

High levels of phenological asynchrony between specialized pollinators and plants with short flowering phases

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Abstract. Species phenology plays a key role in determining mutualistic interactions, such as those between plants and pollinators. Notably, temporal synchrony shapes the patterns of interactions by influencing the probability of encounters between interacting partners; thus, species phenology greatly contributes to structuring ecological communities. In these communities, specialized species are expected to show a high level of synchrony with their partners; however, the relationship between species phenology and specialization remains largely unexplored. In three localities in the tropical mountains of Costa Rica, we quantified the level of phenological synchrony in plant–pollinator networks and tested whether phenological synchrony is associated with the degree of pollinator specialization on plant partners. We also tested the relationship between pollinator specialization and the length of the flowering phase of the visited plants. Across all three studied networks, our results show a strong asynchrony between interacting plant and pollinator species. We also found that more specialized pollinators were more asynchronous with their plant partners and, moreover, that specialized pollinators preferably visited plant species with shorter flowering phases compared to generalized pollinators. These patterns suggest that specialized pollinators may be more vulnerable to mutualistic disruptions because they depend primarily on short-lived resources and have a high risk of phenological mismatch. This discovery has important consequences for specialized species' potential to survive and adapt to changes in the phenology of their interacting partners, which is highly relevant in a time characterized by changing climates and associated shifts in species phenology.

Key words: *mutualistic interactions; phenological synchrony; pollination; pollinator vulnerability; specialization; temporal asynchrony.*

INTRODUCTION

Phenology is the study of organisms' seasonal activities such as timing of flowering or insect emergence. For species engaging in mutualistic interactions, such as flowering plants and their pollinators, phenology is a crucial aspect of their ecology. Notably, phenological synchrony, i.e., temporal matching, is a requirement for species to interact in a pairwise relationship (Olesen et al. 2008, Vázquez et al. 2009). In the current context of climate change, the study of phenological synchrony between interacting species has become highly relevant

because climate-mediated shifts in species' phenologies may lead to altered probabilities of pairwise interactions and eventually the reshaping of mutualistic networks in the future (Schmidt et al. 2016). Phenological asynchrony (i.e., lack of temporal overlap between plants and pollinators) may affect the reproduction of plants by reducing pollinator visitation and pollen deposition, and pollinators may experience reduced food availability (Kjøhl et al. 2011). Given the importance of temporal matching in determining patterns of interactions and the associated reproduction and survival of species (Vázquez et al. 2009), it is crucial to identify the characteristics of species that are more asynchronous with their mutualistic partners. Notably, it would be important to know whether specialized pollinators are phenologically more in synchrony or asynchrony than generalized pollinators are with their plant partners.

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Ecological specialization of consumers is characterized by using a small and/or unique proportion of the available resources in a given community (Blüthgen et al. 2007). The level of specialization is generally believed to influence species co-existence, with species-rich communities often being more specialized than species-poor communities (Dalsgaard et al. 2011, Maglianesi et al. 2015). The level of specialization is, therefore, an important characteristic of ecological communities. Noticeably, as specialization can vary considerably among species within the same network (Maglianesi et al. 2014, Dalsgaard et al. 2018), analysis of specialization at the species level is required to understand how communities are structured (e.g., Waser and Ollerton 2006). Because ecologically specialized species have fewer interacting partners, there is less redundancy in their interactions compared to generalists. Thus, specialists may be impacted more by the loss of their partners (Schleuning et al. 2016, Dalsgaard et al. 2018) and, consequently, they may be more sensitive to phenological asynchrony (Memmott et al. 2007, Miller-Rushing et al. 2010). Understanding the link between phenology of interacting species and ecological specialization is therefore crucial to anticipate changes in interaction patterns that may lead species to be vulnerable to mutualistic disruption.

Despite the importance of temporal matching between plants and pollinators from which the patterns of interactions emerge, little is known about how species phenology and specialization are interrelated. This relationship may be especially relevant for species at high altitudes in the tropics, as many of these only occur in a restricted range (Sonne et al. 2016, Dalsgaard et al. 2018) and have to cope with low resource availability and a low predictability of flowering phenology (Ornelas et al. 2007, Wright et al. 2015). In the Neotropics, the páramo is a mountaintop ecosystem inhabited by a variety of flower visitors such as hummingbirds, bees, flies, and butterflies; these taxa include the most important pollinators in mountain ecosystems and vary in their level of specialization (Lehmann et al. 2019). Thus, plant–pollinator networks in the páramo represent an important and highly suitable study system to examine how species phenology relates to specialization.

