Phylogenetic trait-based analyses of ecological networks

Nicole E. Rafferty[1] and Anthony R. Ives

Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706 USA

Abstract. Ecological networks of two interacting guilds of species, such as flowering plants and pollinators, are common in nature, and studying their structure can yield insights into their resilience to environmental disturbances. Here we develop analytical methods for exploring the strengths of interactions within bipartite networks consisting of two guilds of phylogenetically related species. We then apply these methods to investigate the resilience of a plant–pollinator community to anticipated climate change. The methods allow the statistical assessment of, for example, whether closely related pollinators are more likely to visit plants with similar relative frequencies, and whether closely related pollinators tend to visit closely related plants. The methods can also incorporate trait information, allowing us to identify which plant traits are likely responsible for attracting different pollinators. These questions are important for our study of 14 prairie plants and their 22 insect pollinators. Over the last 70 years, six of the plants have advanced their flowering, while eight have not. When we experimentally forced earlier flowering times, five of the six advanced-flowering species experienced higher pollinator visitation rates, whereas only one of the eight other species had more visits; this network thus appears resilient to climate change, because those species with advanced flowering have ample pollinators earlier in the season. Using the methods developed here, we show that advanced-flowering plants did not have a distinct pollinator community from the other eight species. Furthermore, pollinator phylogeny did not explain pollinator community composition; closely related pollinators were not more likely to visit the same plant species. However, differences among pollinator communities visiting different plants were explained by plant height, floral color, and symmetry. As a result, closely related plants attracted similar numbers of pollinators. By parsing out characteristics that explain why plants share pollinators, we can identify plant species that likely share a common fate in a changing climate.

Key words: climate change; interaction network; linear mixed models; phenology; phylogenetic signal; plant–pollinator interactions.

Introduction

Currently, there is much concern that climate change-induced phenological shifts could disrupt ecological interactions, such as those between plants and pollinators (Hegland et al. 2009). Many plants are flowering earlier in response to warmer temperatures (Bradley et al. 1999, Fitter and Fitter 2002, Miller-Rushing and Primack 2008), and some pollinators have also exhibited shifts in the timing of life-history events (Roy and Sparks 2000, Stefanescu et al. 2003, Gordo and Sanz 2005). There is a growing number of empirical examples of temporal mismatches between plants and pollinators (Wall et al. 2003, Kudo et al. 2004, Doi et al. 2008, McKinney et al. 2012), and simulations and models of phenological shifts (Memmott et al. 2007, Gilman et al. 2012) also point to the potential for disrupted interac-

tions under climate change. A central concern is that temporal mismatches between plants and pollinators become sufficiently extreme that coextinction occurs (reviewed by Hegland et al. [2009] and Kiers et al. [2010]).

To date, the primary tools used to analyze plant–pollinator interactions have included ordination methods (e.g., principal components analyses), measures of community similarity, and, more recently, tools from network analysis. These techniques have been used to explore how pollinator communities vary across time (Alarcón et al. 2008, Petanidou et al. 2008), space (Yates et al. 2007, Davis et al. 2008, Kaiser-Bunbury et al. 2009), and plant species (Bosch et al. 1997, Wilson et al. 2004, Morales and Aizen 2006, Kimball 2008, Lázaro et al. 2008, Winfree et al. 2008, Ollerton et al. 2009). Some studies have linked variation in pollinator community composition with explanatory factors (Bosch et al. 1997, Potts et al. 2003, Morales and Aizen 2006, Lázaro et al. 2008), but only a few have incorporated phylogenetic relationships among plants or pollinators (Rezende et al. 2007, Vázquez et al. 2009). The inclusion of phylogeny is important because it not only reveals important drivers of community patterns but also removes potentially

[1] Present address: Department of Ecology and Evolutionary Biology and Center for Insect Science, University of Arizona, Tucson, Arizona 85721 USA.
E-mail: nrafferty@email.arizona.edu

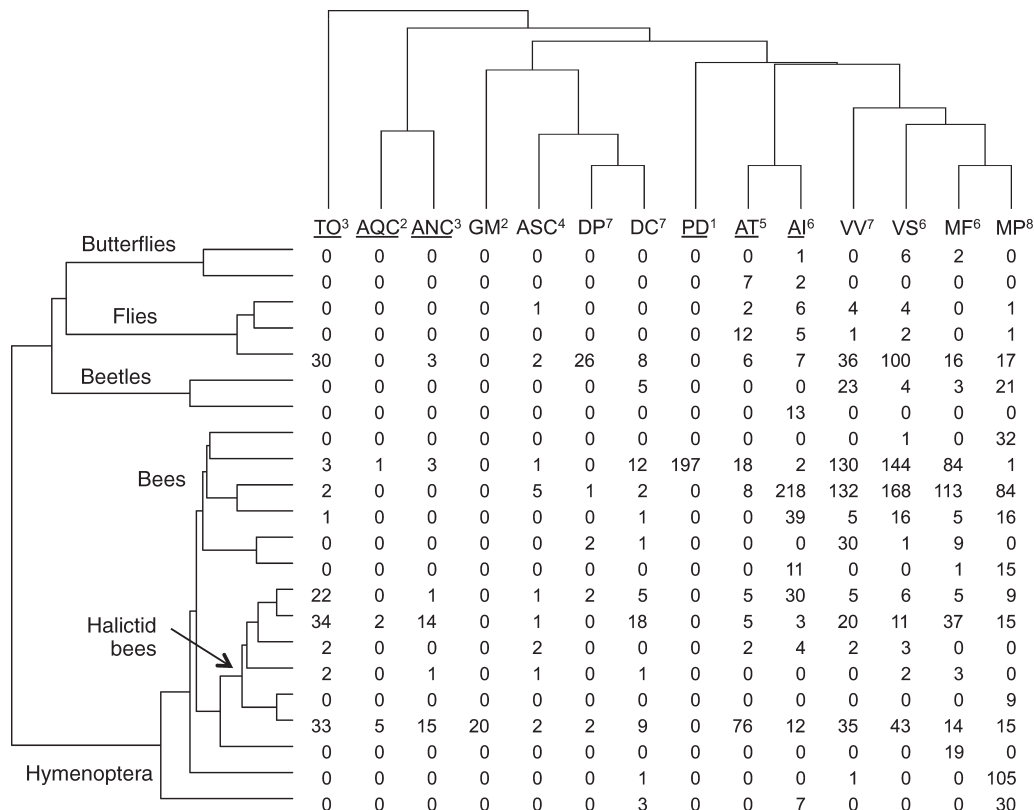|	TO[3]	AQC[2]	ANC[3]	GM[2]	ASC[4]	DP[7]	DC[7]	PD[1]	AT[5]	AI[6]	VV[7]	VS[6]	MF[6]	MP[8]
Butterflies	0	0	0	0	0	0	0	0	0	1	0	6	2	0
	0	0	0	0	0	0	0	0	7	2	0	0	0	0
Flies	0	0	0	0	1	0	0	0	2	6	4	4	0	1
	0	0	0	0	0	0	0	0	12	5	1	2	0	1
Beetles	30	0	3	0	2	26	8	0	6	7	36	100	16	17
	0	0	0	0	0	0	5	0	0	0	23	4	3	21
	0	0	0	0	0	0	0	0	0	13	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	1	0	32
Bees	3	1	3	0	1	0	12	197	18	2	130	144	84	1
	2	0	0	0	5	1	2	0	8	218	132	168	113	84
	1	0	0	0	0	0	1	0	0	39	5	16	5	16
	0	0	0	0	0	2	1	0	0	0	30	1	9	0
	0	0	0	0	0	0	0	0	0	11	0	0	1	15
Halictid bees	22	0	1	0	1	2	5	0	5	30	5	6	5	9
	34	2	14	0	1	0	18	0	5	3	20	11	37	15
	2	0	0	0	2	0	0	0	2	4	2	3	0	0
	2	0	1	0	1	0	1	0	0	0	0	2	3	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	9
	33	5	15	20	2	2	9	0	76	12	35	43	14	15
	0	0	0	0	0	0	0	0	0	0	0	0	19	0
Hymenoptera	0	0	0	0	0	0	1	0	0	0	1	0	0	105
	0	0	0	0	0	0	3	0	0	7	0	0	0	30

