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Research

Floral uniformity through evolutionary time in a species-rich tree lineage

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Summary

• Changes in floral morphology are expected across evolutionary time and are often promoted as important drivers in angiosperm diversification. Such a statement, however, is in contrast to empirical observations of species-rich lineages that show apparent conservative floral morphologies even under strong selective pressure to change from their environments.

• Here, we provide quantitative evidence for prolific speciation despite uniform floral morphology in a tropical species-rich tree lineage. We analyse floral disparity in the environmental and phylogenetic context of *Myrcia* (Myrtaceae), one of the most diverse and abundant tree genera in Neotropical biomes.

• Variation in floral morphology among *Myrcia* clades is exceptionally low, even among distantly related species. Discrete floral specialisations do occur, but these are few, present low phylogenetic signal, have no strong correlation with abiotic factors, and do not affect overall macroevolutionary dynamics in the lineage.

• Results show that floral form and function may be conserved over large evolutionary time scales even in environments full of opportunities for ecological interactions and niche specialisation. Species accumulation in diverse lineages with uniform flowers apparently does not result from shifts in pollination strategies, but from speciation mechanisms that involve other, nonfloral plant traits.

Introduction

Tropical forests harbour the most species-rich biomes on Earth (Brown, 2014). These lush environments provide endless opportunities for interspecific relationships, powerful sources of selective pressure enhancing species and phenotypic diversity into different ecological niches (Schemske *et al.*, 2009). In angiosperms, a constant cycle of ecological niche opening and filling has resulted in the evolution and diversification of floral strategies (Endress, 1994).

The evolution of the flower and the relationship between plant and pollinator is considered one of the major key innovations in angiosperm evolutionary history (Vamosi & Vamosi, 2010; Van der Niet & Johnson, 2012; Barrett, 2013; Sauquet & Magallón, 2018). Characters of the floral phenotype are tightly linked to pollination efficiency and consequently to overall plant reproductive success (Rosas-Guerrero *et al.*, 2014). As pollinators positively select specific floral traits across evolutionary time (Gervasi & Schiestl, 2017), flowers are under constant and strong selective pressure to change. In this sense, shifts in floral strategy are often observed over an evolutionary time scale (Stebbins, 1970; e.g. O'Meara *et al.*, 2016). In the context of a single lineage, these shifts are frequently linked to changes in species diversification dynamics, accelerating speciation rates if the new floral features increase fitness in a given environmental context (e.g. O'Meara *et al.*, 2016; for a review see Armbruster, 2014). This principle has been key to arguments that changes in floral strategy are among the most important drivers in bursts of angiosperm species diversification (Vamosi *et al.*, 2018).

The appearance of these novel traits, in addition to environmental changes, promotes or demotes lineages in macroevolutionary adaptive landscapes, affecting rates of species diversification and extinction (Sanderson & Donoghue, 1994). The identification of novel traits in the flower that lead to such changes has therefore been central to many plant evolutionary studies in the last decade (e.g. Silvestro *et al.*, 2014; de Vos *et al.*, 2014; Sauquet *et al.*, 2017; Vamosi *et al.*, 2018). Changes in floral strategy and their effect on species turnover (i.e. cycles of species diversification and extinction) can be inferred by examining extant floral morphological diversity (i.e. disparity) relative to

molecular-based phylogenetic trees (e.g. Lagomarsino *et al.*, 2016). Consequently, the link between morphological changes of the flower and accelerated species diversification rates is frequently presented in the literature, with numerous studies emphasising this connection (e.g. Van der Niet *et al.*, 2014; Lagomarsino *et al.*, 2017; Serrano-Serrano *et al.*, 2017).

Contrary to expectation, however, it is notable that a large number of angiosperm lineages have apparently uniform floral morphologies. This trend includes species-rich lineages of woody plants such as *Myrcia*, *Eugenia* (Myrtaceae; Vasconcelos *et al.*, 2018), *Croton* (Euphorbiaceae; Webster, 1993), *Mimosa* (Fabaceae; Barneby, 1991), *Solanum* (Solanaceae; Symon, 1979), some Malpighiaceae (Anderson, 1979), Sapotaceae (Chartier *et al.*, 2017) and *Miconia* (Melastomataceae; Renner, 1989), to cite just a few. These groups are crucial components of the woody tropical flora in both abundance and diversity of species (e.g. Bernacci *et al.*, 2004; Murray-Smith *et al.*, 2009) and the uniformity of their floral morphologies, despite considerable species diversification, may be more common than previously thought. Nevertheless, studies have neglected these cases and there is a lack of quantitative studies that investigate floral uniformity over long evolutionary time in species-rich lineages.

In this study, we present quantitative evidence for considerable species diversification in a tropical tree genus without radical changes in flower morphology. We contrast multivariate and macroevolutionary dynamics analyses to demonstrate floral uniformity through evolutionary time in one of the most speciose and abundant Neotropical genera. *Myrcia* (Myrtaceae, Myrtales) is an angiosperm genus of *c.* 700 species (WCSP, 2017) and is characterised by inconspicuous and fairly unspecialised flowers that are mostly self-incompatible, are pollinator-dependent and do not offer nectar, relying only on pollen as a pollinator reward (Fig. 1; NicLughadha & Proença, 1996; Gressler *et al.*, 2006).

Myrcia consistently features among the most species-rich tree genera in biodiversity hotspots of South America (e.g. Cerrado savanna biome: Françoso *et al.*, 2016; and Atlantic Rainforest: Oliveira-Filho & Fontes, 2000). After *c.* 30 Myr of evolution in these species-rich environments (Mannion *et al.*, 2014; Santos *et al.*, 2017) and assuming that morphological changes arise as lineages diverge in ecological niches (Pfennig & Pfennig, 2009), *Myrcia* would be expected to have developed several specialised floral strategies (e.g. Junker *et al.*, 2013; but see Tobias *et al.*, 2014) and changes in macroevolutionary dynamics. To better understand the absence of these expected evolutionary patterns in the genus, we analyse floral disparity for over 140 species in the macroevolutionary context of *Myrcia*.

Materials and Methods

Unless otherwise stated, all analyses were performed using the software R v.3.4.0 (R Core Team, 2017). Functions are referred to as follows: *function name*{*package name*}.

