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Temporal stability of pollinator preference in an alpine plant community and its implications for the evolution of floral traits

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Abstract A traditional view of diverse floral traits is that they reflect differences in foraging preferences of pollinators. The role of pollinators in the evolution of floral traits has been questioned recently by broad community surveys, especially studies concerning variation in pollinator assemblages and visitation frequency, which suggest a diminished role of pollinators in floral evolution. Here, we investigate the relationships between six categories of floral traits of 29 species and 10 pollinator functional groups in an alpine meadow in the Hengduan Mountains of China, over three consecutive years. Simpson's diversity index was used to estimate the level of pollinator generalization of each plant species by considering both pollinator groups and their relative visitation frequencies. Multivariate analyses indicated that eight of the ten pollinator groups showed constant preferences for at least two floral traits, leading to a relatively stable level of ecological generalization for most floral traits (two out of three categories), despite the fact that the level of generalization of the entire community varied across years. Shape preferences of butterflies, honeybees and beeflies varied such that open flowers exhibited a lower level of ecological generalization in 2007 than closed flowers, in contrast with the other 2 years. These results suggest that temporally stabilized preferences of diverse pollinators may contribute to the evolution of specialized versus generalized floral traits; however, their role may be moderated by variation in community structure, including both the composition and abundance of plants and pollinators.

Keywords Floral traits · Temporal variation · Ecological generalization level · Generalization and specialization · Visitation frequency

Introduction

Floral trait evolution is thought to be influenced by diverse selective factors, including the abiotic environment, floral herbivory and pollinators (Faegri and van der Pijl 1979; Fenster et al. 2004; Strauss and Whittall 2006). Convergence in floral traits is common in groups of plants with a similar suite of pollinators (Faegri and van der Pijl 1979; Fenster et al. 2004). For example, bird-pollinated flowers are often red with little odor while moth-pollinated flowers are often white with a strong odor (Faegri and van der Pijl 1979). This association between pollen vectors and floral traits is thought to provide a conceptual framework for understanding floral evolution (e.g., Herrera 1988a, b; Bosch et al. 1997).

Studies on plant–pollinator interactions at the community level have questioned the role of pollinators in the evolution of floral traits (Waser et al. 1996; Johnson and Steiner 2000; Fenster et al. 2004). Plants with specialized flowers are sometimes visited by generalist pollinators not predicted by their floral syndrome (Herrera 1988a, b; Ollerton et al. 2007, 2009). It is clear that networks of plant–pollinator interactions are usually asymmetrical, and that there exist spatial and temporal variation in the links between plants and pollinators (Alarcón et al. 2008; Olesen

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et al. 2008; Petanidou et al. 2008; Dupont et al. 2009). Such variation in pollinator assemblages and visitation frequency may diminish the role of pollinators in floral evolution (Herrera 1988a, b; Schemske and Horvitz 1989; Pettersson 1991; Eckhart 1992). Most previous studies at the community level have documented the species links between plants and pollinators (Alarcón et al. 2008; Olesen et al. 2008; Petanidou et al. 2008; Dupont et al. 2009), but the relative importance of variation in visitation frequency of different pollinator groups in molding the evolution of floral traits has been largely ignored (but see McCall and Primack 1992; Lázaro et al. 2008). Through quantitative investigations of spatial variation in the frequency of insect visits to flowers across communities, McCall and Primack (1992) and Lázaro et al. (2008) found evidence of stable relationships between pollinators and floral traits, supporting the classic pollination syndrome hypothesis. However, they also demonstrated that some preferences of pollinators changed spatially, showing an interesting community-context dependence of pollinator preferences. To our knowledge, only Nakano and Washitani (2003) have quantified temporal variation in pollinator visitation frequency at the community level, showing a relative stability of bumblebee preference in a maritime grassland. To quantify the importance of various pollinators in the evolution of floral traits, it is important to consider temporal variation in both the identity of pollinating visitors and their visitation frequency (Wilson and Thomson 1991; Sahli and Conner 2006; Reynolds and Fenster 2008; Ne'eman et al. 2010).

