

Research article

Diversification rates in Antirrhineae (Plantaginaceae): The contribution of range shifts and pollination modes



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ABSTRACT

The relationship between range and diversification is an enduring evolutionary question. The rich angiosperm diversity stems from several factors including variations in geographical distribution and floral morphology, which tend to correlate with different pollinator preference in many taxa. Pollinator diversity varies among different regions, thus range shifts can play an important role in increasing divergence by predisposing clades to floral shifts. Antirrhineae is an ideal model to examine the effect of range shifts and pollination modes on diversification, because the tribe is distributed in the Old World and the New World with representatives exhibiting hummingbird pollination in the New World, and bee pollination in the Old World and the New World. Using ancestral reconstructions and phylogenetic modeling, this study investigated the effects of geographic distribution and pollination modes on the macroevolution of the tribe Antirrhineae. Our age-range correlation analysis showed young nodes had more range overlap than older nodes in the phylogeny, suggesting sympatric speciation as a contributing factor to the diversification of the tribe. We observed similar rates of diversification throughout time with no evident rate shifts within the tribe. Old World and New World lineages have similar speciation rates in Antirrhineae, and pollination mode did not have a significant effect on the diversification of the tribe. However, we found evidence for increased diversification rates through long-distance dispersal events, which occurred between the Old World and the New World four times throughout the evolutionary history of Antirrhineae. Therefore, we conclude that long-distance dispersal was a strong contributor to the diversity within Antirrhineae.

1. Introduction

With over 300,000 species, angiosperms form an extraordinarily species-rich group. This exceptional diversity has generated a wealth of explanatory mechanisms, many of which are not mutually exclusive (Dodd et al., 1999; Chase et al., 2010; Vamosi and Vamosi, 2011; Armbruster, 2014; Tank et al., 2015). Early theories of diversification of angiosperms posited that reproductive features involved with seed production and dispersal were key factors in the evolutionary success of angiosperms (Stebbins, 1981). It has also long been suggested that interactions between flowering plants and pollinators contribute to this diversity (Stebbins, 1981; Kiestler et al., 1984; Armbruster, 2014; Forest et al., 2014; Van der Niet et al., 2014). These interactions include lineage splitting in angiosperm taxa due to pollinator shifts (Forest et al., 2014); pollinator-mediated floral trait divergence and reproductive isolation (Van der Niet et al., 2014); and evolutionary success of angiosperm taxa associated with specialized pollination systems (Hu et al., 2008; Armbruster, 2014).

Underlying these biotic influences on diversification, such as plant-pollinator interactions described above, geographical isolation presents another strong force on diversification (Anacker and Strauss, 2014). The geographical extent of a clade has a significant impact on its diversity, and it is shaped by several factors including geological processes such as continental drift, glaciation, and mountain formation; the clade's ecological niche; and dispersal events (Wiens and Donoghue, 2004). When species are exposed to different environmental factors upon a range shift, they often become subject to different selective forces, favoring a different set of characters than the ones in their previous environment (Sexton et al., 2009). Previous studies have found many factors may increase diversification rates in a lineage upon dispersal into a new region (Moore and Donoghue, 2007). The biotic factors for a plant lineage include the exposure of the species to new ecological niches (Linder, 2008), or simply the lack of competitors in the new region (Sexton et al., 2009). Abiotic factors include a more favorable climate decreasing the environmental stress on the species, thus reducing the risk of extinction caused by harsh environments

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(Cracraft, 1985). Alternatively, allopatric speciation may be facilitated by the availability of fragmented heterogeneous microhabitats in the new environment (Hughes and Eastwood, 2006). It was recently shown that high diversification rates in angiosperms are correlated with dispersal into new regions (Uribe-Convers and Tank, 2015). One of the key traits associated with increased speciation rates in angiosperms is biotic pollination (Dodd et al., 1999). This is suggested to be due to reproductive isolation facilitated by the visitation of different pollinator groups (Kay and Sargent, 2009). The diversity of functional groups of pollinators varies in different regions; making the likelihood that diversification is increased when dispersal is accompanied by exposure to new suites of pollinators, particularly when there is selection for pollinator specialization.

Changes in the composition or abundance of pollinators have been shown to promote angiosperm radiation (Kiestler et al., 1984) through shifts toward alternate pollinators or a reduce reliance of pollinators (Foxe et al., 2009). Plants can be pollinator generalists that attract a broad range of visitors, or pollinator specialists that attract a narrow set of visitors (Waser et al., 1996). If the specialized pollinator is the most abundant or the most efficient pollinator in the area, specialization can be advantageous for plants (Waser et al., 1996). Shifts between specialized systems can promote speciation in flowering plants (Hardy and Otto, 2014). In several angiosperm clades, shifts from bee to hummingbird pollination have been observed in the New World (Beardsley et al., 2003; Wilson et al., 2007; Lara and Ornelas, 2008), and pollinator shifts were associated with high rates of diversification both in the New World (Kay et al., 2005; Schmidt-Lebuhn et al., 2007), and the Old World (Forest et al., 2014).

Depending on the geographic distribution of pollinators, range shifts in plant lineages may be accompanied by shifts in pollination mode. Bees are the most common pollinators in the world, and they were estimated to have originated in the early to mid-Cretaceous, which also corresponds to the early diversification of the angiosperms (Danforth et al., 2006). Hummingbirds on the other hand, experienced a late diversification about 22 mya in South America, and expanded their geographic range to North America and to the Caribbean relatively recently (McGuire et al., 2014). When compared to the bees as a broad functional group that has a worldwide distribution, hummingbirds have a more narrow distribution, limited to the New World (Bleiweiss, 1998). Hummingbirds are effective pollinators with high visual ability and long-term memory, and they are capable of flying long distances, especially in adverse weather conditions when bees are unable to serve as effective pollinators (Cronk and Ojeda, 2008). However, the advantages of hummingbird pollination come with a cost, as hummingbird pollinated plants tend to have showy flowers with large volumes of dilute nectar (Pacini et al., 2003; Cronk and Ojeda, 2008). The costs and benefits of each functional group of pollinators make for greater potential for speciation via pollinator shifts (Forest et al., 2014).

It was previously assumed that the allopatric speciation events represent the dominant speciation mode (Futuyma and Mayer, 1980), but more studies show non-allopatric speciation modes are also common, especially in plants (Savolainen et al., 2006; Grossenbacher and Whittall, 2011). Reproductive isolation is necessary for sympatric species to generate and maintain enough divergence to prevent gene flow large enough to lapse back into a single species (Rieseberg and Willis, 2007). In many cases, closely-related species are isolated by multiple reproductive barriers (Chari and Wilson, 2001). Nonrandom mating due to pollinator preference is a common reproductive isolation mechanism that can facilitate sympatric speciation in flowering plants (Kiestler et al., 1984; Sexton et al., 2013; Forest et al., 2014; Ferris et al., 2014; Van der Niet et al., 2014).

With 27 genera representing 240 species (The Plant List; <http://www.theplantlist.org>), Antirrhineae Dumort. is one of the larger tribes under the family Plantaginaceae Juss (Albach et al., 2005). Antirrhineae has a cosmopolitan distribution, and the tribe is represented in the Old World and the New World (Fig. 1). As one of the biggest

angiosperm diversity hotspots (Thompson, 2005), the Mediterranean Basin has the highest Antirrhineae diversity and abundance in the world (Ogutcen and Vamosi, 2016). In the New World, most of the Antirrhineae taxa are concentrated in the California coast, which also has a Mediterranean climate. Bee pollination is the most common pollination mode in Antirrhineae both in the Old World and the New World, but hummingbird pollination is also observed in several genera in the New World (Elisens and Freeman, 1988).

The wide range of geographical distribution patterns and different pollination modes make Antirrhineae a useful group to study the relationship between long-distance dispersal and pollination in macroevolution, and to understand whether changes in the environment triggers speciation and trait evolution. Using phylogenetic comparative methods, we aim to (i) quantify the evolutionary rate heterogeneity within Antirrhineae to determine whether any taxa experienced different rates than the rest of the tribe; (ii) test whether range overlap increases or decreases with time since divergence within the tribe; and (iii) examine the effects of geographical distribution and pollination syndrome on diversification rates. We hypothesize that transitions from bee to hummingbird pollination, which promotes reproductive isolation thus facilitating sympatric speciation, may be an important factor in Antirrhineae diversification upon dispersal from the Old World to the New World.