Study group

We selected *Myrcia* as study group because: (1) it has a central ecological role in the biomes in which it is most diverse (Neotropical rainforests and savannas), presupposing high levels of interspecific interactions (e.g. it is one of the richest pollen sources for pollinators (Wilms *et al.*, 1996) and fruit sources for vertebrates (Staggemeier *et al.*, 2017) in these biomes); (2) the availability of a series of recent systematic revisions that have significantly increased taxonomic stability (e.g. Lucas *et al.*, 2016, 2018; Santos *et al.*, 2016); and (3) *Myrcia* has diversified into one of the most species-rich areas on the globe, most probably after the establishment of the modern latitudinal

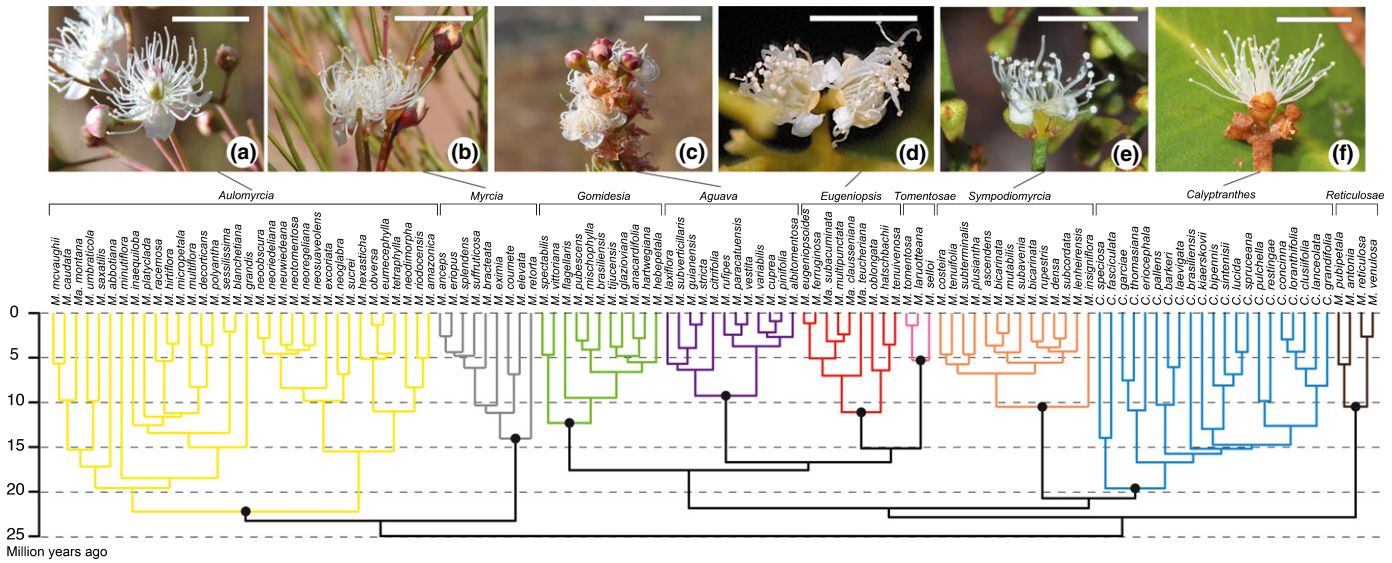

Fig. 1 Floral similarity across the *Myrcia* phylogeny (phylogeny based on Santos *et al.*, 2017). Section names are given for the nine clades with consistent bootstrap and posterior probability support (crown nodes marked with black dots). (a) *Myrcia myrtillifolia* (section *Aulomyrcia*); (b) *M. linearifolia* (section *Myrcia*); (c) *M. nivea* (section *Aguava*); (d) *M. multipunctata* (section *Eugeniopsis*); (e) *M. mutabilis* (section *Sympodiomyrcia*); (f) *Calyptranthes brasiliensis* (section *Calyptranthes*). Bars: (a–f) 5 mm. Species name abbreviations: M., *Myrcia*; Ma., *Marlierea*; C., *Calyptranthes*.

gradient of species diversity (Mannion *et al.*, 2014; Santos *et al.*, 2017).

Myrcia is subdivided into nine sections corresponding to clades that have received strong support in independent phylogenetic analyses (e.g. Lucas *et al.*, 2011; Staggemeier *et al.*, 2015; Wilson *et al.*, 2016; Santos *et al.*, 2017; see Fig. 1). Reliable estimates of species numbers are available for these nine clades (Lucas *et al.*, 2011, 2018), which is necessary for the evaluation of diversification rates in incomplete phylogenetic datasets (e.g. Rabosky *et al.*, 2014).

Sampling strategy

Species were selected according to the most recent phylogeny for the genus (Santos *et al.*, 2017), based on systematic revisions (Lucas *et al.*, 2018) to represent the broadest possible phylogenetic diversity and geographical distribution. Each clade was represented by at least 10% of its species diversity in the morphological diversity analysis. We included additional samples of some widespread species complexes (e.g. *Myrcia tomentosa*, *M. splendens*) in the morphological diversity analysis; these were not considered pseudoreplicates for the question addressed because high phenotypic plasticity in these complexes suggests that species delimitation is not clear (e.g. Lima *et al.*, 2015). In total, 161 species were sampled (120 of which were also represented in the phylogenetic analyses, see below), corresponding to 22% of *Myrcia* species diversity (Table 1, Supporting Information Methods S1). For a full list of vouchers see Dataset S1.

Trait measurements and environmental data

After a preliminary survey, we chose a series of floral traits based on the following criteria: (1) the selected traits clearly vary among species, (2) it is possible to record the trait in question for every species, (3) traits can be measured with a dissecting microscope and (4) they have or may have relevance in reproductive strategy, based on reproductive biology surveys (such as NicLughadha & Proença, 1996; Gressler *et al.*, 2006).

Morphological trait measurements were made on specimens from the Royal Botanic Gardens Kew Herbarium, using, where possible, the same vouchers as used in the phylogenetic reconstruction. Specimens used in the phylogenetic analysis that did not bear buds or flowers were substituted with flowering specimens from similar geographical locations and identifications were confirmed by specialists. We selected an average of three buds and three recently opened flowers from each specimen. Buds and flowers were boiled in water for 10 min, left to cool overnight and then fixed in 70% ethanol for longer preservation. Each bud and flower was cut longitudinally (Fig. 2ai) and structures were measured using a Nikon ShuttlePix model P-400R digital microscope. Sixteen floral traits were measured (A–P, Fig. 2aii) and the final measurement for each trait corresponds to the mean measurement of that structure among all measured buds and flowers per specimen. We chose this approach to ease the effect of post-anthetic distortions in floral structures. We also recorded three inflorescence traits (estimated number of flowers; length of main axis; flowers clustered or scattered) and the presence/absence of oil glands on the anthers (a proxy for flower specialisation; Armbruster, 2012) from herbarium specimens.

