

## Specialization and phenological synchrony of plant–pollinator interactions along an altitudinal gradient

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### Summary

1. One of the most noticeable effects of anthropogenic climate change is the shift in timing of seasonal events towards earlier occurrence. The high degree of variation in species' phenological shifts has raised concerns about the temporal decoupling of interspecific interactions, but the extent and implications of this effect are largely unknown. In the case of plant–pollinator systems, more specialized species are predicted to be particularly threatened by phenological decoupling, since they are assumed to be less flexible in the choice of interaction partners, but until now this hypothesis has not been tested.

2. In this paper, we studied phenology and interactions of plant and pollinator communities along an altitudinal gradient in the Alps as a model for the possible effects of climate change in time.

3. Our results show that even relatively specialized pollinators were much more flexible in their use of plant species as floral resources than their local flower visitation suggested. We found no relationship between local specialization of pollinators and the consistency of their visitation patterns across sites, and also no relationship between specialization and phenological synchrony of pollinators with particular plants.

4. Thus, in contrast to the conclusions of a recent simulation study, our results suggest that most pollinator species included in this study are not threatened by phenological decoupling from specific flowering plants. However, the flexibility of many rarely observed pollinator species remains unknown. Moreover, our results suggest that specialized flower visitors select plant species based on certain floral traits such as the length of the nectar holder tube. If that is the case, the observed flexibility of plant–pollinator interactions likely depends on a high degree of functional redundancy in the plant community, which may not exist in less diverse systems.

**Key-words:** climate change, ecological network, elevation, flowering, mutualistic interaction, phenological decoupling, phenological mismatch, phenological shift, pollination

### Introduction

Climate change generally advances the timing of phenological events in temperate regions of the world, but the magnitude of shifts in phenology varies greatly among species (Fitter & Fitter 2002; Parmesan 2006). As a result, previously existing species interactions may be disrupted when the species involved respond differently to changing

environmental conditions (Stenseth & Mysterud 2002). While a small number of such phenological desynchronizations have been documented (Visser & Holleman 2001; Edwards & Richardson 2004; Winder & Schindler 2004; Both *et al.* 2006), for most systems observations detailed enough to detect the existence and consequences of phenological mismatches are lacking (Miller-Rushing *et al.* 2010). Mutualistic systems are expected to be particularly vulnerable to phenological desynchronization, since in these systems, both partners benefit from the interaction and are likely to be negatively affected by its loss (Bartomeus *et al.* 2011). For plant–pollinator systems, one of

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the most common mutualistic interactions in terrestrial ecosystems (Ollerton, Winfree & Tarrant 2011), it is currently uncertain whether phenological desynchronization constitutes a major threat (Hegland *et al.* 2009; Willmer 2012). Of the limited number of studies available, some report similar rates of advance of flowering phenology and pollinator appearance (Bartomeus *et al.* 2011), while others suggest that either flower-visiting insects (Gordo & Sanz 2005) or flowering plants (Forrest & Thomson 2011) respond more strongly to climate change. However, even if mean rates of phenological shifts of plant and pollinator communities do not differ substantially, a critical question is to what extent each species depends on the availability of particular mutualists (Willmer 2012). Phenological synchrony with one or a few species of mutualists should be important for species that are specialized in the sense that their performance (rate of survival and/or reproduction) is much higher in the presence of these particular mutualists compared to others. While most plant–pollinator networks exhibit a moderate degree of specialization at the community level (Blüthgen *et al.* 2007), species-level specialization can vary considerably even within the same network, ranging from entirely opportunistic behaviour to highly specific associations (Vazquez & Aizen 2003; Waser & Ollerton 2006). Until now, studies of the effects of climate change on pollination mutualisms have mostly focussed on a limited number of generalist pollinators, providing a somewhat incomplete and possibly biased picture (Bartomeus *et al.* 2011; Forrest & Thomson 2011; Willmer 2012). An exception is the simulation study by Memmott *et al.* (2007), which covered a large pollination network and predicted reduced availability of floral resources for up to 50% of all flower visitor species under simulated climate change scenarios. However, these simulations were based on the assumption that each pollinator species can only visit plant species that it has been previously observed to visit. For many rarely observed pollinators, this approach underestimates the true range of interaction partners (Blüthgen 2010). Moreover, many species may be able to use new floral resources when they become available through phenological shifts. Thus, it is likely that Memmott *et al.* (2007) overestimated the negative effects of climate change on pollinators.

In the current study, we examined data on phenology and interactions of plant and pollinator communities to assess the extent of phenological synchrony with particular plant species among pollinators of different degrees of specialization. Since sufficiently long time series of plant and pollinator phenology in combination with recordings of their interactions are currently unavailable, we used data collected at six sites along an altitudinal gradient in the Alps as a space-for-time substitution. Provided that the results are interpreted with some caution, the climatic changes along altitudinal gradients can serve as a useful model for the effects of climate change in time (Fukami & Wardle 2005). Since temperature has

been found to be the main determinant of insect phenology and one of the two main drivers of plant flowering phenology (together with photoperiod; reviewed by Forrest & Thomson 2011), the change in temperature along an altitudinal gradient should result in phenological shifts similar to those caused by climate change (see Cornelius *et al.* (2013) for a recent study of temperature-related changes in plant phenology along an altitudinal gradient in our study area). For the current study, we monitored flowering phenology of insect-pollinated plants and insect flower visitation at each site weekly over the course of a season. We expected to observe an overall shift of plant and pollinator phenology towards earlier occurrence and an increase in the length of the season with decreasing altitude.

In our analyses, we took the perspective of the insects, since we assumed that flower-visiting insects are more vulnerable to changes in availability of their mutualistic partners compared to plants. Most insect-pollinated plant species are buffered against fluctuations in pollination success by a variety of traits such as self-pollination, vegetative reproduction, seed banks and iteroparity (Bond 1994). By contrast, obligate flower visitors, particularly bees, but also numerous species of butterflies and flies, depend on the availability of nectar and/or pollen for survival (and reproduction in the case of bees) on a shorter time-scale. Consequently, the selection pressure on insects to synchronize with the phenology of their floral resources should be stronger than the reciprocal pressure on plants, although long-term reductions in pollinator availability are likely to be detrimental to most plant species as well.

Note that in this paper, we use the terms ‘pollinator’ and ‘flower visitor’ interchangeably, although we are well aware that flower-visiting insects contribute to plant reproduction to varying degrees, and not all flower visitors are legitimate pollinators (e.g. Schemske & Horvitz 1984). Since this study focuses on dependence of insects on floral resources and not the reciprocal dependence of plants on insect pollination, its conclusions should equally apply to non-pollinating insects.