2. Materials and methods

2.1. Taxon sampling and phylogenetic analyses

We recently generated an extensive phylogeny of Antirrhineae (Supplementary Figure S1; Ogutcen and Vamosi, 2016). Out of 240 species from 28 genera within the tribe (The Plant List; <http://www.theplantlist.org>), we sampled 146 species (61% coverage) from 24 genera. Node ages were inferred using an uncorrelated lognormal relaxed clock, and the tree was calibrated using the divergence between *Cheanorhinum* and *Linaria* (mean of 23 million years ago with the standard deviation of 4 million years) estimated in a previous study based on fossil data (Bell et al., 2010). We used the Maximum clade credibility (MCC) tree generated from the Bayesian analysis of five molecular markers (*ITS*, *ndhF*, *rbcL*, *rps16*, and *trnL-F*) in all the analyses in this study (see Ogutcen and Vamosi, 2016 for further details about the phylogenetic reconstruction of the tribe).

2.2. Age-range correlations

We collected occurrence data from the Global Biodiversity Information Facility (GBIF) database (www.gbif.org). We excluded duplicate records, records with missing coordinates, and those having coordinates with less than two significant digits after the decimal. We also removed the data points that were outside the native range of a species. After these quality control procedures, we had with 104,318 geographic coordinate data points for 80 out of the 146 species represented in the current phylogeny (55% coverage). Data points were visualized on a world map using the R package ggmap (Kahle and Wickham, 2013). We calculated age-range correlations with the Fitzpatrick-Turelli algorithm (Fitzpatrick and Turelli, 2006) using an R script provided by Dena Grossenbacher (University of Minnesota; Turelli et al., 2014). The environmental data were scaled to 1 km². After generating a geographic range overlap matrix, we used a two-tailed permutation test (Fitzpatrick and Turelli, 2006) to assess the significance of the correlation between the node age and the geographic range overlap.

In order to detect regional differences in age-range correlations, we also analyzed the Old World and the New World taxa separately. Analysis of covariance (ANCOVA) was used to determine whether the degree of age-range overlap was different between the two regions. We set range overlap as the dependent variable, age as the continuous

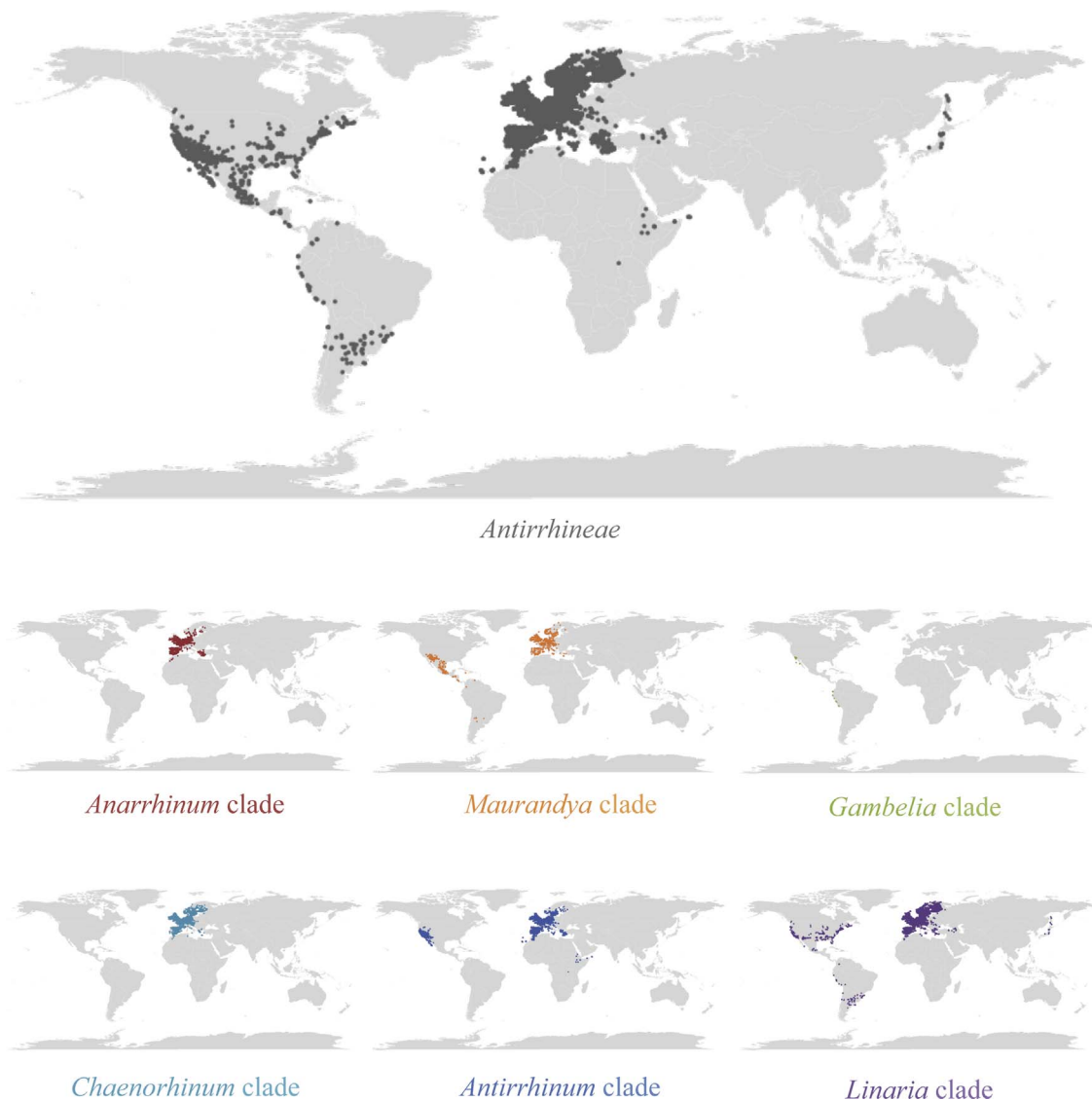


Fig. 1. Geographic distribution of the tribe Antirrhineae. Top panel: distribution map for the tribe as a whole (in gray). Bottom panel: distribution map for each clade. *Anarrhinum* clade: red, *Maurandya* clade: orange, *Gambelia* clade: green, *Chaenorhinum* clade: cyan, *Antirrhinum* clade: blue, *Linaria* clade: purple. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

covariable, and region (Old World versus New World) as the categorical factor. In order to estimate the function of the regression without making an a priori assumption that there is a linear relationship between age and range overlap, we also employed nonparametric regression using locally weighted polynomial regression (LOESS) method (Fox and Weisberg, 2011). We performed Analysis of variance (ANOVA) to determine whether the regression lines for the Old World and the New World were different when compared to each other and to the overall regression line within the tribe.

2.3. Evolutionary rate analysis

We used the Bayesian Analysis of Macroevolutionary Mixtures (BAMM; Rabosky, 2014) program to examine the rates of evolution, and to determine whether the evolutionary rates vary among the clades. In order to account for incomplete taxon sampling, we set the global sampling fraction to 0.61 (146 out of 240 species in Antirrhineae). We executed the speciation/extinction analysis using MCMC simulation with 10,000,1000 generations, and used the R package BAMMtools (Rabosky et al., 2014) to visualize and analyze the BAMM data. We used the R package coda (Plummer et al., 2006) to confirm the effective

size for the MCMC cycles were higher than 200 in order to ensure each run reached convergence to equilibrium. Using the posterior probability of changes in speciation rates within the phylogeny, we generated a set of rate shift configurations, which account for the 95% of the probability of the data. We selected the configuration with the highest posterior probability to determine the number of rate shifts within the tribe, and examined the rate through time plot to observe the speciation rates through evolutionary time.