To determine the role of pollinators in the divergence of floral traits, an initial step is to quantify the preferences of diverse pollinators for particular floral traits. Experimental studies showing selection by pollinator preference on floral evolution have mainly focused on one or a few plant species. For example, in two closely related *Mimulus* species, the predominant preference of bees for *M. lewisii* in contrast to the hummingbird preference for *M. cardinalis* are largely responsible for the adaptive divergence of floral color, floral size and nectar production (Schemske and Bradshaw 1999). However, in addition to visiting plants predicted by classic pollination syndromes, specialized pollinators may also visit other sympatric flowering species (see Pauw et al. 2009), and plants are often pollinated by a variety of pollinator types (Waser et al. 1996). To understand how pollinators respond to the various floral traits of co-occurring species, a whole-community perspective provides more relevant information than studies focusing on just one or a few plant species (e.g., Nakano and Washitani 2003; Lázaro et al. 2008). Therefore, studies at the community level are needed to understand how diverse pollinator preferences contribute to the divergence of floral traits, and particularly, how temporal and spatial variation in community structure affects plant–pollinator interactions (Armbruster et al. 2000; Fenster and Dudash 2001; Waser 2006).

Given that pollinators belonging to the same functional group may exert similar selection pressures on floral traits (Fenster et al. 2004), the method of characterizing ecological generalization in plant pollination systems (Sahli and Conner 2006; Lázaro et al. 2008) provides an index allowing us to estimate the role of pollinator functional groups in the evolution of floral traits. By calculating the level of ecological generalization, taking into account both the identity of pollinators and their relative frequency in the long term, one can infer past and present selection on focal floral traits (Herrera 1995; Sahli and Conner 2006). Using this index, we examined temporal variation in pollinator preference in a whole alpine community in Hengduan Mountains, China. We address the following questions. (1) Do pollinator functional groups have constant preferences for specific floral traits or trait combinations during the study years? (2) Are the floral traits included in our analyses consistently related to specialized versus generalized pollination systems (by calculating ecological generalization level quantitatively)?

Closed and brightly colored flowers (purple and pink in our study) are often preferred by bees, whereas open and pale colored (white and yellow) flowers tend to be visited by a wider range of pollinator groups, including flies, beetles and ants (Faegri and van der Pijl 1979; Pellmyr 2002). For this reason, we predicted that the level of ecological generalization would be greater in open and pale colored flowers than that of closed and brightly colored flowers across flowering seasons. We discuss the implications of our results with respect to understanding the evolution of floral traits.

Materials and methods

Study community and data collection

To quantify pollinator preference for different plants, we recorded pollinators and their frequency in an alpine meadow in Shangri-La Alpine Botanical Garden, southwest China (27°54′230″N, 99°38′298″E; 3,250 m altitude). The garden is located in the Hengduan Mountains region, recognized as one of the world's biodiversity hotspots. As details of the study community, including pollinator observations and calculations of pollinator generalization (PG) have been previously described by Gong and Huang (2009), we present only a brief description here. During July and August of 2006, 2007 and 2008, we investigated 12 permanent 2 × 2 m^2 plots for pollinator visits using 30-min observation periods. Plots were positioned in four rows and three columns with 10 m separating adjacent plots in a row and 20 m in a column. In total, 64 observation periods were conducted in 2006 (i.e. 1,920 min in total), and 108 periods (3,240 min) in 2007 and 2008. Correspondingly, in the 3 years, we observed 8,486, 19,043 and 35,890 floral visits by ten pollinator functional groups including bumblebees (*Bombus* spp.), muscoid flies (Muscidae), solitary bees (Halictidae), hoverflies (Syrphidae), ants (Formicidae), butterflies (Lepidoptera), beeflies (*Bombylius minor*), wasps (Ichneumonidae), beetles (Coleoptera) and honeybees (*Apis* spp.) (Table 1; note honeybees were absent in 2008). We used functional groups instead of pollinator species because members of each functional group behaved in a similar way in the flowers, and thus might exert similar selection pressures (Fenster et al. 2004). Based on the percentages of the ten pollinator groups, we used Simpson's (1949) diversity index to measure pollinator generalization level for each plant species. This index includes both richness and evenness in estimating generalization, making it useful for broad community comparisons (Sahli and Conner 2006). Those plant species that were observed in fewer than six periods or received fewer than five visits throughout the study period were excluded from the final analysis to avoid inaccurate estimation of specialization levels (Hingston and McQuillan 2000; Lázaro et al. 2008). Therefore, we yielded 29 plants species in total and 19, 23 and 25 plant species in 2006 (Simpson's diversity index = 6.77), 2007 (11.73) and 2008 (8.05), respectively (Electronic Supplementary Material, Table S1). The 29 flowering species in this herbaceous community were from 15 families, dominated by Fabaceae (5 species), Lamiaceae (5), Asteraceae (3) and Orobanchaceae (3), followed by Apiaceae (2) and Rosaceae (2) and 1 species each from Boraginaceae, Campanulaceae, Caryophyllaceae, Convolvulaceae, Dipsacaceae, Gentianaceae, Onagraceae, Orchidaceae and Polygonaceae. The study plant species, total hours spent recording pollinators, total numbers pollinator visits and the main pollinator for each study plant species are shown in Table S1. We also counted floral visual units of each plant species (see Gong and Huang 2009) within the plots before each observation period to account for plant species' abundance.