To assess the relationship between species phenology and specialization of pollinator species, we collected data on phenology, species abundance, and interactions in plant–pollinator networks in the páramo during an entire year. As pollinators might have evolved under strong selective forces to respond similarly to climatic cues as their plant partners, we expected a high temporal overlap between interacting species (Rafferty et al. 2015). We especially expected a high degree of phenological synchrony between specialized pollinators and the visited plant species, which may result in an increased fitness for both partners (Schenk et al. 2017). To increase our knowledge on the association between species phenology and specialization, we specifically investigated (1) the extent of phenological synchrony between plants and pollinators, and (2) whether

phenological synchrony between interacting species and the length of the flowering phase are associated with the degree of ecological specialization of pollinator species. Contrary to our hypothesis, we found strong asynchrony between interacting species, especially for ecologically specialized pollinators. Moreover, specialized pollinator species visited plant species with shorter flowering phase than did generalised pollinators. This indicates that specialized pollinators may be more sensitive to mutualistic disruptions than predicted by the level of specialization alone.

MATERIALS AND METHODS

Study area and sampling design

The study was conducted in central–southern Costa Rica on the Caribbean and Pacific slopes of the Talamanca mountains, a volcanic mountain range that forms the spine of the Central American isthmus from central Costa Rica through western Panama. This area includes the Cerro de la Muerte with the largest extent of páramo ecosystem in the country (09°60' N, 83°76' W), which is a grass- or shrub-dominated ecosystem in the cool and wet upper slopes of Neotropical mountains. Field data collection was conducted at three sites of about 12 ha each: Cerro las Vueltas Biological Reserve, Cerro Sakira, and Cerro Buena Vista (hereafter Reserve, Sakira, and Buena Vista, respectively) at elevations between 3,000 and 3,400 m above sea level. In the Costa Rican páramo, the mean annual temperature is 9.7°C with temperatures that can drop below 0°C (Kappelle and Horn 2016). The dry season lasts from December to April and the wet season reaches a peak during October. The páramo has a wet to pluvial climate with daily abrupt changes in cloud cover, rain, fog, and relative atmospheric humidity. The mean annual precipitation is 2,800 mm and relative humidity ranges from 70 to 85% (Kappelle and Horn 2016). The low temperatures together with strong daily fluctuations in temperature and humidity cause harsh conditions for most organisms, being challenged by frost, water deficits, and droughts. Seasonality in temperature and precipitation are thought to be the main drivers of flowering phenology in the páramo (Contreras Arias and Méndez-Estrada 2014). In the three study sites, we collected data on species phenology and abundance, and plant–pollinator interactions. Because the two types of studied organisms (plants and mobile pollinators) differ in space use, we used a combination of transects and plots distributed across each study site (see Appendix S1: Fig. S1). Data collection was conducted about every 12 d at each study site over an entire year (i.e., covering the intra-annual variation in the dynamic of plant–pollinator networks) from January to December 2016.

Data collection

Plant–pollinator interactions.—We attempted a comprehensive sampling of mutualistic plant–pollinator

interactions in the whole community, that is, recording diurnal animal visitation for all plant species. Thus, flower visitation by hummingbirds and insects (hereafter *pollinators*) was monitored separately by direct sighting from 7:00 to approximately 13:00, that is, when sunshine and pollinator activity were more likely in the páramo. Observations were conducted within haphazardly chosen plots, which we selected by walking in the study area and selecting plots around plant individuals that were in bloom. To avoid spatial pseudo-replication, selected plots were separated by a minimum distance of 30 m for hummingbird and 20 m for insect plots. Within these plots, 1–5 flowering plant individuals belonging to the same and/or different species were observed simultaneously to record pollinator visitation. All hummingbirds were identified to species level, whereas insects were classified to species level or to morphospecies at genus level. Plant–hummingbird interactions were recorded within 3×3 m plots during 20 min and from a distance of about 8 m, whereas plant–insect interactions were recorded within 2×2 m plots during 10 min from a distance of about 1 m (Appendix S1: Fig. S1). As these plots cover a small area, we established on average 23 plots each day of sampling throughout the area in order to have a representative sample. For analyses, we considered only legitimate interactions between plants and pollinators, that is, when animal visitors used the corolla opening to access food resources and likely touched the reproductive parts of the flower; we thus excluded nectar larceny when visitors used the base of the corolla to access nectar in a nonmutualistic relationship. Based on this criterion, we excluded 33 out of 68 insect morphospecies, assuming that these do not act as legitimate pollinators. In support of this, none of the 33 morphospecies had been previously reported as potential pollinators. Instead, the excluded morphospecies most likely visited the flowers for purposes other than feeding on pollen/nectar, such as phytophagous or predatory beetles and coprophagous or necrophagous flies (Reina-Ávila et al. 2013, Lefebvre et al. 2018). Removing these species from the analysis makes it more likely that all included insects contribute to plant pollination. Our sampling effort for species interactions consisted of about 5,280 plant individuals observed in 2,280 plots during about 1,410 h distributed over 98 d and 23 sampling periods. To evaluate the sufficiency of our sampling, we used an approach based on rarefaction curves applied to interaction networks (Jordano 2016). The rarefaction curves showed similar shapes (slopes) for the three study sites and a trend toward saturation, which indicates an adequate sampling effort that captured the majority of plant–pollinator interactions at each site (Appendix S1: Fig. S2).

