

SPECIALIZATION CLINES IN THE POLLINATION SYSTEMS OF AGAVES (AGAVACEAE) AND COLUMNAR CACTI (CACTACEAE): A PHYLOGENETICALLY CONTROLLED META-ANALYSIS¹

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The biogeography of plant–animal interactions is a novel topic on which many disciplines converge (e.g., reproductive biology, biogeography, and evolutionary biology). Narrative reviews have indicated that tropical columnar cacti and agaves have highly specialized pollination systems, while extratropical species have generalized systems. However, this dichotomy has never been quantitatively tested. We tested this hypothesis using traditional and phylogenetically informed meta-analysis. Three effect sizes were estimated from the literature: diurnal, nocturnal, and hand cross-pollination (an indicator of pollen limitation). Columnar cactus pollination systems ranged from purely bat-pollinated in the tropics to generalized pollination, with diurnal visitors as effective as nocturnal visitors in extratropical regions; even when phylogenetic relatedness among species is taken into account. Metaregressions identified a latitudinal increase in pollen limitation in columnar cacti, but this increase was not significant after correcting for phylogeny. The currently available data for agaves do not support any latitudinal trend. Nectar production of columnar cacti varied with latitude. Although this variation is positively correlated with pollination by diurnal visitors, it is influenced by phylogeny. The degree of specificity in the pollination systems of columnar cacti is heavily influenced by ecological factors and has a predictable geographic pattern.

Key words: Agavaceae; agave; Cactaceae; columnar cactus; comparative method; generalization; meta-analysis; phylogenetic meta-analysis; pollen limitation; pollination system; specialization.

A major question in plant biology is how plant–animal interactions have evolved (specialized vs. generalized) and how the outcome of these interactions varies geographically (Thompson, 2002). The study of the geographic patterns of plant–animal interactions is interesting to a wide group of plant biologists studying the biogeography, ecology, evolution, and reproductive biology of plants. The plant–pollinator interaction is one of the most studied of these interactions because of its importance in the reproduction of wild plants and crops. The predictive value of floral syndromes and its implicit trend toward an association with specialized pollinators was, however, seriously questioned in the mid-1990s (Waser et al., 1996), leading to an intense discussion among pollination biologists. The debate has focused on the frequency of occurrence of specialized pollination systems in flowering plants. While more classical views held that there should be a general trend toward specialization in angiosperms because floral syndromes constrain the assem-

blage of flower visitors, others argued that “generalization in pollination systems appears to be the rule more than the exception” (Waser et al., 1996; Olesen and Jordano, 2002). An emerging view in pollination biology claims that plants occupy virtually every point along the continuum from extreme generalization to extreme specialization (Johnson and Steiner, 2000) and that the spatiotemporal predictability of pollinators has the most important effect on the degree of specificity in pollination systems (Gómez and Zamora, 2006).

There has been a considerable number of studies on the mechanisms that shape pollination systems over the last few years (see Waser and Ollerton, 2006 and references therein). However, geographic correlates of specificity in pollination systems have received little attention. Rigorous quantitative approaches were offered by Olesen and Jordano (2002) and Ollerton and Cranmer (2002). These authors came to completely opposite conclusions despite their use of a similar data set. Using 29 published and unpublished data sets for plant–pollinator networks, Olesen and Jordano (2002) stated that tropical plants were indeed more specialized than those of higher latitudes, while Ollerton and Cranmer (2002) concluded that the apparent trend toward ecological specialization in tropical latitudes was an artifact of systematic variation in sampling effort at both the community (29 communities) and species level (103 species of asclepiads). The analyses of Olesen and Jordano (2002) focused on the connectance of plant–pollinator networks (the proportion of all possible interactions within a network that are actually established) as an overall measure of generalization in a given community, but the usefulness of this variable as an indicator of generalization has been vigorously questioned (Kay and Schemske, 2004). The geographic trends presented in both studies (Olesen and Jordano, 2002; Ollerton and Cranmer,

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2002) used the number of visitor species as a measure of pollination system specificity. It is well known that the number of visitors to flowers overestimates the number of effective pollinators because visitors differ greatly in their effectiveness as pollinators (e.g., Adler and Irwin, 2006). Also, these studies did not consider phylogenetic relatedness among species. We would expect more realistic patterns of the geographic distribution of pollination systems to emerge when only those visitors that affect plant reproductive success are taken into account and a comparative approach is used.