Table 1 Pollinator assemblages in the study community over 3 years

Pollinator	2006	2007	2008
Bumblebees	89.4	26.0	36.1
Muscoid flies	2.8	11.3	34.8
Solitary bees	1.4	45.2	7.0
Hoverflies	2.5	7.6	17.4
Ants	0.3	7.1	3.2
Butterflies	3.0	0.6	0.4
Beeflies	0.1	0.1	0.2
Wasps	0.2	0.3	0.6
Beetles	0.1	1.5	0.3
Honeybees	0.2	0.2	0.0
Total visits recorded	8,486	19,043	35,890

Data are the percentage of visits (of the total visits recorded) made by each pollinator group in each of the 3 years

In each plant species, we recorded 14 floral traits belonging to six categories (see details in Table S1): (1) floral clustering; species with individual florets grouped together (e.g., Asteraceae), so that they could not easily be observed and counted in the field, were regarded as inflorescences; others were considered as solitary flowers; (2) floral color; the most attractive parts in flowers were evaluated by the human eye as four types, purple or blue (purple hereafter), pink or red (pink hereafter), white and yellow; (3) floral orientation; for the orientation of flowers relative to the horizontal axis, we distinguished three categories: up, horizontal and down; (4) floral shape; we classified species as open or closed flowers (including semi-closed flowers, e.g., Lamiaceae); (5) floral symmetry; we defined species as bilateral or radial flowers; and (6) corolla tube length; we measured one flower from each of 20 randomly selected individuals of each species using digital calipers and a portable anatomical lens in the field. Tube lengths of open flowers were classified as zero directly without measurement.

Statistical analyses

Multivariate analyses (performed by CANOCO version 4.5) were used to investigate the relationships between categories of floral traits and pollinator assemblages for each year separately. To decide on an ordination method, we first conducted detrended correspondence analyses (DCAs). All 3 years' maximum gradient lengths were shorter than 3.0, indicating that linear methods are most appropriate for our data (Ter Braak and Smilauer 2002). Therefore, we conducted redundancy analyses (RDAs) with plant species as samples, percentage of each pollinator group on a particular plant species as response variables and categories of floral traits as explanatory variables. Total visits recorded for each plant species and plant species' abundance were entered as covariables to prevent possible under-sampling effects and their influence on the composition of the visitor assemblage (Herrera 2005; Lázaro et al. 2008). Log $(y + 1)$ was used to transform the response variables. Tube length was input as a continuous variable and other categories of floral traits were re-coded into dummy variables. However, tube length, floral orientation and floral symmetry were just projected passively into the biplots without including them in the calculations because they were strongly correlated with floral shape (Ter Braak and Smilauer 2002). A total of 1,000 Monte Carlo permutations were used, firstly to test the statistical significance of the full model, and then to assess significance of the relationships between each category of floral traits and the pollinator assemblages separately.

To test the interactions between floral traits and year (dummy variable), we conducted a joint RDA including 3 years' data together. Variables were the same as in the previous analyses but also included variables contributing to the interactions as covariables, to test for the consistency of relationships between floral traits and pollinator assemblages across years (for details, see Table 2). Following the procedure used by Lázaro et al. (2008), the percentage of visits was recalculated since we only included the pollinator functional groups that occurred in all 3 years (honeybees were excluded from this analysis because they were absent in 2008). Again, 1,000 Monte Carlo permutations were used to assess statistical significance.

Relationships between specific floral traits and pollinator groups can be interpreted in the RDA biplots (Fig. 1). Following instructions by Ter Braak (1994), one can project the solid triangles or arrow tip of tube length (representing floral traits) perpendicularly onto the line running to the arrow of each pollinator group; the direction of the arrow indicates the increase in the degree of associations between floral traits and the pollinator group. However, the biplots only display the ranking of relationships between response variables and explanatory variables without definite lines (Ter Braak 1994). For more accurate results, we performed an alternative analysis of tests of nominal variables (McDonald 2008; see below for details). If both analyses indicate that the proportion of some pollinator groups' visits were higher for specific floral traits, they were included in the final results.