Flower abundance and flowering phenology.—To estimate the abundance of floral resources and flowering phenology independent of pollinator phenology, we established three permanent 50×5 m transects at each study site, with transects being separated from one another by at

least 200 m to achieve statistical independence. Transect locations were chosen to cover a representative sample of the páramo vegetation. Along these transects, open flowers of all plant species were counted (see Appendix S1). Because certain plant species have an aggregated distribution or grow in specific microhabitats, we occasionally found some plants flowering out of the transects at a given sampling period. To avoid missing abundance and phenological data for these species during such sampling periods, we counted all flowers in a 2×2 m area of the first plant individual found after having completed the flower counts in the three fixed transects (i.e., flowers for these plant species were counted following a standardized method). Both transects and plots for counting flowers allowed us to carry out an exhaustive sampling of food resource for pollinators. We used the total number of flowers per plant species summed across all transects and plots (i.e., the overall flower abundance) as an estimate of plant species-specific resource availability for pollinators at each study site and sampling period. We did not use the same plots where we recorded plant–pollinator interactions for counting flowers because we wanted to quantify flower abundance independently of its use by pollinators for a better estimation of phenological synchrony in plant–pollinator interactions and ecological specialization of pollinator species.

A high frequency and intensity of sampling allowed us to obtain more than 276,000 flower counts for flowering phenology and pollinator's resource abundance analyses. To quantify the variability of the floral resources available for pollinators in space and time, we used analysis of variance to test for variation in log-transformed flower abundances across plant species, study site, and sampling period.

Pollinator abundance and phenology.—We estimated the abundance and phenology of hummingbird species by using both observation and capture data. We used the total number of individuals observed in three 100×20 m fixed transects plus those captured in mist nets as an independent measure of hummingbird species abundance at each site. To avoid overestimation of hummingbird abundance, transects were established outside of the mist-netting area and recaptured individuals were excluded from abundance estimates. As a proxy for the abundance and phenology of insect pollinator species, we used interaction frequency, which is often used in other studies (Benadi et al. 2013, Maldonado et al. 2013) and a good approach according to Vázquez et al. (2009). Previous research has shown that the total number of interactions of animal pollinators largely corresponds to independently estimated pollinator abundances in the community (Maruyama et al. 2018). We performed an additional analysis to corroborate that using different measures of abundance for hummingbirds and insects did not influence the level of phenological asynchrony between plants and pollinators (Appendix S2).

We found that 98% of all plant species visited by pollinators in the study had a flowering phase lasting >47 d, with a single species lasting less than that (21 d), representing the plant species with the shortest flowering phase. Regarding pollinators, 92% of the total species occurred on >49 d, where the shortest occurrence was 20 and 21 d for two pollinator species. Therefore, a sampling frequency of about 12 d allowed us to obtain reliable phenology estimates, because it is unlikely that we have overlooked any species. As phenology varies not only over time but also over space, it is important to highlight that our sampling design based on a combination of field methods, transects, and plots (Appendix S1: Fig. S1) allowed us to include uncommon plant species and those irregularly distributed in space. We thus accounted for habitat heterogeneity at each study site. Consequently, our sampling provides good representation of the plant and diurnal pollinator species across the páramo ecosystem in Costa Rica, and it is unlikely that the observed phenological patterns were influenced by sampling artifacts that may result in an inflated asynchrony between interacting species.

Statistical analysis

Specialization of pollinator species.—Based on matrices built from plant–pollinator interaction data, we quantified ecological specialization of pollinator species with two different measures: the effective number of partners (e^H) and the mean standardized Kullback–Leibler distance (index d'). We used the exponential of the Shannon diversity index e^H to compute the effective number of plant species visited by each pollinator species (Jost 2006) where we included the total number of visits of a pollinator to a plant species in a given community (Dormann 2011). This measure e^H ranges from 1 (maximum specialization) to >1, with high values indicating many plants being visited relatively evenly by a given pollinator species (i.e., lower specialization). Index d' measures how strongly a given species deviates from an expected random choice of available interaction partners and ranges from 0 to 1, indicating low and high specialization, respectively (Blüthgen et al. 2006, Appendix S3). This index has the advantage over other indices in that it is robust to differences in the number of observations per species and is largely unaffected by the number of interacting species and differences in sampling intensity (Blüthgen et al. 2006). Even so, we corroborated to what extent estimates of ecological specialization were influenced by species rarity, because singletons in particular (those species with a single observed interaction) may appear highly specialized. Thus, we performed a Pearson correlation analysis between both metrics of specialization (e^H and d' values) and log-transformed marginal totals (the total number of observed interactions per pollinator species). We found a strong positive association between e^H and marginal totals ($r = 0.84$, $P < 0.001$), indicating that more abundant pollinator species interact