Columnar cacti and agaves are distributed throughout the tropical and extratropical regions of the New World. Using bibliographical and herbarium data as well as available studies of columnar cactus pollination biology in the southwestern United States (Alcorn et al., 1959, 1961, 1962; McGregor et al., 1959), Mexico (Valiente-Banuet et al., 1996), and Venezuela (Sosa and Soriano, 1992), Valiente-Banuet et al. (1996) pointed out that the relationship between nectar-feeding bats and columnar cacti was stronger and more tightly coupled in the tropics than in the extratropical deserts of North America, where a more diverse array of visitors had been reported. In that study, the authors also identified chiropterophily as the most common syndrome in columnar cacti and expressed doubt about the migratory latitudinal movements of nectar-feeding bats at tropical latitudes. When we speak of chiropterophily, chiropterophilous syndrome, or chiropterophilous plants, we are referring to species with nocturnal anthesis and whitish, bowl-shaped flowers that produce a strong unpleasant or neutral odor at night and abundant nectar. However, these traits do not imply exclusive pollination by bats. The observations of Valiente-Banuet et al. (1996) were reproduced at other latitudes, and this novel pattern of geographic variation in the columnar cactus pollination system rapidly gained support (Fleming et al., 1996, 2001; Nassar et al., 1997; Casas et al., 1999; Molina-Freaner et al., 2004). The dichotomy between tropical and extratropical columnar cacti with chiropterophilous flowers has been explained as a consequence (ultimate cause) of latitudinal variation in the predictability of pollinator availability throughout the year (Valiente-Banuet et al., 1997a, b), i.e., cacti that are able to receive pollinator service from diurnal visitors and from bats are selected over those whose flowers are only available for nocturnal pollinators, when they face unreliability in the availability of bats, the main pollinators. Such predictability is lower outside the tropics, where nectar-feeding bats are seasonal migrants. In contrast, conspicuous nectar-feeding bats (especially *Leptonycteris* spp.) have resident populations in the tropics (Rojas-Martínez et al., 1999). The degree of specialization in chiropterophilous columnar cacti seems to be related to flower longevity and nectar production, i.e., flowers that are open and produce nectar only at night are limited to nocturnal pollinator guilds, while flowers with more extended longevity and greater nectar production allow both diurnal and nocturnal visitors to pollinate the flowers (proximate causes; Valiente-Banuet et al., 2002). The proximate causes of pollination system divergence have not been tested formally yet. Because agaves overlap extensively in their distribution with columnar cacti and several species have chiropterophilous flowers, some authors have suggested that agaves might follow the same geographic pattern as columnar cacti and that this pattern is explained by similar mechanisms (Molina-Freaner and Eguiarte, 2003). However, latitudinal patterns in the specificity of the pollination systems of agaves and columnar cacti have not been statistically tested.

In contrast to previous attempts to identify geographic trends in the pollination systems of other plant species, exclusion experiments (excluding diurnal or nocturnal pollinators) have been used to assess the ecological specificity in the pollination systems of agaves and columnar cacti, and either fruit set or seed set has been used as unbiased evidence of pollinator effectiveness. When diurnal visitors are excluded, mainly bats pollinate the chiropterophilous flowers of columnar cacti and agaves, and the exclusion of nocturnal visitors generally allows two functional groups (birds and bees) to effectively visit flowers during the day. Chiropterophilous cacti that rely exclusively on nocturnal pollinators are considered bat-specialized, while the contribution of both nocturnal and diurnal visitors to fruit set provides direct evidence of generalization in this pollination system. This pattern has been the focus of nonquantitative reviews (narrative reviews in meta-analysis jargon) for both groups: columnar cacti (Valiente-Banuet, 2002; Valiente-Banuet and Godínez-Alvarez, 2002; Valiente-Banuet et al., 2002) and agaves (Eguiarte et al., 2000; Slauson, 2001; Rocha et al., 2006). Although most studies support the higher degree of specialization to bats in the tropics, recent studies show evidence of the opposite in both agaves and columnar cacti (Ornelas et al., 2002; Estrella-Ruiz, 2005; Rivera-Marchand and Ackerman, 2006). Is the pattern consistent in spite of the exceptions? A meta-analytical approach will allow for the quantitative evaluation of the evidence from available studies and help answer this question.

We analyzed latitudinal variation in the degree of specificity of pollination systems in columnar cacti and agaves. Because their pollination system has been approached experimentally with almost the same procedure and several species and regions have been studied, the studies on agaves and columnar cacti offer an excellent resource for addressing this problem with a meta-analytic approach. Meta-analysis is clearly the appropriate statistical tool when the overall prevalence for a particular trend is to be examined by synthesizing findings across a set of independent studies (Arnqvist and Wooster, 1995). However, because species are not independent from their common ancestry, it is also necessary to incorporate phylogenetic information into this analysis. Until recently, it was not possible to incorporate phylogenetic information into a meta-analysis, but a novel approach has been developed by Verdú and Traveset (2004, 2005). Here, effect sizes are permuted across phylogeny tips and then the permuted values are compared with the real effect size to assess significance. Phylogenetically controlled meta-analysis is without doubt a powerful analytical tool for drawing high-order conclusions about general trends and patterns in a group of phylogenetically related species.

The goal of this study was to test rigorously potential correlates in the degree of specificity in the pollination systems and pollen limitation of agaves and columnar cacti with latitude using fruit set data from different visitors and within a phylogenetic framework. The potential role of floral traits (longevity, nectar production, and morphology) in the definition of pollination system specificity was also explored.