Tests of nominal variables were performed to ascertain whether each pollinator group preferred a particular floral trait of one category (e.g., open-shaped). We conducted the analyses for each category and each study year separately. The null hypothesis was that the total number of

Table 2 Results of the redundancy analysis used to test the relationships between pollinators and floral traits (see text for details)

Year	Effect	Covariables	*V* (%)[a]	Trace	*F*	*P*
2006	Full model	V_i, Ab	66.2	0.577	4.307	0.001
	Shape	V_i, Ab	40.6	0.354	10.267	0.001
	Color	V_i, Ab	34.4	0.300	2.272	0.031
	Clustering	V_i, Ab	20.2	0.176	3.790	0.011
Total variance = 1.000; sum of all canonical eigenvalues/sum of all eigenvalues = 0.577/0.872						
2007	Full model	V_i, Ab	53.5	0.491	3.464	0.001
	Shape	V_i, Ab	24.0	0.220	6.017	0.003
	Color	V_i, Ab	38.4	0.352	3.540	0.002
	Clustering	V_i, Ab	7.7	0.071	1.598	0.167
Total variance = 1.000; sum of all canonical eigenvalues/sum of all eigenvalues = 0.491/0.917						
2008	Full model	V_i, Ab	75.5	0.626	10.484	0.001
	Shape	V_i, Ab	65.6	0.544	40.007	0.001
	Color	V_i, Ab	42.2	0.350	4.627	0.005
	Clustering	V_i, Ab	7.3	0.061	1.666	0.190
Total variance = 1.000; sum of all canonical eigenvalues/sum of all eigenvalues = 0.626/0.829						
All years	Year × shape	V_i, Ab, Yr, Sh	6.5	0.035	4.191	0.003
	Year × color	V_i, Ab, Yr, Co	0.9	0.005	0.493	0.780
	Color	V_i, Ab, Yr	34.6	0.297	7.682	0.001
	Year × clustering	V_i, Ab, Yr, Cl	1.1	0.009	0.686	0.533
	Clustering	V_i, Ab, Yr	7.8	0.067	5.120	0.007
	Year	V_i, Ab	8.4	0.079	2.855	0.026

Data are shown for 2006, 2007, 2008 (using ten pollinator groups), and all three study years, using the nine shared pollinator groups as responses. Main effects are tested only when their interactions are not significant

V_i Visits recorded, *Ab* plant species abundance, *Yr* year, *Sh* shape, *Co* color, *Cl* clustering, *V* (%) percentage of variance explained by the different explanatory variables

[a] *V* (%) Trace/sum of all eigenvalues; the sum of variances explained by the factors is higher than the variance explained by the full model due to shared variance