FIG. 1. Data for the number of visits to different plant species by different pollinator taxa. The phylogeny of the pollinators is given on the left, and the phylogeny of the plants is given along the top. Plant species are as follows: PD, *Phlox divaricata*; AQC, *Aquilegia canadensis*; GM, *Geranium maculatum*; ANC, *Anemone canadensis*; TO, *Tradescantia ohiensis*; ASC, *Astragalus canadensis*; AT, *Asclepias tuberosa*; AI, *Asclepias incarnata*; MF, *Monarda fistulosa*; VS, *Verbena stricta*; DC, *Dalea candida*; DP, *D. purpurea*; VV, *Veronicastrum virginicum*; and MP, *M. punctata*. Underlined species are those that are flowering significantly earlier. Superscripts indicate the order of flowering onset by week; species that share numbers begin flowering in the same week.

spurious associations that arise from phylogenetic nonindependence (Felsenstein 1985, Garland et al. 1992).

Here, we develop methods for analyzing bipartite ecological networks, incorporating information on the phylogenies of the two guilds and species traits that could affect the interactions between guilds. Our approach uses linear mixed models (LLM; Gelman and Hill 2007, Bolker et al. 2009) that are tailored for phylogenetic analyses. These phylogenetic linear mixed models (PLMMs) treat the strengths of pairwise interactions (e.g., visitations of a pollinator species to a plant species) as the dependent variable, and incorporate phylogenies as anticipated covariances among these interactions. Because PLMMs are model based, parameter estimation and statistical inference (hypothesis tests, confidence intervals, etc.) can be performed using maximum likelihood. Thus, PLMMs give a statistically robust approach for analyzing bipartite networks.

We use PLMMs to explore a community of pollinators of prairie wildflowers, specifically addressing the potential vulnerability of this community to climate-driven shifts in phenology. The data come from an experiment designed to assess the potential for phenological mismatches between pollinators and the plants (Rafferty and Ives 2011). Of the 14 perennial plant species, six have exhibited earlier flowering over the last 70 years ("advanced-flowering species"), whereas the remaining eight have not (Fig. 1). We experimentally manipulated flowering onset in greenhouses and then placed plants in the field before natural flowering would have begun. We found that, of the six advanced-flowering species, five experienced more pollinator visits when experimentally forced to flower even earlier, whereas of the eight historically unchanged plants, only one experienced greater pollinator visits to earlier flowers. Therefore, although there is a risk of phenological mismatches for those six advanced-flowering species, this risk was not realized for five of the species because their pollinators were common even earlier in the season (Rafferty and Ives 2011). We use PLMMs to ask whether differences in the pollinator species visiting different plants can explain the apparent resilience of the six advanced-flowering plants to mismatches. Do these six species have a distinct subcommunity of pollinators

that are available early in the season? And are these pollinators phylogenetically related to each other? We also ask whether phylogenetically related plants, or plants sharing the same traits, are more likely to have the same pollinator communities; this will identify subsets of plants that, by sharing the same pollinators, will likely share the same fate if climate change were to affect pollinator phenology.

Methods

We begin by constructing a simulation model of plant–pollinator community assembly that incorporates phylogenetic and plant trait information; although we describe the model in terms of plants and pollinators, it would equally apply to any bipartite community. We use the simulations both to show how ecological processes can generate patterns in community assembly and to provide data to test the PLMM methods. We then present the PLMMs and apply them to the simulated data. The first PLMM with plant traits mirrors the simulation model, whereas the second PLMM includes only phylogenetic associations; these associations are generated by the plant traits in the simulation model, but no information on plant traits is available for statistical fitting. Therefore, fitting the simulated data with the second PLMM tests the ability of the model to detect emergent phylogenetic patterns from underlying trait-based processes. Finally, we apply the methods to the real plant–pollinator data.

Simulation model

The simulation model is based on the experimental data with $n = 22$ pollinator and $m = 14$ plant species with phylogenies given in Fig. 1. It has the form of a regression of interaction strengths on plant trait values

$$y = a_{pol} + b_{pol}x_{plt} + e. \qquad (1)$$

Here, y is a measure of the strength of interaction between pollinator (pol) and plant (plt) species, such as the log number of pollinator visits to a plant. We assume that each plant has a trait value x_{plt}, and that closely related plant species can be more likely to have the same values; specifically, we assume the variance of x_{plt} is given by an m-dimensional Gaussian random variable with mean 0 and covariance matrix $\sigma_K^2 I_m + \sigma_L^2 V_m$, where I_m is the $m \times m$ identity matrix, and V_m is a covariance matrix that contains phylogenetic correlations among species. The terms σ_K^2 and σ_L^2 scale variances so that when $\sigma_L^2 = 0$, there is no phylogenetic correlation among values of x_{plt}, while when $\sigma_K^2 = 0$ the covariances are all phylogenetic. To derive a form for V_m, we assume that, when $\sigma_K^2 = 0$, x_{plt} evolves according to a Brownian-motion model of evolution, so the theoretical covariance in values of x_{plt} between two plants is proportional to their shared branch length on the phylogenetic tree (Martins and Hansen 1997); specifically, the element v_{jk} of the matrix V_m is proportional to the height of the most recent node shared by taxa j and k.

Similar to x_{plt}, there is both independent and phylogenetic variation in the mean abundances of pollinators, with the intercept a_{pol} having a Gaussian distribution with covariance matrix $\sigma_A^2 I_n + \sigma_B^2 V_n$. If $\sigma_B^2 = 0$, all variation among pollinators is independent, whereas when $\sigma_A^2 = 0$, all variation is phylogenetic. We assume that the phylogenetic covariance matrix for the n pollinators, V_n, is generated by Brownian motion evolution up the pollinator phylogenetic tree. We also assume that the slope of response of pollinators to variation in plant trait values, b_{pol}, varies among pollinators; b_{pol} has a Gaussian distribution with mean β and covariance matrix $\sigma_C^2 I_n + \sigma_D^2 V_n$. Thus, pollinators may respond differently to the plant trait, and these differences may show phylogenetic signal. Finally, residual (uncorrelated) variation in y is given by e, which is assumed to be an independent Gaussian random variable with mean 0 and variance σ_e^2.

