006).

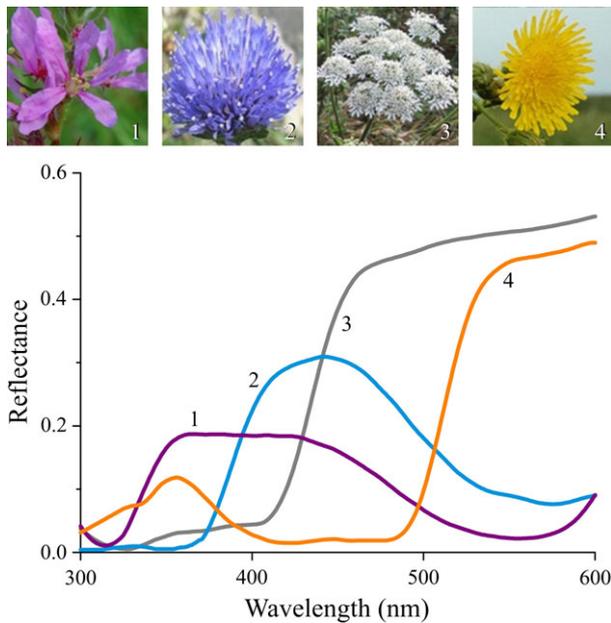
## RESULTS

### Specialists and generalists

Throughout our study period, 39 simultaneously flowering plant species (from 14 angiosperm families) were frequently visited by insects (Table S1). We excluded 49 plant species that did not occur frequently and were thus only rarely visited by insects, or because they were visited mostly by unidentified insect species (Table S2). A total of 17 frequently occurring plant species were visited by many different insects, and we subsequently considered these plant species as generalist (Table 1; Figure S1). Despite its apomictic mode of reproduction, *Taraxacum officinale* was included in the generalist guild because it affects the community's colour composition due to its frequent occurrence and high number of pollinator visitations in our study area (Table S1). A total of 22 frequently occurring plant species were, at least 40%, visited by one pollinator, and we accordingly assigned these plant species to one of the six specialist pollinator guilds (Table 1; Figure S1). The degree of specialisation of specialist plant species to an insect

**Table 1.** Assignment of generalist and specialist plant species to different pollinator guilds and the degree of specialisation (in parentheses).

guild	plant species
generalists	<i>Achillea millefolia</i> ; <i>Aegopodium podagraria</i> ; <i>Angelica sylvestris</i> ; <i>Chamerion angustifolium</i> ; <i>Cirsium arvense</i> ; <i>Cirsium palustre</i> ; <i>Epilobium hirsutum</i> ; <i>Eupatorium cannabinum</i> ; <i>Glechoma hederacea</i> ; <i>Jasione montana</i> ; <i>Lamium album</i> ; <i>Lychnis flos-cuculi</i> ; <i>Lycopus europaeus</i> ; <i>Lythrum salicaria</i> ; <i>Mentha aquatica</i> ; <i>Rorippa amphibia</i> ; <i>Taraxacum officinale</i>
<i>Bibio</i>	<i>Heraclium sphondylium</i> (47); <i>Hieracium pilosella</i> (45)
<i>Musca</i>	<i>Filipendula ulmaria</i> (47); <i>Hieracium aurantiacum</i> (59)
<i>Eristalis</i>	<i>Nasturtium officinale</i> (47); <i>Sonchus arvensis</i> (62); <i>Succisa pratensis</i> (69); <i>Valeriana officinalis</i> (46)
<i>Rhingia campestris</i>	<i>Ajuga reptans</i> (86); <i>Phyteuma spicatum</i> (60); <i>Silene dioica</i> (59)
<i>Bombus pascuorum</i>	<i>Galeopsis tetrahit</i> (52); <i>Linaria vulgaris</i> (43); <i>Lotus corniculatus</i> (59); <i>Stachys palustris</i> (50); <i>Symphytum officinale</i> (62); <i>Trifolium pratense</i> (55); <i>Trifolium repens</i> (51); <i>Vicia cracca</i> (93); <i>Vicia sativa</i> (82)
<i>Bombus terrestris</i>	<i>Lupinus polyphyllus</i> (86); <i>Rhinanthus angustifolius</i> (68)