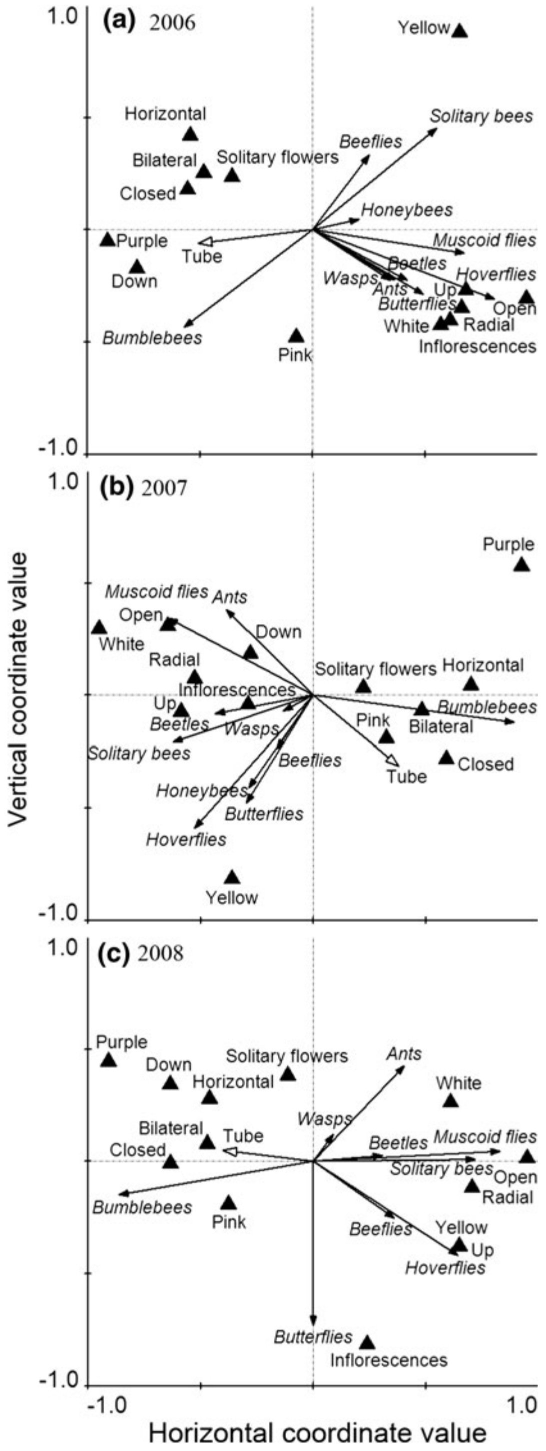

Fig. 1 Biplots showing results of redundancy analyses (RDAs) determined for each of 3 years, to illustrate relationships between the pollinator groups (*closed arrows*) and floral traits (*triangles* indicate dummy variables and one *open arrow* represents the continuous variable of tube length). See text for details of analytic process and interpretation of results

flower visits (V_{in}) by pollinator group i to the plant species possessing the floral trait n of one category was determined by:

$$V_{in} = \frac{F_n V_i}{F},$$

where F_n is the total number of observed flowers in a particular state of a categorical floral trait n (e.g., 1 and 2 represent open and closed floral shape, respectively; tube length was not included in the analyses because it was a continuous variable), F is the total number of flowers observed for insect visitation, V_i is the total number of recorded visits by pollinator group i to the category. A G test of goodness-of-fit was used when the maximum $n \geq 2$ and $V_i > 1{,}000$; when the maximum $n = 2$ and $V_i < 1{,}000$, the exact test of goodness-of-fit was used; and when the maximum $n > 2$ and $V_i < 1{,}000$, we used randomization tests of goodness-of-fit (McDonald 2008). If the null hypothesis was violated, we considered that there was an association between pollinator group i and floral trait n.

DISTLM (distance-based multivariate analysis for a linear model) conducted by the DISTLM v.5 FORTRAN computer program (Anderson 2001, 2004) was used to test the relationships between floral traits and the pollinator generalization level (PG), as well as their temporal dynamics across 3 years. We used this non-parametric analysis because assumptions of homogeneity and normality were violated. The XMATRIX FORTRAN computer program (Anderson 2003) was used to generate design matrices corresponding to the factor in our unbalanced ANOVA design used in the DISTLM v.5 FORTRAN computer program. The index of PG was the response variable, and floral shape, floral color, floral clustering and year were the crossed factors. Tube length, floral symmetry and floral orientation were not included in the analysis because of their strong correlations with floral shape. Due to lack of replicates to test higher order interactions, only the interactions of each floral trait with year and their main effects were tested. Analyses were conducted on Bray–Curtis distance with 4,999 permutations. When the effects were statistically significant ($P < 0.05$), pair-wise comparisons were also carried out using DISTLM (Anderson 2001, 2004).

Results

The results of all 3 years' RDA models were significant (Table 2), indicating that categories of floral traits included in our analyses explain a significant proportion of the variance in the composition of pollinator groups. Except for floral clustering (only significant in 2006), all other categories of floral traits included in the calculations showed consistently significant effects on the composition of pollinator groups (Table 2). Temporal stability of the relationships between floral traits and pollinator assemblages is shown in the RDA biplots (Fig. 1). As shown in the biplots and the results of tests of nominal variables (Table S2), bumblebees preferred to visit plant species with closed, bilateral, purple and pink, horizontal flowers in all 3 years. The proportion of bumblebee visits also increased continuously with tube length (Fig. 1; Table S2). In addition, bumblebees preferred solitary flowers in 2008 (Fig. 1c; Table S2). In 3 years, muscoid flies, hoverflies, solitary bees and beetles mainly visited open, radial, upward, and white inflorescences (Fig. 1; Table S2). Furthermore, yellow flowers were preferred by solitary bees in all 3 years and hoverflies in 2007; downward flowers were preferred by beetles in 2007 (Fig. 1; Table S2). Wasps and ants showed the same preference as muscoid flies except for inflorescences in 2008 (Fig. 1; Table S2). In 2007, butterflies and honeybees showed the same preference for open, radial, upward, and yellow inflorescences, but butterflies only preferred to visit upward inflorescences in 2008 and inflorescences were the only preference for honeybees in 2006 (Fig. 1; Table S2). Except for the consistent preference for upward inflorescences, open, white and pink flowers were more related to butterflies in 2006 (Fig. 1; Table S2). Only beeflies did not prefer the same type of flowers consistently; they preferred horizontal inflorescences in 2006, but upward, open, radial flowers in the following 2 years. Additionally, beeflies preferred to visit yellow flowers in 2007 but white inflorescences in 2008 (Fig. 1; Table S2).

