

# Friends or foes? Plant-animal coevolutionary history is driven by both mutualistic and antagonistic interactions

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## Abstract

Coevolution played a central role in shaping biodiversity. However, coevolutionary events driving reciprocal diversification between interacting partners lack empirical evidences. Examples of diversification arising from mutualisms and antagonisms at different trophic levels are scarce, which limits our understanding on how complex relationships between species arise within communities. By adopting a cophylogenetic framework, we investigated whether congruence in plant-lemur phylogenies are driven by mutualisms and antagonisms in Madagascar, where endemic species have evolved within a unique isolated biogeographical context. Although we found weak support for coevolution, this is not to say that lemurs and plants did not share evolutionary history. Weak cophylogenetic signals do not necessarily imply lack of co-diversification. Rather, our results suggest that vertebrates and plants influenced each other evolution, but in a multi-specific context where the cophylogenetic process led to spatio-temporal asymmetries and shifts between periods of coevolution and independent evolution, ultimately resulting in a weak, continuous and diffuse process.

## INTRODUCTION

Coevolution—the reciprocal adaptation between two or more interacting organisms (Hembry et al. 2014)—is considered to have played a central role in shaping the diversity of life on Earth (Laine 2009). Yet, empirical evidence that coevolution is the main mechanism driving reciprocal diversification among interacting clades lack alternative explanations (Medina et al. 2022). Most examples of diversification driven by coevolution involves cases of resource competition between species during adaptive radiations (reviewed in Hembry et al. 2014). In contrast, examples of diversification arising from mutualistic and antagonistic interactions at different trophic levels are comparatively rare (e.g. Hutchinson et al. 2017; Fuzessy et al. 2022). The lack of attention to such pervasive and relevant types of interactions not only leads to a biased view of the importance of coevolution in diversification processes, but also limits our understanding on how complex relationships between species arise within communities (Thompson 1994).

A major obstacle to generate empirical evidence for coevolutionary history in antagonistic/mutualistic in-

teractions has been ruling out alternative explanations of diversification (Althoff et al. 2014; Medina et al. 2022). Although often taken as evidence for coevolution, reciprocal adaptation can emerge through adaptation by one of the interacting species alone or through neutral processes, such as genetic drift (Yoder & Nuismer 2010). Plant-animal interactions are recognized as important drivers of one-sided evolution (Strauss & Irwin 2004; Hembry & Weber 2020; Johnson et al. 2021). The correlation in features of interacting pairs, such as floral structures that match traits of pollinators (Nilsson 1988), color vision phenotypes that shape fruit color (Valenta et al. 2018), or defenses against predators that are met by counter defenses (Brodie & Ridenhour 2003), may also arise through correlated selection (Nuismer et al. 2010). Phylogenetic congruence between the interacting lineages, another major expectation of coevolutionary processes, is neither exempt of problems. While often interpreted as evidence of co-speciation, in nature, other types of evolutionary events often act concurrently, so a perfect cophylogenetic fit is rarely observed (Hutchinson et al. 2017; Fuzessy et al. 2022). Congruence may result from a combination of events unrelated to co-speciation, such as phylogenetic tracking (when one taxon tracks the evolution of its interaction partner), shared biogeographic histories (like vicariance, where common biogeographic events cause the subdivision of the distribution range of both interacting partners) or from parallel evolution driven by climate changes and other broad selective agents (Nuismer et al. 2010).

Demonstrating that interactions shape concomitant diversification on both sides has also been challenging due to two main constraints. First, interacting species on both sides are on a continuum of generalist-specialist interactions, which strongly affects the expected selective pressure each party exerts on the other, and hence the overall selective regime. In plant-animal interactions, phylogenetic tracking by adaptive switching behaviors, the ability of generalists to adaptively increase the consumption of one resource at the expense of decreased consumption of an alternative resource (Carnicer et al. 2008), is expected to be particularly relevant in creating phylogenetic congruences that can be confounded as coevolutionary diversification. This is because switching behaviors should not only facilitate animals to change feeding habits to new plants at the expense of others, but morphology and other constraints should also increase the chances that changes will favor plants that are closely related (and thus more similar) to those previously used as a resource (see Carnicer et al. 2008). The second constrain is that mutualism and antagonism themselves are only two ends of a single continuum, where no individual acts exclusively as an antagonist or a mutualist. For example, animals that feed on flowers can carry a significant amount of pollen in their body, acting simultaneously as florivores and pollinators (Kress et al. 1994). In the same vein, when feeding from fruits, many frugivores transport the seeds far from parent trees, acting as seed dispersers (Schupp et al. 2010). However, since not all seeds are defecated intact, seed dispersing animals can also act as seed predators (Norconk 2020). Due to these constrains and the difficulties to rule out alternative explanations, the role of coevolutionary processes in antagonistic/mutualistic interactions is currently backed by limited evidence (but see Hutchinson et al. 2017; Fuzessy et al. 2022).