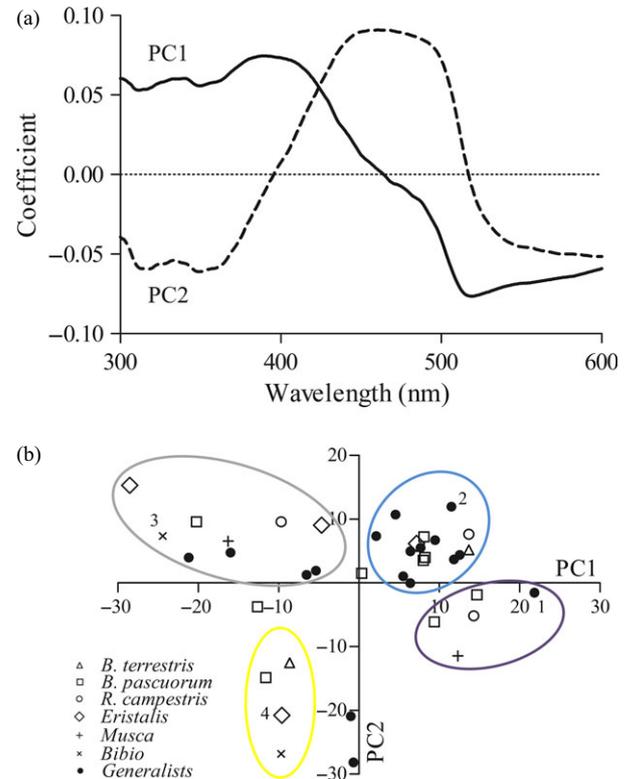
**Fig. 1.** Flower reflectance spectra of four exemplar plant species: *Lythrum salicaria* (1), *Jasione montana* (2), *Heracleum sphondylium* (3) and *Sonchus arvensis* (4).

guild ranged from 43% (moderately specialised) to 93% (highly specialised).

### Floral reflectance analysis

Flowers that appear white to the human eye are always low in ultraviolet reflectance (Kevan *et al.* 1996), but spectra with peaks in the medium or long wavelength range can co-occur with high reflectance in the ultraviolet wavelength range (depending on the nature of the pigment; Grotewold 2006; Lee 2007; van der Kooij *et al.* 2014, 2015). The PCA of flowers in our study area yielded two principal components, PC1 and PC2, which accounted for 92.4% of the total variation in the reflectance spectra (53.7% and 38.7%, respectively). The two principal components strongly depended on the different wavelength ranges of the reflectance spectra (Fig. 2a). Flowers with reflectance peaks in the ultraviolet or short wavelength range yielded a high PC1 score, and had low, medium or high PC2 scores. Flowers with high reflectance in the medium and long wavelength range yielded low PC1 scores and high and low PC2 scores, respectively. A plot of PC2 *versus* PC1 thus formed a reflectance scatterplot, with different floral reflectance spectra clustering in different sections of the scatterplot (Fig. 2b).

To quantify the spectral differences, we calculated the MPD between plant species belonging to the same pollinator guild. We calculated the MPD using both the PCA-transformed spectra and the raw spectra (see Figure S2). This yielded very similar results. The MPDs within all six specialist guilds were larger than MPDs within the generalist guild (Fig. 3a), which is significant according to a binomial test ( $P = 0.017$ ). Furthermore, the average MPD between the specialist guilds and the generalist guild was 7.1, which yielded a  $P$ -value of 0.050 according to our randomisation test (Fig. 3b). Similarly, the results based on the raw spectra were significant, with a  $P$ -value of 0.052.