In addition to simulations of y as a continuous variable, we also simulated the case in which only the presence/absence of interactions between plants and pollinators are known. We simulated these binary data by generating values of y using Eq. 1, computing the inverse logit of y, $\mu = e^y/(1 + e^y)$, and then selecting 1 with probability μ and 0 otherwise.

PLMM with plant traits

The PLMM we used to test for an effect of plant traits on the strengths of interactions between plants and pollinators is

$$Y_i = \alpha_{pol[i]} + B_{pol[i]}x_{plt[i]} + e_i$$

$$B_{pol[i]} = \beta + C_{pol[i]} + D_{pol[i]}$$

$$C \sim \text{Gaussian}(0, \sigma_C^2 I_n)$$

$$D \sim \text{Gaussian}(0, \sigma_D^2 V_n)$$

$$e \sim \text{Gaussian}(0, \sigma_e^2 I_{nm}). \qquad (2)$$

This is similar in structure to the simulation model. The value Y_i gives the interaction strength between a plant and a pollinator species for observation i in the data set, so i takes values from 1 to nm. The trait values for plant species are given in the independent variable $x_{plt[i]}$, which is written using the function plt[i] that gives the identity of the plant species corresponding to observation i in the data set (Gelman and Hill 2007:251–252). Pollinators are assumed to have intercepts $\alpha_{pol[i]}$ that are estimated as separate values for each species ("fixed effects" in the lexicon of mixed models); therefore, there are n estimated values summarized by $\alpha_{pol[i]}$, where pol[i] maps the appropriate pollinator species onto the datum

i. The slopes $B_{pol[i]}$ that give the pollinator-specific responses to plant trait $x_{plt[i]}$ is a Gaussian random variable ("random effect") with species-wide mean β and covariance matrix $\sigma_C^2 \mathbf{I}_n + \sigma_D^2 \mathbf{V}_n$. As in the simulation model, we assume that $\mathbf{V}_n$ is the $n \times n$ covariance matrix corresponding to the pollinator phylogeny under the assumption of Brownian motion evolution. Finally, residual variation is given by e_i, which is assumed to be an independent Gaussian random variable with mean 0 and variance σ_e^2.

PLMM for phylogenetic associations

We built a second PLMM to investigate patterns reflecting the phylogenetic relationships among pollinators and the phylogenetic relationships among plants. This PLMM does not include plant traits, although it identifies the existence of patterns that could be driven by unknown traits

$$Y_i = \alpha + a_{pol[i]} + b_{pol[i]} + c_i + d_{plt[i]} + f_{plt[i]} + g_i + h_i + e_i$$

$$a \sim \text{Gaussian}(0, \sigma_a^2 \mathbf{I}_n)$$

$$b \sim \text{Gaussian}(0, \sigma_b^2 \mathbf{V}_n)$$

$$c \sim \text{Gaussian}\left(0, \text{kron}(\mathbf{I}_m, \sigma_c^2 \mathbf{V}_n)\right)$$

$$d \sim \text{Gaussian}(0, \sigma_d^2 \mathbf{I}_m)$$

$$f \sim \text{Gaussian}(0, \sigma_f^2 \mathbf{V}_m)$$

$$g \sim \text{Gaussian}\left(0, \text{kron}(\sigma_g^2 \mathbf{V}_m, \mathbf{I}_n)\right)$$

$$h \sim \text{Gaussian}\left(0, \text{kron}(\sigma_h^2 \mathbf{V}_m, \mathbf{V}_n)\right)$$

$$e \sim \text{Gaussian}(0, \sigma_e^2 \mathbf{I}_{nm}). \tag{3}$$

The constant α gives the global mean strength of interactions. The following three random variables $a_{pol[i]}$, $b_{pol[i]}$, and c_i incorporate variation among pollinator species; the three random variables $d_{plt[i]}$, $f_{plt[i]}$, and g_i incorporate variation among plant species; h_i contains interactions between the phylogenies of pollinators and plants; and e_i gives the residual variation. In more detail, the n values of $a_{pol[i]}$ give differences among pollinators in overall interaction strengths and are assumed to be drawn independently

from a Gaussian distribution with mean 0 and variance σ_a^2. The n values of $b_{pol[i]}$ are similar, except they are assumed to be drawn from a multivariate Gaussian distribution with covariance matrix $\sigma_b^2 \mathbf{V}_n$ reflecting the pollinator phylogeny. Incorporating both random and phylogenetic variation among pollinators gives a way to assess the strength of phylogenetic signal in the data; the correlation between pollinators j and k is $(\sigma_b^2 v_{jk})/(\sigma_a^2 + \sigma_b^2)$, so the greater the value of σ_b^2 relative to σ_a^2, the larger the phylogenetic covariances among species. The term c_i assesses whether phylogenetically related pollinators are more likely to visit the same plant species. The covariance matrix for c_i is constructed using the Kronecker product, $\text{kron}(\mathbf{I}_m, \sigma_c^2 \mathbf{V}_n)$. This sets the covariance between pollinators j and k to $\sigma_c^2 v_{jk}$ for visits to the same plant species, but to 0 otherwise. Because variation in the mean value of Y among pollinators is already incorporated into $a_{pol[i]}$ and $b_{pol[i]}$, c_i includes only that covariance between pollinators visiting the same plant that cannot be attributed to similarities in their visitation frequencies. The random terms for plant species, $d_{plt[i]}$, $f_{plt[i]}$ and g_i, are defined in the same way as those for pollinators. The term h_i depends on the phylogenies of both pollinators and plants given through the matrix $\text{kron}(\sigma_h^2 \mathbf{V}_m, \mathbf{V}_n)$. If v_{jk} is the jkth element of $\mathbf{V}_n$, and u_{qr} is the qrth element of $\mathbf{V}_m$, then h_i incorporates the correlation between pollinator j on plant q and pollinator k on plant r as the product $v_{jk}u_{qr}$; this "coevolutionary" model of species interactions is the same as that used by Ives and Godfray (2006).

From Eq. 3, a statistical test for phylogenetic differences in mean visitation frequencies among pollinators is whether $\sigma_b^2 > 0$, and a test for whether phylogenetically related pollinators are likely to visit the same plant is whether $\sigma_c^2 > 0$. Similarly, a test for phylogenetically related plant species attracting greater abundances of pollinators is whether $\sigma_f^2 > 0$, and a test for phylogenetically related plants attracting similar abundances of a given pollinator is whether $\sigma_g^2 > 0$. Finally, a test of whether the visitation frequency of pollinators depends simultaneously on the pollinator and plant phylogenies is whether $\sigma_h^2 > 0$. Eq. 3 could be modified in numerous ways. For example, if we were not interested in the plant phylogeny and wanted to factor out differences among plants in order to focus on the pollinators, we could replace the constant α with a categorical variable $\alpha_{plt[i]}$ for plants (i.e., a set of m constants, one for each plant) and then remove the terms $d_{plt[i]}$, $f_{plt[i]}$, g_i, and h_i. Ives and Helmus (2011) present additional model structures for presence/absence data that could be modified for continuous-valued data as analyzed here.