Our main aim in this study was to test the hypothesis that specialized flower visitors show a higher degree of phenological synchrony with the plant species they visit than generalized visitors. At a given time and location, a specialized pollinator should use a relatively small fraction of all available flowering plant species. Moreover, the sets of plant species visited by a specialized pollinator at different sites should be similar. Thus, we expected to be able to identify specialists both by their local specialization and the consistency of their visitation patterns across sites. Regarding phenology, according to our hypothesis, a specialized pollinator should show a high degree of phenological overlap with the plant species it depends on at a given site. In addition, if the specialist occurs at more than one altitude, its phenology should closely track the phenological shift of the relevant plant species from one site to the next. In summary, we expected to find a

positive relationship between local and inter-site specialization and phenological synchrony.

## Materials and methods

### DATA COLLECTION

The data used in this paper were recorded in the National Park Berchtesgaden in the German part of the Alps. Six grasslands in the central valley of the Park ('Wimbachtal') at altitudes between 950 m and 2020 m a.s.l. were selected for data collection (see Supporting Information, Table S1, for precise locations and sampling periods, and Fig. S1 for monthly mean temperatures at different altitudes in the park). Linear distances between neighbouring sites were between 2.2 and 0.5 km. These sites were neither mown nor grazed by cattle during the period of data collection. Only after the flowering season, the three lowest sites were grazed extensively. Hence, the flowering phenologies recorded in this study are not influenced by human land use. At each of the six sites, five rectangular transects of  $30 \times 4$  m were established. Transect locations were chosen so as to cover a representative sample of the local vegetation. Data collection at the lowest site started on 8 May 2010. Sampling at higher sites began as soon as they were free of snow and the first plants started to flower. Whenever possible, each site was visited once a week during dry weather to record open flowers and visiting insects. However, due to long-lasting rain and bouts of snowfall at higher altitudes, this was not always possible. As a result, between 13 (site 1: 950 m) and 9 (site 6: 2020 m) censuses were taken at each site over the course of the season. Field work was carried out by three observers (G.B. and two students; see Acknowledgements).

When visiting a site, all open flowers of insect-pollinated species (excluding grasses) were counted in 5 quadrats of  $2 \text{ m}^2$  that were placed at equal distances along each transect. Thus, quadrats covered  $5 \times 2 \text{ m}^2 \times 5 = 50 \text{ m}^2$  or  $1/12$  of the total area of all transects at a site. Flowers of rarer plant species that were not found in at least one of the quadrats were counted separately. Diameters of flowers of all plant species found at a site were measured in the laboratory with a digital calliper to the nearest 0.1 mm and converted to flower area by assuming a circular shape. Between 1 and 10 flowers per species were measured (mean: 7.1 flowers), and the mean of their diameters taken as a basis for the calculation of flower areas. For zygomorphic flowers, the mean of length and width of a flower was used as diameter of the circle. Strongly compact inflorescences (e.g. those of Asteraceae) were treated as single flowering units both in counting and measurement of diameters. For each plant species, the total flower area per site was calculated by multiplying the number of counted flowers in quadrats by the average area of a single flower or flowering unit and by extrapolating from the quadrat samples to the total area covered by all transects at a site ( $600 \text{ m}^2$ ).

In addition to the monitoring of open flowers, transect walks were carried out for five to seven hours (mean: 6 : 12 h) between 9 a.m. and 6 p.m. at each sampling date. During transect walks, the observer walked along the middle line of the long side of a transect, and recorded flower-visiting insects within two metres to the left and to the right of this line. Only taxonomic groups comprising obligate flower visitors were included in insect sampling: Bees (Hymenoptera: Apidae), flies (Diptera: Brachycera), and

butterflies and moths (Lepidoptera). Although not all brachyceran flower visitors obligatorily depend on floral resources, we decided to nevertheless include the whole group in our study. Beside the typical flower visitors such as hover flies and bee flies, members of other fly families can be important pollinators especially in high-altitude areas (e.g. Kearns 1992). By including insect group as an additional factor in our analyses (see below), we were able to separately consider the relationship between specialization and phenological synchrony for known obligatory flower visitors (bees and lepidopterans) and the fly group which contained an unknown proportion of non-obligatory flower feeders.

Except for a few very common and easily identifiable species [e.g. *Apis mellifera* (L.), *Episyrphus balteatus* (De Geer)], all recorded flower visitors were caught with a sweepnet and kept in individually labelled test tubes for later identification. Fly specimens were killed and preserved in 70% alcohol, while bees and lepidopterans were killed with ethyl acetate and stored in dry tubes. All captured insect specimens were later identified to species level with the help of taxonomists (see Acknowledgements).

To examine the relationship between specialization and floral morphology, we took measurements of the length of the floral tube from the same flowers used to determine flower areas (see above). The procedure was identical to the one described by Stang, Klinkhamer and van der Meijden (2006).

### STATISTICAL ANALYSES

#### Phenological estimator

For all statistical analyses of insect and plant phenology, we used the weighted mean day of occurrence (WMD) as a phenological estimator. The WMD is the arithmetic mean of all dates on which the species in question was observed, weighted by its abundance on each date. In a simulation study comparing ten phenological estimators, Moussus, Julliard and Jiguet (2010) found that WMD and a second estimator based on generalized additive modelling were the most accurate, unbiased and robust measures of phenophase, while commonly employed metrics such as first appearance dates performed poorly. In our analyses, abundances of insect and plant species were quantified as counts of individuals per hour of observation and flower area ( $\text{m}^2$ ) per  $\text{m}^2$  transect area, respectively.

#### Overall phenological shift

To describe the overall shift of plant and insect phenology with increasing altitude, we used a linear mixed-effects model with WMD per species and site as response variable, and altitude and guild ('plant' or 'insect') as explanatory variables. To account for the non-independence of data points of the same species at multiple sites, species identity was included as a random factor. In a first step, a choice was made between a model without the random factor, a model with random intercepts and a model with random intercepts and slopes for each species. The best model of this choice was then subjected to model selection of the fixed effects. In both cases, we selected the best model based on Akaike's Information Criterion (AIC). Diagnostic plots were examined to check for heteroscedasticity and normality of errors. We used the R statistical software version 2.14 (R Development Core Team 2011) for all data analyses and production of graphs.

For linear mixed-effects models, we used the 'lme' function included in the R package 'nlme' (Pinheiro *et al.* 2011).