The joint RDA including 3 years' data together suggested that both floral color and clustering had consistently significant effects on the composition of pollinator visits across 3 years in our study community (Table 2). Floral color explained a high amount of variance in the composition of pollinators (Table 2). However, the effect of floral shape was different in 3 years (Table 2), which might be due to the different preferences of butterflies, honeybees and beeflies between years. Butterflies preferred to visit open flowers in 2006 and 2007, but showed no preference for open flowers in 2008; honeybees showed no preference for open flowers in 2006, but preferred to visit open flowers in 2007; beeflies preferred open flowers in 2007 and 2008 but showed no preference for open flowers in 2006 (Fig. 1; Table S2).

According to the results of DISTLM, floral shape had a significant effect on pollination generalization level ($F_{1,66} = 47.36$, $P < 0.001$), but this effect varied across years because the interaction between floral shape and year was also significant ($F_{2,66} = 4.53$, $P = 0.012$). In 2006 and 2008, the level of generalization was higher for open flowers. However, in 2007, there was no significant difference in generalization level between open and closed flowers (Fig. 2a). The other two categories of floral traits also affected generalization level significantly (color, $F_{3,66} = 7.24$, $P < 0.001$; clustering, $F_{1,66} = 13.51$, $P = 0.001$), and they showed consistent effects across years (interaction color × year, $F_{6,66} = 0.48$, $P = 0.842$; clustering × year, $F_{2,66} = 0.72$, $P = 0.501$). Compared to purple flowers, white and yellow flowers were more generalized in all 3 years (Fig. 2b). The generalization level was also consistently higher for flowers clustered in inflorescences than solitary flowers (Fig. 2c). In addition, all three separate DISTLM analyses suggested that the generalization level of the entire community varied across years, with PG higher in 2007 than in the other 2 years ($P < 0.05$).

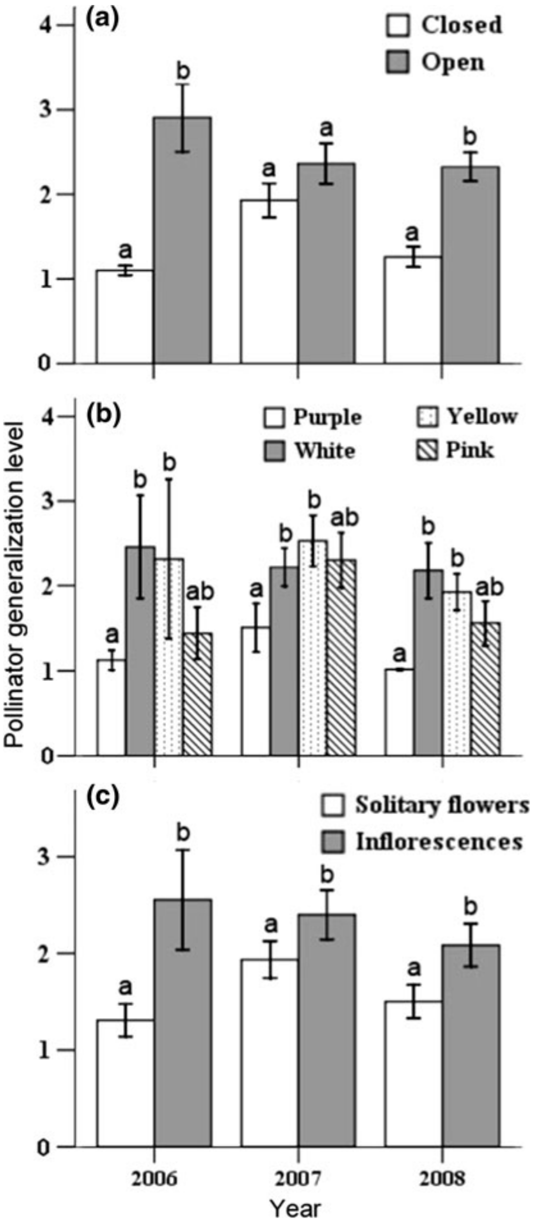

Fig. 2 Pollinator generalization level for plant species with different **a** floral shapes, **b** floral color and **c** floral clustering in 3 years. *Different letters* indicate significant differences between two groups. Values are mean ± SE ($n = 29$ species)