with more plant partners, which is biologically plausible. In addition, we found that index d' and marginal totals were weakly and negatively correlated ($r = -0.19$, $P = 0.080$), suggesting that rare species were not more ecologically specialized compared to commonly observed species.

Species phenology.—We used the weighted mean day of occurrence (WMD) as a species phenology estimator that consists of the arithmetic mean of all dates on which a given species was observed, weighted by its abundance on each date (Gillespie et al. 2016). This is an unbiased and robust measure considered a better estimator of phenophase compared to other metrics commonly employed, such as first appearance dates (Moussus et al. 2010, CaraDonna et al. 2014). Although WMD can be considered as a point measure, it is important to note that this metric is based on the complete species phenology including relative abundance at each observation date, unlike first occurrence dates and other phenological metrics. For pollinators highly dependent on particular plant species, the WMD of each pollinator is expected to be close to the WMD of the plant species it uses (Benadi et al. 2013). Therefore, at each study site we calculated the absolute difference between the WMD of a given pollinator species and the collective WMD of all the plant species it visited as a measure of *absolute asynchrony* (Fig. 1). The collective plant' WMD for each pollinator species was calculated by summing the flower abundance of all plant species it visited at each date and study site. Because a close match between WMDs of plants and pollinators may be less important for pollinator species that visit plant species with a long flowering phase, we also calculated a *z-score* as an additional measure of phenological asynchrony (Fig. 1). The *z-score* that we named *relative asynchrony* consists of the absolute asynchrony divided by the standard deviation of the collective plant species' WMD (Benadi et al. 2013). This standardized measure of phenological asynchrony takes into account the length of the flowering phase of plant species, because the difference in WMDs is expressed relative to a measure of the length of the plants' flowering period (i.e., the standard deviation of WMD). To test whether WMD of pollinators and plants differ significantly, we performed a linear mixed effect model (LMM) with WMD of pollinators as response variable, WMD of plants as fixed effect, and site and species identity as random effects to account for variation in these two factors. We also calculated a measure of the phenological breadth of plant species based on the number of days between the 5 and 95% quantile of flowering plant observations. We did not consider the entire range, to avoid the influence of extreme values that could lead to misinterpretation of the results.

Relationship between species phenology and specialization.—We fitted in total six separate LMMs for the three phenological estimators (i.e., *absolute* and *relative*

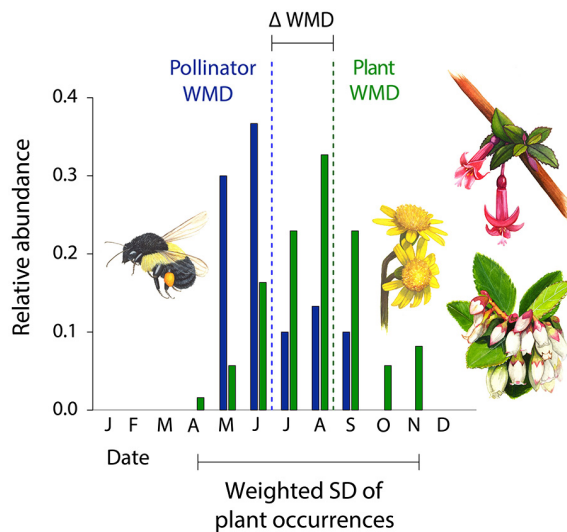


FIG. 1. Phenology of a hypothetical pollinator species and all the flowering plant species it visited in a given community. Information relative to the pollinator is indicated in blue and to the plants with green. Bar plots show the relative abundance of the pollinator and the flowers of plant species visited over time (months of the year). Dashed lines mark the weighted mean day of occurrence (WMD) of the pollinator species and the collective WMD of the plant species visited. The difference between these two WMDs (Δ WMD) indicates the absolute asynchrony in number of days between the pollinator and plant species. The weighted standard deviation (SD) of the visited plant occurrences is used as a measure of the length of the plants' flowering period. The relative asynchrony (z -score) is a standardized measure, because the absolute asynchrony is expressed in terms of the flowering length (Δ WMD/SD of the plant's WMD). If the pollinator visited a distinct set of plant species with the same collective WMD of the plant species visited, but larger SD than the set of plant species depicted in the plot, the result would be a lower relative asynchrony, showing that it is less important in this case whether the WMD of the pollinator exactly matches the WMD of the plants.