While generating macroevolutionary evidence for coevolutionary diversification in antagonistic/mutualistic interactions remains challenging, recent advances in analytical tools developed for testing phylogenetic congruence provides a stronger framework to rule out alternative explanations of diversification (Balbuena et al. 2013; Blasco-Costa et al. 2021). A great advantage of these cophylogenetic methods (so called pattern-based methods, Dismukes et al. 2022) is the ability to use phylogenies where interacting species may have multiple pairs, which is the case of most plant-animal interactions. This allows to accommodate variation among the interacting species along the generalist-specialist continuum while considering phylogenetic uncertainties. With this framework, it is also possible to evaluate the contribution of individual links between interacting partners to the overall congruence (Hutchinson et al. 2017), and to test the extent to which the patterns of co-diversification and/or coevolution can be explained by phenotypic traits (Blasco-Costa et al. 2021).

Here, we adopt the above cophylogenetic framework to investigate macro-coevolutionary hypotheses for phylogenetic congruence of plant-lemur interactions in Madagascar. The flora and fauna of Madagascar provides an interesting study case to test such macro-coevolutionary hypotheses. About 82% of vascular plants (Callmander et al. 2011) and 84% of vertebrates (Goodman & Benstead 2005) are endemic, and hence have evolved under similar climatic conditions within a relatively small island, and have had ample opportunity

for interaction and coevolution (Génin et al. 2022). Lemurs, in particular, diverged from Haplorrhini primates about 68 Ma, having originated from an ancestor on the African mainland, and occurring only in Madagascar (Pozzi et al. 2014). They arrived in the island earlier than other mammalian orders, resulting in a variety of adaptations to diverse niches and feeding habits, which include the consumption of fruits, leaves, flowers, nectar, bark and insects (Richard & Dewar 1991; Dewar & Richard 2012).

Previous work suggests that lemur colonization and subsequent diversification may have been facilitated by the divergence of endemic Malagasy angiosperms. Based on physiognomic and chronological reconstructions, Génin et al. (2022) concluded that the generalization of mutualistic interactions (such as frugivory and nectarivory) might have boosted the coevolution of modern lemurs and at least 10 new angiosperm families. However, observations were based on physiognomic and chronological reconstructions, and empirical evidence remains elusive both for pattern and process. Also, we still lack clear evidence for the potential of mutualistic and antagonistic interactions in generating congruence in the phylogenies of lemurs and Malagasy angiosperms. Here we address these issues by testing for cophylogenetic signals across a spectrum of plant-lemur interactions, including frugivory, folivory, florivory, granivory and nectarivory. To support a role for coevolutionary processes, we first expect phylogenies of lemurs and plants to be congruent. This is to be expected if mutualistic and antagonistic interactions shape reciprocal evolution regardless of interaction type. We also expect to identify putative coevolving phenotypic traits that may have propelled diversification (Blasco-Costa et al. 2021). If both predictions met, we finally expect to find a positive correlation between phylogenetic distances and dissimilarities in sets of interacting partners for both lemurs and plants. This would help to rule out the possibility that the observed cophylogenetic patterns were driven by phylogenetic tracking or vicariance instead of coevolution (Kahnt et al. 2019).

## METHODS

### Database

#### *Plant-lemur interactions*

We used comprehensive information on plant-lemur interactions from the Lemur Food Plants database (LFP, Steffens 2020), updated with new information published between October 2019 and August 2022 (SM Table S1.1). The final dataset comprised 6,061 pairwise interactions (SM Table S1.2), where same species were recorded interacting with distinct types of resources (fruits, flowers, nectar, leaves). This is reflected in the dataset, which included 3,317 frugivory interactions (1,519 unique), 2,249 folivory interactions (1,056 unique), 717 florivory interactions (367 unique), 207 nectarivory interactions (50 unique) and 131 granivory interactions (58 unique). Rarefaction curves of sampling effort are presented in SM Figure S5.

#### *Plant and lemur traits*

To test our coevolutionary hypothesis, we used a functional trait approach. For plants, we obtained information on traits related to fruit-consumption (fruit length and width, seed length and width, number of seeds per fruit and diaspore color) from Albert-Daviaud et al. (2021) (SM Table S2). For lemurs, we obtained information on morphological traits known to be related to food consumption (body mass, body length, and tail length) (Mittermeier et al. 2010; SM Table S3).