From specimen labels, we extracted information on plant height and two environmental variables: altitude and vegetation type (rainforest vs savanna). Relative investment in inflorescences was estimated by dividing the mean length of the main inflorescence axis by the plant's mean height. This may be considered a coarse estimate, but the consistent paniculate pattern of the inflorescence in addition to observations in the field show that this approximation makes empirical sense. Additional label data and inflorescence traits were recovered directly from herbarium material. See Methods S1 for details of data collection.

Five per cent of the data set (236 entries) was scored as 'missing data' (NA), corresponding to the few cases where no suitable material was available. Because most continuous trait analyses do not allow missing data, missing data were substituted by the corresponding mean trait values for the whole data set. This imputation method is considered impartial for data sets with NA values below 10% (Shrive *et al.*, 2006).

Phylogenetic reconstruction

Our phylogenetic reconstruction is based on one nuclear (ITS) and four chloroplast (psbA-trnH, trnQ-rps16, trnL-trnF, ndhF) markers from the study of Santos *et al.* (2017; see original publication for GenBank accession numbers). This molecular matrix

Table 1 Results from npMANOVA showing degree of dissimilarity between clades (sections) based on morphospace analyses.

	ret	cal	sym	myr	gom	tom	agu	eug	aul
ret	na	3.542	2.115	0.86	1.572	4.376	2.433	1.577	4.957
cal	ns	na	0.208	4.269	6.632	8.978	1.316	0.276	3.829
sym	ns	ns	na	3.029	3.986	5.597	0.987	−0.072	1.223
myr	ns	ns	ns	na	3.132	8.879	2.322	2.447	9.151
gom	ns	*	ns	ns	na	18.253	5.485	3.615	8.627
tom	ns	ns	ns	ns	ns	na	4.779	4.569	10.294
agu	ns	ns	ns	ns	ns	ns	na	0.306	5.101
eug	ns	ns	ns	ns	ns	ns	ns	na	1.767
aul	ns	ns	ns	*	*	*	ns	ns	na

Values above the diagonal represent *F* values and those below the diagonal show relationships that are not significantly different (ns); asterisks mark those with $P < 0.01$ (significantly distinct clades). Abbreviations: agu, *Aguava*; aul, *Aulomyrcia*; cal, *Calyptranthes*; eug, *Eugeniopsis*; gom, *Gomidesia*; myr, *Myrcia*; ret, *Reticulosae*; sym, *Sympodiomyrcia*; tom, *Tomentosa*.

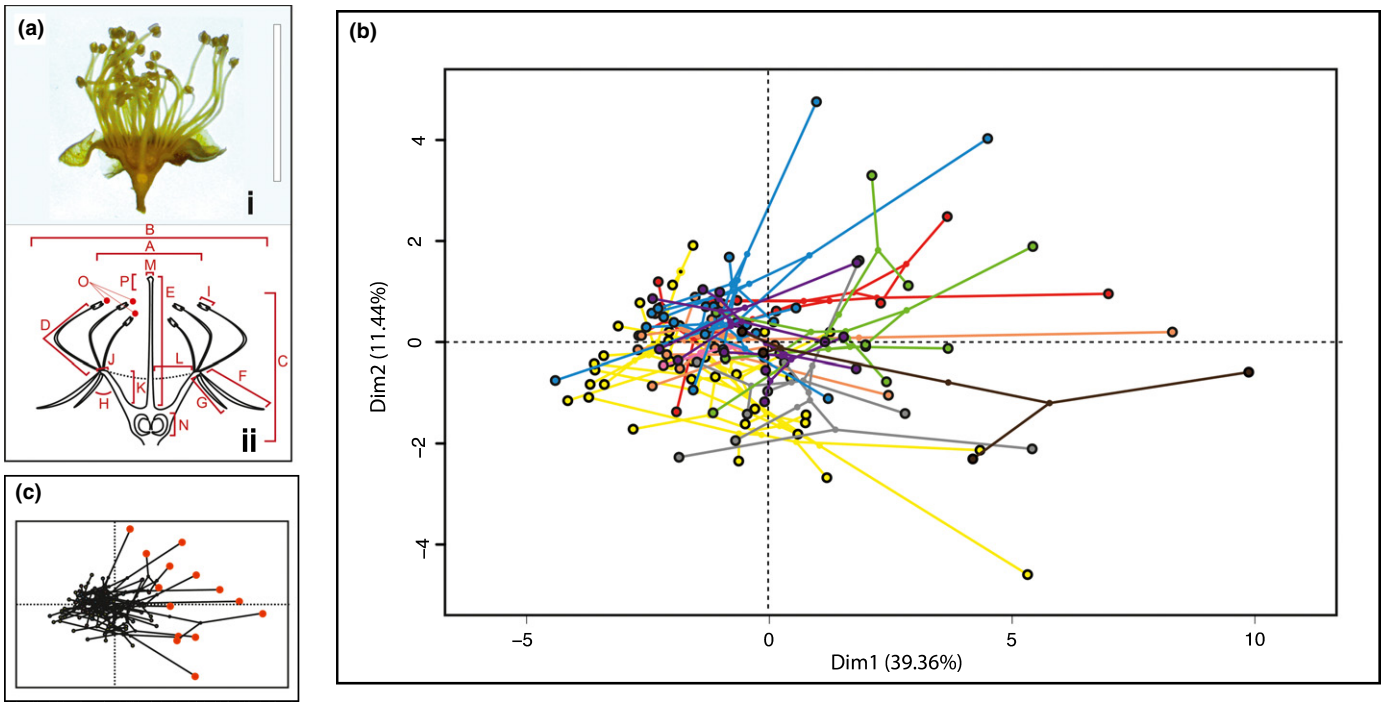

Fig. 2 Change of floral form through evolutionary time in *Myrcia*. (a) Floral measurements: (a-i) flower of *Myrcia rubella* in longitudinal section, (a-ii) schematic drawing of flower showing the 16 (A–P) traits measured. (b) Floral phylomorphospace showing distribution of species in multivariate space according to flower structure and phylogenetic relationships. (c) Twelve species placed at the periphery of the morphospace are shown in red. Bar: (a) 5 mm. The nine infrageneric sections are colour-coded in (b) as follows: yellow, *Aulomyrcia*; blue, *Calyptranthes*; grey, *Myrcia*; pink, *Tomentosae*; black, *Reticulosae*; green, *Gomidesia*; orange, *Sympodiomyrcia*; purple, *Aguava*; red, *Eugeniopsis*.