asynchrony, and flowering phenological breadth) and the two measures of ecological specialization (index d' and e^H). In these models, we used the phenological estimator as response variable, and ecological specialization and pollinator order as fixed effects. We accounted for random variation among the three networks by including site and pollinator identity as random effects in all six models. We used square-root-transformed *absolute asynchrony* and *relative asynchrony* to normalize model residuals. We used automated selection to identify the most parsimonious model according to the corrected Akaike information criterion (Δ AIC_c to all other models >2). For each of the six LMMs, we fitted a full model that included the two fixed effects (specialization and pollinator order) and the interaction term. We compared the performance of the full model to all possible alternative model combinations with different fixed effect structures. For model comparisons, parameters were estimated with maximum-likelihood approximation, whereas for the best-fitted model, restricted maximum

likelihood was used (Cousineau and Allan 2015). We used *pseudo-R*² as goodness of fit statistic (Nakagawa and Schielzeth 2013) where marginal $R^2_{(m)}$ indicates the variance explained by the fixed effects and conditional $R^2_{(c)}$ the variance explained by both fixed and random effects. To corroborate whether species rarity could have influenced our results, we performed the same models excluding the five singletons in the study and found that the overall relationship between phenology and ecological specialization remained similar (Appendix S2: Table S1). All statistical analyses were conducted in the R programming environment (version 3.4.0, R Development Core Team 2015); functions and packages are listed in Appendix S4.

RESULTS

Patterns of species interactions and specialization

We observed a total of 2,300 plant–pollinator interactions between 59 plant and 39 pollinator species (see Appendix S5: Tables S1, S2, for complete species lists). Of these, four were hummingbird species and 35 insect morphospecies, with 13 and 15 species that occurred in two and three sites, respectively. Networks at each site included 36×28 , 29×27 , and 42×27 (plant \times pollinator) species at Reserva, Sakira, and Buena Vista, respectively. Specialization of pollinator species per site ranged from completely generalized ($d' = 0.003$) to specialized ($d' = 0.689$) and the effective number of partners (e^H) ranged from 1 to 14 (Appendix S5: Table S2).

Phenological synchrony between plants and pollinators

Flower abundance varied significantly among plant species, study sites, and sampling periods ($F_{94, 1,604} = 29.82$, $F_{2, 1,604} = 8.42$ and $F_{22, 1,604} = 3.58$, respectively, $P < 0.001$ for all three variables). The phenological estimator WMD of pollinator species and the collective WMD of the plant species they visited were significantly different, with pollinator species overall occurring 26 d earlier than their main plant species ($\beta = 0.62$, $t = 4.60$, $P < 0.001$). The average differences between both WMDs were 17 ± 11 , 24 ± 17 , and 38 ± 13 d (estimate \pm SE) at Reserva, Sakira, and Buena Vista, respectively. We did not detect differences in asynchrony among pollinator orders, that is, the difference between the WMD of pollinators and plants (*absolute asynchrony*) as well as the asynchrony relative to the flowering length (*relative asynchrony*) was similar among the four pollinator orders (Fig. 2).

Species phenology and specialization

We found the general pattern that more ecologically specialized pollinator species were more asynchronous and visited plant species with shorter flowering phases compared to generalized pollinator species. Specifically,