We also gathered complementary information on lemur activity patterns and feeding guilds, which are related to feeding behavior (Mittermeier et al. 2010; Razafindratsima et al. 2018; Galán-Acedo et al. 2019; SM Table S3), and on plant traits that may be related to selection by lemurs according to interaction types: frugivory, folivory and florivory. Full description of plant traits is available in Table 1 and the complete list of traits is available in the Table S4. The complementary information on fruit, leaf and flower traits were available for a restricted number of plant species for which we had information on lemur-plant interactions. Thus, this complementary information was used only to test for their possible influence on the contribution to the cophylogenetic signal according to each type of interaction they are related (see ‘Individual contributions to the CS’ section below).

**Table 1** - Full description of plant traits

Interaction type	Plant trait	Measure	Description	
Frugivory	Fruit size	Length, width	Length and width measures	
	Seed size	Length, width	Length and width measures	
	Seeds/fruit	# seeds/fruit	Count of number of seeds per fruit	
	Fruit scent	Quantitative Chemical		
	Fruit color		Subjective color	Human-defined subjective color categories (contrast, black, red, green, yellow, purple)
			Hue	Hue subdivisions converted into a continuous variable relative to the background
			Chromatic distances	Chromatic contrasts between fruits and background leaves
	Fruit type	Fruit type	Classification based on morphology of the gynoecium and fruit shape	
	Nutritional content		Nitrogen	First and second axis of a PCA <sup>2</sup> (content of N, soluble protein)
			Protein	
		Sugar		
		Fat		
Folivory	Dispersal mode	Lemur/mixed	Whether if lemur-dispersed or dispersed by lemurs and other animals	
	Leaf form	Leaf area	Area of the smallest lamina unit (leaf or leaflet, in mm <sup>2</sup> )	
		Leaf thickness		
		Leaf mass	LMA	Leaf mass per leaf area (in g/mm <sup>2</sup> )
	Water content	Water	Water content per leaf dry mass (in ml/g)	
	Nutritional content	Nitrogen	N content per dry mass (in mg/g)	
Phosphorous		P content per dry mass (in mg/g)		
Florivory	Flower color	Subjective color	Human-defined subjective color categories (white, pink, red, yellow, purple)	
	Flower shape	Symmetry	Whether flower perianth can be divided into two or more parts	

\*Number of plant species for which each trait was available (total number of species used in the PACo analysis for cophylogenetic signal)

<sup>1</sup>A trichromatic phenotype was simulated based on S, M and L cones, with peak spectral sensitivities ( $\lambda_{max}$ ) of 425 and 53, respectively. The peak sensitivities selected roughly correspond to a typical old world monkey phenotype, as well as many Strepsirrhines (Nevo et al. 2018)

<sup>2</sup>First and second axis explained 64% of the variance in nutritional content of fruits (Supplementary Material S7).

### Phylogenetic reconstruction

We used ultrametric time-calibrated phylogenies and reconstructed both plant and lemur phylogeny at the species-level. For plants, we used two phylogeny sets: (a) complete, including all plant species in our dataset, and (b) native, excluding plant species considered introduced and/or invasive, following Kull et al. (2012) (SM Table S4). After updating the plant taxonomy nomenclature (WFO 2022), species relationships were obtained using ‘V.Phylomaker’ package in R, the most extensive phylogeny reconstruction tool covering the highest below family-level plant diversity, and with accurate and updated age estimates for internal nodes (Jin and Qian 2019). Lemur phylogeny was obtained from Bray et al. (2019), and taxonomic resolution was supplemented with additional references (Pastorini et al. 2001; Markolf et al. 2013; Lei et al. 2017).

### Cophylogenetic signal (CS)

To test for a CS between Angiosperms and lemurs, we implemented a Procrustean Approach to Cophylogeny (PACo; Balbuena et al. 2013). The PACo approach assesses phylogenetic congruence by optimizing the topological fit of two phylogenies using interaction graphs derived from the matrix of interactions (see Supplementary Material S6 for further details). It quantifies topological similarity by the extent to which each internal node and branch-length in a phylogenetic tree maps to a corresponding position in the other phylogenetic tree (Blasco-Costa et al. 2021). When co-phylogenetic signal is high, diversification patterns

of associated organisms over evolutionary time tend to be dependent, and congruence (i.e. topological similarity) between the phylogenies of interacting clades is expected to occur.