### Local specialization

In order to quantify the degree of specialization of insect species on flowering plants at a given date and site, we calculated the  $d'$  index for species-level specialization in bipartite interaction networks (Blüthgen, Menzel & Blüthgen 2006; Blüthgen *et al.* 2008). This metric describes the deviation of the observed flower visits of a focal pollinator species from the expected distribution of visits based on resource availability. Its value ranges from zero (most generalized) to one (most specialized). While a number of other metrics of specialization at the species level have been proposed (Dormann 2011; Poisot *et al.* 2012), the  $d'$  index has two advantages over alternative indices: It considers the visitation pattern of a species in relation to resource availability and is robust to differences in numbers of observations per species (Blüthgen, Menzel & Blüthgen 2006). Thus, singleton observations do not automatically count as maximum specialization, when in fact the sample size is too low to judge the range of resources used by a species (see the Supporting Information, Fig. S3 for an analysis of the relationship between  $d'$  and number of observations for our data).

For the calculation of  $d'$ , the relative availability of floral resources of different plant species can be assessed in two ways: Either the distribution of total visitor numbers on plant species can be taken as estimates of resource availability or a measure of floral abundance such as flower area may be used instead (Dormann 2011). While external flower abundances have the advantage of being independent of the overall visitation patterns of the pollinator community, using the plant abundance distribution as a null expectation for the distribution of flower visits may lead to unrealistic assumptions. For example, the theoretical maximum of specialization is reached when all visits of a pollinator species are to the least abundant plant species in the community, but for a highly abundant pollinator it is unrealistic to assume that all individuals could visit a plant with only one or two flowers at the site. For our calculations of  $d'$ , we used the total number of visits to each plant as a null expectation, because in our opinion, these visitation totals give a better estimate of resource availability or floral attractiveness. Using  $d'$  values based on flower area instead did not qualitatively change the results of our analyses (Supporting Information, Table S6). For 60 out of 66 date-site combinations, flower areas and total numbers of visits per plant species were significantly positively correlated (mean of Kendall's  $\tau$ : 0.49).

In a first step, we compiled separate interaction networks for all date-site combinations and calculated  $d'$  values for all insect species in each network. In this way, we avoided the problem of impossible interactions ('forbidden links') in aggregated networks due to separation of species in time or space. To obtain one overall specialization value for each insect species, we then calculated the mean of all  $d'$  values of a species weighted by number of observations. Index values based on single observations of a species were excluded. For comparison with other studies of mutualistic networks, we also calculated the  $H_2'$  index for specialization at the network level (Blüthgen, Menzel & Blüthgen 2006). As the  $d'$  index, the  $H_2'$  index is derived from Shannon entropy and describes the deviation of the observed visitation patterns from the null expectation of random associations. The index is

standardized to a value between 0 (maximal generalization) and 1 (maximal specialization). Both  $d'$  and  $H_2'$  were calculated using the 'bipartite' package for R (Dormann, Gruber & Fründ 2008).

We conducted a simple ANOVA followed by Tukey's post hoc test for pairwise comparisons to test for a difference in specialization between taxonomic groups (bees, flies and butterflies and moths). The analysis of the relationship between specialization and tube length of flowers visited by each insect species was split into two parts. For each insect species, we calculated the mean floral tube length of all plant species visited, with plant species weighted by numbers of visits. Since there was a high number of zero values in the data set (i.e. insect species visiting only flowers with openly accessible nectar), we first applied a Generalized Linear Model (GLM) to tube length as a binary variable (zero / non-zero values) with local specialization (weighted mean  $d'$ ) and insect group as explanatory variables. We then performed a second analysis on the subset of data with nonzero tube lengths, this time treating tube length as a continuous variable. As the subsets of data of the three taxonomic groups showed different distributions and normality could not be achieved by applying a transformation to the whole data set, we fitted separate linear models to the data of each insect group, and applied log transformation only to the fly data.

### Consistency of flower visitation across altitudes

To assess the similarity of groups of plant species visited by an insect species at two altitudes (hereafter termed 'visitation consistency'), we used the Bray–Curtis index of community similarity (Bray & Curtis 1957; Legendre & Legendre 1998) as an index of visitation consistency. Given two vectors of standardized species abundances  $X_j$  and  $X_k$  with elements  $x_{ij}$  and  $x_{ik}$  for species  $i$  at site  $j$  and  $k$ , respectively, the Bray–Curtis similarity  $S$  is calculated as

$$S = \frac{2 \sum_{i=1}^N \min(x_{ij}, x_{ik})}{\sum_{i=1}^N x_{ij} + \sum_{i=1}^N x_{ik}}$$

where  $N$  is the number of species. The index ranges between zero (no overlap in species occurring at the two sites) and one (all species with identical relative abundances). To compare the flower choices at two sites, we used the relative frequency of visits of the focal insect species to each plant species instead of the abundances  $X_j$  and  $X_k$  in the calculation of  $S$ . For this purpose, all observations of the focal insect from different dates were pooled. Depending on the number of sites at which each insect species was found, one to fifteen comparisons of plant species visited by an insect species at two sites could be made. However, in order to avoid pseudoreplication due to non-independence of pairwise comparisons, we only included comparisons between the lowest site at which a species occurred and all other sites in the analyses. Thus, one to five comparisons per insect species were made. Using only comparisons between neighbouring sites produced qualitatively and quantitatively similar results. Visitation consistency was calculated for all 56 insect species that were found at two or more sites with at least five individuals per site.

In order to test whether a value of  $S$  as high or higher than the observed value could have occurred by chance, we performed randomization tests. For this purpose, we drew random samples from all plant species available to the focal insect at each site. In a first step, samples were taken separately for each date at which the insect species was observed. The sample size was equal to the



number of flower visits of the insect species observed at that date, while the probability of choosing a certain plant species equalled the proportion of visits of all insects to this plant species at that date and site. In a second step, all plant visitation samples of the separate days were summed to obtain an overall sample of flower visits as large as the total number of visits of the focal insect observed at the site. The similarity of these overall plant visitation samples of the two sites was assessed in the same way as for the real visitation data. The procedure was repeated 1000 times. A *P*-value was then calculated as the proportion of all similarity values as high or higher than the observed value (i.e. the value obtained from real visitation data). Following Manly (2007), the observed value of *S* was included in both the numerator and the denominator of the proportion. Significance was assessed at the 5% level in all randomization tests.

As in the case of floral tube lengths, due to an excess of zero values, the statistical analysis relating visitation consistency to local specialization (*d'*) and taxonomic group was divided into two stages. First, consistency was analysed as a binary variable (zero/nonzero consistency). In addition to *d'* and order, we included the mean consistency of random plant samples (mean of 1000 replicates) and sample size (minimum number of observations at the two sites) as covariates in the model. We then built a second model including only nonzero consistency values, this time treating consistency as a continuous variable. In both cases, we first compared the fit of a mixed-effects model with species as a random factor to a (G) LM with only fixed effects. Since for the continuous data, the mixed model did not perform significantly better in a likelihood ratio test, we proceeded with a linear model, whereas for the binary data, a GLMM was used. The GLMM was fitted using the function 'lmer' included in the R package 'lme4' (Bates, Maechler & Bolker 2012).