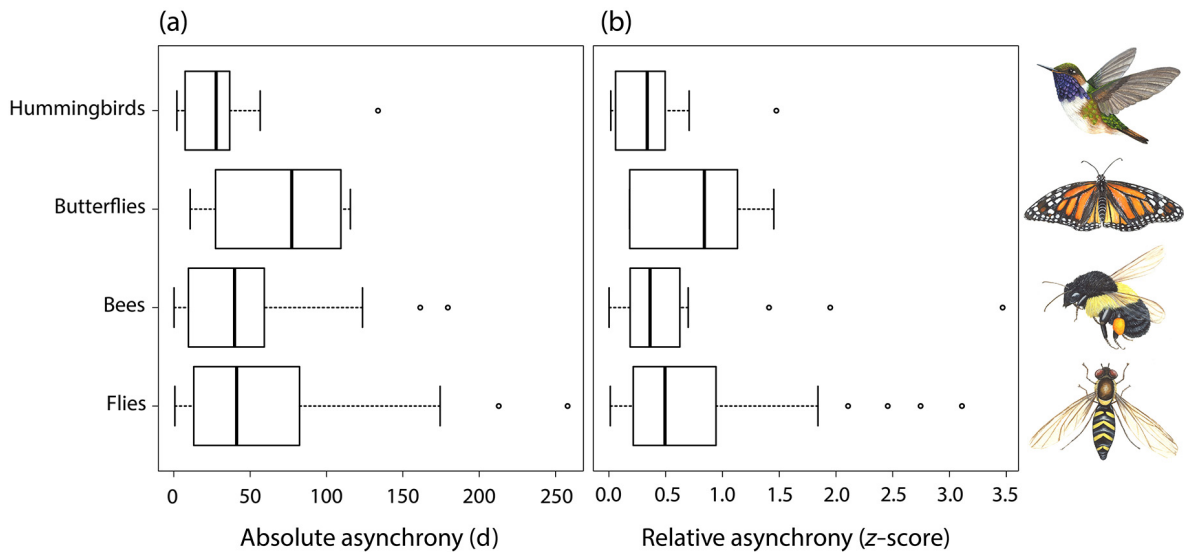


FIG. 2. Phenological asynchrony between pollinator species and the plants they visited at three study sites in a neotropical mountaintop ecosystem, Costa Rica. Box plots represent (a) the absolute asynchrony as the number of days and (b) the relative asynchrony as z-score values of each species at a given site ($n = 39$ species; 13 and 15 species occurred at two and three sites, respectively). There were no differences in the level of absolute or relative asynchrony among pollinator orders. The absolute asynchrony is given by the difference between the weighted mean day of occurrence (WMD) of each pollinator species and the collective WMD of the plant species it visited (see Fig. 1). The relative asynchrony is expressed in terms of the flowering length of the plant species visited and, thus, calculated as the absolute asynchrony divided by the standard deviation of the collective WMD of the plant species. Zero values indicate perfect synchrony, whereas values larger than zero indicates pollinators occurring asynchronously regarding the flowering plants visited. Data are pooled by pollinator order for concision. Thick lines across boxes are medians; boxes indicate 25th and 75th percentiles; whiskers indicate the data range and circles are outliers.

TABLE 1. Statistics of best models of the relationship between species phenology and ecological specialization of pollinator species in plant–pollinator networks in a neotropical mountaintop ecosystem, Costa Rica.

Phenological estimator	Measure of ecological specialization of pollinator species									
	Index d'					Effective number of partners (e^H)				
	β	P	$R^2_{(m)}$	$R^2_{(c)}$	VR (%)	β	P	$R^2_{(m)}$	$R^2_{(c)}$	VR (%)
Absolute asynchrony	1.30	0.073	0.03	0.62	62	-0.08	0.047	0.07	0.52	46
Relative asynchrony	2.00	0.005	0.08	0.65	64	-0.13	0.002	0.15	0.51	38
Phenological breadth	-3.23*	<0.001	0.21	0.56	18	-0.03	0.391	0.01	0.42	27

Notes: We used three estimators of species phenology and two measures of pollinator specialization. Identity of pollinator species ($n = 39$) and the three study sites were included as random effects to account for random variation among the three networks; 13 and 15 species occurred in two and three sites, respectively. One of the six best models included pollinator order as predictor variable (*); only statistics of the main factor are reported in this case. Parameter estimates are based on scaled values (mean zero and unit variance) to make models more comparable. Shown are marginal and conditional R^2 as goodness of model fit and variance of the random effect pollinator species identity (VR). Models with significant relationships between phenological estimator and specialization index are shown in bold ($P < 0.05$).

we found that *relative asynchrony* was significantly associated with both measures of specialization (Table 1, Fig. 3a, b). Moreover, we found a negative significant association between *absolute asynchrony* and the effective number of plant partners (e^H) of pollinator species, whereas a positive, but nonsignificant result was observed for the relationship between *absolute asynchrony* and specialization index d' (Table 1). In addition, we found a negative significant relationship between

flowering phenological breadth and index d' , that is, more specialized pollinator species visited plant species with shorter flowering phases compared to generalized species (Table 1, Fig. 3c).

DISCUSSION

Based on detailed field data and robust phenological measures, we found a pattern of temporal asynchrony