We calculated one CS value for each interaction type in our dataset: frugivory, folivory, florivory, nectarivory and granivory. PACo returns a quantification of the global fit based on observed interactions as the sum of squared residual distances ( $SS = \sum r^2$ ) between phylogenetic-interaction graphs (Balbuena et al. 2013). As in any regression analysis, the smaller the residual distances (Procrustean residuals), the better the fit of the two phylogenies, and the more support for a hypothesis of CS (Hutchinson et al. 2017). CS values were considered to be significant whenever it was smaller than 95% of the values obtained from 1000 randomizations of each interaction matrix. The randomization approach implemented in PACo (and other global-fit methods) maintains the topology of the phylogeny of each group while shuffling the associations (i.e., interactions) between species to generate random instances of the observed data (Balbuena et al. 2013, Hutchinson et al. 2017). To ease interpretation of results, CS was assessed as the Procrustes coefficient of determination ( $R^2 = 1 - SS$ ) (Legendre & Legendre 2012), following Soares et al. (2023). Thus, congruence expresses the extent to which each node in the phylogenetic tree of lemurs corresponds to a position in the phylogenetic tree of plants. A perfect congruence is obtained when  $R^2 = 1$  (i.e.,  $SS = 0$ ), and can be interpreted as a strong cospeciation evidence, which may or may not result from coevolutionary mechanisms.

### Drivers of CS

We estimated the phylogenetic signal in species interactions (PSI) associated to the calculation of CS to investigate the main drivers of the observed cophylogeny and ask whether closely related species interact with similar sets of partners. Following Russo et al. (2018) we used Mantel tests to assess if closely related lemur or plant species were more likely to interact with similar set of partners than expected by chance (e.g. Kahnt et al. 2019). For each of the interacting clades, the distance matrices compared were the between-species phylogenetic distance and the between-species dissimilarity (Jaccard distances) in interactions with the other clade. A significant CS (i.e. overall phylogenetic congruence) as well as a significant PSI for both lemur and plants were interpreted as a case of coevolution. Instead, if phylogenies were congruent but PSI was only significant for one of the interacting clades, this would indicate phylogenetic tracking. Finally, a significant CS but non-significant PSI for both lemurs and plants would suggest that the interactions were little important for the evolution of both clades, highlighting the importance of vicariance or other evolutionary processes. We used the function ‘`phylosignal_network`’ from the *R*-package RPANDA to estimate PSI values and estimate significance through permutations (Perez-Lamarque et al. 2022). In order to preserve any influence of the degree distribution of species, we used a conservative approach and set the permutation argument to ‘`nbpartners`’. This keeps constant the number of partners per species and shuffle at random their identity.

### Quantitative cophylogenetic spatial framework

To obtain a deeper understanding of the coevolution of interacting clades, we asked whether speciation in one of the partners influenced morphology in the other. To this purpose, we estimated distance matrices based on morphological traits for both plant and lemur species. Next, we superimpose each matrix with the phylogenetic distances of the interacting partner (plants when morphology was from lemurs, and lemurs when morphology was from plants) using a procrustean approach (Blasco-Costa et al. 2021). Coordinates derived from principal component analyses (PCA) were used to compute morphological distances between species for lemurs and plants (Supplementary Material S7). For plants, we only had complete data for fruit and seed length and width, so we restricted the analyses to frugivory interactions, while for lemurs we had body mass, body length, and tail length for all the species. Congruence values were considered to be significant whenever it was smaller than 95% of the values obtained from 1000 randomizations of each interaction matrix. A significant congruence of plant phylogeny with lemur morphology would suggest that plant speciation determined lemur body size, while a significant congruence of lemur phylogeny with plant morphology would be more consistent with the view that lemur speciation determined fruit and seed sizes.