### Phenological synchrony

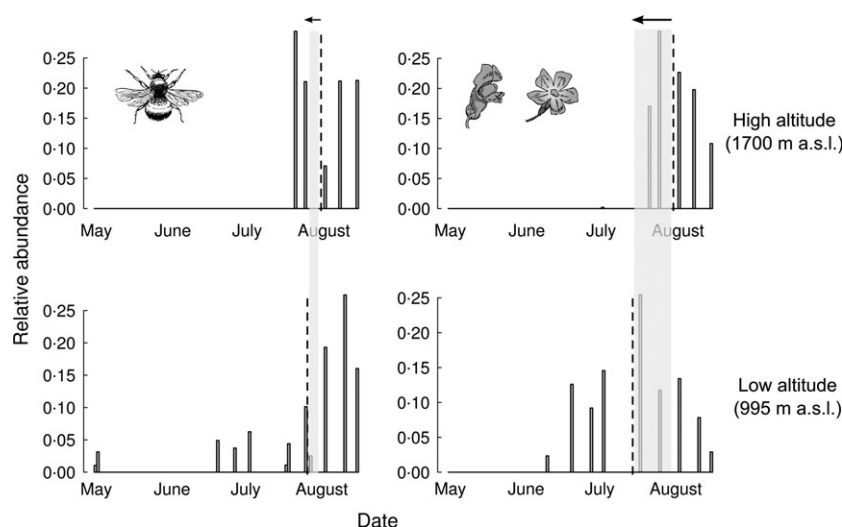
For flower visitors that depend on particular sets of plant species for survival and/or reproduction, we expected that the WMD of each flower visitor should be close to the WMD of the plant species it uses. Therefore, we used the absolute difference between the WMD of an insect species and the collective WMD of the plant species it visited as a measure of phenological asynchrony at one site. The collective WMD of all visited plant species was calculated by summing the plants' abundances at each date and

calculating the WMD of the time series of summed abundances. However, one could argue that a close match between weighted mean occurrences of insects and plants is of less importance if the flowering phase of the plant species is long. Hence, we also calculated a *z*-score (absolute difference in WMD divided by the standard deviation of the distribution of summed plant abundances) as an alternative measure of phenological asynchrony. All insect-site combinations with at least five observations were included in the analysis. Overall, the data set comprised 286 asynchrony values of 138 insect species. Since a mixed model with species identity as a random factor did not result in a better fit, we employed a linear model to test for an effect of local specialization (*d'*) and taxonomic group.

For insect species that occurred at more than one site, we examined the shift of WMD between sites in relation to the shift of the group of plant species visited by the insect at one of the two sites (Fig. 1). Using the higher altitude as a reference, we considered the phenology of all plant species that were visited by the insect at the reference site and also occurred at the lower site. Phenological asynchrony was defined as the absolute difference between the insect's and plants' shifts. The higher altitude was chosen as a reference since a shift from high to low altitude corresponds to the direction of climate change. However, using the lower site as a reference did not qualitatively change the results of the analyses. The procedure for statistical tests of asynchrony of shifts in phenology in relation to insect specialization and order was the same as for synchrony at one site. As in the case of visitation consistency (see above), we calculated phenological shifts between the lowest site at which each insect species occurred and all other sites. Using combinations of neighbouring sites instead yielded qualitatively similar results. All combinations of insect species and pairs of sites with at least five observations of the insect were included in the analysis, resulting in 138 data points of 54 insect species.

Since several insect species peaked in abundance towards the end of the season (e.g. *Bombus pascuorum*, Fig. 1), it is possible that the WMD estimates of these species were affected by truncation of the field season. To test whether this might have affected our results, we repeated the analyses of phenological synchrony using only data of early-occurring insect species (i.e. those with their WMD before 7 July 2010). Exclusion of late-occurring species did not qualitatively change the results (Supporting Information, Table S5).

**Fig. 1.** Synchrony of the phenological shift of an insect species (*Bombus pascuorum*) and the flowering plants it visited. Barplots show the relative abundance of the bumblebee (left) and the flowers (right) over time. The flowering phenology comprises all plant species that were visited by the bumblebee at the higher altitude and occurred at both altitudes. Dashed lines mark the weighted mean day of occurrence (WMD) of the bumblebee and plant species, respectively. Grey rectangles indicate the magnitude and arrows the direction of shifts in phenology between the two sites.



### Effects of environmental variation and observer identity on pollinator abundance

Visitor numbers at flowers tend to fluctuate with current environmental conditions and may therefore not always reflect actual population densities. Although all our observations were carried out under dry and relatively warm conditions (at least 12 °C), it is likely that some of the observed variation in insect abundance was due to the weather being more or less favourable for flower visitation. Moreover, since data collection for this study was carried out by three different observers, recorded visitor numbers may have been affected by interobserver differences in rates of detecting and catching insects from flowers. To examine the influence of environmental conditions and observer identity on visitor numbers per day (all species combined), we employed a Poisson GLM with mean daily temperature (°C) and humidity (%), observation time (hours), total flower area (m<sup>2</sup>/m<sup>2</sup> transect area), altitude (m a.s.l.) and observer as explanatory variables. Since exploratory data analyses suggested a nonlinear relationship between flower area and visitor number, flower area was log-transformed to linearize the relationship. In addition, a quadratic effect of altitude was included in the model. The final model showed significant positive effects of humidity and flower area, a marginally significant positive effect of observation time and a significant hump-shaped relationship with altitude (Table S4, Supporting Information). Since one of the three observers (L.M.) recorded significantly lower individual numbers compared to the other two, we multiplied all visitor numbers by a correction factor to account for this interobserver difference and repeated the analyses presented in this paper. As correction factor, we used the inverse of the effect of L.M. in the final model relative to visitor numbers recorded by G.B. (estimated effect of L.M. on the response scale: 0.500, SE 1.138,  $P < 0.001$ ). Visitor numbers recorded by the third observer (S.N.) did not differ significantly from those observed by G.B. (estimate: 1.142, SE 1.084,  $P = 0.104$ ). Using the correction factor did not qualitatively change the results of the analyses.