2.4. Ancestral state reconstruction for pollination

We collected pollination data from various resources based on direct observations, historical surveys, and occasionally, inferences from floral morphology and pollinator community within the studied region (see Appendix A; Proctor and Yeo, 1973; Elisens, 1985, 1986; Elisens and Freeman, 1988; Sutton, 1988; Kampny, 1995; Ghebrehiwet et al., 2000; Fernández-Mazuecos et al., 2013). Bee pollination is the more common pollination mode in the tribe, and bees also visit many “hummingbird pollinated” species. For simplicity, we categorized pollination data into two groups: bee pollination and hummingbird pollination. The bee pollination category represents species that are

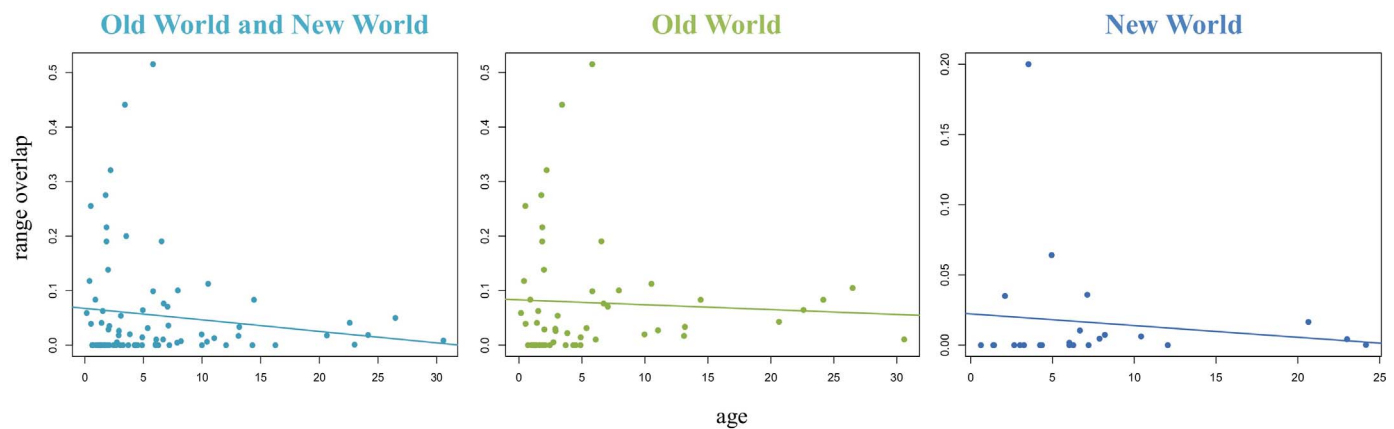


Fig. 2. Age range correlation in Antirrhineae. Data points represent geographic range overlap of the sister taxa and the age (in million years) for each node in the phylogeny.

visited only by bees, whereas the hummingbird pollination category represents species that are visited by hummingbirds and also by bees to some extent.

In order to characterize the pollination history of Antirrhineae, we performed ancestral state reconstructions in Mesquite 3.03 (Maddison and Maddison, 2014). We performed both unordered maximum parsimony (MP) and maximum likelihood (ML) reconstruction. For the ML reconstruction with Markov-k state 1 model, we denoted the presence of hummingbird pollination as 1, and exclusive bee pollination (no hummingbird pollination) as 0.

2.5. Trait-dependent diversification analyses

In order to determine the differences in speciation and extinction rates within the Old World and the New World, we used the “GeoSSE” function (Geographic State Speciation and Extinction; Goldberg et al., 2011) within the R package diversitree (FitzJohn, 2012). We listed species under two categories: New World distributed (A) and Old World distributed (B). The parameters were: speciation within New World (sA), speciation within Old World (sB), between-region speciation (sAB), extinction from New World (xA), extinction from New World (xB), dispersal from New World to New World (dA), and dispersal from Old World to New World (dB). Our sampling was unbiased in terms of the ratio of the Old World species to the New World species. Old World and New World species represent 78% and 22% of our phylogeny respectively. This ratio is comparable to the ratio of the whole tribe (77% Old World species, 23% New World species).

Next, we examined how pollination mode correlates with diversification rates in Antirrhineae using the “BiSSE” function (Binary State Speciation and Extinction; Maddison et al., 2007) within the R package diversitree (FitzJohn, 2012). We coded bee pollination as 0, and hummingbird pollination as 1. We estimated speciation rates in taxa with both traits (λ_0, λ_1), extinction rates in taxa with both traits (μ_0, μ_1), and character state shift rates from one trait to another trait (q_{01}, q_{10}). We tested constrained models with (i) equal speciation rates ($??1 = ??0$), (ii) equal extinction rates ($??1 = ??0$), and (iii) equal character state shift rates ($??10 = ??01$) to test the effects of pollination mode on diversification. We performed an ANOVA to determine whether the effects of different pollination modes on diversification were significant.

In order to examine the effects of incomplete sampling (FitzJohn et al., 2009), we ran the analysis using six different settings: (i) in the “original” setting, the available pollinator data was used without incomplete taxon sampling correction; (ii) in the “phylo” setting, we used the available pollinator data was used with incomplete taxon sampling correction independent of the character states of the missing taxa. The following settings incorporate state-dependent incomplete

taxon sampling corrections based on different assumptions: (iii) in the “realistic” setting, pollination mode was kept consistent throughout a genus (i.e., if a single pollination mode is observed in the genus, then all the species within the genus were assumed to have the same pollination mode. Alternatively, if there are both bee and hummingbird pollination within a genus, then the ratio of bee:hummingbird pollination was kept constant); (iv) in the “more bee” setting, all the unknown pollinators in the New World were assumed to be bee, (v) in the “less bee” setting, all the unknown pollinators in the New World were assumed to be hummingbird; and (vi) in the “all NW bird” setting, all New World species are assumed to be hummingbird pollinated, even if they are observed to be bee pollinated. Two more assumptions were made for all the state-dependent settings: (i) All Old World species are bee pollinated; and (ii) two species that were not in the phylogeny (*Mabrya coccineae* and *Mabrya geniculata*) are hummingbird pollinated (assumption based on Elisens, 1986).

3. Results

3.1. Age-range correlations in Antirrhineae

Age-range correlation analysis showed a significant decrease in range overlap over time in Antirrhineae ($p = 0.0100$; Fig. 2, Table 1). When examined separately, both the Old World and the New World taxa showed negative age-range correlations, but they were not significant ($p = 0.572$ and $p = 0.144$ respectively; Fig. 2, Table 1). The regression lines for the Old World and the New World taxa are significantly different from each other (ANCOVA, $p = 0.0149$; Table 1), with the New World taxa having a steeper decrease in range overlap ($r = -0.00083$) than the Old World taxa ($r = -0.00076$) over time. However, non-parametric regression analysis showed no significant difference between the Old World and the New World Antirrhineae ($p = 0.0998$; Table 1).

3.2. Speciation rates in Antirrhineae

Antirrhineae experienced a slow increase in speciation rates through time (Fig. 3). BAMM analysis revealed three possible configurations for the speciation rate shifts within the tribe (Fig. 3): the no rate shifts configuration was the most probable one ($f = 0.72$), followed by the configuration where *Lafuentea* had decreased speciation rates ($f = 0.15$) and the third configuration where the rest of the tribe (with *Lafuentea* as an outgroup) had increased speciation rate ($f = 0.071$).

3.3. Geographical distribution and pollination mode in Antirrhineae

Out of 146 species in the phylogeny, we retrieved pollinator data for

Table 1

Age-range correlation analysis results. (A) Regression slopes and p values for the age-range correlations. OW: Old World, NW: New World, All: Old World and New World. (B) Age-range correlation comparison between regions. * indicates significance $p < 0.05$. (C) Non-parametric regression age-range correlation comparison between regions.

A.					
	Regression slope			<i>p</i>	
OW	− 0.000755			0.572	
NW	− 0.00829			0.144	
All	− 0.00210			0.0100*	
B.					
	d.f.	Sum sq.	Mean sq.	<i>F</i>	<i>p</i>
OW vs. NW	1	0.0566	0.0566	6.207	0.0149*
Adjusted error	75	0.684	0.00912		
C.					
	ENP	RSS	<i>F</i>	<i>p</i>	
All	4.65	0.744			
OW	4.90	0.623			
NW	4.53	0.0352			
All vs. OW			0.376	0.995	
All vs. NW			1.267	0.173	
OW vs. NW			1.513	0.0998	

97 of them (Supplementary Table S1). 85 species are bee pollinated (58%), 12 are hummingbird pollinated (8%), and the rest of the taxa had no pollination data available (34%; Fig. 4). In terms of geographic distribution, 112 of the 146 species were distributed in the Old World (78%), whereas 22 species were distributed in the New World (22%). In examining the distribution of predominant pollinators in the two geographic regions, we find that bee pollination was observed in 100% of the Old World species, and hummingbird pollination was observed in 44.44% of the New World species.