### Individual contributions to the CS

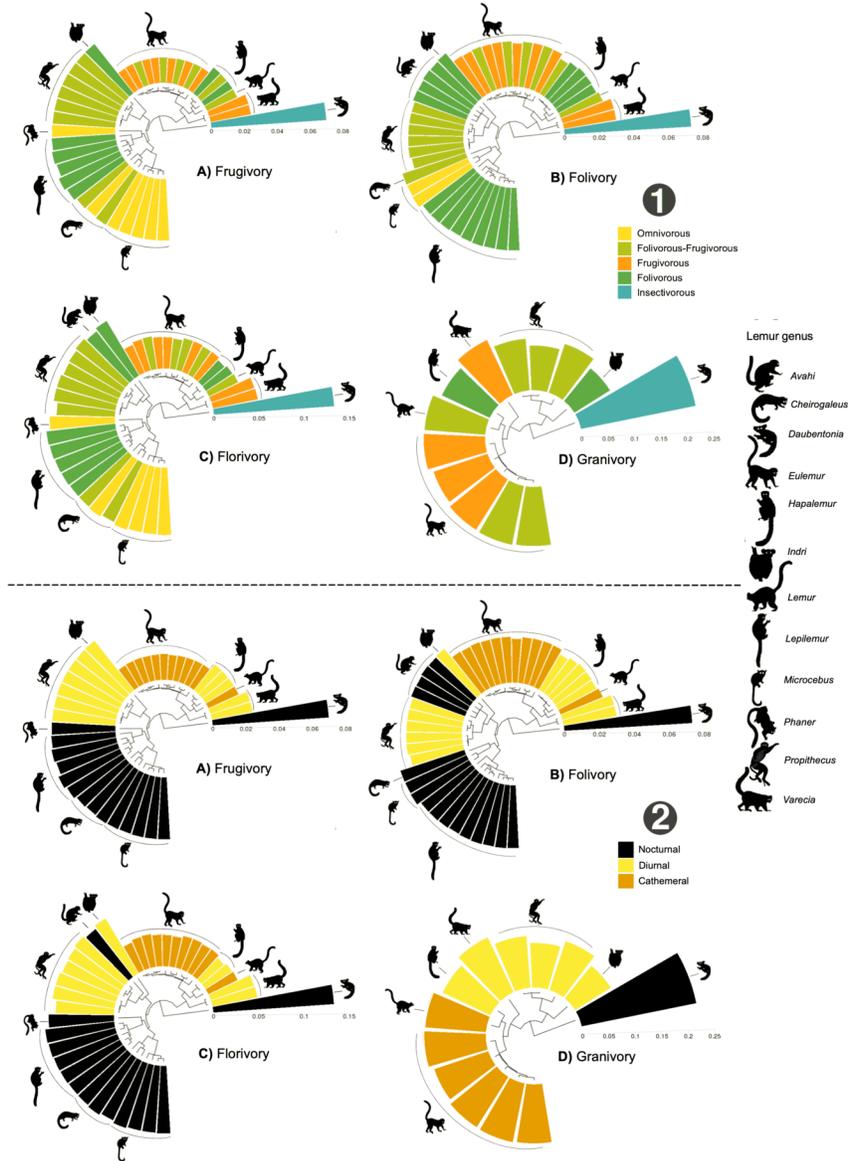
To test whether the obtained complementary information on lemur and plant traits influence the magnitude of the CS, we fitted linear mixed models with a lognormal distribution, using a Bayesian approximation (details in Supplementary Material S9). We fitted separated models for lemurs and plants, and separated models for each interaction type where CS was significant (see Supplemental Material for details).

For lemur traits, we had complete information for all lemur species included in our analyses. We fitted four models, one for each interaction type: frugivory, folivory, florivory and granivory. In each model, we included activity pattern (diurnal, nocturnal, cathemeral) and feeding guild (nectarivorous, omnivorous, folivorous-frugivorous, folivorous, frugivorous, insectivorous) as independent variables, and Procrustean residuals calculated by the PACo function as response variables. Abundant species tend to be generalists, so to account for the variation in geographic distribution area among lemur species, we used geographic range as a proxy of their density, given the lack of abundance data for each specific site. This approach is supported by strong associations between species distribution ranges and population sizes (Estrada et al. 2017). Data on lemur distribution range (in km<sup>2</sup>) were extracted from IUCN maps (IUCN 2022) and added as random effect to models. To account for the possible influence of sample size of interactions, the total number of studies reporting each interaction was incorporated as a random effect to the models. To control for the possible influence of phylogenetic relatedness, we added lemur phylogeny as a covariate in our models.

For plant traits, we had information for only few species, and each trait was available for different sets of plants (Table 1). In order to take better advantage of the available information, we fitted separated models for each trait, correlating it to the respective interaction type. To account for the possible influence of sample size of interactions, the total number of studies reporting each interaction was considered as random effect in the models. To control for the possible influence of phylogenetic relatedness, we added plant phylogeny as a covariate in our models.