We fit the PLMMs (Eqs. 2 and 3) to both simulated and real data using restricted maximum likelihood (REML). Estimation can be performed using software such as the lme function in the nlme package of the R programming language. We performed the analysis in Matlab (MathWorks 2005) (Supplement). Statistical

significance of the variance estimates σ^2 was determined with a likelihood ratio test. Because estimates of σ^2 are constrained to be nonnegative, the ratio of likelihoods between models with and without σ^2 is a 50:50 mix of χ_0^2 and χ_1^2 distributions (Self and Liang 1987, Stram and Lee 1994); thus, the P values given by the constrained likelihood ratio test are one-half the values that would be calculated from a standard likelihood ratio test using χ_1^2.

Data set

We analyzed data from an experiment on prairie flowers in which potted plants from 14 species were forced to flower in a greenhouse and placed in the field; pollinator visits to plants were then observed (see Plate 1). The analyses here focus on the pollinator communities, resolving these communities to a finer taxonomic level than done in the previous analysis that focused on visitation rates aggregated among pollinators (Rafferty and Ives 2011).

Data on pollinator visitation rates were collected in a restored tallgrass prairie at the University of Wisconsin Arboretum in Madison, Wisconsin, USA (43.04° N, 89.43° W). Observations were conducted for 51 days from 15 April to 6 August 2009. Individual experimental plants were observed continuously for 10 minutes typically at least twice per day, 08:30 to 17:00, for 3 days per week. A pollinator was defined as an insect that contacted the anthers, stigma, and/or nectar of a flower. Potential pollinators that could not be identified in the field were given a morphospecies code and, when possible, captured for identification. Voucher specimens were deposited in the Insect Research Collection of the Department of Entomology, University of Wisconsin, (Madison, Wisconsin, USA).

A total of 61 species and morphospecies were observed. We refined the data set by excluding species for which eight or fewer individuals were observed, known florivores/pollen-feeding beetles (*Popillia japonica* and weevils), and nonflying insects (ants). We aggregated some species (e.g., species in the genus *Bombus*) due to their ecological similarity and the difficulty of distinguishing them during field observations. Thus, the pollinator taxa represent a mix of species and aggregations of species (Appendix A). For each plant species, we combined the pollinator records over the 2–7 weeks (depending on species) they were in the field, giving a 22 pollinator by 14 plant table of visitation frequencies of pollinators (Fig. 1). These data do not represent the communities of pollinators visiting plants over the entirety of their flowering periods but instead just several weeks as flowering commences. Phylogenies for the plant and pollinator taxa were compiled from primarily molecular data in the literature (Appendix B).

In comparing the communities of pollinators among plants, we were interested in the relative visitation frequencies of pollinators rather than the absolute numbers of visits. For example, if one plant was visited by 1, 10, and 100 individuals of pollinators A, B, and C, and a second plant was visited by 10, 100, and 1000 individuals from the same pollinator taxa, then the analysis should show that the communities have the same visitation-weighted composition of pollinator taxa. Taking the $\log_{10}$ of visitation frequencies for both communities, the values for pollinators A, B, and C in the first are 0, 1 and 2, and in the second they are 1, 2 and 3. Therefore, the differences between $\log_{10}$ visitation frequencies of pollinators are the same in both communities, and the data can be analyzed using a linear model. Throughout the analyses, before log-transforming the data we replaced zeros with 0.01, 0.001, or 0.0001 as required to normalize the residuals. To test for normality, we computed Cholesky residuals that orthogonize the residuals through the fitted covariance matrix (Houseman et al. 2004); the covariances among Cholesky residuals are zero. We then applied a Jarque-Bera normality test (Jarque and Bera 1987) that assesses a distribution by its skewness and kurtosis.

We investigated eight plant traits to determine which could generate differences among the pollinators visiting different plant species (Appendix C). Two traits involved phenology: (1) phenological shift (whether plants are flowering significantly earlier) and (2) date of first bloom (mean week of flowering onset; Fig. 1). We also analyzed six morphological traits: (3) plant height, (4) flower color, (5) floral symmetry (whether flowers are actinomorphic), (6) floral display size (mean number of flowers or inflorescences per plant), (7) nectar volume, and (8) nectar concealment (whether flowers have concealed nectar). We measured these traits directly from the experimental plants. To facilitate comparisons among the effects of plant traits, we standardized values for each trait to have mean 0 and variance 1.

We also tested whether phylogenetically related plants share similar trait values. For continuously valued variables, we used RegressionV2.m (Lavin et al. 2008) and report the value of the estimated parameter d, which equals 0 if there is no phylogenetic signal and 1 if the strength of phylogenetic signal equals that under a Brownian motion model of evolution. For binary traits (phenological shift, floral symmetry, and nectar concealment) we used PLogReg.m (Ives and Garland 2010) and report $\exp(a)$, which equals 0 if there is no phylogenetic signal.

Results

Simulations

We simulated three cases using the model in Eq. 1: (1) pollinators show random variation in visitation frequency ($\sigma_A^2 = 1$, $\sigma_B^2 = 0$), and all pollinators respond in the same way to plant trait x ($\beta = 1$, $\sigma_C^2 = \sigma_D^2 = 0$); (2) pollinators show phylogenetic variation in visitation frequency ($\sigma_A^2 = 0$, $\sigma_B^2 = 1$), and pollinator response to x
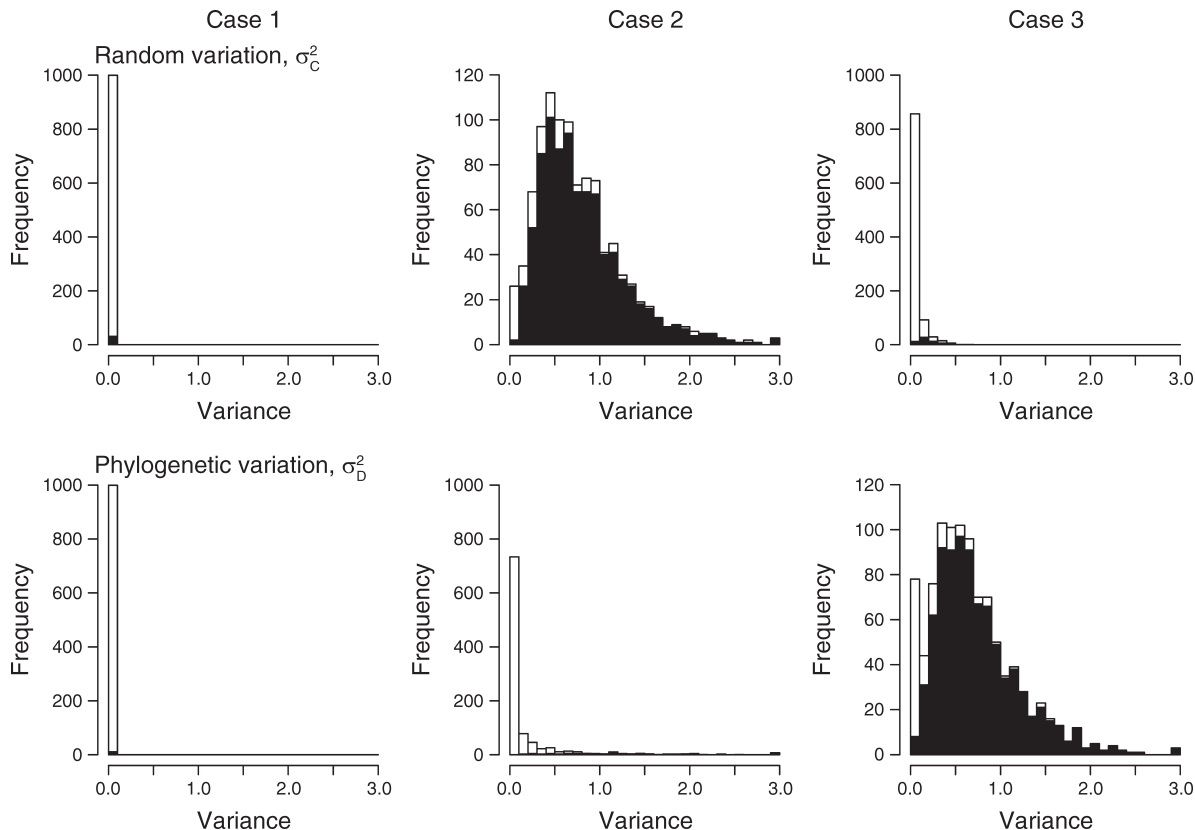
FIG. 2. Estimates of random (σ_C^2) and phylogenetic (σ_D^2) variation among pollinators in their responses to plant trait x from 1000 simulations using Eq. 1. Open bars give all estimates of the variances, and black bars give those estimates that are statistically significantly greater than zero in a constrained likelihood ratio test with $\alpha = 0.05$. In cases 1 and 2, the fractions of simulations giving estimates of σ_D^2 that are nominally significant are 0.011 and 0.044, respectively. Simulation cases 1, 2, and 3 are described in *Results: Simulations*.