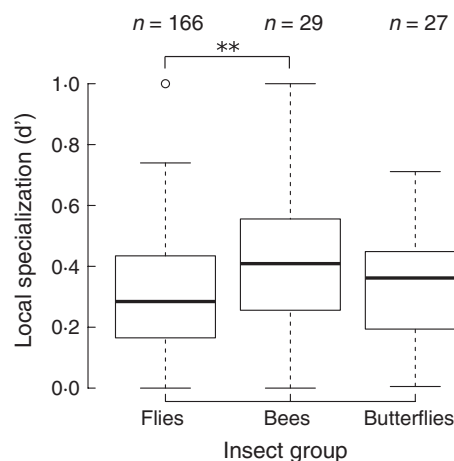
## Results

### OVERALL PATTERNS

In total, we observed 10 504 interactions between 166 flowering plant species and 444 insect species. Flower-visiting insects included 326 species of flies, 45 bees and 73 butterflies and moths. White and yellow flowers of disc or bowl shape dominated the plant community, but nearly all other flower types and colours were represented, as well (Supporting Information, Fig. S2 and Table S2). The data set comprised 67 interaction networks, of which 59 were significantly specialized (mean  $H_2' = 0.554$ , SD = 0.149).

### LOCAL SPECIALIZATION

At the species level, flower visitors were on average moderately specialized (overall mean of species' weighted mean  $d'$  values = 0.332, SD = 0.208). Bees were significantly more specialized than flies, with lepidopterans at an intermediate level of specialization (Fig. 2). The



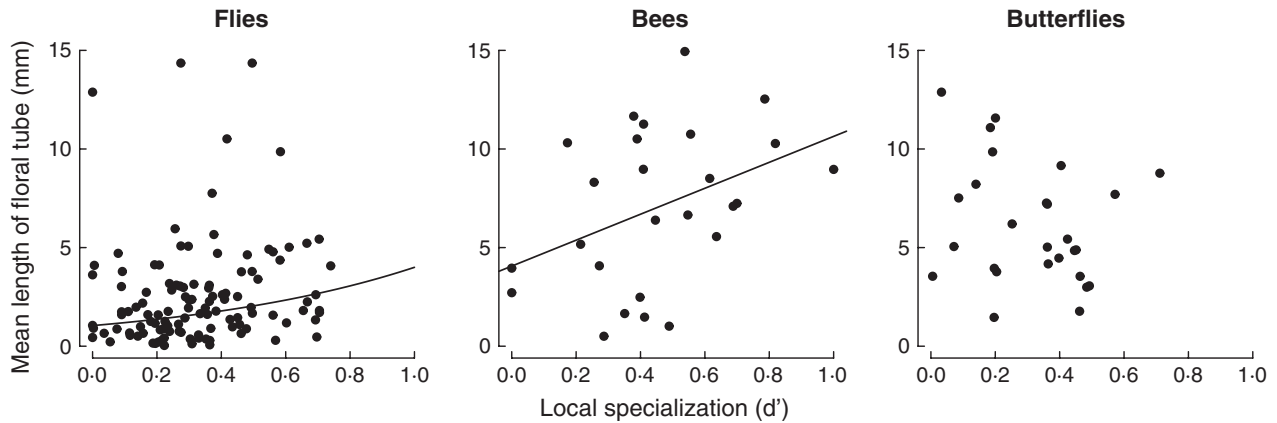
**Fig. 2.** Local specialization of insect taxonomic groups. Sample sizes given above each box are species numbers. Each data point is the mean  $d'$  value over all networks in which a species was observed weighted by number of observations. Single observations were excluded from the calculation. Overall effect of taxonomic group:  $F_{2,219} = 4.399$ ,  $P = 0.013$ . Asterisks indicate a significant difference ( $P < 0.01$ ) determined by Tukey's post hoc test for pairwise comparisons.

average number of plant species visited by an insect species (all sites and dates pooled, singletons excluded) was 6.53 (flies: 6.62, bees: 7.62, butterflies and moths: 4.77).

Mean tube length of flowers used was generally positively related to local specialization of insects, but we found differences between insect groups. In the analysis of tube length as a binary variable (flowers without or with tubes), the proportion of species visiting flowers with nonzero tube lengths was highest for lepidopterans (100%,  $n = 27$ ), followed by bees (92.8%,  $n = 28$ ) and flies (69.3%,  $n = 166$ ). Both the effects of taxonomic group [ $P < 0.001$ , estimate on the logit scale: Flies 0.207 (SE 0.293), bees 1.541 (0.761), butterflies and moths 17.732 (0)] and of  $d'$  [estimate: 2.104 (SE 0.876),  $P = 0.012$ ] on the probability of visiting flowers with tube lengths greater than zero were significant, with no significant interaction between the two variables. With tube length as a continuous variable (flowers with openly accessible nectar excluded), mean floral tube lengths increased strongly with insect specialization for bees and to a lesser extent for flies, but not for butterflies and moths (Fig. 3).

### VISITATION CONSISTENCY

In addition to the local specialization of insects on flowering plants at a given date and site, we calculated the Bray–Curtis similarity of sets of plant species visited by each insect species at two sites (its visitation consistency) to assess the degree of dependence of insect species on particular plant species. For 51 out of 148 insect-altitude combinations, we found that the plant species visited were significantly more similar than expected by chance. Thus, the proportion of significant tests was much higher



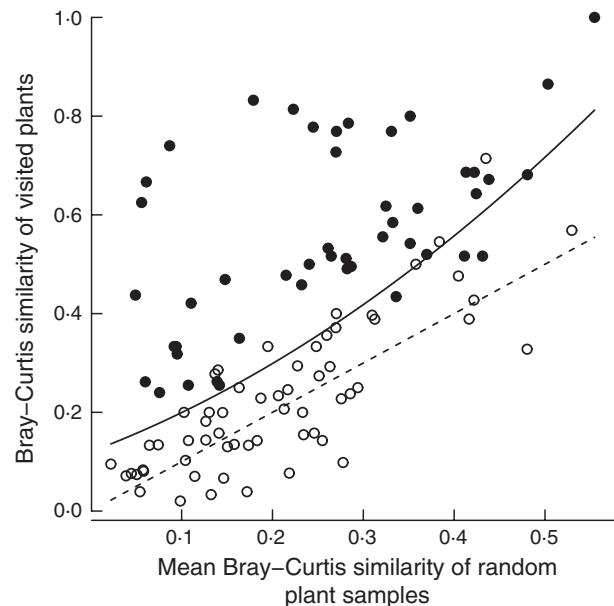
**Fig. 3.** Relationship between pollinator specialization and mean tube length of flowers visited. Tube lengths are means of flowers visited by an insect species weighted by number of visits. Lines indicate the prediction of linear regressions: Flies:  $\log(y) = 1.342x + 0.044$ ,  $P = 0.015$ ,  $R^2 = 0.04$ ; Bees:  $y = 6.578x + 4.061$ ,  $P = 0.045$ ,  $R^2 = 0.12$ . The regression for butterflies and moths was not significant ( $P = 0.25$ ).