## RESULTS

Our analyses of mutualistic and antagonistic interactions between lemurs and plants showed co-phylogenetic signals (CS), providing evidence for phylogenetic congruence between clades. The co-phylogenetic signal was generally low, but significant, for all interaction types (frugivory, folivory, florivory and granivory) except nectarivory, regardless of whether the interaction was antagonistic or mutualistic (Table 2). The pattern also held true when removing introduced and/or invasive plant species (SM Table S8), suggesting either a deep conservatism of interactions or that invasion rates were low in Madagascar. In all interaction types where we found significant CS, the contribution of lemurs to CS was determined by both feeding guild and activity pattern. In all cases, insectivores and nocturnal lemurs showed the largest Procrustean residuals, and contributed the least to the CS (Fig. 1, Supplementary Material S9-A). As for plant traits, we found little evidence that fruit, leaf and flower traits defined individual contribution of plants to the CS (Supplementary Material S9-B).



**Figure 1** – Contribution of each lemur genus to the overall cophylogenetic signal in (A) frugivory, (B) folivory, (C) florivory, and (D) granivory, measured as Procrustean residuals. Colors highlight differences among (1) feeding guilds and (2) activity pattern. Silhouettes were built by the authors. Procrustean residuals measure the variation in the topological fit that is not explained by the cophylogenetic structure of the interaction matrix. Interactions with small values contribute the most to CS, whereas those deviating more from the shared phylogenetic history contribute the less to CS.

To more accurately demonstrate coevolution, we also need evidence that phylogenetic distances and the dissimilarity between species in sets of interacting partners co-vary for both plants and lemurs (PSI). Contrary to our expectation of the existence of phylogenetic signal in species interactions for both plants and lemurs, we found that cophylogenetic patterns observed for frugivory, florivory and granivory emerged from similar lemurs (in terms of the between-species dissimilarities in sets of interacting partners) interacting with phylogenetic-related plants, with no reciprocal trend (i. e. plant diversification was unaffected by lemurs).

In the case of folivory, however, phylogenetic congruence was associated with significant phylogenetic signals for both plants and lemurs. Nectarivores were the only group that did not show any evidence of PSI for either plants or lemurs (Table 2).

Although the observed cophylogenetic patterns for frugivory do not appear to reflect strong coevolution, we further investigated this possibility by taking advantage of the existence of functionally relevant morphological traits for both plants and lemurs. We found that, in addition to the congruence between plant and lemur phylogenies (significant CS), there was also congruence between lemur phylogeny and plant morphology, and between plant phylogeny and lemur morphology (Table 2). This result suggests that, to some extent, plant speciation shaped lemur morphology (notably body size), at the same time that lemur speciation somehow affected diaspore morphology (fruit and/or seed sizes).

**Table 2** – Cophylogenetic signal (CS) and Phylogenetic signal in the interactions (PSI) between plants and lemurs

Interaction type	Cophylogenetic signal (CS)	Phylogenetic interactions (PSI)*	Phylogenetic interactions (PSI)										
	# lemurs spp.	# plants spp.	SS	L	P	R <sup>2</sup>	R <sup>2</sup>	R <sup>2</sup>	p-value	p-value	p-value	Plants	Plants
Frugivory	40	735	0.99	0.99	0.99	0.01	0.01	0.01	<b>0</b>	<b>0</b>	<b>0</b>	0.03	<b>0.00</b>
Folivory	44	679	0.98	-	-	0.02	-	-	<b>0</b>	-	-	0.03	<b>0.00</b>
Florivory	38	269	0.96	-	-	0.04	-	-	<b>0</b>	-	-	0.04	<b>0.00</b>
Nectarivory	7	40	0.89	-	-	0.11	-	-	0.2	-	-	0.04	0.12
Granivory	3	53	0.86	-	-	0.14	-	-	<b>0</b>	-	-	0.20	<b>0.00</b>

SS – sum of squares

R<sup>2</sup> – Procrustes coefficient of determination

CS – cophylogenetic signal (congruence between plant and lemur phylogenies)

L – lemur interactions (congruence of lemur phylogeny with differences in plant morphology)

P – plant interactions (congruence of plant phylogeny with differences in lemur morphology)

\*Interpretation of PSI: a p-value > 0.05 for plants means that similar lemurs tend not to interact with closely related plant species. Similarly, a p-value > 0.05 for lemurs means that similar plants tend not to interact with closely related lemurs. If p-values for both plants and lemurs are > 0.05 (as is the case of nectarivory), it means that there is no significant phylogenetic signal in species interactions.