MATERIALS AND METHODS

Study species—*Agave* is the largest genus in the Agavaceae family with 166 species distributed from USA to northern South America; most of its species occur in Mexico (García-Mendoza, 2002). The genus is divided in two subgenera: *Agave* (paniculate agaves) and *Littaea* (spicate agaves). Although in the past *Agave* and *Littaea* were thought to be pollinated by bats and insects,

respectively, recent evidence shows that bats also visit agaves from the *Litsea* subgenus (Rocha et al., 2005). Most species bear protandrous, perfect flowers that live 4–6 d. These plants are typically semelparous at the individual rosette level (Slauson, 2001). Columnar cacti belong to the Cactoidae subfamily (Cactaceae). This group of plants is represented by at least 170 species belonging to 25 genera and four tribes (Browningieae, Cereae, Pachycereae, and Trichocereae). Its distribution ranges from USA to southern South America (Fleming and Valiente-Banuet, 2002). Typically, columnar cactus flowers are chiropterophilous and hermaphroditic, although there are noteworthy exceptions: trioecy in *Pachycereus pringlei* (Watson) Britton & Rose (separate males, females and hermaphrodites; Fleming et al., 1994) and androdioecy in *Neobuxbaumia macrocephala* (Weber ex Schum) Dawson (separate males and hermaphrodites; Valiente-Banuet et al., 1997a). Flowers live less than 24 h, but flower longevity is influenced by environmental temperature and flowers may last longer when the temperature is cooler (Holland and Fleming, 2002). Columnar cacti are iteroparous.

Data set—We searched online data bases (Current Contents: http://www.thomsonreuters.com/products_services/science/science_products/scholarly_research_analysis/research_discovery/current_contents; Scopus: <http://www.scopus.com>) for all published studies using combinations of keywords on the topic (e.g., agave, columnar cactus, pollination, pollination system). Once we had acquired a paper, the references therein were also examined. We also asked colleagues for unpublished data or theses and for their permission to use their data. Our own unpublished data on the pollination biology of *Pilosocereus leucocephalus* (Poselger) Byles & G. D. Rowley were included in the data set.

From the pool of studies, we selected those with data on fruit set under natural conditions (open pollination or control group), from diurnally (nocturnal pollination) or nocturnally bagged flowers (diurnal pollination), and from hand cross-pollinated flowers. Data on flower length (mm), perianth width (mm), flower longevity (the time during which flowers are open, in hours for columnar cacti and in days for agaves), period of nectar production (the time of uninterrupted nectar production by flowers, in hours), and nectar concentration (% sucrose) were also recorded. When the results were graphically presented, we extracted data using a caliper. When latitude was not provided, we obtained it from topographic maps. Because studies reporting significant differences are more likely to be published (Gurevitch and Hedges, 1999), we assessed potential publication bias with funnel plots where a strong deviation from symmetry suggests publication bias. When repeated measures were performed on the same population or on populations that were very close together (less than 6° latitude apart) in a given study, only one cumulative effect size and one mean latitude were calculated. In a study (Casas et al., 1999) in which the authors were interested in populations with different levels of human intervention, we selected the wild populations. We only used the data for individuals with hermaphroditic flowers. With the exception of *Pachycereus pecten-aboriginum* (Engelm. ex Watson) Britton & Rose, and *Stenocereus griseus* (Haw.) Buxb., only one effect size was calculated per cactus species. Two populations of *P. pecten-aboriginum* and *S. griseus* were used. However, they were separated by a considerable distance (9 and 11 degrees of latitude respectively). For *Agave*, we only used two populations for *A. chysantha* Peebles. Thus, we consider our study robust to any deviation from statistical independence because there are only a few species for which more than one population was used, and those populations are sufficiently far away from each other. Because the resolution of our study is at the species level, more than one effect size was calculated from those studies giving information for more than one species (e.g., Valiente-Banuet et al., 1997b).

To test for any effect using meta-analysis, it is necessary to get experimental fruit set data as well as data for the respective control group, in this case, open-pollinated flowers. We used information from 28 populations and 26 species of columnar cacti to test the effect of diurnal pollination, 27 populations and 25 species to test the effect of nocturnal pollination, and 27 populations and 25 species to test the effect of hand cross-pollination. For agave, 12 and 11, 12 and 11, and 12 and 12 sets of populations and species were selected to test, respectively, the effect of diurnal, nocturnal, and hand cross-pollination; these data include *Manfreda virginica* (L.) Salisb ex Rose. The genus *Agave* is paraphyletic to the genus *Manfreda* (Bogler and Simpson, 1996; Good-Avila et al., 2006). Of the 27 species of columnar cactus in the data set, 22 have typical chiropterophilous flowers. Of the 12 different agave species; seven belong to the *Agave* subgenus, four to *Litsea*, and one to *Manfreda*. All *Agave* and *Manfreda* species considered in this study have nocturnal anthesis. Data on flower length, perianth width, flower longevity, duration of nectar secretion, and nectar concentration, were available for 25 and 24, 24 and 23, 27 and 25, 26 and 25, and 23 and 21 populations and species of columnar cactus, respectively. For

agaves, data were available for 20 and 17, 14 and 7, 15 and 14, 19 and 18 and 18 and 17 populations and species, respectively. Sample sizes varied because some studies did not report all the variables we took into account.

