

Associated evolution of fruit size, fruit colour and spines in Neotropical palms

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Abstract

Understanding how ecological interactions have shaped the evolutionary dynamics of species traits remains a challenge in evolutionary ecology. Combining trait evolution models and phylogenies, we analysed the evolution of characters associated with seed dispersal (fruit size and colour) and herbivory (spines) in Neotropical palms to infer the role of these opposing animal–plant interactions in driving evolutionary patterns. We found that the evolution of fruit colour and fruit size was associated in Neotropical palms, supporting the adaptive interpretation of seed-dispersal syndromes and highlighting the role of frugivores in shaping plant evolution. Furthermore, we revealed a positive association between fruit size and the presence of spines on palm leaves, bracteas and stems. We hypothesize that interactions between palms and large-bodied frugivores/herbivores may explain the evolutionary relationship between fruit size and spines. Large-bodied frugivores, such as extinct megafauna, besides consuming the fruits and dispersing large seeds, may also have consumed the leaves or damaged the plants, thus simultaneously favouring the evolution of large fruits and defensive structures. Our findings show how current trait patterns can be understood as the result of the interplay between antagonistic and mutualistic interactions that have happened throughout the evolutionary history of a clade.

KEYWORDS

frugivory, herbivory, macroevolution, phylogeny, seed-dispersal syndromes, spines

1 | INTRODUCTION

Understanding how ecological interactions have shaped trait evolution in plants over time is a central challenge in evolutionary ecology (Strauss & Irwin, 2004). Animal–plant interactions, including mutualisms such as seed dispersal and antagonisms such as herbivory and seed predation, affect the fitness of individual plants. Seed dispersal by frugivores increases the fitness of plants by increasing the chances of seedling establishment on sites with high probability of survival (Wenny, 2001) and reducing density-dependent and distance-dependent mortality of seedlings (Connell, 1971; Janzen, 1970). On the other hand, herbivory decreases the fitness of plants by reducing plant growth, survival or reproduction (Strauss & Agrawal, 1999). These fitness consequences at the individual level may scale up, affecting the macroevolutionary patterns and dynamics of plant traits within clades (Agrawal, 2007; Onstein et al., 2017; Serrano-Serrano et al., 2015). Consequently, the extant morpho-physiological diversity that we observe in a given clade is shaped not only by abiotic environmental factors but also by biotic pressures that acted over individuals, moulding trait distributions in a clade over long timescales (Forest, Chase, Persson, Crane, & Hawkins, 2007).

The evolution of fruit characters, in particular, is thought to be highly responsive to interactions with frugivores. Different recurrent combinations of fruit size and colour presumably associated with the morphology and behaviour of different seed dispersers are termed seed-dispersal syndromes (Gautier-Hion et al., 1985; Janson, 1983). Fruit size constrains the ingestion of fruits by frugivores, so that large fruits with large seeds are usually consumed by large-bodied species (Fuzessy, Janson, & Silveira, 2018). Fruit colours affect attractiveness and detectability, with conspicuous colours that contrast against the background (red, black and purple) being more attractive to frugivorous birds, whereas dull-coloured fruits (green, yellow, brown and pale-orange) are often consumed by frugivorous mammals (Cazetta, Schaefer, & Galetti, 2009; Gautier-Hion et al., 1985; Janson, 1983). Recent phylogenetic studies support seed-dispersal syndromes as an adaptive outcome of plant–vertebrate interactions (Lomáscolo & Schaefer, 2010a; Nevo, Razafimandimby, et al., 2018; Valenta et al., 2018). For instance, fruit size and fruit colour of 64 fig species seem to have undergone correlated evolution as predicted by the seed-dispersal syndrome hypothesis (Lomáscolo, Speranza, & Kimball, 2008). Nevertheless, the support for the evolutionary interpretation of seed-dispersal syndromes as the result of selection by frugivores is mixed. Among fruit traits, fruit size seems to be more responsive to selection by frugivores than other traits (Jordano, 1995a). A recent macroevolutionary study found that fruit size of > 400 plant species across the Indo-Malay Archipelago was evolutionary related to bird and mammal diversity, but found no association between vertebrate diversity and fruit colours (Brodie, 2017). Thus, it remains unclear whether the evolutionary trajectories of fruit size and colour are associated within lineages and how general the adaptive signature of seed-dispersal syndromes across clades and regions is.