(34.4%) than expected if the null hypothesis were true in all cases (5%). In the analysis of visitation consistency as a binary variable (consistency equal to zero or higher than zero), significant effects of taxonomic group [ $P < 0.001$ , estimates on logit scale: Flies  $-6.648$  (SE  $1.550$ ), bees  $1.164$  ( $2.060$ ) and lepidopterans  $-7.088$  ( $2.018$ )] and of the mean similarity of random plant samples (square root-transformed,  $29.965$  ( $6.354$ ),  $P < 0.001$ ) were found. The effect of local specialization ( $d'$ ) on visitation consistency was not significant. On the other hand, when visitation consistency was treated as a continuous variable (zero values excluded), only the mean similarity of random plant samples had a significant effect (Fig. 4). Moreover, the within-species variation in visitation consistency was high in relation to the between-species variation. Specifically, the average standard deviation of consistency values of the same insect species was approximately 1.5 times as large ( $0.232$ ) as the standard deviation of all species means ( $0.15$ ).

#### PHENOLOGICAL SHIFTS AND ASYNCHRONY

When assessing the overall shift in phenology with increasing altitude, model selection resulted in a linear mixed-effects model of WMD against altitude and guild without an interaction between the two explanatory variables (Fig. 5). Thus, the population-level estimate of the mean shift of WMD with increasing altitude was the same for plants and insects (estimate  $\pm$  SE:  $1.34 \pm 0.16$  days/100 m,  $P < 0.001$ ). The estimated mean WMD of insect species was approximately 6 days later than the plants' mean ( $5.91 \pm 2.09$  days,  $P = 0.005$ ).

The average difference in WMDs of insects and the plant species they visited at one site was  $11 \pm 9.5$  days (median: 8.4 days). We found no significant effect of local specialization and no difference between insect groups regarding the degree of phenological asynchrony at one altitude (Fig. 6, panels (a) and (b), Table 1). The same



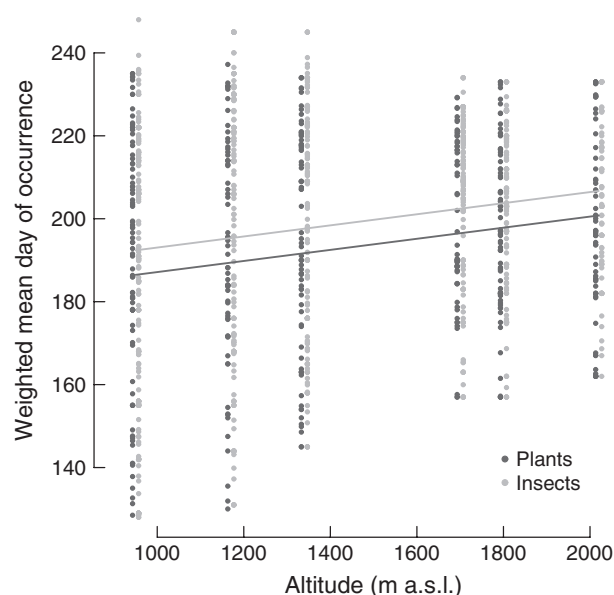
**Fig. 4.** Similarity of flower selection across sites. The Bray-Curtis similarity of plant species visited by an insect species at two altitudes is plotted against the mean similarity of random samples of all flowering plants available to the insect at the two sites. The dashed diagonal line marks the expected similarity of visited plant species under the null hypothesis of random selection of flowers. Filled circles indicate significantly higher similarity of visited plants than expected by chance, as determined in randomization tests. Open circles indicate non-significant tests. The solid line is the prediction of a linear regression:  $\sqrt{y} = 1.167x + 0.336$ ,  $P < 0.001$ ,  $F_{1,105} = 51.28$ ,  $R^2 = 0.32$ .

was true for synchrony of shifts in phenology of insect species and the plants they visited (Fig. 6, panels (c) and (d), Table 1).

#### Discussion

In order to be able to predict the effects of climate change on plant-pollinator interactions via shifts in phenology, it

is important to understand to what extent pollinator species depend on phenological synchrony with specific plant species as floral resources. The current study sheds light



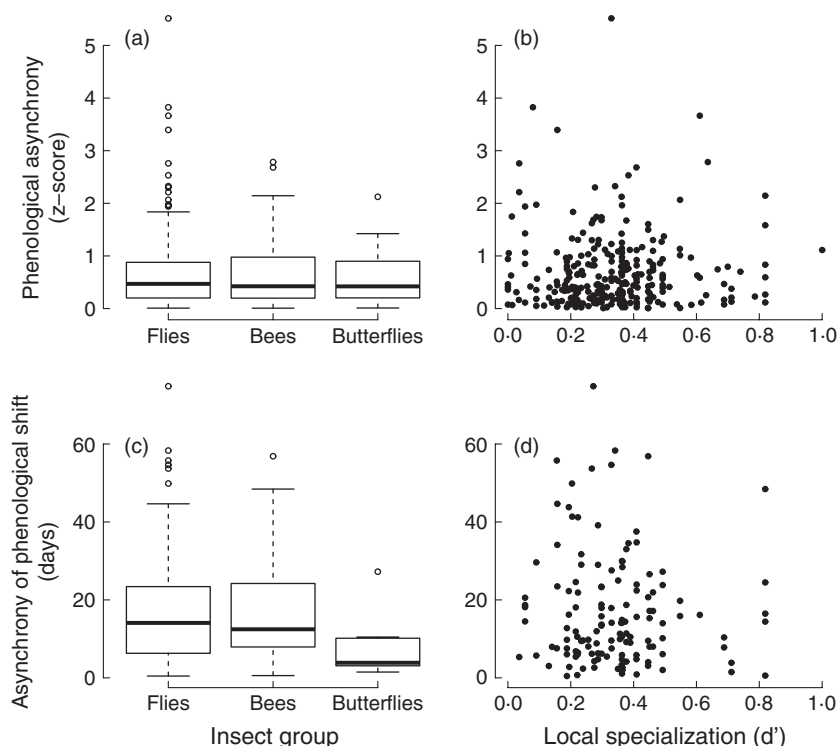
**Fig. 5.** Shift of flowering phenology and flower visitor occurrence with increasing altitude. For each species at each site, the weighted mean day of occurrence (WMD) is plotted against the site's altitude. Day 1 refers to 1 January 2010. Sampling at the lowest altitude began on 8 May (day 128). For clarity, plant data have been plotted 7 m below their true altitude, insect data 7 m above. The straight lines indicate the population-level prediction of a linear mixed-effects model of weighted mean day against altitude and guild ('plant' or 'insect'), with species as a random factor.

on this question by considering the relationship between pollinator specialization and phenological synchrony with flowering plants along an altitudinal gradient. Contrary to our expectation, we found that specialized pollinators did not show a closer phenological match with the plant species they visited than generalized pollinators. Moreover, the similarity of plant species visited by the same pollinator species at different altitudes ('visitation consistency') was highly variable within species and showed no relationship with local specificity of flower visitation at a given time and location.