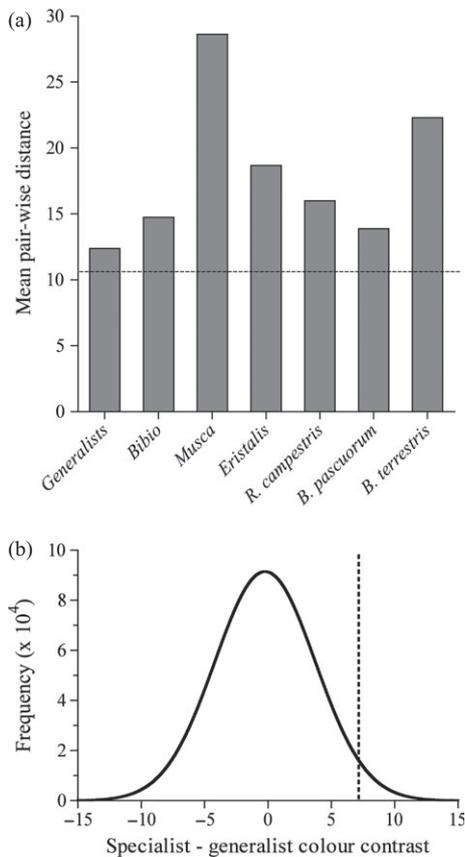


**Fig. 2.** (a) Coefficients of the first two principal components of the PCA performed on the spectra of the species listed in Table 1. PC1 represents the relative amount of short to long wavelength reflectance. PC2 represents the relative amount of medium wavelength reflectance to both short (<400 nm) and long (>500 nm) wavelength reflectance. (b) Flower reflectance scatterplot based on the PC1 and PC2 values of our 39 plant species. Similarly as perceived by the human eye, different floral reflectance spectra cluster in different sectors of the scatterplot, as indicated by ellipses. The positions of the four exemplar plant species with different spectra (see Fig. 1) are indicated with numbers (1–4).

### DISCUSSION

The present investigation of the spectral properties of co-flowering plants within pollinator guilds lends support to the hypothesis that flower communities are, at least in part, structured by plant–pollinator interactions. The results obtained for lowland flowers are in fair agreement with earlier findings in a subalpine community (McEwen & Vamosi 2010) and also with a recent study on hummingbird-pollinated Solanaceae (Muchhala *et al.* 2014).

We could distinguish several pollinator guilds in our study area. Some plants were pollinated by various insect species, others mainly by insects of one of six specialist pollinator guilds (Table 1). Three of six specialist guilds are defined to the insect genus level, meaning that within these specialist guilds, plant species were visited by different species from that genus. Nevertheless, a plant species visited by various insect species of one insect genus with similar ecologies is more specialist than a plant species visited by only a few insect species from distinct lineages with different ecologies (Gómez & Zamora 2006). The pollinator guilds observed in our study can thus be regarded as functional pollinator groups.



**Fig. 3.** Observed mean pair-wise distance (MPD) between the PCA-transformed floral reflectance spectra within each pollinator guild. (a) All plant species within specialist guilds have more spectral contrast than generalist plants. The MPDs between plant species within each guild were calculated using the Euclidean distance based on the 39 principal components describing the spectral properties of the flowers. (b) Distribution of specialist-generalist spectral differences, calculated over the 1,000,000 communities with randomly assigned guilds. The observed spectral contrast (vertical line) is higher than the spectral contrast expected by chance.

The generalist flowers in our study are visited by a large array of insect species (Table S1). These generalists are not restricted to a specific region in the reflectance scatterplot, indicating that the generalist guild comprises plant species with different spectral properties (Fig. 2b). Spectral dissimilarity among generalists seems to contradict our hypothesis that generalist plants are more similar in spectral reflectance than specialists. However, the MPD value of the generalists is smaller than in any of the specialist groups (Fig. 3), meaning that within the generalist guild the spectral properties are more similar than among specialists. Furthermore, the derived spectral dissimilarities are larger than expected for a random assembly. The same spectral dissimilarities were obtained based on distance between the raw spectra, and thus the transformation of spectra into principal components did not significantly alter our results. Interestingly, for generalists, spectral dissimilarity might not be a prerequisite for survival in the community – in fact, the opposite might be true – because similar reflectance spectra might increase visitation from generalist pollinators (e.g. Schiestl & Johnson 2013).

Furthermore, generalist plants may have converged in floral colouration, yet can be specialist in e.g. floral morphology (Eaton *et al.* 2012; Low *et al.* 2015).