Whereas fruit traits are mainly associated with seed-dispersal mutualisms, the evolution of defensive traits is primarily shaped by antagonisms. Mechanical defensive traits like spines and thorns are a widespread defence strategy of plants against mammalian herbivores (Grubb, 1992), and leaves protected with spines tend to suffer less herbivory by mammals than unprotected ones (Cooper & Owen-Smith, 1986). Producing spines constitutes resource allocation costs to plants (Goheen, Young, Keesing, & Palmer, 2007), whereby resources that otherwise would be devoted to growth or reproduction are used to produce defensive structures. For example, in an exclusion experiment, *Acacia* individuals consumed by larger mammalian herbivores produced larger spines and less seed biomass than *Acacia* individuals that did not suffer attack by herbivores (Goheen et al., 2007). Thus, defensive traits and fruits may be involved in a trade-off in energy allocation. If this trade-off is consistent over time, it could lead to a negative association between the presence of spines and fruit size.

Alternatively, both fruit and defensive traits may respond to selection imposed by interactions with the same animal species if these interactions generate both negative and positive impacts on plant fitness. For example, African elephants are key seed dispersers for many plant species (Chapman, Chapman, & Wrangham, 1992; Sekar & Sukumar, 2015), but they often consume vegetative parts or damage the plant when reaching for fruits (Ihwagi, Vollrath, Chira, Douglas-Hamilton, & Kironchi, 2010; Owen-Smith, 1992). By investing in fruits that attract mammals and defences, such as spines in the stem or leaves that could deter them, the plant may increase seed dispersal while protecting itself, which could produce a positive association between spinescence and fruit size in a clade.

Here, we study trait evolution of Neotropical palms (Henderson, Galeano-Garces, & Bernal, 1995) to explore the evolutionary consequences of ecological interactions (frugivory, herbivory) in shaping the association between seed dispersal and defensive traits. The palm family (Arecaceae) is a species-rich plant family with more than 2,500 species worldwide. The palms are a characteristic element of tropical habitats (Couvreur & Baker, 2013; Kissling et al., 2012) and their fruits are a keystone resource for the tropical fauna (Fleming & Kress, 2013). The great morphological diversity in palm fruits and seeds (Figure 1a–c) (Henderson et al., 1995) allows seed dispersal by a wide range of frugivores, from small songbirds to elephants and tapirs (Muñoz, Trøjelsgaard, & Kissling, 2019; Zona & Henderson, 1989). This variety of morphological characteristics and interactions can be roughly mapped into two main seed-dispersal syndromes: small-colourful fruits dispersed mainly by birds and large-dull-coloured fruits dispersed mainly by mammals. The taxonomic diversity of palms seems to have been influenced by fruit and seed size, with speciation being greater in lineages of small-fruited palms (Onstein et al., 2017) and extinction being more likely in large-fruited ones (Onstein et al., 2018). Another conspicuous feature of many palm species is the presence of spines (Figure 1d–e; Henderson et al., 1995). Palms can have long spines on the trunk, as well as smaller densely clustered spines in the bractea and leaves. Several studies on palms show that spinescence reduces herbivory by large mammals (Beck, 2006; Berry, Gorchov,



FIGURE 1 Fruits and spines of different palm species exemplifying the variation in fruit and defence traits. (a) Variation in fruit size of palms. (b) Hard-shelled fruits of *Attalea brasiliensis*. (c) Conspicuous coloured fleshy fruits of *Aiphanes horrida*. (d) Spines at the base of the leaves of *Copernicia baileyana*. (e) Spines distributed on the stipe of *Aiphanes horrida*. Images: LFN; B - E in Jardim Botânico Plantarum—Nova Odessa, SP, Brazil

Endress, & Stevens, 2008; Gödel, Araujo, Kissling, & Svenning, 2016), suggesting that herbivory could have been an important selective force in the clade. Hence, palms are a suitable model system for testing how mutualistic and antagonistic plant–vertebrate interactions in tropical ecosystems have shaped the evolution of plant seed dispersal and defence traits.

In this study, we compiled a comprehensive dataset on Neotropical palm traits and used the most recent species-level palm phylogeny (Faurby, Eiserhardt, Baker, & Svenning, 2016) to examine the evolutionary trends of fruit size, fruit colour and spines, and to investigate whether certain character states have been favoured during the evolutionary history of the clade. We expected to detect associated evolution of fruit traits related to seed-dispersal syndromes, so that small fruits and fruit colours related to frugivory by birds would be evolutionarily linked, whereas large fruits would be associated with dull colours as they are predominantly consumed by frugivorous mammals. Additionally, we expected to find a negative association between fruit size and defensive traits as a result of resource allocation trade-offs, or a positive association in case large fruits and spines evolved as a result of positive and negative effects of interactions with large frugivores.