Statistical analyses—Meta-analysis requires the results of each experiment to be summarized with an estimate of the magnitude of response to the manipulation: the size of the effect (effect size for short in meta-analysis terminology). As data from columnar cacti and agaves are usually presented in different ways, we used two different metrics. The log of odds ratio was used for columnar cactus data because it is appropriate for fruit set data when presented as the proportion of flowers that set fruit after applying any treatment. And the standard difference was used for agaves because the data given in the research results are mean fruit set per plant. The log-transformed odds ratio estimates the probability of flowers—treated in a particular way—of setting fruit, relative to the observed probability of setting fruit in open-pollinated flowers (control group). The mean standard difference is the difference between the treatment and control means divided by the pooled sample standard deviation. A review of the metrics of effect size that are typically used in meta-analysis is provided by Osenberg et al. (1999). Effect size was weighted by the inverse of its variance. Weighting is the most effective solution when faced with the heteroscedasticity that is typical of ecological meta-analyses (Gurevitch and Hedges, 1999). The log of odds ratio and the standard difference metrics of effect size rank values from negative to positive. When it is close to zero, the effect of treatment is negligible. Positive values indicate that the treatment increases fruit set relative to the control group. When negative, the treatment decreases fruit set. We decided to perform a random-effects meta-analysis. In these models, the true effect size is expected to differ among studies, and the goal of the analysis is to quantify the variation in the effect parameters. Random-effects models are more appealing to ecologists than fixed-effects models because the real effect is often expected to vary among studies (Gurevitch and Hedges, 1999).

We examined three effects: those of diurnal pollination, nocturnal pollination, and hand cross-pollination on fruit set. As in previous studies (Fleming et al., 2001), we took the latter effect to be an estimate of pollen limitation. However, pollen limitation could be confounded with resource reallocation when hand pollination is not performed at the plant level for several reproductive seasons (Knight et al., 2006). This type of treatment is not feasible with giant columnar cacti that can live hundreds of years. In addition, Sutherland (1987) and Holland and Fleming (2002) did not find any evidence of resource reallocation after performing several hand pollination experiments on one species of agave (*A. mackelveyana* Gentry) and one species of columnar cactus (*Pachycereus schottii* Hunt), respectively. The results of these studies led us to think that resource reallocation is not an invariable characteristic of these succulent species. Agaves bloom only once, so allocation to flowering cannot occur between different years. After obtaining the effect size, we modeled its variance using typical metaregressions, where the explanatory variable (or moderator variable in meta-analysis terminology) was latitude (north or south). As meta-analytic data exhibit unequal error variance among studies, in contrast to conventional regression analysis, metaregression models account for this by incorporating a vector of weights—in this case, the reciprocal of the sampling error variance (Hedges and Olkin, 1985). It has been suggested that the pollination system of angiosperms on the whole are more specialized in tropical than temperate regions (Olesen and Jordano, 2002). However, the pattern of latitudinal variation in columnar cactus pollination systems as initially described (Valiente-Banuet et al., 1996) only included chiropterophilous cacti. Because there are some other experimental studies on nonchiropterophilous cacti, we performed the analyses using two subsets of data: the full data set and data on chiropterophilous columnar cacti only, to test whether this pattern could be extended beyond chiropterophilous cacti. We included a single subset for agaves with the factor inflorescence (with two levels, paniculate and spicate) in the model. Because of the phylogenetic position and inflorescence morphology of *Manfreda*, we classified the flowers of these species as spicate. The flowers of agaves and columnar cacti are primarily nocturnal. The role of the floral traits as facilitators of generalization in pollination systems was addressed by running metaregressions between diurnal effect size (response variable) and each floral trait (explanatory variables: flower length and width, flower longevity, duration of nectar production and nectar concentration) in both groups (agaves and columnar cacti).

Descriptive data on floral biology could not be analyzed by meta-analysis because they did not come from experimental or correlational studies. Instead, in separate linear regression models, we used raw data for flower morphology (total length and perianth diameter) and flower longevity, as well as nectar concentration and period of production as the response variable to explore their

relationship to latitude. For columnar cacti, the two subsets of data previously mentioned were used. In all cases, we evaluated models by analyzing residual plots (Crawley, 2002). Typical meta-regressions were performed using R 2.5.1 (R Development Core Team, 2007) helped by the “meta” library developed by Guido Schwarzer (freely available online at <http://www.r-project.org>). See Supplemental Data with the online version of this article for the database of effect sizes and studies (Appendix S1) and their authorship (Appendix S2). Published raw data are available upon request. We are not authorized to distribute unpublished raw data; unpublished data can be obtained by contacting the original authors (contact information is given in Appendix S2).

We also conducted a phylogenetically informed meta-analysis following the approach of Verdú and Traveset (2004, 2005). Incorporation of phylogenetic relatedness is mandatory when several species are being considered to address a research question. Phylogenetic information was implemented in the meta-analysis models helped by the `lm.phylog` function in the PHYLOGR of R software written by R. Díaz-Uriarte and T. Garland (available at website <http://www.r-project.org>). This function allowed us to perform weighted least squares analyses in which the phylogenetic information is included as a variance-covariance matrix. The sizes of the effect (diurnal, nocturnal, and hand cross-pollination) were used as the response variable, and the reciprocal of the effect size variance was incorporated as the vector of weights. Significance tests were obtained by contrasting the observed values against simulated distributions after 1000 permutations of effect size shuffled across the tips of the phylogenetic trees using the PDSIMUL module of the PDAP program (Garland et al., 1992). Because the relationships between floral traits and latitude were not addressed with meta-analysis, we corrected these by using phylogenetically independent contrasts outlined by Felsenstein (1985). Using this procedure, we obtained positivized contrasts that were fitted to a linear regression through the origin (Garland et al., 1992). Because several species considered in our analysis have not been considered in published phylogenies, the real branch length is unknown; therefore, we assigned an arbitrary value of one.