3.4. Ancestral pollination mode in Antirrhineae

The ancestral pollination mode was inferred to be bee pollination in both MP and ML ancestral state reconstruction analyses (bootstrap value: BS = 93.68%; Fig. 5) Antirrhineae experienced three switches from bee to hummingbird pollination (Fig. 5): (i) *Gambelia-Galvezia* lineage (BS = 53.67%) between 24.69 and 12.35 mya, (ii) *Lophospermum-Mabrya* lineage (BS = 99.44%) between 11.71 and 7.29 mya, and (iii) *Sairocarpus-Galvezia* lineage (BS = 97.04%) between 9.77 and 5.98 mya. One hummingbird to bee pollination transition was observed within the *Gambelia-Galvezia* lineage, when the bee pollinated species *Schweinfurthia papilionaceae* split from its sister species *Gambelia speciosa* 8 mya (Fig. 5). The ancestor of these two sister species were inferred to be hummingbird pollinated (BS = 53.49%).

When the floral color transitions are examined, the switches from bee to hummingbird pollination were mostly accompanied by changes from purple to red floral color (data not shown). Out of 12 hummingbird-pollinated species, nine of them have red-dominant (crimson, red, magenta; collectively called red from now on) flowers, one has yellow-dominant (cream, yellow, brown, orange; collectively called yellow from now on) flowers, and two have purple-dominant (pink, mauve, lilac, violet, purple, blue; collectively called purple from now on)

flowers (Fig. 6). In *Gambelia-Galvezia* lineage, a switch from purple to red flowers was observed along with a bee to hummingbird transition at the ancestral node. The splitting of bee-pollinated *Schweinfurthia papilionaceae* from its sister species *Gambelia speciosa* was coupled with a red to yellow transition. *Lophospermum-Mabrya* lineage was observed to have similar transitions; a purple to red transition at the base, and a red to yellow transition at the node splitting *Mabrya acerifolia* (the only hummingbird pollinated species with yellow flowers) from its sister species *Lophospermum scandens*. In *Sairocarpus-Galvezia* lineage, however, purple remained the floral color for the hummingbird-pollinated *Sairocarpus* species, but *Galvezia juncea* had a switch from purple to red.

3.5. Trait-dependent diversification patterns in Antirrhineae

GeoSSE analysis showed the speciation rates in the Old World were not significantly different from that in the New World ($p = 0.426$; Table 2, Fig. 7). Between-region speciation rates were higher than within-region speciation rates, and dispersals between the Old World and the New World had significant effects on diversification within the tribe ($p = 0.00914$; Table 2). Overall extinction rates were inferred to be low, and the New World extinction rates were shown to be zero. Dispersals were only observed from the Old World to the New World. No significant difference in extinction or dispersal rates between the two regions was observed (Fig. 7).

In terms of the effects of pollination mode on diversification rates, BiSSE analysis showed that the rates of speciation, extinction, and character shifts were not significantly different between bee pollinated and hummingbird pollinated lineages in Antirrhineae, and that employing different settings that incorporated the potential for sampling bias did not have any effect on the results (Table 3).

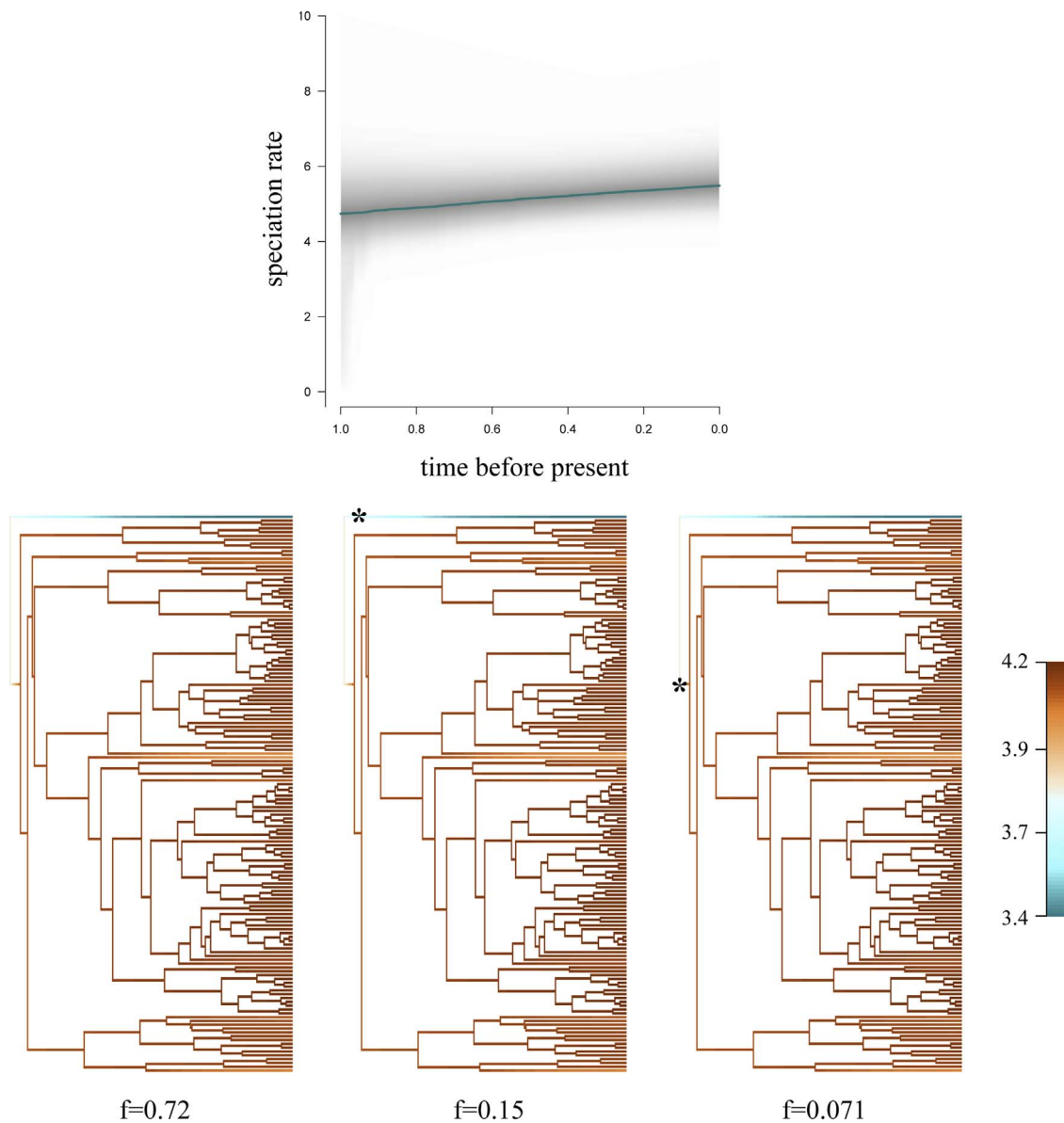


Fig. 3. Speciation rates in Antirrhineae. Top panel: Speciation rate through time. The gray shade around the plot line represents the 95% confidence interval. For simplicity, the node age for the tribe was set to 1. Bottom panel: The most probable speciation rate shift configurations for the tribe. Color scale represents speciation rates. Asterisks indicate where speciation rate changes occurred on the phylogeny. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Discussion

4.1. Sympatric versus allopatric speciation in Antirrhineae

The observed trend that younger nodes have more geographic overlap than older nodes suggests that sympatric speciation may be a significant factor shaping the evolutionary history of Antirrhineae. Because chromosome count changes are common in Antirrhineae (Ogutcen and Vamosi, 2016), sympatric speciation may be facilitated by chromosome count changes acting as a reproductive barrier. While Antirrhineae has also experienced a number of long-distance dispersal events (Ogutcen and Vamosi, 2016), which contributed to allopatric speciation, our analysis reveals that allopatric speciation alone is unlikely to account for all the speciation events in the tribe.