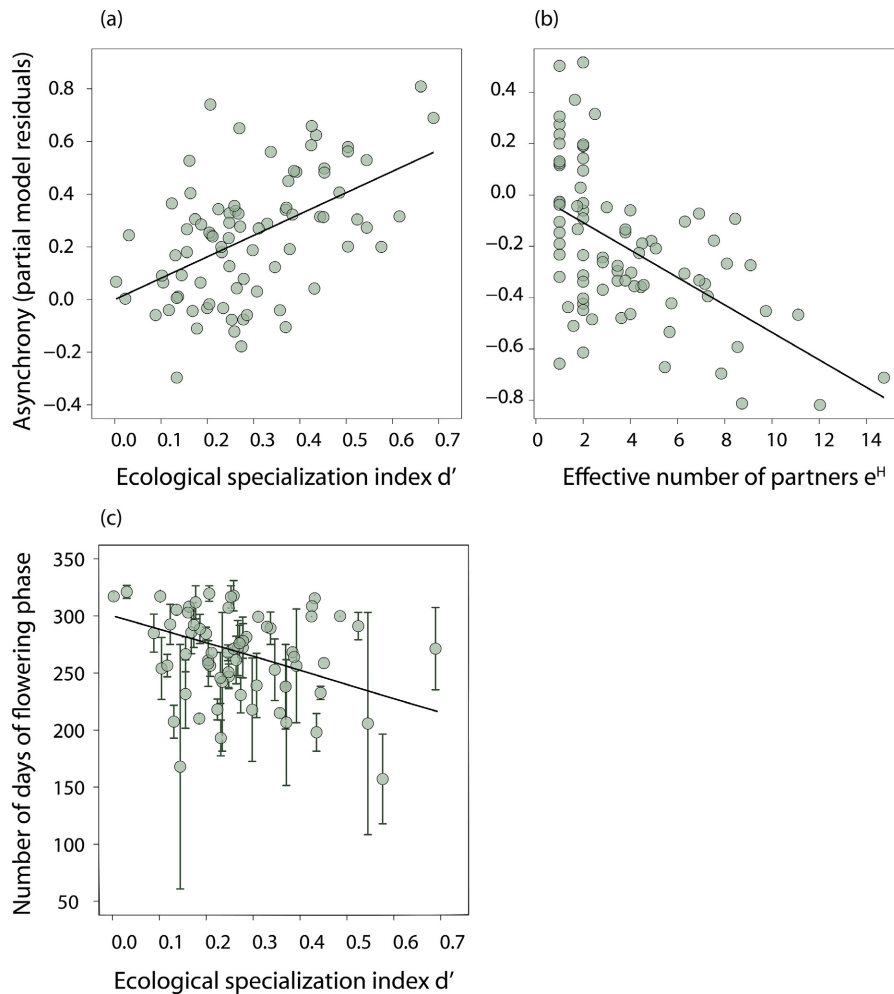


FIG. 3. Relationship between different measures of species phenology and ecological specialization of pollinator species at three communities in Costa Rica. Each data point represents one pollinator species at a given site ($n = 39$; 13 and 15 species occurred at two and three sites, respectively), whereas lines depict the estimates of the best model. (a,b) To better represent the relationship between relative asynchrony and both indices d' and e^H , given the high influence of species identity as random effect we had in these two models, we plotted the partial residuals from the LMM against the predictor variable instead of the observed values. The partial residuals were calculated by the formula: $r_i + e^*x_i$, where r_i is the residual for the i th observation of phenological asynchrony, e is the model estimate for ecological specialization (index d' or e^H) and x_i is the i th observation of index d' or e^H . (c) We computed the mean number of days between the 5th and 95th quantiles of the flowering phase of plant species across all observed interactions at a given site against the respective pollinator specialization value. Standard deviation (\pm SE) of the mean values is shown for pollinator species with more than one observed interaction, and the absence of SE corresponds to singleton species.

between interacting species in plant–pollinator networks with pollinators overall occurring 17–38 d earlier than the flowering of the main plant species they visited. Our findings also reveal that ecologically more specialized pollinator species were more asynchronous with their food plants. Moreover, ecologically specialized pollinators visited plant species with relatively shorter flowering breadth compared to generalized pollinators. Taken together, these findings point toward ecologically specialized species being at greater risk of experiencing phenological mismatch.

Albeit asynchronous phenophases between plant species and their pollinators have been documented in some

studies, we show higher levels of phenological asynchrony in plant–pollinator networks compared to previous research where the level of asynchrony was 10–11 d (e.g., Benadi et al. 2013, Kudo 2014). Although Benadi et al. (2013) found plants that occurred ahead of their pollinators, our results are consistent with Kudo (2014), where pollinators occurred earlier than their plants. It has been found that phenological shifts are highly species specific with some species advancing their activities and some delaying (Solga et al. 2014). Therefore, there is no clear pattern of divergence between plants and pollinators (Bartomeus et al. 2011) and the direction of

phenological asynchrony will greatly depend on whether interacting species respond similarly to the same environmental cues. The high level of asynchrony found in our study may be a consequence of a less predictable occurrence of food resources for pollinators in mountain-top ecosystems resulting from more changeable and extreme environmental conditions compared to lowlands (Wright et al. 2015). Our findings of high spatial and temporal variation in flower abundance may be the result of the highly variable climate conditions in the páramo (Kappelle and Horn 2016). Other studies have also found great variation in flowering phenological patterns in highlands and suggested unreliable food scenarios for pollinators (Fagua and Gonzalez 2007, Wright et al. 2015). It is an open question whether plant and pollinator species in the highlands are adapted to such a level of phenological asynchrony, or whether current climate change has already exaggerated the asynchrony, as observed in some high-latitude regions (Kudo and Ida 2013, Schmidt et al. 2016). Whether pollinators in the páramo might be experiencing a climate-change-induced phenological advance greater than their plants should be addressed with multi-year phenological and climate data.