shows random variation ($\beta = 0$, $\sigma_C^2 = 1$, $\sigma_D^2 = 0$); and (3) pollinators show phylogenetic variation in visitation frequency ($\sigma_A^2 = 0$, $\sigma_B^2 = 1$), and pollinator response to x shows phylogenetic variation ($\beta = 0$, $\sigma_C^2 = 0$, $\sigma_D^2 = 1$). In all cases, trait x varied phylogenetically among plants ($\sigma_K^2 = 0$, $\sigma_L^2 = 1$), and there was unexplained variation ($\sigma_e^2 = 0.5$). Presence/absence data were simulated for the same cases, but all nonzero values of σ_A^2, σ_B^2, and σ_D^2 were decreased from 1 to 0.25 to reduce the extreme values of y, and σ_e^2 was set to zero.

The first PLMM (Eq. 2) should identify whether the 22 simulated pollinators differed in their responses to a plant trait x, and if so, whether phylogenetically related pollinators responded in a similar way. For simulated case 1 with no variation in response among pollinators, the estimates of σ_C^2 and σ_D^2 were 0 or close to 0 (Fig. 2). At a significance level of $\alpha = 0.05$, the constrained likelihood ratio test rejected the null hypotheses $H_0 : \sigma_C^2 = 0$ in 3.1% of the data sets, and $H_0 : \sigma_D^2 = 0$ in 1.1%; these imply that the test is not giving false positives, although suggesting less than optimal power. For case 2 with random variation among pollinators, estimates of σ_C^2 were generally greater than 0 while estimates of σ_D^2 were near 0. The null hypotheses $H_0 : \sigma_C^2 = 0$ was rejected in

88% of the data sets, and $H_0 : \sigma_D^2 = 0$ in 4.4%, demonstrating an appropriate rate of false positives for the α level. Finally, for case 3 with phylogenetic variation among pollinators, estimates of σ_C^2 were near 0 while estimates of σ_D^2 were generally greater than 0, and $H_0 : \sigma_C^2 = 0$ and $H_0 : \sigma_D^2 = 0$ were rejected in 10.9% and 68% of the data sets; thus, there was a falsely high rejection rate for non-phylogenetic variation in pollination response to plant trait values, in contrast to the correct rejection rate for phylogenetic variation. Overall, the PLMM was able to identify the existence and type of variation among pollinators in their response to a plant trait.

We repeated this exercise for simulated presence/absence data (Fig. 3). Although the PLMM assumes Gaussian variation, it nonetheless performed similarly on presence/absence data as it did on continuous data. For case 1 ($\sigma_C^2 = \sigma_D^2 = 0$), the null hypothesis $H_0 : \sigma_D^2 = 0$ was rejected in 1.4% of the data sets, suggesting loss of power. For case 2 ($\sigma_C^2 > 0$, $\sigma_D^2 = 0$), the null hypothesis $H_0 : \sigma_D^2 = 0$ was rejected in 6.6% of the data sets; this raises the risk of false positives, which was not found for the continuous simulated data, although this false positive rate is not very high. The good performance
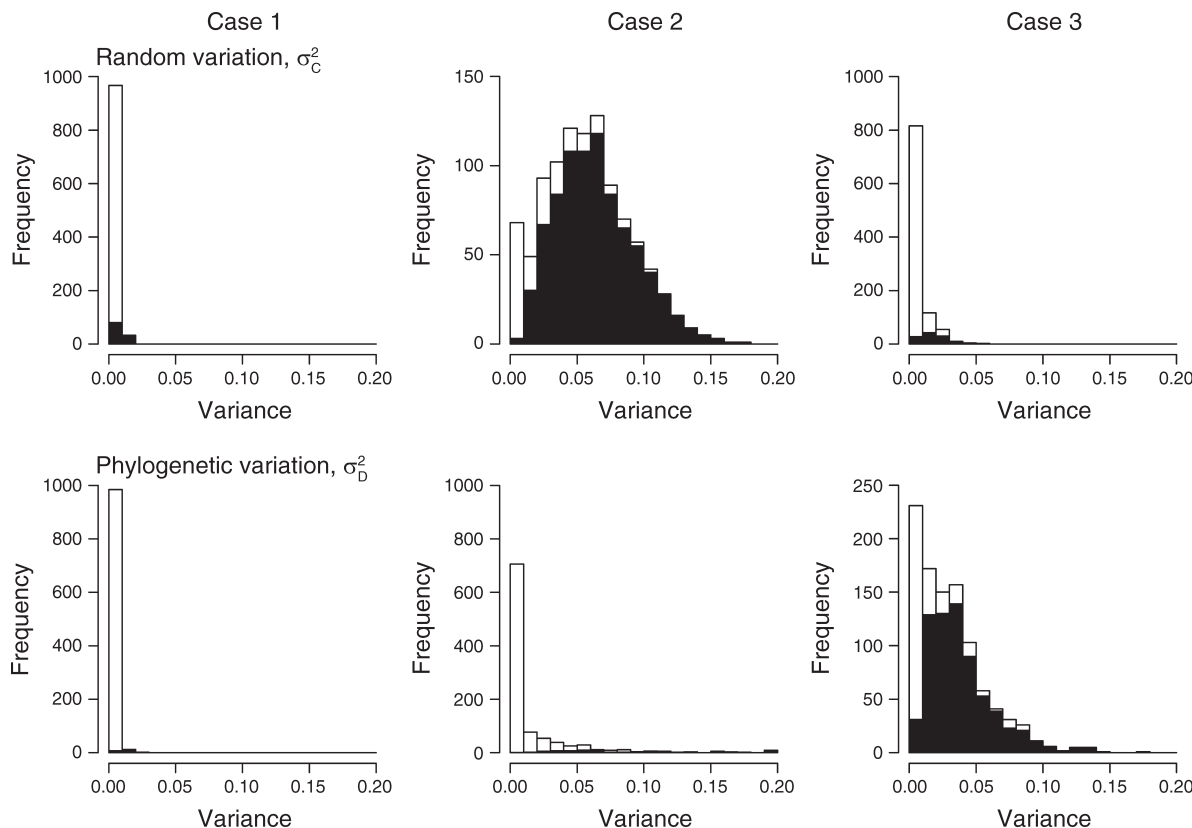
Fig. 3. Estimates of random (σ_C^2) and phylogenetic (σ_D^2) variation among pollinators in their responses to plant trait x from 1000 simulations using Eq. 1 in which data were transformed to presence/absence of interactions. Open bars give all estimates of the variances, and black bars give those estimates that are statistically significantly greater than zero in a constrained likelihood ratio test with $\alpha = 0.05$. In cases 1 and 2, the fractions of simulations giving estimates of σ_D^2 that are nominally significant are 0.014 and 0.066, respectively. Simulation cases 1, 2, and 3 are described in *Results: Simulations*.