We constructed two composite phylogenies for agaves and cacti, based on published phylogenies and personal communications with specialists. For Cactaceae, we used the phylogenies presented in Arias et al. (2003), Taylor and

Zappi (1989), and Nyffeler (2002). Relationships among agaves are even more poorly understood than those among cacti, so we used the classification scheme of Gentry (1982) for the sections and phylogenies presented in Bogler and Simpson (1996) and Good-Avila et al. (2006). Unknown relationships among species were included as soft polytomies at the generic level. These polytomies reflect the lack of information about the branching pattern instead of a true instantaneous speciation, and they can be handled by correcting the degrees of freedom of the model (Garland and Díaz-Uriarte, 1999) in the PDAP analyses. The phylogenetic trees of *Agave* (Appendix S3) and columnar cacti (Appendix S4) used in this study are available online as supplementary appendices.

RESULTS

All funnel plots were approximately symmetrical, suggesting that there is no strong publication bias for any of the three effect sizes evaluated in agave and columnar cactus. Funnel plots are available as online supplementary material (Appendix S5). Heterogeneity among studies was significant in all cases (agaves: $Q = 43.23 - 53.56$, all P values < 0.01 ; columnar cacti: $Q = 124 - 202$, all P values < 0.01). Therefore, a random-effects model is justified.

Considering the full data set for columnar cacti, the general sizes of the effects (and their 95% confidence intervals) for diurnal, nocturnal, and hand cross-pollination treatments on fruit set were -0.87 ($-1.11, -0.64$), -0.27 ($-0.45, -0.11$) and -0.11 ($-0.34, -0.14$), respectively. Using the subset of chiropterophilous cacti, the effect sizes were greater: diurnal, nocturnal, and hand-cross pollination effect sizes were -0.96 ($-1.26, -0.65$), -0.34 ($-0.58, -0.09$), and -0.17 ($-0.51, 0.17$), respectively. Only the general effect of nocturnal and diurnal pollination

TABLE 1. Results of traditional meta-regressions and phylogenetically controlled meta-regressions (phylogenetic meta-regressions), fitted to assess the relationship between effect size and latitude in two groups of New World succulents: agaves and columnar cacti. For agaves, the factor inflorescence type (spicate or paniculate) was also included in the model. Three effect sizes were examined as the dependent variable: the effect of nocturnal pollinators (“nocturnal”, exclusion of diurnal visitors), diurnal pollination (“diurnal”, exclusion of nocturnal visitors) and hand cross-pollination (“cross”, hand pollination with exogenous pollen). The latter is a potential indicator of pollen limitation. Two data sets—full data and plant species with chiropterophilous flowers—were used for the columnar cacti. Only the full data set was used for analyses with the agave data. The R^2 and slopes are shown only when the results are statistically significant.

Plant group	Analysis	Effect	Data set	Source of variation	Statistics	Slope	R^2
Cacti	Meta-regression	Diurnal	Full	Latitude	$F_{1,26} = 8.31$ **	0.06	0.24
		Nocturnal		Latitude	$F_{1,25} = 1.2$ n.s.		
		Cross pollination	Chiropterophilous	Latitude	$F_{1,25} = 5.67$ *	0.04	0.19
		Diurnal		Latitude	$F_{1,21} = 14.18$ **		
		Nocturnal		Latitude	$F_{1,20} = 2.06$ n.s.		
		Cross pollination		Latitude	$F_{1,21} = 5.06$ *	0.04	0.15
	Phylogenetic meta-regression	Diurnal	Full	Latitude	$F = 30.64$ *		
		Nocturnal		Latitude	$F = 79$ n.s.		
		Cross pollination	Chiropterophilous	Latitude	$F = 54.51$ n.s.		
		Diurnal		Latitude	$F = 31.90$ *	0.02	
Nocturnal	Latitude	$F = 88$ n.s.					
Cross pollination	Latitude	$F = 47$ n.s.					
Agaves	Meta-regression	Diurnal	Full	Latitude	$F_{1,9} = 1.09$ n.s.		
				Nocturnal	Inflorescence		
		Cross pollination		Latitude	$F_{1,9} = 0.35$ n.s.		
				Inflorescence	$F_{1,9} = 0.20$ n.s.		
		Diurnal		Latitude	$F_{1,9} = 1.77$ n.s.		
				Inflorescence	$F_{1,9} = 0.83$ n.s.		
Agaves	Phylogenetic meta-regression	Diurnal	Full	Latitude	$F = 57.27$ n.s.		
				Nocturnal	Inflorescence		
		Cross pollination		Latitude	$F = 37.94$ n.s.		
				Inflorescence	$F = 18.33$ n.s.		
		Diurnal		Latitude	$F = 1.36$ n.s.		
				Inflorescence	$F = 0.59$ n.s.		