Contrary to our expectation, our results show that more specialized pollinator species were more asynchronous with the plant species they visited (had less temporal overlap). These results contrast with earlier studies where generalized and specialized pollinator species showed similar levels of asynchrony (Benadi et al. 2013, Donoso et al. 2016). It is important to notice that ecologically specialized pollinators prefer specific resource types (Pemberton 2010, Temeles et al. 2010, Dalsgaard et al. 2018). This suggests that ecologically specialized species are more adapted to their partners because of a tight trait matching between them (Temeles et al. 2010, Maglianesi et al. 2014) and, hence, may not be able to easily switch to other resources (Dalsgaard et al. 2018). Thus, phenological asynchrony to the resource plants is more likely to occur for specialized than generalized species (Memmott et al. 2007, Miller-Rushing et al. 2010). This lack of flexibility in establishing new interacting partners may cause specialized pollinators to visit particular plant species, even if these plants have a low phenological synchrony with those specialized pollinators.

We also found that ecologically specialized pollinators visit plant species with short flowering phases. This finding is closely related to the fact that more specialized species were significantly more asynchronous when we used relative asynchrony but not when we used absolute asynchrony. Because specialized pollinator species visit plants with shorter collective phenophases compared to generalized species, relative asynchrony of specialized species will be larger. It is important to highlight that the risk of phenological mismatch for pollinators increases when flowering periods of interacting plant species are short (Miller-Rushing et al. 2010). Hence, if species phenologies change within a short time period, specialized

pollinators also have a greater probability of phenological mismatch, especially as they are expected to have a reduced potential to switch to other plant partners (Memmott et al. 2007, Dalsgaard et al. 2018). Ecologically specialized pollinator species are therefore more sensitive to uncoupling with their food resources.

In previous research, it has been proposed that the severity of the consequences of asynchrony for pollinators is likely to be amplified by the degree of specificity in resource use (Hodkinson et al. 2011). This suggests that even small changes in environmental conditions could enhance phenological asynchrony between specialized species and their plants, which might translate into severe consequences in terms of both plant and pollinator fitness (Kudo and Ida 2013, Schenk et al. 2017). This stresses that different risks are additive; that is, specialists are prone to extinction because of multiple threats and potentially strong constraints in resource use (Biesmeijer et al. 2006, Memmott et al. 2007, Schleuning et al. 2016, Dalsgaard et al. 2018).

Even though we attempted to sample species phenology and interactions comprehensively, we acknowledge that it is difficult to achieve given the complexity of ecological communities. A limitation in our study was a low number of observations for some pollinator species. Estimates of phenology may be rather coarse for these species, which could explain the high amount of variation explained by species identity in some of the statistical models. In addition, specialization might be overestimated for these species, because of insufficient sampling of rare interactions (Vázquez and Aizen 2003, Dorado et al. 2011). However, we consider it very unlikely that species rarity has influenced the results of our study because (1) we had fewer singletons (13%) compared to other studies (e.g., 23%, Olesen et al. 2008; 46%, Bartomeus 2013); (2) we used a quantitative approach that has been shown to generate less biased specialization metrics (Blüthgen et al. 2007); (3) the robust measure index d' we used to quantify ecological specialization already accounts for differences in sample size; (4) we performed additional analyses to test for the influence of species rarity on specialization and its relationship with species phenology. We also observed few species in two of the four animal orders, which is a consequence of the low species diversity in highland ecosystems compared to the species-rich tropical lowlands (Maglianesi et al. 2015). Therefore, both few individual and species observations reflect the natural situation in tropical mountaintop ecosystems.

CONCLUSIONS

Our findings reveal that phenological synchrony between interacting partners differs between specialized and generalized species in mutualistic interaction systems. These findings suggest an increased vulnerability of specialized pollinator species to mutualistic disruption, because of high levels of phenological asynchrony

with their floral resources. In a time characterized by climate-induced phenological changes, this knowledge is highly important to assess the risk of mutualistic disruption between species and for unraveling how ecological specialization and phenological asynchrony might constrain the potential for reorganization of plant–pollinator interactions in the future.

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