The number of potential pollinators in a community is reduced if the morphology of the plant requires a specific morphological fit to the pollinator (Cresswell 1998; Schemske & Bradshaw 1999; Gómez & Zamora 2006; Murúa & Espíndola 2015). In other words, a ‘complex’ morphology might render a plant specialist. Complex morphologies are often associated with specific angiosperm families, e.g. in Fabaceae and Lamiaceae, where anthers and stigmas are not as easily accessible as in members of the Apiaceae and Asteraceae. In addition, occurrence of floral pigments, and hence floral reflectance, might be conserved within families (Levin 1985; Grotewold 2006). Floral reflectance and morphology might thus potentially be linked through ancestry. However, generalist plant species are located throughout the reflectance scatterplot, and both the generalist and specialist guilds include multiple plant families, indicating that phylogenetic constraints do not exist (Fig. 2b). This is corroborated by many studies that report phylogenetic effects on floral reflectance are negligible in large communities (e.g. Schemske & Bradshaw 1999; Sargent & Otto 2006; McEwen & Vamosi 2010; Eaton *et al.* 2012; Muchhala *et al.* 2014).

We note that a theoretical framework for the effects of plant-pollinator relations on plant community composition is not fully developed. A theoretical framework might provide a more solid basis for the criteria by which plant species can be grouped into generalist and specialist guilds. In the present study, the grouping of plant species proved to be rather straightforward, since plants were either visited by numerous different insect species or predominantly by a single group together with a limited number of other species. However, the chosen minimal percentage of at least 40% visits covered by the principal pollinator is somewhat arbitrary and has no fundamental justification.

We conclude that spectral dissimilarity is most likely a prerequisite for pollinator-competing plants. When flowers of different species within a community have dissimilar reflectance spectra, pollinators are less likely to switch between species (Dyer & Chittka 2004), and thereby interspecific pollen transfer is reduced. Even though our results are not highly significant, pollinators are documented as capable of detecting small spectral differences (e.g. Papiorek *et al.* 2013; Renoult *et al.* 2013). Clearly, for specialists, spectral dissimilarity is an efficient signalling cue, as it can be perceived by insects from far longer distances than is the case with morphological traits such as corolla length (Schemske 1976). Interestingly, a study performed on a South African flower community showed that flowers with similar spectral properties carry a fitness cost, yet co-flowering plant species had rather similar spectral characteristics (de Jager *et al.* 2011). The latter might be explained by a high prevalence of a particular type of pollinator or by the species composition in that specific community, as it largely consisted of plant species that belong to the same genus. This might indicate that the specific set of conditions present in the Drentsche Aa nature reserve (e.g. plant and pollinator species composition) create a level of competition for pollinators in which character displacement can be observed.

The dissimilarity in spectral properties between co-flowering plants in specialised pollinator guilds that we encountered in our study area might also occur in other communities.

Comparisons with other habitats are particularly relevant, as plant reproductive strategies often differ between habitats (Linhart & Feinsinger 1980; Waser & Ollerton 2006). In addition, since both plant and insect diversity often depend on the environment, the selective pressures on floral traits, such as spectral reflectance, might be different between environments (Gumbert *et al.* 1999; Lázaro *et al.* 2015). Future studies on plant–pollinator interactions will help explain the differences in plant reproductive strategies between populations.

## ACKNOWLEDGEMENTS

The authors thank Frank Hoffman for kindly providing the Drentsche Aa dataset and Adrian G. Dyer, Aidan Vey and three referees for their suggestions for improvements. We also thank Ger Telkamp, Hein Leertouwer and Jacob Hogendorf for assis-

tance in acquiring samples of the plant species and their practical support.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Relative pollinator visitation of plants used in this study.

**Figure S2.** Raw reflectance spectra for the plants within each pollinator guild.

**Table S1.** Total insect visitation counts (obtained from Hoffmann 2005) of the 39 plant species investigated in this study.