For a long time, allopatric speciation was accepted to be the main mode of geographic speciation, and sympatric speciation remained a controversial concept (Mayr, 1942; Coyne and Orr, 2004). It recently became clear that geographic speciation is not bimodal, but a contin-

uous spectrum (Butlin et al., 2008), and several cases of non-allopatric speciation have been documented in a variety of taxa including ants (Savolainen and Vepsäläinen, 2003), birds (Sorenson et al., 2003), palms (Savolainen et al., 2006), and monkeyflowers (Grossenbacher and Whittall, 2011). However, because geographic ranges can shift over time, the geographic speciation mode can be obscure (Anacker and Strauss, 2014), and making once sympatric species currently allopatric (Graham and Lundelius, 1984), or vice versa (Berlocher, 1998). Throughout its evolutionary history, Antirrhineae has experienced four independent cross-continent dispersal events (Ogutcen and Vamosi, 2016), which might influence the correlation between time since divergence and range overlap. These ancient splits that have zero range overlap will skew the analysis to have the signature of sympatric speciation, and mask the influence of allopatric speciation in the tribe. The presence of Antirrhineae is very dense in Europe, where the most recent glaciation eradicated plant communities in the North, forcing them to shift their ranges to the South in the Quaternary Glacial Period (Takhtajan, 1969). These historical range shifts and local extinctions

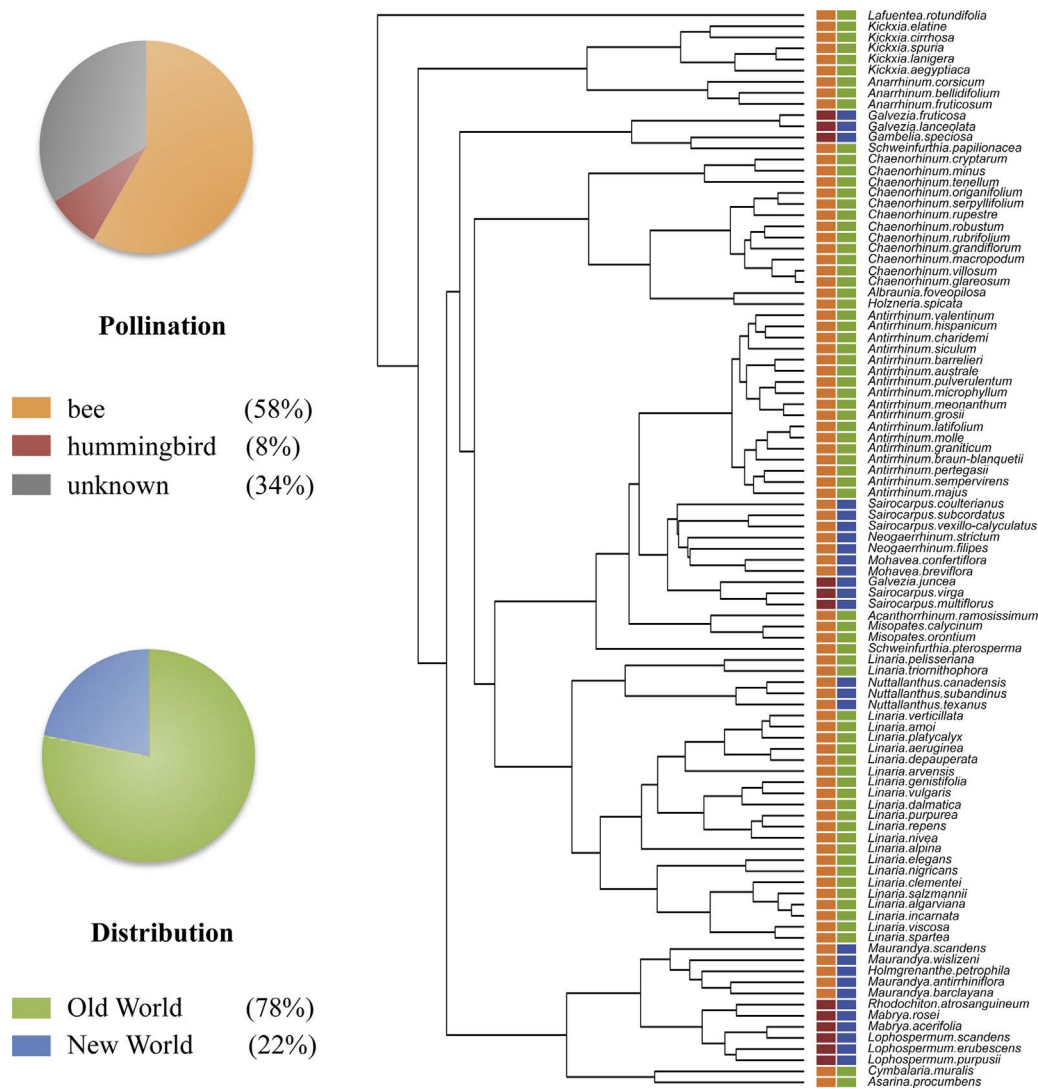


Fig. 4. The pollination modes and the geographic distributions of the species in the Antirrhineae phylogeny. Left panel: for pollination mode, 34% of the species had no available data. Bee pollination and hummingbird pollination percentages are shown on the top pie chart; Old World distribution and New World distribution percentages are shown on the bottom pie chart. Right panel: pollination and distribution states for each tip on the Antirrhineae phylogeny.

may also skew the age-range correlation within the tribe, masking the true speciation mode in the region. In addition to these factors explained above, undersampling can be another source of error. “Absence” of a species in a particular region can either mean the area is not within the species’ range, or simply the species has not been sampled in that area. Finally, as previously discussed by other authors (Phillimore et al., 2008; Hodge et al., 2013). The age-range analysis is accurate only when one mode of speciation is dominant, but most clades experience a mixture of different geographic speciation modes throughout their evolutionary history, making the majority of age-range analysis results inconclusive (Bolnick and Fitzpatrick, 2007). Therefore, whereas it is still worthy to note the significant impact of sympatric speciation on the diversification of Antirrhineae, the assumption that sympatric speciation is the dominant speciation mode in the tribe should be approached cautiously.

4.2. Diversification patterns in the Old World and the New World

The GeoSSE results indicate that between-region speciation is significantly higher than the within-region speciation in the tribe. In other words, dispersals between the Old World and the New World are

large contributors to Antirrhineae diversification. Upon entering into a new region, a clade may experience increased sympatric speciation rates due to the availability of new niches to exploit within the region, but the rates will gradually decrease, because fewer niches will be available to fill (Coyne and Orr, 2004). However, testing this hypothesis is difficult, because diversification rates are determined not only by speciation rates, but also by extinction rates. Unfortunately, measuring extinction rates can be difficult, especially when working with extant taxa that have no fossil record. For this reason, extinction rates usually tend to be underestimated, and this underestimation skews diversification rates (Etienne and Apol, 2008; Rabosky, 2010; Stadler and Bokma, 2013). In Antirrhineae, the estimated extinction rate is zero in the New World, and very low in the Old World. Therefore, “high” diversification rates may be an artifact of underestimated extinction rates. Overall, GeoSSE results suggest between-region speciation is the major factor in the Plantaginaceae diversification, whereas regional differences in diversification are not significant.