was used to reconstruct a dated phylogeny in BEAST (Drummond *et al.*, 2012). Substitution models were based on Santos *et al.* (2017) and calibration parameters followed those of the pollen-fossil approach and secondary calibration points of Vasconcelos *et al.* (2017). The final topology is similar to those found in previous studies (Staggemeier *et al.*, 2015; Wilson *et al.*, 2016; Santos *et al.*, 2017). The resulting tree contains 146 taxa, including 133 ingroup and 13 outgroup taxa, and is available in Methods S1. For phylogenetic signal analysis, the final tree was pruned (using function *drop.tip*{*ape*}; Paradis *et al.*, 2004) to exclude outgroups.

Phylomorphospace and morphological diversity (disparity)

A representation of the floral morphospace for 146 species of *Myrcia* was built with a principal component analysis (PCA) on the 16 continuous floral measurements in millimetres using the function *PCA*{*FactoMineR*} (Lê *et al.*, 2008). This analysis allowed us to score the effect of each trait on the morphospace distribution. To visualise phylogenetic relationships over the PCA plot, we used the function *phylomorphospace*{*phytools*} (Revell, 2012). This function is based on Sidlauskas (2008) and creates a projection of the phylogenetic tree into a morphospace. In this way, it is possible to visualise how phylogeny tips diverge and converge from ancestral nodes in the morphospace along evolution. Morphological differences among clades were tested with a nonparametric multivariate analysis of variance (npMANOVA) using the function *adonis*{*vegan*} (Oksanen *et al.*, 2018). This allowed us to show which clades are significantly different from others in the phylomorphospace.

To test for dependence between measurements and phylogenetic relationships, values of Pagel's *lambda* were estimated for each continuous trait with the function *fitContinuous*{*geiger*} (Harmon *et al.*, 2008). Values of *lambda* closer to 1 indicate stronger phylogenetic signal (Pagel, 1999), i.e. a strong dependence between trait and phylogeny. Finally, a Mantel test (function *mantel*{*vegan*}) was used to compare morphological and phylogenetic distances to identify patterns of phylogenetic signal in our floral data set. For this test, a Euclidean distance matrix was built from the continuous morphological traits and a phylogenetic dissimilarity matrix was estimated using the function *cophenetic.phylo*{*ape*} (Paradis *et al.*, 2004).

To be able to include all traits into the disparity analysis (measurements A–P, plus anther gland and inflorescence categorical traits) a second distance matrix was calculated using the *mean character difference* index (Foote, 1997), following Chartier *et al.* (2017). Disparity was calculated for each clade on this matrix as the mean pairwise morphological distance between pairs of species belonging to a given clade. For each clade, disparity was further tested for correlation against age and species number using Spearman's rank correlations in the function *cor.test*{*stats*}.

Correlations between traits and environmental variables

Morphological variation was investigated in relation to altitude and vegetation type, according to herbarium label (see

Methods S1). Traits tested included floral shape (i.e. the 'filling' of the morphospace, represented as the PCA of 16 floral measurements in millimetres, as seen above), presence of oil glands on anthers, relative investment in inflorescence (i.e. inflorescence length divided by plant height) and inflorescence display (flower number and arrangement on panicle (clustered or scattered)), and plant height (in metres). npMANOVA (function *adonis*{*vegan*}; Oksanen *et al.*, 2018) was used to test whether species of similar altitude/vegetation occupy significantly different areas in the morphospace. Kruskal–Wallis rank sum tests (function *kruskal.test*{*stats*}) were used to test for the correlation between vegetation/altitude, relative investment in inflorescence and number of flowers per inflorescence, and plant height. Correlation between vegetation/altitude and presence/absence of anther glands and flowers organised in clusters on the inflorescence was tested with chi-squared tests.

Interpretation of phylogenetic heterogeneity

Analysis of phylogenetic branching patterns allows for the estimation of areas of the phylogenetic tree that show significant variation in diversification or extinction rates (Rabosky, 2006). Increased availability of phylogenetic tree data has been accompanied by increased statistical power to analyse such rate heterogeneity in ultrametric trees (see summary in Tess vignette, Höhna *et al.*, 2015), although not without controversy (e.g. Moore *et al.*, 2016). To infer patterns of phylogenetic heterogeneity, two methods were contrasted; a Bamm analysis (v.2.5; Rabosky *et al.*, 2014, 2017) was used to identify significant rate shifts that could be associated with cryptic key innovative phenotypic characters highlighted by morphological analyses. Empirical priors were generated based on the *Myrcia* phylogeny pruned for outgroups and an estimated total diversity of 700 species (WCSP, 2017). Sampling estimates per clade are based on Lucas *et al.* (2011) and can be accessed in Methods S1. Tess (Höhna *et al.*, 2015) was used to estimate changes in speciation and extinction rates over time and to calculate the number of rate shifts based on marginal likelihood and Bayes factors. For Tess, the original phylogeny was rescaled to minimise the effects of clade over-representation; tips were randomly pruned from over-sampled clades prior to analysis (eight from section *Sympodiomyrcia*, five from section *Guianensis* and four from section *Eugeniopsis*).

Results

Phylomorphospace and phylogenetic signal of floral traits

The phylomorphospace reconstructed on the PCA based on 16 floral traits shows no visible trend of morphological diversification, with phylogenetic trajectories of the nine subgeneric clades of *Myrcia* overlapping each other (Fig. 2b; see Notes S1). In addition, all ($n = 36$) but four pairwise comparisons (*post hoc* tests) among the nine clades were nonsignificant (overall npMANOVA, $P = 0.014$; *post hoc* tests: see Table 1), meaning that no group was significantly morphologically different from all the others. The handful of species falling at the edge of the morphospace (highlighted in Fig. 2c) increase the overall disparity of the genus, but belong to different clades in the phylogeny. There is, thus, no clade that presents any distinct new combinations of features; new combinations of features are present in a few species scattered throughout the phylogeny. This is confirmed by phylogenetic signal estimates, which are low for most floral trait measurements (all except four traits score Pagel's *lambda* < 0.6; traits F, I, L and O score Pagel's *lambda* < 0.8; see Notes S1), and a lack of correlation between pairwise morphological dissimilarities and phylogenetic dissimilarities (Mantel statistic, $r = 0.01496$; significance = 0.3249). Floral morphological diversity is therefore not correlated with phylogenetic distance, which further underlines the lack of a phylogenetic pattern in the evolution of floral shape.