As always when a statistical test fails to reject the null hypothesis, there are several alternative explanations. One possibility is that the proposed relationship is truly absent, but the data used may also simply be inadequate to evaluate the hypothesis in question, or the effect may be weak, highly nonlinear, or confounded with other factors. Below, we explore some of the possible explanations for the lack of a relationship between the two aspects of specialization and between specialization and phenological synchrony in greater detail.

#### WHY DID WE NOT FIND A POSITIVE RELATIONSHIP BETWEEN LOCAL SPECIALIZATION AND VISITATION CONSISTENCY?

Highly specialized pollinators should always visit the same flowering plant species, and thus appear specialized under both criteria. However, since the measure of local specialization used in this study ( $d'$ ) is based on a comparison of observed and expected distributions of flower visits among plant species, it is not well suited to detect specialization on abundant and frequently visited plant species. Other



**Fig. 6.** Phenological asynchrony of insects and the plants they visited. Boxplots in panels (a) and (c) display the relationship between phenological asynchrony and taxonomic groups of insects, scatterplots in (b) and (d) the relationship with local specialization of insect species. In (a) and (b), asynchrony is measured as the absolute difference of the weighted mean days of occurrence (WMD) of insects and plants at one site divided by the standard deviation of the plant WMD. Panels (c) and (d) show the asynchrony of shifts in WMD of insects and the plants they visited with changing altitude. See Table 1 for results of the statistical analyses.



**Table 1.** Results of linear models of phenological asynchrony at one altitude and asynchrony of phenological shifts between altitudes as a function of degree of local specialization ( $d'$ ) and taxonomic group (insect order)

Response variable	Explanatory variable(s)	d.f.	F	P
Asynchrony at one site	Taxonomic group	2, 280	0.16	0.851
	Specialization	1, 280	0.35	0.557
	Taxonomic group $\times$ Specialization	2, 280	0.76	0.471
Asynchrony of shifts between sites	Taxonomic group	2, 132	1.96	0.145
	Specialization	1, 132	0.05	0.831
	Taxonomic group $\times$ Specialization	2, 132	0.22	0.802

measures [e.g. Normalized Degree (Dormann 2011), Resource Range or Paired Difference Index (Poisot *et al.* 2012)] may indicate a high degree of specialization even if all visits of a pollinator species are to the most abundant plant species, but this detection ability comes at the cost of many false positives – pollinators without any preference for specific plant species are treated as specialists, simply because their visitation patterns follow the skewed abundance distribution of the plant community, and only a subset of their interactions have been sampled (Blüthgen, Menzel & Blüthgen 2006; Dormann 2011). Therefore, we preferred to use a conservative measure of specialization such as  $d'$ , which admittedly fails to detect true specialists under some circumstances.

Of the three insect groups included in this study, the flower visitation patterns of bees have received the most attention in the literature. Traditionally, bee species have been classified as either oligolectic or polylectic, depending on whether they collect pollen from flowers of one or more plant families. Most bee species included in the current study are classified as polylectic in the literature (Westrich 1989). Of the oligolectic species, *Bombus gers-taeckeri* (Morawitz) (specialized on flowers of monkshood, genus *Aconitum*) had a high mean  $d'$  value (0.79), while *Chelostoma florissomme* (L.) (a specialist on the genus *Ranunculus*) and *Panurginus montanus* (Giraud) (a specialist on Asteraceae, particularly the subfamily Cichorioideae) scored low on the  $d'$  scale, because the flowers they visited were common and visited by many other insect species, particularly by flies who constituted by far the most abundant visitor group. Hence, it is likely that several other insect species were wrongly classified as generalists by our approach. On the other hand, several bee species known as polylectic in the literature received high  $d'$  values in our study. The fact that  $d'$  was positively related to floral tube length suggests that many insect species classified as specialized based on  $d'$  visited flowers of multiple plant species, but were selective with respect to the floral traits of the species they visited. This would explain the high degree of intraspecific variation in visitation consistency. For example, for *Bombus wurflenii*

(Radoszkowski), a bumblebee species with a high mean  $d'$  value (0.82) that occurred at all six sites, seven out of fifteen pairwise comparisons of visited plant species showed significant visitation consistency, while in the remaining eight cases, the similarity of plant species visited by the bumblebee was even lower than the mean of randomly chosen plant samples. Yet the species consistently selected flowers with mean tube lengths greater than expected at all six sites (Table S3, Supporting Information). Thus, it seems that *Bombus wurflenii* is a specialist with respect to certain floral traits and that the bumblebee did not visit the same plant species throughout its altitudinal range because the traits it prefers were present in multiple plant species of which a subset occurred at each site.

#### WHY DID WE NOT FIND A POSITIVE RELATIONSHIP BETWEEN SPECIALIZATION AND PHENOLOGICAL SYNCHRONY?

Regarding the synchrony of phenological shifts of flower visitors and the plants they visited, one possible explanation for the lack of a relationship with specialization is that those species that were both unable to shift their phenology in synchrony with the relevant flowering plants and not flexible enough to use other floral resources simply did not occur at more than one altitude. Since only 56 out of 444 flower visitor species were found at more than one site in sufficient numbers to be included in the analysis, this argument does not seem unreasonable. If highly specialized species have trouble adjusting to the conditions at different altitudes, we would expect that species occurring at more than one altitude should on average be less specialized than single-site species, or at least have a lower variance of degrees of specialization. While the mean degree of specialization did not differ significantly between the two groups (generalized least-squares; estimates  $\pm$  SE: single site  $0.312 \pm 0.022$ ,  $n = 119$ ; multiple sites  $0.334 \pm 0.027$ ,  $n = 103$ ;  $P = 0.407$ ), insects found at a single site did indeed show a higher variance of  $d'$  compared to species found at multiple sites (Fligner–Killeen test of homogeneity of variances; single-site variance: 0.056; multiple-site variance: 0.028;  $P < 0.001$ ). However, this difference in variances may simply be due to a sampling effect, since the average number of observations per species was significantly lower for species that occurred only at one site (generalized least-squares; estimates  $\pm$  SE: single site  $3.296 \pm 0.210$ ; multiple sites  $6.050 \pm 0.050$ ;  $P = 0.005$ ). On the other hand, as discussed above, some specialized species almost certainly remained undetected by our approach. Thus, the possibility that less flexible pollinator species did not occur at more than one site cannot be ruled out based on our data.