**Table S2.** Overview of plant species that were excluded from the analysis and the reason for exclusion.

## REFERENCES

- Armbruster W., Edwards M., Debevec E. (1994) Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology*, **75**, 315–329.
- Arnold S.E., Faruq S., Savolainen V., McOwan P.W., Chittka L. (2010) FRiD: the floral reflectance database – a web portal for analyses of flower colour. *PLoS One*, **5**, e14287.
- Ashman T., Arceo-Gómez G. (2013) Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany*, **100**, 1061–1070.
- Barth F.G. (1991) *Insects and flowers. The biology of a partnership*. Princeton University Press, Princeton, NJ, USA.
- Briscoe A.D., Chittka L. (2001) The evolution of color vision in insects. *Annual Review of Entomology*, **46**, 471–510.
- Brown J.H., Kodric-Brown A. (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology*, **60**, 1022–1035.
- Burd M., Stayton C.T., Shrestha M., Dyer A.G. (2014) Distinctive convergence in Australian floral colours seen through the eyes of Australian birds. *Proceedings of the Royal Society Series B: Biological Sciences*, **281**, 20132862.
- Caruso C.M. (2000) Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution*, **54**, 1546–1557.
- Caruso C.M. (2001) Differential selection on floral traits of *Ipomopsis aggregata* growing in contrasting environments. *Oikos*, **94**, 295–302.
- Clement S.L., Hellier B.C., Elbertson L.R., Staska R.T., Evans M.A. (2007) Flies (Diptera: Muscidae: Calliphoridae) are efficient pollinators of *Allium ampeloprasum* L. (Alliaceae) in field cages. *Journal of Economic Entomology*, **100**, 131–135.
- Cresswell J. (1998) Stabilizing selection and the structural variability of flowers within species. *Annals of Botany*, **81**, 463–473.
- Cuthill I.C., Bennett A.T.D., Partridge J.H.C., Maier E.J. (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *The American Naturalist*, **153**, 183–200.
- D'Arcy-Burt S., Blackshaw R. (1991) Bibionids (Diptera, Bibionidae) in agricultural land – a review of damage, benefits, natural enemies and control. *Annals of Applied Biology*, **118**, 695–708.
- Dyer A.G., Chittka L. (2004) Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. *Journal of Comparative Physiology A*, **190**, 105–114.
- Dyer A.G., Boyd-Gerny S., McLoughlin S., Rosa M.G., Simonov V., Wong B.B. (2012) Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision. *Proceedings of the Royal Society Series B: Biological Sciences*, **279**, 3606–3615.
- Eaton D.A., Fenster C.B., Hereford J., Huang S., Ree R.H. (2012) Floral diversity and community structure in *Pedicularis* (Orobanchaceae). *Ecology*, **93**, S182–S194.
- Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R., Thomson J.D. (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution and Systematics*, **35**, 375–403.
- Garibaldi L.A., Steffan-Dewenter I., Winfree R., Aizen M.A., Bommarco R., Cunningham S.A., Kremen C., Carvalheiro L.G., Harder L.D., Afik O., Bartomeus I., Benjamin F., Boreux V., Cariveau D., Chacoff N.P., Dudenhoefler J.H., Freitas B.M., Ghazoul J., Greenleaf S., Hipolito J., Holzschuh A., Howlett B., Isaacs R., Javorek S.K., Kennedy C.M., Krewenka K.M., Krishnan S., Mandelik Y., Mayfield M.M., Motzke I., Munyuli T., Nault B.A., Otieno M., Petersen J., Pisanty G., Potts S.G., Rader R., Ricketts T.H., Rundlof M., Seymour C.L., Schuepp C., Szentgyorgyi H., Taki H., Tscharntke T., Vergara C.H., Viana B.F., Wanger T.C., Westphal C., Williams N., Klein A.M. (2013) Wild pollinators enhance fruit set of crops regardless of honeybee abundance. *Science*, **339**, 1608–1611.