4.3. Pollination mode in Antirrhineae

Antirrhineae flowers are visited by a variety of pollinators from the

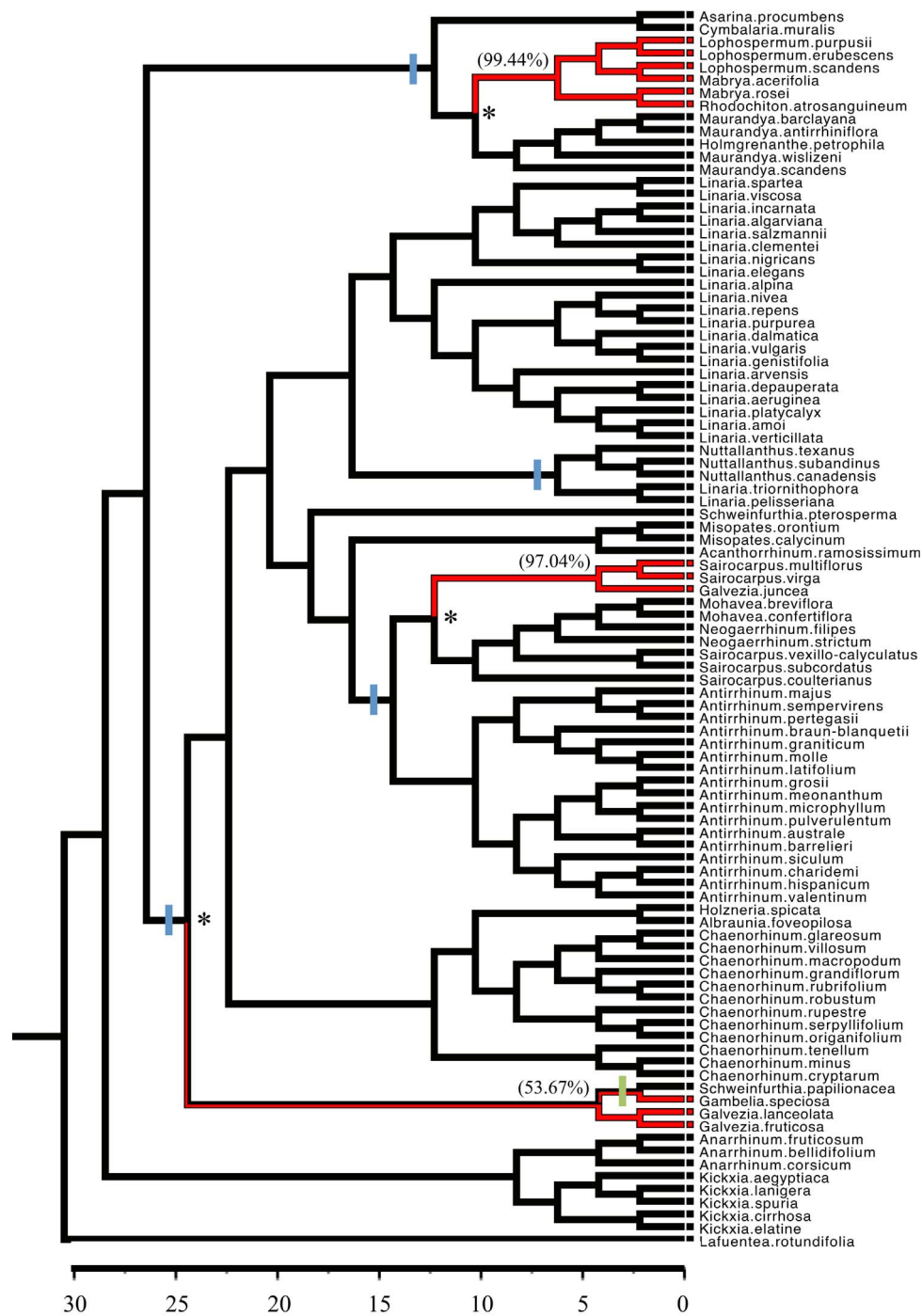


Fig. 5. Ancestral state reconstruction of pollination mode in Antirrhineae. Red: hummingbird pollination, black: bee pollination. Asterisks represent the switches from bee to hummingbird pollination. Bootstrap values for hummingbird pollination are shown in parentheses. Blue lines represent long-distance dispersals from the Old World to the New World, and green lines represent long-distance dispersals from the New World to the Old World. Time scale is in million years. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

insect orders Hymenoptera, Lepidoptera, Diptera, and Coleoptera, and the hummingbird family Trochilidae. We focused on bee versus hummingbird pollination, because (i) it is the main difference between the Old World and the New World in terms of pollination modes, and (ii) they are the two pollination modes with the most reliable data. Other pollinators (e.g.: beetles, butterflies, flies) are only documented as “visitors” in very few studies (Guzman et al., 2015), and it is not well known whether they are effective pollinators. Similar to previous studies (Ghebrehiwet et al., 2000), the current study inferred bee

pollination as the ancestral pollination mode in the tribe, which was expected, as it is the most common pollination mode in Antirrhineae, and the origin of the tribe was the Old World (Ogutten and Vamasi, 2016), where hummingbirds are absent. There were three transitions from bee to hummingbird pollination, all of which occurred shortly after the long-distance dispersals from the Old World to the New World. The switches in the *Lophospermum*-*Mabrya* and *Sairocarpus*-*Galvezia* lineages have high support values (BS > 95%). In contrast, the bee to hummingbird switch in the *Gambelia*-*Galvezia* lineage has a lower

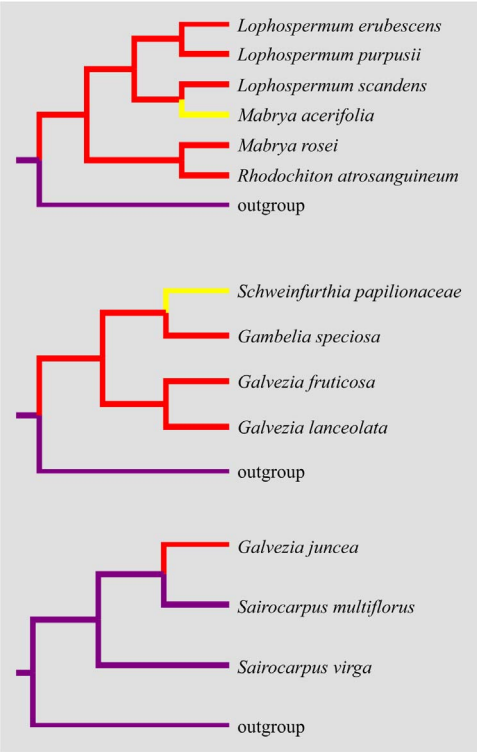


Fig. 6. Floral color transitions in the hummingbird-pollinated lineages in Antirrhineae. The branch colors represent the floral color of the species, and the outgroup represents the sister clade for the hummingbird-pollinated lineage on each tree.

support value (BS = 54%), which could be due to the presence of a bee-pollinated species (*Schweinfurthia papilionacea*) within the lineage. *S. papilionacea* experienced a transition from hummingbird to bee pollination, which was in conjunction with the long-distance dispersal event from the New World to the Old World. Therefore, our finding supports that transition from bee to hummingbird pollination is not an irreversible process (Tripp and Manos, 2008).

In Antirrhineae, transitions from bee to hummingbird pollination were often, but not always, accompanied by changes from purple to red flowers. Switching from purple to red floral color has generally been observed to be the more common direction than the reverse due to the biochemical nature of the anthocyanin production (Zufall and Rausher, 2003). Transitions from purple to red are known to be associated with

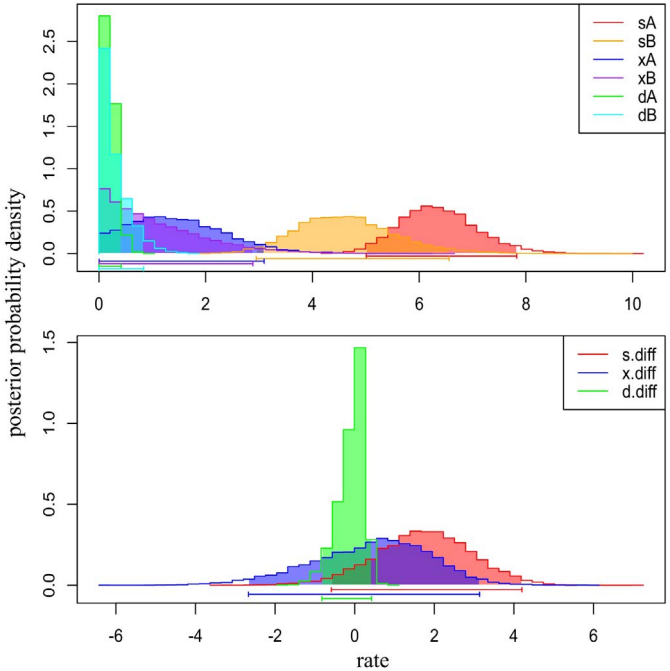


Fig. 7. GeoSSE posterior probability distribution for Antirrhineae. Top panel: posterior probability densities for the six parameters used in the analysis. sA, xA, sB, and xB represent speciation rates in the Old World, extinction rates in the Old World, speciation rates in the New World, and extinction rates in the New World respectively. dA and dB represent dispersal from the Old World to the New World and dispersal from the New World to the Old World respectively. Bottom panel: regional differences in the parameters used. s.diff: sA-sB, x.diff: xA-xB, and d.diff: dA-dB.

the inactivation of one or more stages in the anthocyanin production pathway (Rausher, 2008). This transition is virtually irreversible, because restoring purple pigment production would involve simultaneous gain-of-function mutation of several genes in the pathway (Zufall and Rausher, 2003). Previous studies have shown correlations between red flowers and hummingbird pollination (Schemske and Bradshaw, 1999; Rausher, 2008). Considering our finding that red color is not universal in hummingbird-pollinated species, and that all hummingbird pollinated species with red flowers are nested within purple-flowered clades, a pattern that has been shown to be the case in many other clades (Zufall and Rausher, 2003; Rausher, 2008; Thomson and Wilson, 2008), we posit that hummingbird pollination in Antirrhineae likely

Table 2
GeoSSE analysis results. (A) Parameter values are shown for three different models used in GeoSSE analysis. sA: speciation within the Old World, sB: speciation within the New World, sAB: speciation between the Old World and the New World, xA: extinction from the Old World, xB: extinction from the New World, dA: dispersal from the Old World to the New World, dB: and dispersal from the New World to the Old World. The full model was defined as: sA ≠ sB ≠ sAB, xA ≠ xB, dA ≠ dB. no.sAB model assumes no between-region speciation, and eq.div model assumes diversification rate is not region-dependent. (B) ANOVA results comparing the three models. * indicates significance ($p < 0.05$).