Effects of environmental variables on the evolution of floral traits

Given the strong conservation of floral form, null hypothesis significance tests were performed to uncover possible effects of environmental variables (altitude and vegetation) on floral and inflorescence traits. Almost all results receive no statistical support (Fig. 3) and highlight a lack of floral trait variation linked to environmental conditions in *Myrcia*. The only significant correlation shows that the mean relative investment in inflorescence is three times greater in plants occurring in savannas than in rainforests (Fig. 3b, Kruskal–Wallis test: $P < 0.001$). *Myrcia* species growing in savanna environments are shorter, consisting mainly of subshrubs and shrubs (Kruskal–Wallis ANOVA: $P < 0.001$). This shows constraints to change between distinct biomes; that is, *Myrcia* shrubs and subshrubs from savanna vegetation present similar inflorescence displays as trees in rainforests (Fig. 4).

Correlations among disparity, clade age and number of species per clade

Floral morphological disparity does not correlate with species number (Fig. 5a; Spearman's rank correlation, rho = 0.32, $P = 0.41$) or with clade age (Fig. 5b; Spearman's rank correlation, rho = 0.45, $P = 0.23$). However, when excluding the two outliers (and oldest clades) *Aulomyrcia* and *Calyptranthes* from the analyses, disparity significantly increased with clade age (Fig. 5b, Spearman's rank correlation, rho = 0.82, $P < 0.05$). This suggests eventual stabilisation in morphological disparity through time, reinforcing a trend to conserve floral morphology in a lineage. Furthermore, the significant increase of species number per clade relative to clade age (Fig. 5c, Spearman's rank correlation, rho = 0.87, $P < 0.01$) indicates that species richness depends on time for species accumulation rather than accelerated species diversification rates. This is also corroborated by macroevolutionary dynamics analyses (see next section).

Macroevolutionary dynamics constancy

Our analyses of macroevolutionary dynamics in *Myrcia* indicate a general lack of phylogenetic heterogeneity and support a slow

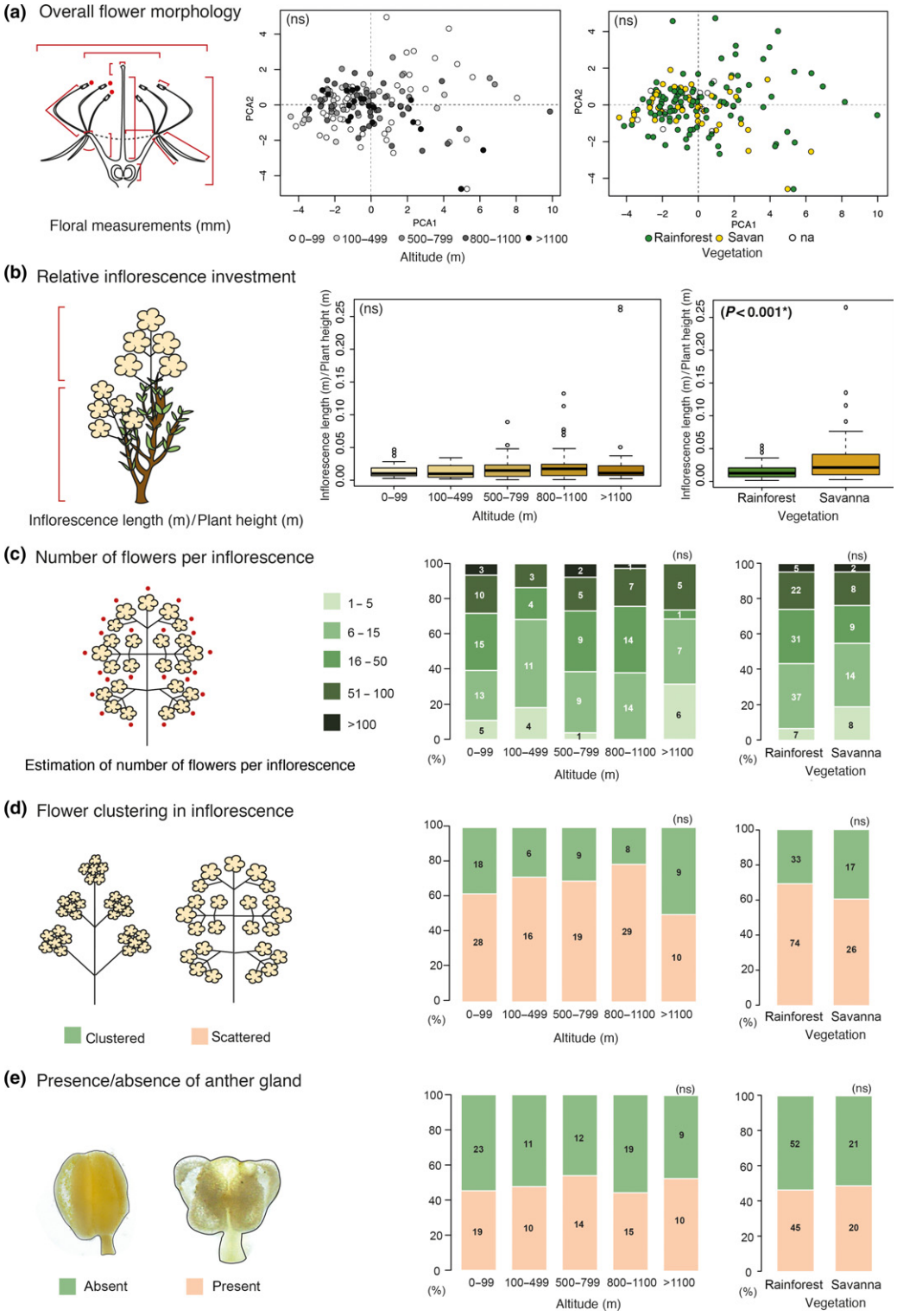

Fig. 3 Correlation between floral traits and environmental variables in *Myrcia*. (a) Species distribution in the morphospace is not correlated with either altitude or the type of vegetation (NA represents missing data for vegetation type); (b) relative inflorescence investment is not correlated with altitude, but significantly increases in savanna vegetation (boxplots: thick bars, median; error bars, range of observations excluding outliers; transparent dots, outliers); (c) estimated number of flowers per inflorescence; (d) flower clustering in the inflorescence; and (e) presence/absence of anther oil gland are not correlated with either altitude or the environment. Analyses of significance in (a) are based on a perMANOVA, in (b) are based on Kruskal–Wallis ANOVA; and in (c–e) are based on chi-squared tests. Non-significance (ns) was considered at $P > 0.05$.