- Goldblatt P., Manning J., Bernhardt P. (2005) The floral biology of *Melasphearula* (Iridaceae: Crocoideae): is this monotypic genus pollinated by march flies (Diptera: Bibionidae)? *Annals of the Missouri Botanical Garden*, **92**, 268–274.
- Gómez J., Zamora R. (2006) Ecological factors that promote the evolution of generalization in pollination systems. In: Waser N., Ollerton J. (Eds), *Plant–pollinator interactions*. University of Chicago Press, Chicago, IL, USA, pp 145–166.
- Grill C.P., Rush V.N. (2000) Analysing spectral data: comparison and application of two techniques. *Biological Journal of the Linnean Society*, **69**, 121–138.
- Grootjans A., Bakker J., Jansen A., Kemmers R. (2002) Restoration of brook valley meadows in the Netherlands. *Hydrobiologia*, **478**, 149–170.
- Grotewold E. (2006) The genetics and biochemistry of floral pigments. *Annual Review of Plant Biology*, **57**, 761–780.
- Gumbert A., Kunze J., Chittka L. (1999) Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society Series B: Biological Sciences*, **266**, 1711–1716.
- Heinrich B. (1975) Bee flowers: a hypothesis on flower variety and blooming times. *Evolution*, **29**, 325–334.
- Heithaus E. (1974) Role of plant–pollinator interactions in determining community structure. *Annals of the Missouri Botanical Garden*, **61**, 675–691.
- Hoffmann F. (2005) Biodiversity and Pollination. Flowering plants and flower-visiting insects in agricultural and semi-natural landscapes. Ph.D. thesis, University of Groningen, the Netherlands. Online access: <http://irs.ub.rug.nl/ppn/289762693>.
- de Jager M.L., Dreyer L.L., Ellis A.G. (2011) Do pollinators influence the assembly of flower colours within plant communities? *Oecologia*, **166**, 543–553.
- Johnson S.D., Steiner K.E. (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution*, **15**, 140–143.
- Kevan P., Baker H. (1983) Insects as flower visitors and pollinators. *Annual Review of Entomology*, **28**, 407–453.
- Kevan P., Giurfa M., Chittka L. (1996) Why are there so many and so few white flowers? *Trends in Plant Science*, **1**, 252–257.
- van der Kooij C.J., Wilts B.D., Leertouwer H.L., Staal M., Elzenga J.T.M., Stavenga D.G. (2014) Iridescent flowers? Contribution of surface structures to optical signaling. *New Phytologist*, **203**, 667–673.
- van der Kooij C.J., Dyer A.G., Stavenga D.G. (2015) Is floral iridescence a biologically relevant cue in plant–pollinator signaling? *New Phytologist*, **205**, 18–20.
- Lázaro A., Lundgren R., Totland Ø. (2015) Pollen limitation, species' floral traits and pollinator visitation: different relationships in contrasting communities. *Oikos*, **124**, 174–186.
- Lee D. (2007) *Nature's palette. The science of plant color*. University of Chicago Press, Chicago, IL, USA.
- Levin D.A. (1985) Reproductive character displacement in *Phlox*. *Evolution*, **39**, 1275–1281.
- Linhart Y.B., Feinsinger P. (1980) Plant–hummingbird interactions: effects of island size and degree of spe-