A.							
	sA	sB	sAB	xA	xB	dA	dB
full	5.667	4.309	18.115	0.049	0.000	0.221	0.000
no.sAB	6.919	4.479	0.000	2.376	0.000	0.189	0.000
eq.div	5.319	5.319	18.402	0.000	0.000	0.22	0.000
B.							
	d.f.	lnLik	AIC	χ^2	p		
full	7	78.665	−143.33	6.795	0.0091-4*		
no.sAB	6	75.267	−138.53				
eq.div	5	77.812	−145.62	1.707	0.426		

Table 3

BiSSE analysis results. Parameter values (A) and ANOVA results (B) were shown for the “realistic” setting. Bee pollination was denoted as 0; hummingbird pollination was denoted as 1. λ : speciation rate, μ : extinction rate, q : character state shift rate. The full model was defined as: $\lambda_0 \neq \lambda_1$, $\mu_0 \neq \mu_1$, $q_{01} \neq q_{10}$. The full model was compared with three different constrained models: (i) $\lambda_0 = \lambda_1$, (ii) $\mu_0 = \mu_1$, and (iii) $q_{01} = q_{10}$. (C) p values for the ANOVA results performed for six different settings.

A.						
λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	
6.255	7.508	0.000	0.000	0.242	0.314	
B.						
	d.f.	ln Lik	AIC	χ^2	p	
Full model	6	40.494	−68.988			
$\lambda_0 = \lambda_1$	5	40.320	−70.640	0.348	0.555	
$\mu_0 = \mu_1$	5	40.494	−70.988	> 0.0001	1.000	
$q_{01} = q_{10}$	5	40.479	−70.957	0.031	0.861	
C.						
	Original	Phylo	Realistic	More bee	Less bee	All NW bird
$\lambda_0 = \lambda_1$	0.7034	1.0000	0.5552	0.3889	0.2812	1.0000
$\mu_0 = \mu_1$	1.0000	1.0000	0.9997	0.9203	1.0000	0.9932
$q_{01} = q_{10}$	0.6702	1.0000	0.8611	0.5793	0.9950	1.0000

evolves in clades with purple flowers and red pigmentation evolves subsequently.

We did not perform a correlation analysis between floral color and pollination modes, because some of the pollination data retrieved for this study were inferred from pollination syndromes (Elisens, 1985, 1986; Elisens and Freeman, 1988), which would give false positive correlation between the two traits. It is also important to note that whereas most of the hummingbird pollinated Antirrhineae species have red flowers, this is not always the case. Some hummingbird-pollinated species are purple (e.g.: *Sairocarpus multiflorus*, *Sairocarpus virga*), and some red flowered species are not pollinated by hummingbirds (e.g.: *Linaria amoi*). It is important to note that bees are able to see red, and that red floral color does not exclude bees as visitors (Chittka and Waser, 1997). These observations suggest that the pollination modes inferred solely by pollination syndromes should be approached with caution, as previously discussed (Waser et al., 1996; Ollerton et al., 2009).

4.4. Trait-specific diversification rates in Antirrhineae: adding pollination into the picture

Pollinator preference promotes reproductive isolation at varying degrees, which may facilitate speciation in flowering plants in sympatry. Following this rationale, we hypothesized that after dispersing to the New World, switches from bee to hummingbird pollination may increase reproductive isolation among the New World clades, thereby increasing sympatric speciation rates in Antirrhineae. However, pollinator shifts in either way do not seem to have an appreciable effect on diversification in Antirrhineae. Our results are in contrast to other studies that have shown increased diversification rates upon switching to hummingbird pollination in neotropical clades (Perret et al., 2003; Barfuss et al., 2005; Kay et al., 2005). It was hypothesized that hummingbirds have originated in the neotropics, and they still have a strong presence in this New World biodiversity hotspot (Bleiweiss, 1998; Rymer et al., 2010). On the other hand, Antirrhineae is predominantly an Old World tribe, and they are densely distributed in the Mediterranean Basin, which is another biodiversity hotspot. During the last glacial period, the Mediterranean Basin served as a refugium for many plant species, and the landmasses in the area have split up and merged several times throughout the history (Thompson,

2005). With spatial and environmental heterogeneity, unique geological properties, and influences from its surrounding floras, the Mediterranean region hosts rich plant diversity (Thompson, 2005). Therefore, dispersing to a different environment may not be advantageous for the Mediterranean clades, because they have traits that are likely adapted for Mediterranean conditions. Most Antirrhineae species have floral traits that are often linked to bee-pollination. The features of typical Antirrhineae flowers include zygomorphy, tubular corollas enclosed by a lip, and nectar containing spurs (Ghebrehiwet et al., 2000; Guzmán et al., 2015). Considering the high frequency of these bee-pollination related traits within the tribe, hummingbird pollination may not be advantageous to a clade that migrated from a bee-dominant region.

The emergence of hummingbird pollination in the New World from bee pollinated Old World ancestors in the Mediterranean was relatively recent in the tribe. Therefore, instead of specializing in hummingbird pollination, The New World Antirrhineae species may simply incorporate hummingbirds into their pollinator suite and become more generalist. Depending on the strength and efficiency of reproductive barriers, sympatric speciation may take a long time, and pollinator preference may be an insufficient mechanism to establish and maintain reproductive isolation between the two sympatric populations (Van der Niet et al., 2014). It was previously shown that pollinator preference alone is usually not strong enough to maintain reproductive isolation, and that additional mechanisms may be necessary to form an effective reproductive barrier between the two diverging populations (Gegeer and Burns, 2007; Armbruster, 2014). It would be interesting to investigate hummingbird-pollinated species at population levels to see whether they show any signatures of population structure hinting at early stages of speciation, and if they do, research should focus on whether there are other modes of reproductive isolation along with pollinator preference to facilitate speciation.

5. Conclusion

In this study, we showed that (i) the evolutionary rate was homogeneous within Antirrhineae; (ii) range overlap between sister taxa decreases over time, suggesting a significant impact of sympatric speciation on the evolutionary history of the tribe; (iii) the Old World and the New World had similar diversification rates, (iv) between-region diversification was the main factor affecting diversification

rates, and (v) pollination mode had no effect on diversification rate in Antirrhineae.

Our results do not support our hypothesis that transition from bee to hummingbird pollination is a driving force in Antirrhineae diversification. State-dependent diversification analyses reveal correlation, but not causation; therefore the presence of a significant relationship between pollination mode and diversification rates would not necessarily provide proof that clades with one pollination mode has higher diversification rates than the other. It should also be noted that these analyses suffer from high rates of type I errors by associating neutral traits with shifts in diversification rates, whereas different diversification rates could also arise due to other traits not included in the analysis (Rabosky and Goldberg, 2015). Checking for diversification rate heterogeneity within the tribe prior to examining the effect of any character states on diversification rates was important to avoid false

positive results in the study. Applying different sampling settings to the analysis with varying assumptions was also crucial to minimize the effect of sample selection bias. Along these lines, a lack of a correlation in any of the studied settings makes the argument that pollination does not have an appreciable effect on diversification in this tribe.

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Appendix A

Geographic distribution and pollination data for the species used in the study. OW: Old World, NW: New World. The question mark (?) indicates pollination data was not available for the species. Note that the hummingbird-pollinated species are also visited by bees, but not vice versa.