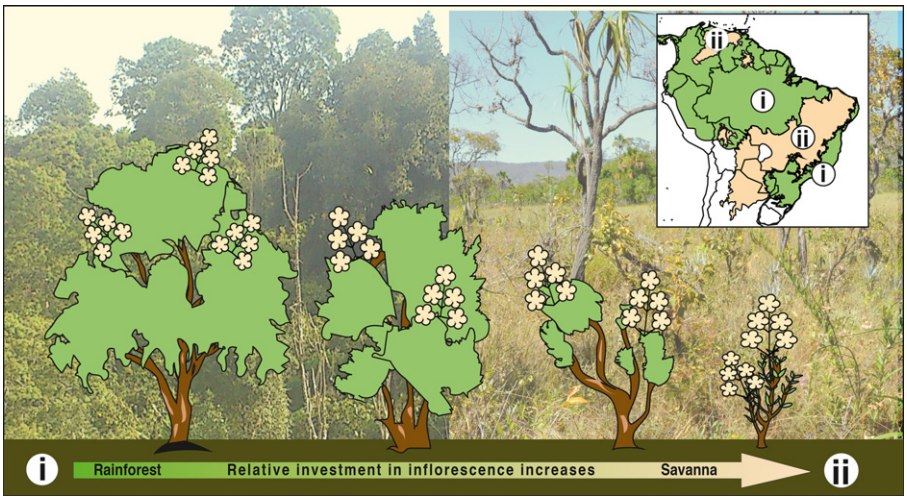

Fig. 4 Biome transition from rainforest (i) to savanna (ii) does not significantly affect floral traits, but plant size decreases substantially in savanna biomes (Kruskal–Wallis ANOVA; $P < 0.001$) increasing investment in inflorescences relative to plant size (see also Fig. 3b).

process of species turnover in the genus resulting from low extinction rates. BAMM estimates of diversification rate shifts show no shift in diversification rates and all parts of the tree share a similar macroevolutionary dynamic (Fig. 6a). TESS results also support a constant moderate speciation rate of 0.3 species per million yr (Myr) and, additionally, low extinction rates of < 0.1 species per Myr through time (Fig. 6b); these results in addition to the strong correlation between age and total species diversity per clade (Fig. 5c) suggest constant and homogeneous accumulation of species diversity throughout the genus over time, without clear increases in rates of diversification or extinction. Despite apparent disparity in species number between clades, variation in species diversity is probably due to the relatively older age of some clades. Additional results regarding macroevolutionary analyses can be found in Notes S1.

Discussion

Innovation is not (always) the key: moving in circles on a long-lasting adaptive peak

The species-rich lineage analysed here presents a highly homogeneous floral morphology, with overlapping clades in the phylomorphospace and no obvious floral specialisations towards different ecological niches. This trend is unexpected after *c.* 30 Myr of evolution (Santos *et al.*, 2017) in the Neotropics, one of the most biodiverse environments on Earth, full of opportunities for interactions with different pollinators (Brockhurst *et al.*, 2014). If a structure crucial for lineage fitness is constrained and does not change over long periods of evolutionary time, as *Myrcia* flowers are, this is interpreted as an adaptive plateau, or a long-lasting peak in an adaptive landscape (Svensson & Calsbeek, 2012). A similar adaptive plateau has also been considered for floral evolution in other diverse Neotropical groups such as Melastomataceae (Renner, 1989) and Malpighiaceae, where Davis *et al.* (2014) call the trend a 'long-term morphological stasis'. Adaptive plateaus in reproductive structures may be crucial to our understanding of why rates of morphological evolution may slow down in certain lineages. This is currently one of the key questions in studies of angiosperm macroevolution (Sauquet & Magallón, 2018).

Examples of morphological stasis such as *Myrcia* flowers are important to showcase, because in contemporary evolutionary studies there has been a constant focus on key innovations and shifts between trait states that change macroevolutionary dynamics (e.g. Hunter, 1998; Silvestro *et al.*, 2014; Lagomarsino *et al.*, 2016; Serrano-Serrano *et al.*, 2017). Focusing only on the high frequency of trait shifts during evolution may lead to the assumption that homogeneous phenotypes such as *Myrcia* flowers do not persist across evolutionary time when a lineage is under strong selection (Schluter, 2000). For that reason, highly diverse groups with homogeneous flowers are sometimes thought to result from recent explosive speciation events where there has not been time for the appearance of clear phenotypic disparity (Stebbins, 1974). Our results reinforce the suggestion that such groups can instead result from a tendency to maintain certain combinations of traits over long periods of time. In *Myrcia*, this evolutionary pattern seems to be associated with a particularly successful eco-evolutionary relationship (i.e. pollen-gathering bee pollination; see section 'The optimum 'unspecialised'' floral strategy of Myrcia' below).

Species with distinct combinations of floral traits also exist in *Myrcia* (i.e. the few points scattered around the periphery of the morphospace), but are rare and not related to any particular lineage. These distinct combinations of traits may be associated with evolutionary dead-ends, conferring a short-term adaptive advantage but leading those lineages to extinction before further speciation events can take place (Barrett, 2013). That is, if floral shape changes radically, the adaptive peak is lost and lineages with distinct morphologies tend to disappear (Schluter, 2000; Barrett, 2013).