- cialization on pollination. *Journal of Ecology*, **68**, 745–760.
- Low S.L., Wong S.Y., Ooi I.H., Hesse M., Städler Y., Schönenberger J., Boyce P.C. (2015) Floral diversity and pollination strategies of three rheophytic Schismatoglottidae (Araceae). *Plant Biology*, doi:10.1111/plb.12320.
- Lunau K. (2014) Visual ecology of flies with particular reference to colour vision and colour preferences. *Journal of Comparative Physiology A*, **200**, 497–512.
- Lunau K., Papiorek S., Eltz T., Sazima M. (2011) Avoidance of achromatic colours by bees provides a private niche for hummingbirds. *Journal of Experimental Biology*, **214**, 1607–1612.
- McEwen J.R., Vamosi J.C. (2010) Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society Series B: Biological Sciences*, **277**, 2957–2965.
- Muchhala N., Potts M.D. (2007) Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the Royal Society Series B: Biological Sciences*, **274**, 2731–2737.
- Muchhala N., Johnsen S., Smith S.D. (2014) Competition for hummingbird pollination shapes flower color variation in Andean Solanaceae. *Evolution*, **68**, 2275–2286.
- Muriá M., Espíndola A. (2015) Pollination syndromes in a specialised plant–pollinator interaction: does floral morphology predict pollinators in *Calceolaria*? *Plant Biology*, **17**, 551–557.
- Ollerton J., Killick A., Lamborn E., Watts S., Whiston M. (2007) Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, **56**, 717–728.
- Papiorek S., Rohde K., Lunau K. (2013) Bees' subtle colour preferences: how bees respond to small changes in pigment concentration. *Naturwissenschaften*, **100**, 633–643.
- Peitsch D., Fietz A., Hertel H., Desouza J., Ventura D., Menzel R. (1992) The spectral input systems of hymenopteran insects and their receptor-based color vision. *Journal of Comparative Physiology A*, **170**, 23–40.
- Pleasants J. (1980) Competition for bumblebee pollinators in Rocky Mountain plant communities. *Ecology*, **61**, 1446–1459.
- R Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Renoult J.P., Thomann M., Schaefer H.M., Cheptou P.O. (2013) Selection on quantitative colour variation in *Centaurea cyanus*: the role of the pollinator's visual system. *Journal of Evolutionary Biology*, **26**, 2415–2427.
- Renoult J.P., Valido A., Jordano P., Schaefer H.M. (2014) Adaptation of flower and fruit colours to multiple, distinct mutualists. *New Phytologist*, **201**, 678–686.
- Richardson D.M., Allsopp N., D'Antonio C.M., Milton S.J., Rejmanek M. (2000) Plant invasions – the role of mutualisms. *Biological Reviews*, **75**, 65–93.
- Sargent R.D., Ackerly D.D. (2008) Plant–pollinator interactions and the assembly of plant communities. *Trends in Ecology & Evolution*, **23**, 123–130.
- Sargent R.D., Otto S.P. (2006) The role of local species abundance in the evolution of pollinator attraction in flowering plants. *The American Naturalist*, **167**, 67–80.
- Schemske D.W. (1976) Pollinator specificity in *Lantana camara* and *L. trifolia* (Verbenaceae). *Biotropica*, **8**, 260–264.
- Schemske D.W., Bradshaw H. (1999) Pollinator preference and the evolution of floral traits in monkey flowers (*Mimulus*). *Proceedings of the National Academy of Sciences USA*, **96**, 11910–11915.
- Schiestl F.P., Johnson S.D. (2013) Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution*, **28**, 307–315.
- Shrestha M., Dyer A.G., Boyd-Gerny S., Wong B.B.M., Burd M. (2013) Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *New Phytologist*, **198**, 301–310.
- Szymank A., Kearns C.A., Pape T., Thompson F.C. (2008) Pollinating flies (Diptera): a major contribution to plant diversity and agricultural production. *Biodiversity*, **9**, 86–89.
- Sun M., Gross K., Schiestl F.P. (2014) Floral adaptation to local pollinator guilds in a terrestrial orchid. *Annals of Botany*, **113**, 289–300.
- Vázquez D.P., Aizen M.A. (2006) Community-wide patterns of specialization in plant–pollinator interactions revealed by null models. In: Waser N.M., Ollerton J. (Eds), *Plant–pollinator interactions. From specialization to generalization*. University of Chicago Press, Chicago, IL, USA, pp 200–219.
- Vogt K. (1989) Distribution of insect visual chromophores: functional and phylogenetic aspects. In: Stavenga D.G., Hardie R.C. (Eds), *Facets of vision*. Springer, Heidelberg, Germany, pp 134–151.
- Waddington K.D. (1979) Divergence in inflorescence height: an evolutionary response to pollinator fidelity. *Oecologia*, **40**, 43–50.
- Waser N. (1983) Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: Jones C., Little R. (Eds), *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, NY, USA, pp 277–293.
- Waser N., Ollerton J. (2006) *Plant–pollinator interactions. From specialization to generalization*. University of Chicago Press, Chicago, IL, USA.
- Wilson E., Brown W. (1953) The subspecies concept and its taxonomic application. *Systematic Zoology*, **2**, 97–111.