of the PLMM presumably reflects the size of the data set (308 points), since the sum of residuals will be approximately Gaussian (Judge et al. 1985: chapter 20).

The second PLMM (Eq. 3) was designed to identify phylogenetic patterns in the interactions between pollinators and plants without using information on plant traits (Fig. 4). We did not assess whether the estimates of the variances were significantly greater than zero, because the simulation model differed substantially from the fitted statistical model. For case 1, estimates from most simulations indicated random variation in the visitation frequency of pollinators (σ_a^2) and phylogenetic variation among plants in the numbers of pollinators they attracted (σ_f^2). This is consistent with the underlying assumptions used in the simulations: that visitation frequency among pollinators varied randomly, that plants exhibited phylogenetic variation in trait x, and that pollinators responded to trait x in the same way. These assumptions generate phylogenetic variation among plants in the numbers of pollinators attracted that were detected by the second PLMM even though this model used no information about plant traits. For case 2, the PLMM identified phylogenetic variation in visitation frequency among pollinators (σ_b^2), and indi-

cated that phylogenetically related plants were more likely to attract the same pollinator species (σ_g^2). This is consistent with the simulation assumptions in which pollinators had phylogenetic variation in visitation frequency, plants had phylogenetic variation in trait x, and pollinators had random variation in their response to trait x. Because phylogenetically related plants had similar trait values, they were more likely to attract the same pollinator species. The analysis of case 3 differed from case 2 by indicating that phylogenetically related plants that attracted one pollinator species were more likely to attract a related pollinator species (σ_h^2); specifically, if plant species q attracted pollinator j, then a phylogenetically related plant species r was more likely to attract pollinator k if it was related to pollinator j. This occurred because in the simulations phylogenetically related pollinators showed similar responses to plant trait x, in contrast to case 2 in which pollinators showed random differences in their responses to x. For case 3, however, some of the estimates of σ_g^2 were positive, and many estimates of σ_h^2 were near 0. Because σ_g^2 and σ_h^2 both depend on the phylogeny of plants and differ only in the inclusion of the pollinator phylogeny
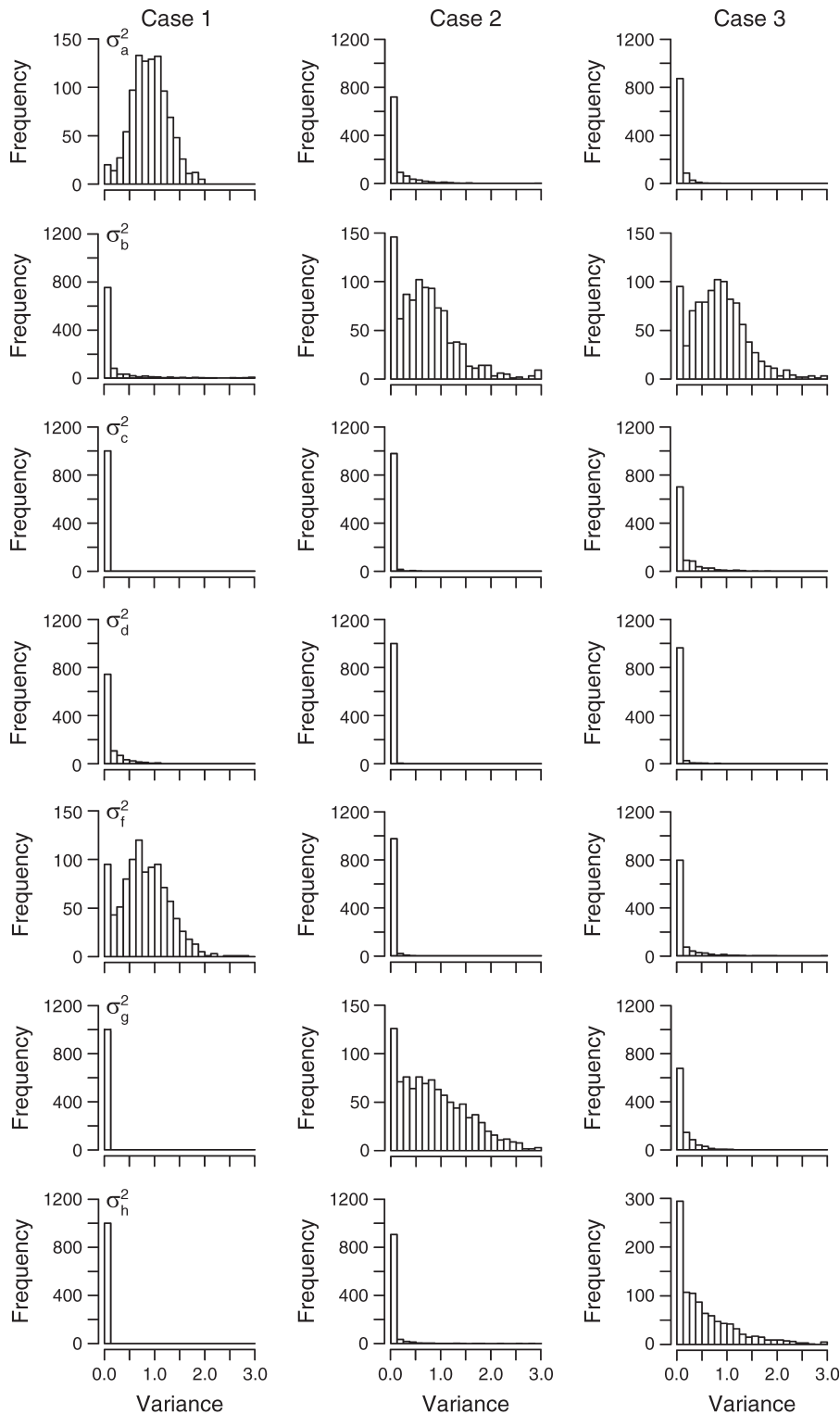
Fig. 4. Estimates of variance components of the second phylogenetic linear mixed model (PLMM; Eq. 3) for 1000 simulations using Eq. 1. Simulation cases 1, 2, and 3 are described in *Results: Simulations*, and definitions of the variance components are given in Table 2.