The presence of macroevolutionary stability (i.e. no significant shifts in diversification rates) also corroborates large-scale stability of overall fitness in these lineages. In this sense, the success of

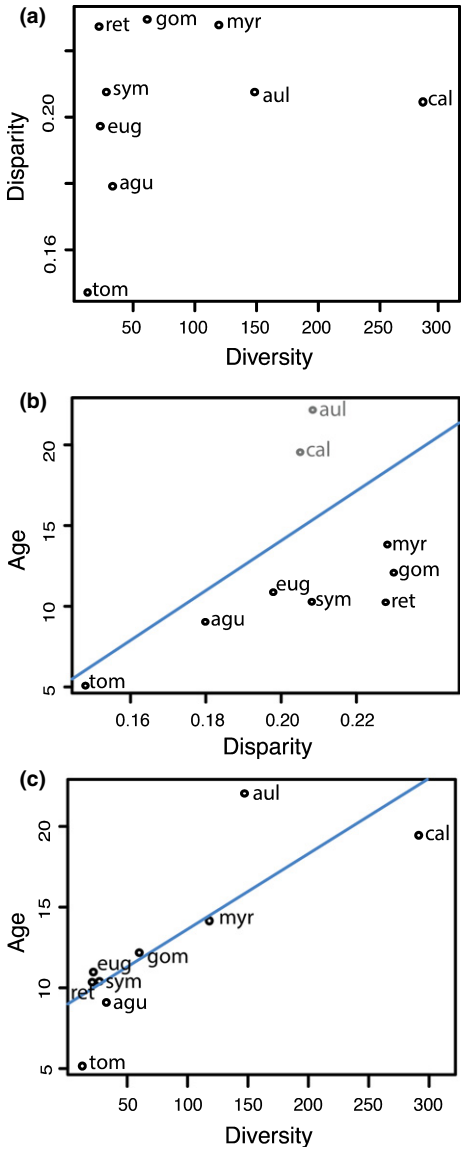

Fig. 5 Spearman's rank correlation contrasting: (a) disparity and species diversity (rho = 0.32, $P = 0.54$); (b) disparity and crown age (rho = 0.45, $P = 0.21$, for all data sets; rho = 0.82, $P < 0.001$, when the two oldest clades (in grey) are excluded); and (c) clade crown age and species diversity (rho = 0.87, $P < 0.01$). Abbreviations for the nine sections in *Myrcia* are as follows: agu, *Aguava*; aul, *Aulomyrcia*; cal, *Calyptranthes*; eug, *Eugeniopsis*; gom, *Gomidesia*; myr, *Myrcia*; ret, *Reticulosae*; sym, *Sympodiomyrcia*; tom, *Tomentosae*.

some of the largest tropical angiosperm lineages may be related to keeping an optimum reproductive strategy over long periods of evolutionary time while being flexible to change in other aspects (see section 'Alternatives to plant–pollinator interaction as the driving force for plant speciation' below) .

The optimum 'unspecialised' floral strategy of *Myrcia*

The adaptive plateau in the floral morphology of *Myrcia* may be related to a particular pollination system that confers reproductive success in multiple geographical and temporal contexts. Distinct clusterings in a floral morphospace are traditionally interpreted as distinct display strategies (Chartier *et al.*, 2014; e.g. Lagomarsino *et al.*, 2017) and a single cluster of species in the phylomorphospace, as observed in *Myrcia*, indicates that a stable mode of floral display is shared among most species. In this case, these are small, polystemonous, white, open flowers distributed in paniculate inflorescences.

Strong selective pressure to maintain this phenotype appears linked to a generalist melittophilous system that relies on pollen-collecting bees as the main functional pollinator. Evidence from reproductive biology studies shows that pollinator guilds and pollination mode are similar throughout the geographical and phylogenetic range of *Myrcia* (see information for 17 species in Notes S1). Bee lineages responsible for successful pollination of *Myrcia* include corbiculates (bumblebees and stingless bees) and, less frequently, the distantly related *Xylocopa* and Halictidae (e.g. Danforth *et al.*, 2006; Fidalgo & Kleinert, 2009; Martins *et al.*, 2014). Stingless bees (Meliponini), the most important pollinators of *Myrcia* flowers, are abundant and conspicuous in the environments where the latter occur. They show social behaviour, requiring large amounts of pollen, frequently collected by buzz behaviour, to maintain their colonies (Wilms *et al.*, 1996; Michener, 2007). The polystemonous, mass-flowering and unspecialised flowers of Myrtaceae (including *Myrcia*) are among the most important pollen sources for these bee lineages in the Neotropics (Wilms *et al.*, 1996; Fidalgo & Kleinert, 2009; Obregon & Nates-Parra, 2014).

This mutualistic bee–flower interaction may have existed since the origin of *Myrcia*, as relevant pollinator groups were already present on South American plateaus (e.g. Brazilian and Guiana shields) during the Oligocene (Rasmussen & Cameron, 2010; Camargo, 2013), potential areas of early diversification in *Myrcia* (Santos *et al.*, 2017). The abundance of these bees throughout the distribution range of *Myrcia* and the success of this relationship may have been the main reason for the maintenance of the uniform floral shape over evolutionary time.

Alternatives to plant–pollinator interaction as the driving force for plant speciation

The optimum floral strategy in *Myrcia* and its association with widespread generalist bees probably allows reproductive success of these plants to be maintained in a multitude of different conditions across geography and time. The remarkable species richness may then have resulted from keeping a constant successful floral strategy that confers lineage growth continuity, corroborated by estimated low extinction rates.

Pollination ecology may explain low extinction rates in *Myrcia* but does not alone explain high species diversity. *Myrcia* presents a net-diversification rate of *c.* 0.28 species Myr^{-1}, with an absolute speciation rate of *c.* 0.3 (Fig. 6). Such numbers are below those estimated for lineages that have undergone recent explosion in speciation rates, such as the Andean Centropogonids (Lagomarsino *et al.*, 2016) and *Lupinus* (Hughes *et al.*, 2006), but are comparable to those of Asterales, which have the highest speciation rates among angiosperm orders (Magallón & Sanderson,