## DISCUSSION

For a deeper understanding of the potential coevolution between lemur and plants, we tested for cophylogenetic signals in plant-lemur interactions, and detected significant, although weak, signals in frugivory, folivory,

florivory and granivory interactions. In the case of frugivory, plant speciation seems to have influenced lemur body size, at the same time that lemur speciation modulated diaspore size. Weak cophylogenetic signals do not necessarily imply lack of evolutionary history shared between symbiotic partners. Indeed, cophylogenetic patterns observed for frugivory, florivory and granivory emerged from lemurs sharing similar sets of partners interacting with phylogenetically-related plants with no reciprocal trend. In the case of folivory, plant diversification was also affected by lemurs. Our results do not give support to a strong coevolutionary diversification between lemurs and plants, in agreement with previous observations for Neotropical primates and Angiosperms (Fuzessy et al. 2022). Instead, our findings reflect that lemur feeding behavior is very generalist, specially of large lemur species, and support the idea that cophylogenetic signals are modulated by processes that are not directly related to strong co-speciation events, such as vicariance, phylogenetic tracking, and diffuse coevolution (Blasco-Costa et al. 2021).

The role of vicariance in coevolution —where common biogeographic events cause subdivision of distribution ranges of interacting partners —is dependent on the geographic scale. Vicariance contributes to coevolutionary processes by promoting diversification of entire populations that become isolated from each other. Over time, as populations diverge and adapt to their respective environments and evolve distinct and specialized structures and traits, including those that are important for their close relationship, they become less compatible. In this sense, vicariance is associated with high cophylogenetic signals (Blasco-Costa et al. 2021), so in the specific case of Madagascar, especially in the context of a relatively small island, it is not expected to play a significant role. Phylogenetic tracking is also usually associated with high cophylogenetic signals. It refers to a tendency of interacting partners to track each other’s evolutionary history, such that closely related species are more likely to interact than distantly related species (Blasco-Costa et al. 2021). Despite our finding of low cophylogenetic signals in lemur-plant interactions, phylogenetic congruence arose even in a supergeneralist system where other plant-consuming animals, such as insects and other vertebrates, may also be shaping plant diversification. The patterns observed here for frugivory, florivory and granivory emerged from similar lemurs interacting with closely related plants with no reciprocal trend (i.e., plant diversification was unaffected by lemurs). In this case, generalist lemurs seem to be tracking temporal fluctuations in resource availability, and increasing the consumption of one plant species at the expense of decreased consumption of an alternative, phylogenetically-related, resource, known as switching behavior (Carnicer et al. 2008). Such low levels of CS associated with a correlation between phylogenetic distances and dissimilarities in sets of interacting plants for lemurs can be interpreted as an indicative case of diffuse coevolution. In this sense, each lemur species interacts with a set of plants, and experiences a complex suite of selective pressures derived from the set of plant species they interact with.

In the case of folivory, similar plants were also found to interact with closely related lemur species, as an indicative of coevolution. Except for the strict folivorous genera *Hapalemur* and *Lepilemur*, lemurs use a variety of resources as feeding items, such as fruits, leaves flowers, seeds, nuts, nectar, and animal prey. Regardless of the main resource, leaves constitute an important resource for most species, reinforcing the idea that lemur-plant interactions are quite generalist. A weak cophylogenetic signals associated with positive correlations between phylogenetic distances and dissimilarities in sets of interacting partners for both lemurs and plants may also be interpreted as a case of diffuse coevolution, since distinct amounts of each feeding item vary across taxa, and other coexisting frugivorous and herbivorous clades act together, thus, favoring a diffuse coevolutionary process (Erikson 2016).

The multi-specific nature of the process delineating cophylogenetic patterns, as observed here, does not necessarily mean either that lemurs and plants have not coevolved, or that coevolution has necessarily been the primary force fueling diversification (e.g. Althoff et al. 2014; Fuzessy et al. 2022). Taken together, our results agree with the notion that vertebrates and plants can influence each other evolution but also highlight that they do not share a very tight coevolutionary history (Valenta & Nevo 2020, Fuzessy et al. 2022), such as those observed in host-parasite interactions (Gandon & Michalakakis 2002) or in some plant-pollinator systems (Herrera 2019). Spatio-temporal asymmetries, disruptions in the strength of the relationships between interacting partners, and shifts between periods of coevolution and independent evolution seem to be leading to adaptive changes, ultimately resulting in a weak and continuous process (Erikson 2016), in agreement