TABLE 1. Pollinator community composition as it depends on plant traits (Eq. 2).

Effects	σ_C^2	P	σ_D^2	P	Phylo. signal
Univariate analyses					
Phenological shift	0.35†	>0.5	0	NS	
Date of first bloom	0.52‡	0.047	0	NS	
Multivariate analysis§					
Plant height	0.36	0.002			0.2 (0, 1.9)
Flower color	0.31	0.009			0
Floral symmetry	0.24	0.046			0.45 (0.02, 4.1)
Floral display size	0.18	0.22			0.82 (0, 3.7)
Date of first bloom	0.18	0.12			1.37 (0, 5.42)
Nectar concealment	0.02	0.46			1.97 (0, 3.12)
Nectar volume	0	NS			0.35 (0, 0.95)
Phenological shift	0	NS			0.31 (0, 1.4)

Notes: Univariate analyses were performed for phenological shift and for date of first bloom; parameters σ_C^2 and σ_D^2 measure variation among pollinator taxa in their responses to the predictor variables that is not or is, respectively, related to the pollinator phylogeny. For the multivariate analysis, all residual variation in pollinator responses to plant traits was assumed to be non-phylogenetic ($\sigma_D^2 = 0$), and "Phylo. signal" gives estimates of the phylogenetic signal for each trait among plant species with 95% confidence intervals. NS, not significant.

† Jarque-Bera Normality test, $P = 0.07$.

‡ J-B test, $P = 0.23$.

§ J-B test, $P = 0.13$.

(σ_h^2), there is limited statistical power to separate these two components of the model.

Data

We first analyzed the effects of plant phenology traits (phenological shift and current date of first bloom) on pollinator composition using the first PLMM (Eq. 2). The log visitation frequencies of pollinators attracted to advanced-flowering plant species were lower than those attracted to species with unchanged flowering ($B_1 = -0.46$), although there was no significant variation among pollinator taxa in response to this trait (Table 1). Therefore, communities of pollinators visiting advanced-flowering plants were not distinct from those visiting plants that are not flowering earlier. More pollinators visited plants that bloomed later in the season ($B_1 = 1.32$), and the estimate of $\sigma_C^2 = 0.52$ ($P = 0.047$) indicates variation among pollinators in response to this trait (Table 1). Note that even though the phylogenetic signal was not found in either analysis ($\sigma_D^2 = 0$), the simulations showed the potential ability of the PLMM to identify phylogenetic signal when it was present (Fig. 2).

To investigate other, non-phenological plant traits that might generate differences in the pollinators they attract, we performed a multiple regression PLMM (Eq. 2) using all eight plant traits (Table 1). We initially included the pollinator phylogeny, but because the estimate of the variance was always 0 ($\sigma_D^2 = 0$), it was omitted. For six traits, the estimates of random variation among pollinator taxa (σ_C^2) were greater than 0, indicating differences in the composition of the pollinator communities visiting different plant species, although only plant height, flower color, and floral symmetry showed statistically significant variation

among pollinators. Furthermore, seven of the eight traits showed nonzero estimates of phylogenetic signal among plants, although only flower symmetry was statistically significant ($P < 0.031$, Table 1).

We used the second PLMM (Eq. 3) to investigate the pattern of interactions between pollinators and plants without regard to plant traits. Although pollinators differed greatly in log visitation frequency ($\sigma_a^2 = 3.59$, $P < 0.001$, Table 2), this variation did not have a phylogenetic component ($\sigma_b^2 = 0$). Furthermore, a given plant species was not more likely to be visited by phylogenetically related pollinators after accounting for differences in mean visitation frequencies ($\sigma_c^2 = 0.04$, $P > 0.5$). We also failed to detect significant pollinator phylogenetic signal in conjunction with the plant phylogeny ($\sigma_h^2 = 0.35$, $P > 0.12$). Nonetheless, pollinators were affected by plant phylogeny; closely related plants were likely to have similar visitation frequencies regardless of species ($\sigma_f^2 = 2.42$, $P < 0.001$). Furthermore, if a given pollinator taxon was attracted to a given plant species, the nonsignificant estimate of $\sigma_g^2 = 0.20$ ($P > 0.5$) indicates that the same pollinator is not more likely be attracted to a closely related plant.

Can variation among pollinator taxa in their responses to plant traits (Table 1) explain the effect of plant phylogeny on the pollinators they attract (Table 2)? To investigate this, we simulated data using Eq. 2 parameterized from the real data and then fit the simulated data using Eq. 3; this procedure is similar to that used for the simulation model (Eq. 1). For fitting the PLMM (Eq. 3), we assumed that there was no effect of pollinator phylogeny ($\sigma_c^2 = \sigma_h^2 = 0$). From 1000 simulations, the mean phylogenetic variation in overall plant attractiveness σ_f^2 was 2.15, close to 2.42 observed in the data. In none of the simulations was the estimate

TABLE 2. Test for the existence of phylogenetic patterns in log visitation frequencies of pollinators distributed among plant species, and ability of trait differences among plants to drive community patterns in plant–pollinator interactions (Eq. 3).

	Data†			Simulation		
Variance component	Estimate	χ^2	P	Mean	66% inclusion	95% inclusion
σ_a^2, random variation among pollinator visitation frequencies	3.59	101.2	<0.001	3.76	(3.2, 4.3)	(2.7, 4.9)
σ_b^2, phylogenetic variation among pollinator visitation frequencies	0	0	NS			
σ_c^2, phylogenetic variation in pollinator attraction to a given plant	0.04	0.02	>0.5	0.47	(0, 0.8)	(0, 1.4)
σ_d^2, random variation in plant attractiveness	0	0	NS			
σ_f^2, phylogenetic variation in plant attractiveness	2.42	77.0	<0.001	2.15	(1.4, 2.9)	(0.8, 3.7)
σ_g^2, phylogenetic variation in plant attractiveness to a given pollinator	0.20	0.04	>0.5	1.13	(0.18, 2.0)	(0, 2.9)
σ_h^2, co-phylogenetic pollinator-plant variation	0.35	0.69	0.12	0.20	(0, 0.49)	(0, 1.3)
σ_e^2, residual variation	5.13	···	···	4.71	(4.0, 5.4)	(3.4, 6.1)

Notes: Statistical significance of the variance components was tested with constrained likelihood ratio tests. To test for the ability of plant traits to explain phylogenetic patterns, 1000 data sets were simulated using Eq. 2 that had been fitted to the real data (Table 1). Each simulation data set was then fit using Eq. 3 that incorporates plant phylogeny but not trait values, with terms involving the pollinator phylogeny excluded ($\sigma_b^2 = \sigma_c^2 = \sigma_h^2 = 0$). The mean values of the estimates from the 1000 simulation data sets are given along with the 66% and 95% inclusion intervals. NS, not significant.