Species	Distribution	Pollination
<i>Acanthorrhinum ramosissimum</i>	OW	bee
<i>Albraunia foveopilosa</i>	OW	bee
<i>Anarrhinum bellidifolium</i>	OW	bee
<i>Anarrhinum corsicum</i>	OW	bee
<i>Anarrhinum fruticosum</i>	OW	bee
<i>Antirrhinum australe</i>	OW	bee
<i>Antirrhinum barrelieri</i>	OW	bee
<i>Antirrhinum braun-blanchetii</i>	OW	bee
<i>Antirrhinum charidemi</i>	OW	bee
<i>Antirrhinum graniticum</i>	OW	bee
<i>Antirrhinum grosii</i>	OW	bee
<i>Antirrhinum hispanicum</i>	OW	bee
<i>Antirrhinum latifolium</i>	OW	bee
<i>Antirrhinum majus</i>	OW	bee
<i>Antirrhinum meonanthum</i>	OW	bee
<i>Antirrhinum microphyllum</i>	OW	bee
<i>Antirrhinum molle</i>	OW	bee
<i>Antirrhinum pertegasii</i>	OW	bee
<i>Antirrhinum pulverulentum</i>	OW	bee
<i>Antirrhinum sempervirens</i>	OW	bee
<i>Antirrhinum siculum</i>	OW	bee
<i>Antirrhinum valentinum</i>	OW	bee
<i>Asarina procumbens</i>	OW	bee
<i>Chaenorhinum cryptarum</i>	OW	bee
<i>Chaenorhinum glareosum</i>	OW	bee
<i>Chaenorhinum grandiflorum</i>	OW	bee
<i>Chaenorhinum macropodium</i>	OW	bee
<i>Chaenorhinum minus</i>	OW	bee
<i>Chaenorhinum organifolium</i>	OW	bee
<i>Chaenorhinum robustum</i>	OW	bee
<i>Chaenorhinum rubrifolium</i>	OW	bee
<i>Chaenorhinum rupestre</i>	OW	bee
<i>Chaenorhinum serpyllifolium</i>	OW	bee
<i>Chaenorhinum tenellum</i>	OW	bee
<i>Chaenorhinum villosum</i>	OW	bee
<i>Cymbalaria aequitriloba</i>	OW	?
<i>Cymbalaria muralis</i>	OW	bee
<i>Cymbalaria pallida</i>	OW	?
<i>Galvezia fruticosa</i>	NW	hummingbird
<i>Galvezia juncea</i>	NW	hummingbird
<i>Galvezia lanceolata</i>	NW	hummingbird
<i>Gambelia speciosa</i>	NW	hummingbird

<i>Holmgrenanthe petrophila</i>	NW	bee
<i>Holzneria spicata</i>	OW	bee
<i>Kickxia aegyptiaca</i>	OW	bee
<i>Kickxia cirrhosa</i>	OW	bee
<i>Kickxia elatine</i>	OW	bee
<i>Kickxia lanigera</i>	OW	bee
<i>Kickxia spuria</i>	OW	bee
<i>Lafuentea rotundifolia</i>	OW	bee
<i>Linaria aeruginea</i>	OW	bee
<i>Linaria algarviana</i>	OW	bee
<i>Linaria alpina</i>	OW	bee
<i>Linaria amethystea</i>	OW	?
<i>Linaria amoi</i>	OW	bee
<i>Linaria angustissima</i>	OW	?
<i>Linaria arenaria</i>	OW	?
<i>Linaria arvensis</i>	OW	bee
<i>Linaria badalii</i>	OW	?
<i>Linaria bipunctata</i>	OW	?
<i>Linaria bordiana</i>	OW	?
<i>Linaria bubanii</i>	OW	?
<i>Linaria caesia</i>	OW	?
<i>Linaria capraria</i>	OW	?
<i>Linaria chalepensis</i>	OW	?
<i>Linaria clementei</i>	OW	bee
<i>Linaria coutinhoi</i>	OW	?
<i>Linaria dalmatica</i>	OW	bee
<i>Linaria depauperata</i>	OW	bee
<i>Linaria diffusa</i>	OW	?
<i>Linaria elegans</i>	OW	bee
<i>Linaria faucicola</i>	OW	?
<i>Linaria flava</i>	OW	?
<i>Linaria genistifolia</i>	OW	bee
<i>Linaria glacialis</i>	OW	?
<i>Linaria glauca</i>	OW	?
<i>Linaria hellenica</i>	OW	?
<i>Linaria hirta</i>	OW	?
<i>Linaria huteri</i>	OW	?
<i>Linaria incarnata</i>	OW	bee
<i>Linaria japonica</i>	OW	?
<i>Linaria latifolia</i>	OW	?
<i>Linaria loeselii</i>	OW	?
<i>Linaria maroccana</i>	OW	?
<i>Linaria micrantha</i>	OW	?
<i>Linaria munbyana</i>	OW	?
<i>Linaria nigricans</i>	OW	bee
<i>Linaria nivea</i>	OW	bee
<i>Linaria oblongifolia</i>	OW	?
<i>Linaria odora</i>	OW	?
<i>Linaria oligantha</i>	OW	?
<i>Linaria pedunculata</i>	OW	?
<i>Linaria pelisseriana</i>	OW	bee
<i>Linaria peloponnesica</i>	OW	?
<i>Linaria pinifolia</i>	OW	?
<i>Linaria platycalyx</i>	OW	bee
<i>Linaria propinqua</i>	OW	?
<i>Linaria purpurea</i>	OW	bee
<i>Linaria reflexa</i>	OW	?
<i>Linaria repens</i>	OW	bee
<i>Linaria rubioides</i>	OW	?
<i>Linaria salzmännii</i>	OW	bee
<i>Linaria saturejoides</i>	OW	?
<i>Linaria saxatilis</i>	OW	?
<i>Linaria simplex</i>	OW	?
<i>Linaria sparteae</i>	OW	bee
<i>Linaria supina</i>	OW	?
<i>Linaria thibetica</i>	OW	?

<i>Linaria thymifolia</i>	OW	?
<i>Linaria triornithophora</i>	OW	bee
<i>Linaria triphylla</i>	OW	?
<i>Linaria tristis</i>	OW	?
<i>Linaria verticillata</i>	OW	bee
<i>Linaria viscosa</i>	OW	bee
<i>Linaria vulgaris</i>	OW	bee
<i>Lophospermum erubescens</i>	NW	hummingbird
<i>Lophospermum purpusii</i>	NW	hummingbird
<i>Lophospermum scandens</i>	NW	hummingbird
<i>Mabrya acerifolia</i>	NW	hummingbird
<i>Mabrya rosei</i>	NW	hummingbird
<i>Maurandya antirrhiniflora</i>	NW	bee
<i>Maurandya barclayana</i>	NW	bee
<i>Maurandya scandens</i>	NW	bee
<i>Maurandya wislizeni</i>	NW	bee
<i>Misopates calycinum</i>	OW	bee
<i>Misopates orontium</i>	OW	bee
<i>Mohavea breviflora</i>	NW	bee
<i>Mohavea confertiflora</i>	NW	bee
<i>Neogaerrhinum filipes</i>	NW	bee
<i>Neogaerrhinum strictum</i>	NW	bee
<i>Nuttallanthus canadensis</i>	NW	bee
<i>Nuttallanthus subandinus</i>	NW	bee
<i>Nuttallanthus texanus</i>	NW	bee
<i>Rhodochiton atosanguineum</i>	NW	hummingbird
<i>Sairocarpus cornutus</i>	NW	?
<i>Sairocarpus costatus</i>	NW	?
<i>Sairocarpus coulterianus</i>	NW	bee
<i>Sairocarpus kingii</i>	NW	?
<i>Sairocarpus multiflorus</i>	NW	hummingbird
<i>Sairocarpus nuttallianus</i>	NW	?
<i>Sairocarpus subcordatus</i>	NW	bee
<i>Sairocarpus vexillo-calyculatus</i>	NW	bee
<i>Sairocarpus virga</i>	NW	hummingbird
<i>Sairocarpus watsonii</i>	NW	?
<i>Schweinfurthia papilionacea</i>	OW	bee
<i>Schweinfurthia pterosperma</i>	OW	bee

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ppees.2017.04.001>.

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