Discussion

Our 3-year investigation of whether pollinator functional groups exhibit preferences for particular floral traits in an alpine community demonstrated that most pollinator groups showed consistent preferences for specific floral traits. The trait combinations, as well as two out of three categories of the floral traits individually, showed significant predictive power for the composition of pollinator groups across years. Specifically, eight of ten pollinator groups showed consistent preferences for at least two floral traits of species from various families; whereas floral traits (two out of three categories) were related to stable ecological generalization levels in all 3 years. The consistent associations between most floral traits and pollinator functional groups have been suggested in several studies (e.g., Momose et al. 1998; Dicks et al. 2002; Nakano and Washitani 2003; Wolfe and Sowell 2006; Lázaro et al. 2008). We found that pollinator preference of some less-abundant pollinator groups varied temporally. For example, shape preferences of butterflies, honeybees and beeflies varied across years, such that in 2007 open flowers did not show a higher level of ecological generalization than closed flowers. This result may be due to variation in the structure of the community (McCall and Primack 1992; Hingston and McQuillan 2000; Ollerton et al. 2007; Lázaro et al. 2008, 2009).

Pollinator preference

Pollinators neither exert selection on just one floral trait (Reynolds et al. 2010) nor on all trait combinations, since floral traits are also subject to selection by other non-pollinator factors (Faegri and van der Pijl 1979; Strauss and Whittall 2006; Sun et al. 2008). This may illustrate why only a certain number of floral traits were included in Faegri and van der Pijl's (1979) classical pollination syndromes. Recent work by Ollerton et al. (2009) showed that very few plants fit into the "phenotype space" defined by combinations of floral traits allegedly preferred by specific pollinator functional groups. Instead, Ollerton et al. recommended a "bottom-up" approach of including those traits that matter most in determining pollinator preferences. Following this recommendation, we included in our analyses floral traits which best explained the empirically observed patterns of plant–pollinator interactions according to previous studies (McCall and Primack 1992; Momose et al. 1998; Hingston and McQuillan 2000; Dicks et al. 2002; Nakano and Washitani 2003; Wolfe and Sowell 2006; Lázaro et al. 2008). All three RDA models for each year are significant, indicating that floral trait combinations used in our analyses are appropriate. Furthermore, most individual categories of floral traits had significant predictive power for the composition of pollinator groups in all 3 years. The only notable exception was floral clustering, which was only significant in 2006. This suggests that the predictive power of floral clustering on the composition of pollinator groups may be less strong than other categories of floral traits included in our analyses. Other floral traits not included in our analysis may also have significant predictive power for the composition of pollinator groups, such as pollen presentation and stigma receptivity strategies, pollen and nectar production, and UV reflection (Faegri and van der Pijl 1979; Lloyd and Yates 1982; Harder and Thomson 1989; Pellmyr 2002; Arnold et al. 2009; Reynolds et al. 2009).

Although the composition of pollinator groups varied among years (Tables 1, 2), most of the pollinator preferences for specific floral traits or trait combinations remained stable across the three study years. Bumblebees consistently preferred to visit plant species with closed, bilateral, purple and pink, horizontal flowers with a long corolla tube, whereas open, radial, white and yellow, upward inflorescences were more consistently attractive to solitary bees. Hoverflies, muscoid flies and beetles had the same constant preference as solitary bees except for yellow flowers. Wasps and ants persistently visited open, radial, white, upward flowers. Butterflies consistently preferred upward inflorescences. Most of these relationships fit the predictions of the classical pollination syndrome hypothesis (Faegri and van der Pijl 1979), which is well summarized in the appendix of Ollerton and Watts (2000), and has been observed in other community studies (e.g., McCall and Primack 1992; Momose et al. 1998; Hingston and McQuillan 2000; Dicks et al. 2002; Nakano and Washitani 2003; Wolfe and Sowell 2006; Lázaro et al. 2008).