† Jarque-Bera Normality test, $P = 0.30$. Empty cells for simulation indicate that no data were possible (estimate = 0); ellipses indicate no data.

of $\sigma_f^2 = 0$, showing a very strong effect of plant traits in explaining why closely related plants had similar visitation frequencies. Finally, the phylogenetic variation in plant attractiveness to a given pollinator σ_g^2 was 1.13; this value differed from the estimate from the real data ($\sigma_g^2 = 0.20$), although the latter was within the 66% inclusion interval of the simulated estimate.

DISCUSSION

For the bipartite ecological network of 14 perennial plants and 22 pollinator taxa analyzed here, we found that, as a group, the six plant species that are flowering earlier did not have a pollinator community distinct from that of the eight plant species with unchanged flowering times. Yet six plant traits, plant height, flower color, floral symmetry, floral display size, date of first bloom, and nectar concealment, were associated with different pollinator communities, with the former three statistically significant. Furthermore, all of these traits except color were distributed among plants in a way that reflected the plant phylogeny. Closely related pollinators were neither more likely to visit plants with similar frequencies nor to visit the same plant species. Conversely, there was a strong signal through the plant phylogeny in that closely related plants were similarly attractive to the pollinator community as a whole, and closely related species were more likely to attract the same pollinator taxa, although the latter was not statistically significant. Finally, analysis of the six plant traits showed that together they explain the similar attractiveness of related plants to the overall pollinator community (Table 2).

These results should be interpreted in the context of the simulations we performed to test the PLMM

methods. Although no effects of the pollinator phylogeny were found in any of the analyses of the data, the simulations showed that phylogenetic patterns in the distribution of pollinators could be detected in the analyses either including (Eq. 2, Fig. 2) or excluding (Eq. 3, Fig. 4) plant trait information. Therefore, the methods are sufficiently powerful that they should have picked up a moderate or strong pollinator phylogenetic signal. The simulations also showed, however, limited power to detect complex phylogenetic interactions; co-phylogenetic patterns involving both plants and pollinators, σ_h^2, were detected in only 30% of the simulations (case 3, Fig. 4). Given the relatively small numbers of species and the statistical challenges of detecting phylogenetic signal (Blomberg et al. 2003), this is not surprising.

In our data set, 59% of the entries were zeros. Nonetheless, the distribution of Cholesky residuals did not show strong deviation from normality (Tables 1 and 2). Furthermore, application of the first PLMM (Eq. 2) to simulated presence/absence data showed the robustness of the approach to even violently nonnormally distributed data (Fig. 3). Given the size of the data set (308 points), this is not surprising. For example, it is well known in standard regression that the least squares estimators of both coefficients and variances asymptotically approach the "correct" (efficient) estimators for nonnormal data (Judge et al. 1985:824). Similarly for LLMs, applying the mis-specified normal likelihood function to nonnormal data leads to estimates that asymptotically converge to their true values (McCulloch et al. 2008:285). For large data sets, even those that are clearly nonnormal, LLMs will often be sufficient to correctly identify statistically significant parameters.

PLATE 1. Bumble bees (*Bombus*) visiting swamp milkweed (*Asclepias incarnata*) in the University of Wisconsin Arboretum (Madison, Wisconsin, USA). Photo credit: N. E. Rafferty.

Our results have implications for conservation of our focal plant species with future climate change. We found that the communities of pollinators visiting advanced plant species did not differ systematically from the communities visiting unchanged species. This suggests that several pollinator taxa buffer the advanced plant species against reductions in pollinator visits when they are forced to flower even earlier. The lack of a single, key pollinator of advanced plant species may increase the resilience of the plant–pollinator network to climate change.

Our results further suggest that basic information on plant traits could be useful in predicting which plants in a community are likely to suffer pollination deficits as a result of phenological shifts. Plant height, flower color, and floral symmetry statistically significantly affected the pollinator community. Foraging height preferences have been documented among various pollinator taxa (Peakall and Handel 1993, Dafni and Potts 2004, Hoehn et al. 2008); in our system, taller plants received visits from a greater number of pollinators. Likewise, flower color has been found to be important in explaining pollinator community composition (Morales and Aizen 2006, Lázaro et al. 2008). In our network, white, purple, or blue flowers attracted a greater abundance of pollinators than did yellow, red, orange, or pink flowers, even though colors as we scored them are not necessarily those that pollinators discriminate (Arnold et al. 2010).

Even in the absence of information on plant traits, phylogenies can be used to infer interaction patterns. We detected strong signal through the plant phylogeny: closely related plants were similarly attractive to the pollinator community as a whole. Thus, if the number of pollinators a plant attracts is predictive of its susceptibility to pollination deficits, then phylogenies could be used to identify plants that are likely to show similar responses.

In a study similar to ours, Vázquez et al. (2009) showed that the number of pollinators visiting different plants depended strongly on the phylogeny of the plants but only weakly on the phylogeny of the pollinators, as have we. The method they used (from Ives and Godfray 2006) is similar in concept but different in statistical approach from that presented here, relying on an Ornstein-Uhlenbeck model of evolution rather than the LMM framework. The LMM structure is more flexible and, as illustrated here, can be extended to ask what plant traits may be responsible for related plants having similar pollinator communities (Eq. 2, Table 1). Both approaches can incorporate phylogenetic information from both pollinators and plants, differentiating them from other phylogenetic methods that have been used to assess plant–pollinator interactions. For example, Rezende et al. (2007) showed that plant phylogeny predicted the number of pollinators that visited a given plant species, and that pollinator phylogeny predicted

the number of plants a given pollinator visited. In this case, the number of pollinators with which a plant interacted was treated as a plant trait, and the number of plants with which a pollinator interacted was treated as a pollinator trait; thus, the analyses of plants and pollinators were separate. In our case, the response variable is the interaction strength between each pair of plants and pollinators, which can be used to ask questions about the overall pattern of the interaction network.

The statistical methods we have deployed allowed us to ask how important phylogenetic signal is in structuring the plant–pollinator interactions in our data set and to explore the role of phenological and other plant traits in shaping pollinator communities. Our data set does not capture the entire spectrum of plants with which pollinators interacted, and our study plants were observed for only a few weeks around the onset of flowering. Nevertheless, our goal was to identify the potential for phenological mismatches; these may be most likely to occur at the onset of flowering due to the absence of pollinators. The general structure of the PLMM can be used to address a broad range of questions incorporating phylogenetic relationships among species in bipartite interaction networks. Thus, the methods illustrated here help to address the need for more flexible phylogenetic statistical approaches.

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SUPPLEMENTAL MATERIAL

Appendix A

List of pollinator taxa used in the phylogeny (*Ecological Archives* E094-214-A1).

Appendix B

Construction of pollinator and plant phylogenies (*Ecological Archives* E094-214-A2).

Appendix C

Plant traits for the 14 plant species (*Ecological Archives* E094-214-A3).

Supplement

Matlab code for phylogenetic linear mixed models (PLMMs) (*Ecological Archives* E094-214-S1).