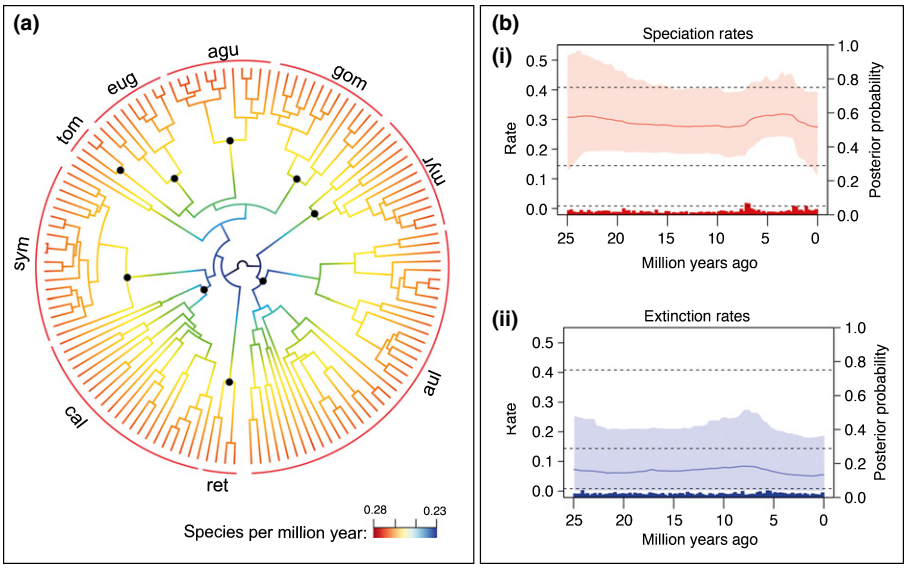

Fig. 6 Speciation rates in *Myrcia*: (a) BAMM phylo-rate showing no evidence for shifts in diversification rates. Clade crown nodes are marked by a black dot. (b) Oscillation in speciation and extinction rates during the last 25 million yr (Myr) in *Myrcia* (inferred by Tess) showing (bi) an intermediate rate of speciation with no significant acceleration over time and (bii) a continuously low extinction rate. Abbreviations for the nine sections in *Myrcia* are as follows: agu, *Aguava*; aul, *Aulomyrcia*; cal, *Calyptranthes*; eug, *Eugeniopsis*; gom, *Gomidesia*; myr, *Myrcia*; ret, *Reticulosae*; sym, *Sympodiomyrcia*; tom, *Tomentosae*.

2001). Because changes in pollination strategy do not appear to be driving diversification in this group, other selective pressures must be examined to explain high speciation rates and species accumulation through time in *Myrcia*. Assuming species estimates are correct (i.e. there is no taxonomic inflation), the elevated number of *Myrcia* species must be explained by flexibility to change in other traits of the plant that allow adaptation to distinct environmental factors (e.g. see Webster, 1993; and Arévalo *et al.*, 2017, for *Croton*); it is likely that speciation mechanisms will be explained by factors unrelated to pollination, as sympatric species of *Myrcia* all share similar pollinators and floral morphological disparity is low.

Reproductive isolation and speciation may be achieved by other means in *Myrcia*. Fruits in *Myrcia* are always fleshy berries and are also not highly variable in shape (Lucas *et al.*, 2011), but changes in epidermal and anatomical composition (Galan *et al.*, 2016) promote variation in colour and texture, subtly changing display and dispersal mode. These fruits are dispersed by a diversity of animals, mainly birds and mammals (Gressler *et al.*, 2006; Staggemeier *et al.*, 2017). Dispersal by vertebrates frequently moves seed germination far from the parental plant, promoting colonisation of new habitats and causing geographical isolation between populations, leading to allopatric speciation (Coyne & Orr, 2004). This mode of prezygotic reproductive isolation, in addition to the apparent lack in niche specificity (as *Myrcia* species are present in most South American biomes, Santos *et al.*, 2017), may be a key driver in the steady speciation rates of *Myrcia*.

Once populations are found in allopatry, other selective forces may act, leading to changes in vegetative traits that make these distinct evolutionary units recognised as different species of *Myrcia*. Vegetative structures, such as leaves, are indeed extremely variable in size, texture and thickness (e.g. Silva Moraes *et al.*, 2017). Growth habit varies from small subshrubs of *c.* 10 cm to trees of 40 m, sometimes even in closely related species (e.g. Santos *et al.*, 2016; Silva Moraes *et al.*, 2017). Furthermore, there is evidence for high levels of diversity of chemical compounds in *Myrcia* leaves (e.g. Stefanello *et al.*, 2011), reflecting selective pressure from herbivores and natural enemies that is very strong in tropical areas (Schemske *et al.*, 2009). Pressures from herbivores as drivers of speciation have been suggested for *Inga* (Fabaceae), a genus of similar floral homogeneity (Kursar *et al.*, 2009) but of much younger age (Richardson *et al.*, 2001). This flexibility in habit and vegetative traits may have been also critical in *Myrcia* species diversifying and colonising even the least hospitable Neotropical biomes (e.g. the 'Dry Diagonal' of South America, Simon *et al.*, 2009). As these newly formed species secondarily expand their distribution and are occasionally found in sympatry again, it is possible that their genetic differences are high enough to prevent gene flow even when occasional cross-pollination occurs between closely related species (i.e. post-zygotic isolation; see similar case in Cozzolino & Widmer, 2005).

Allopatric speciation seems to be a reasonable explanation when closely related species share pollinators, especially when they also present similar flowering phenology, as many *Myrcia* species do (Staggemeier *et al.*, 2010). However, sympatric speciation via subtle changes in reproductive phenology (including both anthesis time and flowering season) cannot be discarded until thorough studies aiming to test these hypotheses are performed (e.g. see Savolainen *et al.*, 2006). Furthermore, actual pollinator observations in the field are indispensable to confirm the speciation mechanisms suggested here.

Conclusion

Previous studies may have placed too much emphasis on the consequences of floral morphological changes for high rates of angiosperm diversification. These changes appear not to be the

strongest driver of plant speciation in many species-rich tropical tree lineages. Species diversification in *Myrcia* and other species-rich lineages with homogeneous flowers seems to be unrelated to shifts in pollination strategy. A highly efficient pollination system has apparently reached an adaptive plateau early during the evolution of the genus, thereby forming the basis for the long-lasting stable diversification process involving various non-floral traits. The origins of high species diversity in the absence of floral change are important when considering evolution of tropical plant diversity. The key to the success of some of the largest Neotropical angiosperm lineages may have been building remarkable species richness via simple variations within a theme on top of an advantageous adaptive plateau.

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Author contributions

TNCV and EL designed the research and generated the data set. TNCV and MC analysed the data. TNCV and EL wrote the paper. MC, ACM, GP, JS and AW contributed with further discussion and writing of the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Dataset S1 Trait data set.

Methods S1 Additional details on methods.

Notes S1 Additional information on data analyses.

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