with previous observations in the Neotropics (Fuzessy et al. 2022). The resemblance between Madagascar and the Neotropics is remarkable, given that the number of frugivorous mammals in Madagascar is low when compared to other tropical communities, which are dominated for frugivorous primates (Fleming et al. 1987; Goodman & Ganzhorn 1997). In Madagascar, there are very few medium-sized strictly frugivorous lemurs (mostly *Varecia* and some species of *Eulemur*), and the proportion of fruits consumed by Malagasy lemurs is low compared with primates in other regions (Fleagle & Reed 1996). The distinctive phenology of Malagasy forests, associated with a great variability in spatio-temporal fruit availability has been proposed as the main drivers of the underrepresentation of frugivores in Madagascar (Dewar & Richar 2007). These patterns highlight potential risks associated with the current disruption of lemur-plant interactions. Despite high levels of generalism, we have shown that extant lemur-plant interactions are phylogenetically constrained, so if a species belonging to an already limited community of frugivores is lost, the probability of other species to fulfill the vacant role is reduced. This issue may be exacerbated if the species lost play the role of both dispersers and pollinators, as is the case between the black-and-white ruffed lemur, *Varecia variegata*, and the traveler palm, *Ravenala madagascariensis* (Kress et al. 1994). Such double mutualisms appear to be highly prevalent in island ecosystems, and pose additional risks for the continuing health of these forests when facing the extinction of frugivorous animal partners (Fuster et al. 2019).

Overall, lemurs consumed plant species that were more closely related than expected by chance, potentially indicating the consumption of plants with similar traits. In addition, invasive plant species have not disrupted the cophylogenetic structure of the interaction networks, suggesting a deep conservatism of interactions (or a low invasion rate in Madagascar). Indeed, we found no clear pattern of the influence of plant traits on the cophylogenetic signals. Nevertheless, to make any precise statement in this regard, we need complete and more detailed information on plant traits, including those related to fruits, leaves, and flowers, which are most frequently consumed by lemurs. Therefore, we encourage future studies to characterize Malagasy flora in terms of functional characteristics related to interactions with mutualistic and antagonistic partners (e. g. Albert-Daviaud et al. 2021).

Lemur feeding preferences and activity patterns likely shaped the congruence between their phylogeny with that of plants. Due to the unique ecology of Madagascar, lemurs evolved to occupy a wide range of niches, from night-living arboreal insectivores to ground-dwelling cathemeral folivores-frugivores, and diurnal frugivores (Gould & Sautner 2006). As expected, nocturnal lemurs and those that include a great amount of insects in their diets (and the least amount of plants), showed the largest Procrustean residuals, and contributed the least to the shared evolutionary history with plants, regardless of interaction type, and in agreement with previous studies (Fuzessy et al. 2022). Overall, diet and activity patterns have been important drivers of lemur evolution, shaping morphology, physiology, and behavior in unique ways that have allowed them to thrive in Malagasy diverse habitats. Dietary adaptations have driven the evolution of morphological and physiological features, such as specialized teeth, tongue, digestive systems, and metabolic rates (Cuozzo & Yamashita 2006; Powzyk & Mowry 2006; Pastor et al. 2021). Over evolutionary time, insectivores such as *Daubetonia*, for instance, have evolved specialized teeth and long fingers that are adapted for catching and handling prey, added to an enhanced night vision and specialized auditory systems to aid in hunting (Goodman et al. 1993; Sterling & McCreless 2006; Lhota et al. 2008). On the other hand, lemurs that consume great amounts of fruits and flowers have evolved color vision to help them distinguish ripe fruits and identify flowers, specially from a conspicuous background (Nevo et al. 2018).

Our study highlighted the important role of evolutionary history in structuring modern interaction patterns between vertebrates and plants. Our cophylogenetic analysis compared phylogenies of interacting partners based on observed extant interactions presented as binary lemur-plant interaction matrices. While this approach provides an important basis for investigating cophylogenetic relationships (Balbuena et al. 2013; Blasco-Costa et al. 2021), it ignores that the strength of associations among interacting partners may affect evolutionary outcomes, and thus precludes a deeper assessment of cophylogenetic processes. In the case of frugivory interactions, in addition to the congruence between plant and lemur phylogenies, we also detected a congruence between lemur phylogeny and plant morphology, and between plant phylogeny and lemur morphology. Although this result suggests that, to some extent, plant speciation shaped lemur body

size, and lemur speciation somehow affected diaspore morphology, we still lack sufficient information on the role of plant traits, including those related to fruits, leaves, and flowers, in shaping the cophylogenetic patterns described. Studies characterizing Malagasy flora in terms of functional characteristics related to interactions with mutualistic and antagonistic partners are urgently required (e. g. Albert-Daviaud et al. 2021). Despite these limitations, results presented here shed unprecedented light on the potential for supergeneralist interactions, including both antagonisms and mutualisms, to shape shared evolutionary trajectories between vertebrates and Angiosperms.

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