The only two pollinator groups that did not show constant preferences for trait combinations were honeybees and beeflies. This may reflect the limited sample sizes for these two groups (Table 1). The preferences of these uncommon pollinators could be affected by the dominance of other pollinator groups in the community, as well as variation in the identity of the abundant pollinators (McCall and Primack 1992; Hingston and McQuillan 2000; Lázaro et al. 2008). For example, Lázaro et al. (2008) suggested that the presence of abundant bumblebees could influence butterflies' preference for floral shape, and McCall and Primack (1992) attributed the community-specific preferences for color by flies to variation in the identities of abundant pollinators across communities. In our community, the dominant pollinator groups were bumblebees in 2006, solitary bees in 2007 and bumblebees along with muscoid flies in 2008 (Table 1). Both their high abundances and variation in dominant position across years might influence the behavior of uncommon pollinator groups.

Pollination generalization level

We found that most floral traits maintained a relatively stable level of ecological generalization over 3 years, in spite of the fact that the generalization level of the entire community varied across years. For example, inflorescences were consistently more generalized than solitary flowers. This contrasts with the findings of Lázaro et al. (2008), who found that inflorescences were more generalized than solitary flowers in one of three study communities. Nakano and Washitani (2003) found that bumblebees consistently preferred purple flowers, corroborating our results, and Lázaro et al. (2008) showed that species with purple flowers were more specialized than yellow and white flowers in two species-rich communities. In our community, purple flowers were also more specialized than white and yellow flowers in all 3 years, suggesting that purple flowers would evolve under selection by bumblebees. In accordance with the common belief that complex flowers should be the most ecologically specialized within a community because their complexity reflects selection by a narrower group of pollinators (Herrera 1988a, b; Johnson and Steiner 2000; Fenster et al. 2004), we found that the pollinator generalization level was lower in bilateral than in radial symmetry species in the community (Gong and Huang 2009). Closed flowers in our community showed higher specialization levels than open flowers in the 2 of 3 years (Fig. 2). This non-significant result in 1 year could be because the realized pollinator generalization level was influenced not only by floral traits but also by community structure (both composition and abundance of plants and pollinators) (McCall and Primack 1992; Hingston and McQuillan 2000; Ollerton et al. 2007; Lázaro et al. 2008, 2009). Lázaro et al. (2008) demonstrated that closed flowers were more generalized than open flowers in one of three Scandinavian plant communities because of the variation in composition of the pollinator assemblage. Temporal variation in pollinator assemblages (Table 1) may explain variation in shape preferences of butterflies, honeybees and beeflies in our community as we mentioned above. Only in 2007 did all three pollinator groups prefer to visit open flowers, leading to a higher level of ecological generalization. In addition, the composition and abundance of plants also varied. For example, three of the most abundant plant species (three *Pedicularis* species), which are also the most specialized closed flowers, were not flowering in 2007, which may have led to the increase of overall generalization level of closed flowers. Similarly, a recent study conducted by Lázaro et al. (2009) demonstrated that the ecological generalization on pollinators can be influenced by variation in the diversity, identity and density of co-flowering neighbors.

Our community survey demonstrated significant pollinator preferences for the floral traits in all 3 years of our study. To our knowledge, such temporal stability of pollinator preference for trait combinations has not previously been documented at a community level. However, the related underlying mechanisms of how pollinator preferences can contribute to the adaptation and divergence of floral traits remain largely unknown. Schemske and Bradshaw's (1999) pioneering work demonstrated the genetic basis of pollinator preferences, whereas Endress (2001) used the methods of molecular developmental genetics to elucidate how pollinators contribute to the evolution of flowers from radial to bilateral symmetry. Our results indicate the role of pollinators in the evolution of floral traits could also be moderated by variation in a community context, by influencing both the level of ecological generalization of floral traits and preferences of pollinator functional groups. Since we did not measure the pollination effectiveness of each insect species, one may question whether pollinator species included in our study are actually pollinator functional groups (Fenster et al. 2004; Ne'eman et al. 2010). This is a limitation of our study. Here, we provide initial evidence for the role of pollinators, as well as variation in community structure, in molding the evolution of floral traits at a community level. To further illustrate the role of diverse pollinators in floral evolution, it is necessary to consider temporal and spatial variation in pollinator preference as well as variation in pollination effectiveness.

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