Notes: ** $P < 0.01$, * $P < 0.05$, n.s. = no significant slope. Sample size = degrees of freedom in denominator of meta-regressions + 2, sample size was the same in the phylogenetic meta-regression. Degrees of freedom and R^2 are not applicable in phylogenetic meta-analysis.

differed statistically from zero ($P < 0.01$) with both data sets. The general sizes of the effects are the cumulative effect sizes from all the studies to assess the overall effect of the treatment without considering the variance explained by any moderator variable (e.g., latitude). Using typical meta-regressions, we found that latitude significantly predicted the effects of diurnal pollination and hand cross-pollination on columnar cactus fruit set. The coefficient of determination of this relationship was greater when using chiropterophilous cacti than with the full data sets (Table 1). The effect size for both diurnal pollination and hand cross-pollination increases as latitude increases. Fruit set resulting from diurnal pollination is much lower than it is in open-pollinated flowers in the tropics, and it increases linearly with latitude (Fig. 1A). Although the effect of nocturnal pollination on fruit set apparently exhibits the opposite trend (to diurnal pollination), this pattern was not statistically significant (Fig. 1B). Apparently, fruit set resulting from hand cross-pollination is almost the same as that observed in open-pollinated flowers in the tropics, but this manipulation consistently produces higher fruit set than open-pollinated flowers as latitude increases (Fig. 1C). When we account for phylogeny, the latitudinal increase in the effect of diurnal pollination on fruit set is consistent considering both data sets: the full data set and chiropterophilous cacti. The latitudinal trend we identified by conventional meta-regression in the effect of hand-cross pollination was no longer significant when we corrected for phylogeny (Table 1).

For the agaves, the general effect sizes of nocturnal, diurnal, and hand cross-pollination were -1.567 (-2.900 , -0.233), -1.796 (-3.242 , -0.349), and 0.336 (-0.207 , 0.879), respectively. Generally, the effects of nocturnal ($P = 0.021$) and diurnal pollination ($P = 0.015$) on the fruit set of agaves were statistically different from zero. However, effect sizes were not explained by latitude or inflorescence type, and these results did not change after phylogenetic relatedness was taken into account (Table 1).

Among the floral traits considered in our analysis, only the variance in flower longevity and the duration of nectar production were significantly and positively explained by latitude for columnar cacti. These relationships are still significant after performing phylogenetically independent contrasts. The determination coefficients are still very similar as well (Table 2). For agaves, only flower length correlates positively with latitude. This pattern is consistent even after conducting the analysis with phylogenetically independent contrasts (Table 2).

The results of the typical meta-regressions suggest that the duration of nectar production by the flowers of columnar cacti was positively related to the effect of diurnal pollination on fruit set, using both data sets. However, when we accounted for phylogenetic relationship among species in the analysis, this relationship was no longer significant. No explanatory variable accounts for the variation in the effect of diurnal pollination on fruit set in agaves (Table 3).

DISCUSSION

Our study is an important contribution to the biogeography of plant–pollinator systems because we used data from effective pollinators rather than visitors and took into account the phylogenetic relatedness among species. This study is a rigorous test of latitudinal patterns in the degree of specificity of the pollination systems of columnar cacti and agaves—two groups

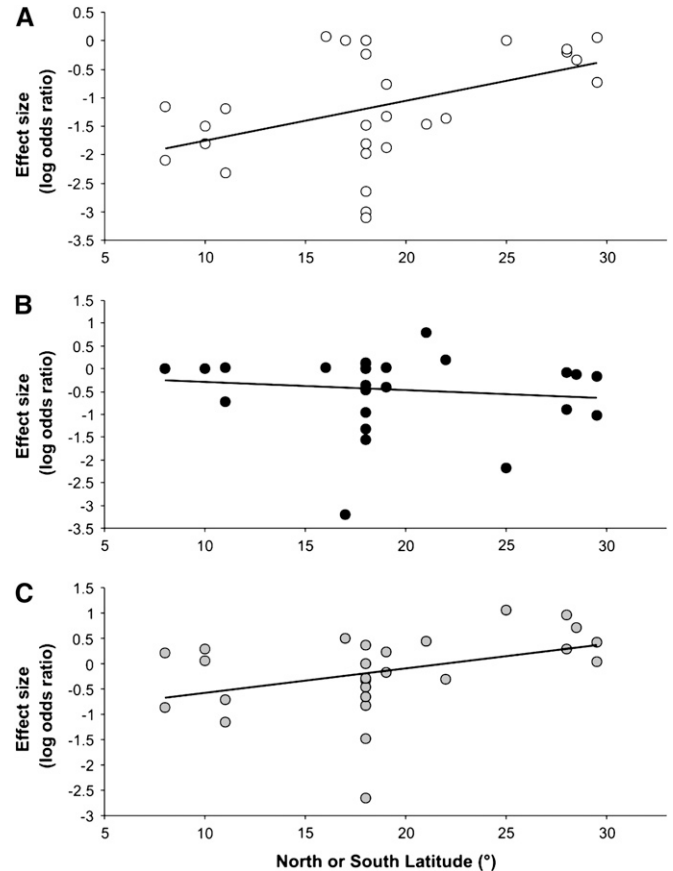


Fig. 1. Linear relationship between latitude and the effect size of (A) diurnal pollination, (B) nocturnal pollination, and of (C) hand cross-pollination. According to typical meta-regressions, for the trends shown in (A) and (C) the slope is different from 0. However, phylogenetically informed meta-regression suggests that only the relationship in (A) has a slope different from 0. Although the slope in (B) appears to be negative, it was not statistically significant using either meta-regression or phylogenetically informed meta-regression. The regression lines were drawn using the full data set.