#### LIMITATIONS OF THE STUDY DESIGN

As in any non-manipulative study, it is difficult to assess to what degree the results presented in this paper were

affected by the specific conditions under which the study was carried out. For example, it is possible that the phenological patterns of 2010 were exceptional, and many pollinator species whose occurrence in the season would normally closely match the flowering phase of the plant species relevant to them were out of synchrony in this particular year. To be sure of the generality of the patterns described here, data should be collected over several seasons.

Furthermore, since our estimates of pollinator phenology were based on observations of insects on flowers, a certain degree of phenological synchrony is a necessary consequence of the sampling design. To date, few data sets of pollinator phenology independent of flowering phenology have been collected (Forrest & Thomson 2011). Even with this constraint in sampling design, the degree of phenological synchrony of insects and flowers may vary considerably, depending on the abundance distribution of each species over time. However, our study design did not allow to detect early appearance of pollinators in spring before the first flowers, or late occurrence in autumn after the end of flowering. It is possible that synchronous peaks in abundance are less fitness relevant for pollinators than synchrony in first appearance. Unfortunately, estimates of first appearance dates of insects from field data are usually very inaccurate due to detection issues (Moussus, Julliard & Jiguet 2010). One solution to this problem is to record emergence dates of adult insects, for example, by using trap nests for bees or ground traps for flies (Forrest & Thomson 2011). However, such studies can usually only cover a small subset of the pollinator community.

Another possible limitation of our study lies in the method of estimation of floral resource availability. Using flower area as a proxy for pollen and nectar availability is common practice in community-wide studies of plant–pollinator interactions (e.g. Klein, Steffan-Dewenter & Tschardt 2003; Herrmann *et al.* 2007; Meyer, Jauker & Steffan-Dewenter 2009), since the time and effort required to directly measure floral resources are usually prohibitive. However, this method causes a certain degree of imprecision, because nectar and pollen standing crop may vary considerably between flowers (e.g. Shmida & Kadmon 1991). Furthermore, our estimates of flower area may themselves be imprecise, since they are based on the simplified assumption of all flowers having a circular shape, and on extrapolation from a few sample quadrats to a larger area.

Finally, as mentioned in the introduction, some caution is needed when drawing conclusion about the effects of climate change in time based on data from altitudinal gradients (Fukami & Wardle 2005). Whereas plant phenology in alpine habitats is primarily affected by local conditions, pollinators experience resource availability and abiotic conditions at a larger spatial scale (Hegland, Grytnes & Totland 2009). Hence, it is possible that some of the more mobile insect species were able to relocate

their foraging ranges along the altitudinal gradient according to resource availability and did not need to synchronize their flight period with the flowering phenology of plants at only one altitude.

## Conclusion

Whereas previous work predicted that, as a consequence of climate change, many pollinator species would suffer from reduced availability of floral resources due to phenological asynchrony with the flowering plants they were previously visiting (Memmott *et al.* 2007), the current study suggests that for most pollinators phenological synchrony with specific sets of plant species is not important. Despite the fact that many pollinator species showed non-random patterns of flower visitation, we found no evidence that insect species that were more selective with regard to the flowers they visited synchronized their flight period with the flowering phase of particular plants. Thus, as Willmer (2012) suggested, plant–pollinator interactions seem to be buffered against climate change by their flexibility in the choice of interaction partners.

A possible explanation for the lack of a positive relationship between pollinator specialization and phenological synchrony lies in the finding that specialized pollinators visited plant species with longer floral tubes than generalists. Since the morphological traits (and possibly other traits such as flower colour and scent) that these specialists preferred were present in multiple plant species of which a subset occurred at each altitude, they did not need to track the phenological shifts of particular plant species. This interpretation highlights the importance of understanding the mechanisms behind ecological specialization. Until now, specialization of plant–pollinator interactions has predominantly been studied from the plants' perspective (Aigner 2001; Fenster *et al.* 2004). While a number of studies have examined pollinator preferences for single floral trait (morphology: Stang, Klinkhamer & van der Meijden (2007), scent: Junker, Höcherl & Blüthgen (2010), colour: Lunau & Maier (1995)), the study of combinations of multiple traits has only just begun (Junker *et al.* 2013).

While our results indicate that at least for the most abundant pollinator species in our study system phenological synchrony is not an issue, we cannot be certain about the generality of these findings. Further investigations are needed to assess the importance of phenological synchrony for the many species of pollinators that were observed too infrequently to be included in the analyses. Since samples of insect communities always contain a large number of singleton observations, this issue can only be addressed by focussing sampling efforts on a small number of species. Likewise, the community approach to estimating specialization employed in this study very likely underestimates the dependence of pollinator species specialized on common and frequently visited plants. Here again, species-level studies are needed

to elucidate the effects of shifts in phenology on extreme specialists such as the oligolectic bee species mentioned earlier. Furthermore, while this study focuses on the insects' perspective, the consequences of phenological shifts on plant reproductive success may be entirely different. These could also be affected by asynchronies with other mutualists such as seed dispersers (e.g. Warren, Bahn & Bradford 2011) or antagonists such as seed predators and pathogens.

Finally, the exceptionally high diversity of plant species found at our study sites may in itself explain the absence of phenological synchrony of pollinators with specific plant species. As mentioned earlier, it appears that even pollinator species with strong preferences for certain floral traits had no need to synchronize with particular plants because the high degree of functional redundancy (Rosenfeld 2002) in the plant community allowed them to visit different plant species at different sites. If this is true, phenological synchrony should be much more important for pollinator species in less diverse systems. Hence, a similar study to ours in an anthropogenically more disturbed habitat with reduced diversity might give very different results. After all, biodiversity may be the best buffer against climate change.

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## Data accessibility

The data associated with this article are publicly accessible in the Dryad data repository, doi:10.5061/dryad.8mn44 (Benadi *et al.* 2014).

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## Supporting Information

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

**Appendix S1.** Study sites and sampling periods

**Appendix S2.** Distribution of floral traits

**Appendix S3.** Selection of floral tube lengths by *Bombus wurflenii*

**Appendix S4.** Effects of observer identity and environmental variables on insect abundance

**Appendix S5.** Models of phenological synchrony without late-occurring insect species

**Appendix S6.** Models of visitation consistency and phenological synchrony with local specialization (d') based on flower area

**Appendix S7.** Relationship between local specialization (d') and number of observations.