of plants sharing the chiropterophilous syndrome—as well as a test of potential causes. The quantitative and comparative analysis of evidence shows that there is a latitudinal and clinal variation in the relative contribution of diurnal visitors to the fruiting success of columnar cacti (but not of agaves): the contribution of diurnal pollinators to columnar cactus fruit set decreases as latitude decreases. However, the relative contribution of nocturnal visitors to fruiting does not have a slope different from zero. The effect of nocturnal visitors on fruit set does not vary in a predictable way, whereas diurnal visitors only play an important role as pollinators in extratropical latitudes. Therefore, extratropical columnar cacti have generalized pollination where bats and diurnal visitors yield fruit, while tropical columnar cacti are bat-specialized. Essentially, those mechanisms that allow the participation of diurnal visitors in reproductive success might be proximate causes of the shift toward generalization in the pollination systems of these plants. Among the floral traits considered in this study, duration of nectar production seems to be a causal factor of the shift toward generalization in the pollination system of columnar cacti. However, the relationship between this trait and the effect of diurnal visitors on fruit set is

TABLE 2. Results of linear statistical modeling to assess the relationship between floral traits (response variable) and latitude (explanatory variable) for two groups of New World succulents: columnar cacti and agaves. Two analyses were done, typical linear regression and phylogenetically independent contrasts fitted to linear models through the origin (PICs). Statistics, slopes, and R^2 (the latter two shown only when the slope was significantly different from 0) are presented for each model.

Plant group	Analysis	Response variable	Statistic	Slope	R^2
Cacti	Linear regression	Flower length	$F_{1,23} = 2.11$ n.s.		
		Perianth width	$F_{1,22} = 1.37$ n.s.		
		Flower longevity	$F_{1,22} = 3.81$ *	0.71	0.14
		Nectar production	$F_{1,24} = 6.58$ **	0.83	0.22
		Nectar concentration	$F_{1,21} = 3.08$ n.s.		
Cacti	PICs	Flower length	$F_{1,16} = 2.36$ n.s.		
		Perianth width	$F_{1,15} = 3.82$ n.s.		
		Flower longevity	$F_{1,18} = 4.08$ *	0.20	0.14
		Nectar production	$F_{1,18} = 8.24$ *	0.32	0.25
		Nectar concentration	$F_{1,14} = 1.09$ n.s.		
Agaves	Linear regression	Flower length	$F_{1,18} = 15.07$ **	-0.27	0.45
		Perianth width	$F_{1,15} = 3.82$ n.s.		
		Flower longevity	$F_{1,13} = 0.01$ n.s.		
		Nectar production	$F_{1,17} = 0.83$ n.s.		
		Nectar concentration	$F_{1,16} = 3.08$ n.s.		
Agaves	PICs	Flower length	$F_{1,7} = 18.57$ **	-1.45	0.51
		Perianth width	$F_{1,3} = 0.37$ n.s.		
		Flower longevity	$F_{1,6} = 0.07$ n.s.		
		Nectar production	$F_{1,8} = 0.83$ n.s.		
		Nectar concentration	$F_{1,7} = 2.41$ n.s.		

Notes: ** $P < 0.01$, * $P < 0.05$, n.s. = no significant slope. Sample size = degrees of freedom in denominator + 2 of linear regressions, sample size in PICs is the number degree of freedom + 2 + number of polytomies in phylogeny.

heavily influenced by phylogeny. Using a novel approach, we have substantiated quantitatively the pattern reported by Valiente-Banuet et al. (1996) on latitudinal variation of columnar cactus pollination system.

Valiente-Banuet et al. (2002) described a latitudinal variation in the degree of specificity of pollination system in columnar cacti as a dichotomy. We showed that this variation is clinal. That means that causal factors must exhibit continuous variation. In their 2002 qualitative review, Valiente-Banuet et al. (2002), on the basis of several field observations of floral biology, proposed (without testing) that two proximate mechanisms underlie the latitudinal dichotomy exhibited by the pollination systems of chiropterophilous columnar cacti: the variation in flower longevity and the duration of nectar production. Certainly, these floral traits do vary with latitude: both flower longevity and the time during which flowers produce nectar increase with latitude (Table 2). However, this relationship does not necessarily imply that these floral traits are associated with variations in the pollination system of columnar cacti. The real causes of variation in the pollination system of cacti must be those related to effect sizes; specifically, those related to the effect of diurnal pollination, which is the only size of the effect that significantly covaries with latitude. Although the duration of nectar production explained the variation in the effect of diurnal visitors on fruit set, this relationship lost significance when species relatedness was taken into account, suggesting that phylogeny is strongly influencing this relationship. Nectar production is probably phylogenetically constrained in cacti, as shown for many other groups of plants (Ornelas et al., 2007).

The significant relationship between latitude and the effect of diurnal pollination on columnar cactus fruit set, showed a better linear fit when only data for the chiropterophilous cacti were used. This improvement in model fit suggests that the geographic cline we identified in pollination systems could be restricted to bat-pollinated cacti, as pointed out by Valiente-Banuet et al. (1996). In contrast, the information available to date does

not support previous suggestions that agaves follow a similar latitudinal pattern (Molina-Freaner and Eguiarte, 2003; reviewed by Rocha et al., 2006). This finding was unexpected because columnar cacti and agaves are mainly bat-pollinated and overlap extensively in their geographic distribution. Contrasting flower longevity between agaves and columnar cacti could have influenced this result. Agave flowers live 4–6 d, and as such it is quite difficult to maintain a specialized pollination system. Even though their flowers produce nectar only at night, some traces of this reward (and perhaps some pollen) may be available to diurnal pollinators in addition to nocturnal ones which could eventually pollinate the flower. We also have to recognize that the number of studies that have been done on the agave pollination system is currently small (13 species). That said, we do not know if new studies on agaves would reveal a trend different from that found in this study. At present, we must conclude that the data currently available do not support a similar pattern between agaves and chiropterophilous columnar cacti for latitudinal variation in their pollination systems. We only found a significant negative relationship between flower length and latitude (Table 3), but this variation seems unimportant for the pollination system of *Agave* as a group. Another novelty of our study is the exploration of potential latitudinal patterns in pollen limitation. While a latitudinal increment in potential pollen limitation was identified for columnar cacti through typical metaregression, this pattern was no longer significant after accounting for phylogenetic relatedness among species. Therefore, the susceptibility of being pollen limited varies among the lineages of columnar cacti. The information available to date for agaves does not indicate that there is a geographical pattern in pollen limitation.

In summary, the effect of nocturnal pollination is latitudinally stable while the effect of diurnal pollination is almost negligible in the tropics but increases steadily as we move into extratropical latitudes. In other words, most columnar cacti have a bat-specialized pollination system that exhibits a clinal

TABLE 3. Results of metaregressions and phylogenetically informed metaregression (phylogenetic metaregression), run to test whether floral traits explain the effect of diurnal pollination on the fruit set of two groups of New World succulent plants: agaves and columnar cacti. The effect size of diurnal pollination was the response variable, the explanatory variables were total flower length (“flower length”), perianth width, flower longevity (“longevity”), time at which flowers produce nectar (“nectar production”), and nectar concentration. Perianth width was not tested for the agaves because of the small sample size (3). The slope and magnitude of R^2 are given when results are statistically significant.

Plant group	Analysis	Data set	Source of variation	Statistics	Slope	R^2	
Cacti	Metaregression	Full	Flower length	$F_{1,20} = 1.40$ n.s.	0.12	0.23	
			Perianth width	$F_{1,20} = 44.08$ n.s.			
			Flower longevity	$F_{1,23} = 0.13$ n.s.			
			Nectar production	$F_{1,22} = 6.76^{**}$			
			Nectar concentration	$F_{1,19} = 2.72$ n.s.			
			Chiropterophilous	Flower length			$F_{1,17} = 1.75$ n.s.
				Perianth width			$F_{1,17} = 0.28$ n.s.
	Flower longevity	$F_{1,19} = 3.39$ n.s.					
	Nectar production	$F_{1,19} = 6.76^{**}$					
	Nectar concentration	$F_{1,17} = 2.57$ n.s.					
	Phylogenetic metaregression	Full		Flower length	$F = 48.03$ n.s.	0.14	0.26
			Perianth width	$F = 44.08$ n.s.			
			Flower longevity	$F = 55.76$ n.s.			
			Nectar production	$F = 60.72$ n.s.			
Nectar concentration			$F = 60.72$ n.s.				
Chiropterophilous			Flower length	$F = 44.17$ n.s.			
			Perianth width	$F = 44.17$ n.s.			
	Flower longevity	$F = 51.97$ n.s.					
	Nectar production	$F = 45.91$ n.s.					
	Nectar concentration	$F = 44.53$ n.s.					
	Agaves	Metaregression	Full	Flower length	$F_{1,5} = 0.13$ n.s.		
Flower longevity				$F_{1,11} = 1.75$ n.s.			
Nectar production				$F_{1,11} = 0.28$ n.s.			
Nectar concentration				$F_{1,9} = 0.01$ n.s.			
Phylogenetic metaregression				Full	Flower length		
		Flower longevity	$F = 63.01$ n.s.				
		Nectar production	$F = 57.33$ n.s.				
		Nectar concentration	$F = 44.18$ n.s.				

Notes: $**P < 0.01$, $*P < 0.05$, n.s. = no significant slope. Sample size = degrees of freedom in denominator of metaregressions + 2, sample size was the same in the phylogenetic metaregression. Degrees of freedom are not applicable in phylogenetic meta-analysis

shift toward a more generalized system for cactus populations further away from the tropics. The time during which a flower produces nectar varies latitudinally and is correlated with the effect of diurnal pollination. However, this variation is also influenced by phylogeny. The latitudinal pattern of pollen limitation revealed for the first time by traditional metaregression was not supported when we took ancestry relatedness among cactus species into account. The latitudinal pattern found in columnar cactus cannot be extended to other chiropterophilous plants with extensive distributions, such as agaves. Thus, the factors affecting pollination systems and the responses of plants are complex. With the exception of two studies in the field of plant biology (Verdú and Traveset 2004, 2005), phylogenetic meta-analysis has been greatly underused, thus we hope that our novel application of this technique may be useful in furthering the debate on the general geographic trend of ecological specificity in angiosperm plant–pollinator systems.

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