2 | MATERIAL AND METHODS

We built a data set on palm traits (available in <https://doi.org/10.5061/dryad.2280gb5nx>) using information from the

literature. Most of the data on traits were obtained from Henderson et al. (1995). In cases where no information from Henderson et al. (1995) was found, we completed the data set with information from Lorenzi, Souza, Costa, Cerqueira, and Ferreira (2004). We systematically searched the literature for information on fruit size, fruit colour and spines for those palm species that were not present in the two key references. We did not consider hybrids and subspecies, identified according to The Plant List (<http://www.theplantlist.org/>), in our analyses. We also excluded *Cocos nucifera* from our analyses because this species is exotic in the Neotropics, and it is not dispersed by animals (Baudouin & Lebrun, 2009), besides being clearly an outlier in terms of fruit size. In total, our data set comprised information on fruit colour for 434 palm species and on spines and average fruit size for 530 out of the approximately 550 species occurring in the Neotropics (Henderson et al., 1995). We considered only the colour of ripe fruits and discarded those species for which the information about colour was ambiguous. We used fruit length as proxy for fruit size in our analyses. Using only one dimension is enough to characterize fruit size in palms because length and diameter are highly correlated in palm fruits (Onstein et al., 2018). For most plants, seed size may be the factor constraining consumption by seed dispersers. Palms, however, are mainly single seeded, or when having multiple seeds, the seeds are often within the same diaspore. Moreover, several previous studies have demonstrated a positive correlation between fruit and seed size in fleshy-fruited plants (Jordano, 2000), especially for single or few seeded fruits (Jordano, 1995b). Thus, we

reasonably assume a strong correlation between fruit size and seed size in Neotropical palms.

We classified palm species into two categories according to the predominant colour of the mature fruit: 'colourful fruits' (red, black and purple) and 'dull-coloured fruits' (green, yellow, brown and orange), reflecting colours that have been related to bird and mammal frugivory, respectively (Gautier-Hion et al., 1985; Nevo, Valenta, et al., 2018). We also classified all species according to the presence ('with spines') and absence ('spineless') of spines. We consider spines to be any stiff sharply pointed structure (Grubb, 1992) that is present either at the stem, peduncular bract, rachis or petioles, which is often associated with defence specifically related to mammalian herbivory (Charles-Dominique et al., 2016).

We used a recently compiled species-level phylogeny of palms (Faurby et al., 2016) for the comparative analyses. Faurby et al. (2016) constructed a variety of time-calibrated super-trees based on all available molecular and morphological characters using a Bayesian approach. Genetic information was available for all 184 genera and for 901 of the 2,539 species included in the phylogeny, and 1,255 species had genetic or morphological data. Taxonomic information (following Govaerts, Dransfield, Zona, Hode, and Henderson (2011)) and indirect morphological knowledge inferred from taxonomy were incorporated as topological constraints to guide the placement of species lacking genetic or direct morphological information (Faurby et al., 2016). Therefore, we performed the analyses on sets of phylogenetic trees that take into account previous morphological knowledge contained in taxonomic information (constraints) and the most up to date world checklist of Arecaceae (Govaerts et al., 2011). To deal with phylogenetic uncertainty, we performed all evolutionary analyses based on sets of 100 trees randomly sampled for the larger sets of trees generated from the posterior distributions obtained by Faurby et al. (2016) and investigated the distributions for the

estimated parameters. Before the analyses, we 'pruned' out those species from the trees for which we did not have information on fruit traits and presence of spines.

To infer the evolutionary histories of fruit colour and presence/absence of spines in Neotropical palms, we performed ancestral state reconstruction using stochastic character mapping (Revell, 2012). Each ancestral state reconstruction for a given phylogeny was obtained from 100 simulations, which were then combined to generate the posterior probabilities of each node being in each trait state (Revell, 2012). This approach therefore incorporates uncertainty in the character history itself (Revell, 2012). To also incorporate the uncertainty related to the phylogenetic uncertainty, we repeated the ancestral state reconstructions for each of the 100 randomly sampled phylogenies, which allowed us to infer the number of evolutionary transitions between trait states during palm evolution. We also registered the proportion of simulations assigning the root a given state to infer how consistent across phylogenies was the state assigned to the most recent common ancestor of all Neotropical palms.

To investigate the evolutionary relationship between (1) fruit length and fruit colour, and (2) fruit length and the presence of spines, we used a maximum-likelihood approach implemented in the 'OUwie' R package (Beaulieu & O'Meara, 2016). The OUwie function uses trait data for the tips of the species-level phylogeny to infer the evolutionary dynamics of continuous traits (fruit size) while considering ancestral reconstructions of categorical traits (i.e. presence/absence of spines and fruit colour). Brownian motion (BM) models assume trait evolution occurs through a random walk governed by the evolutionary rate parameter σ^2 . The Ornstein-Uhlenbeck (OU) models assume a constrained random walk where trait values change towards an optimum θ with strength of selection α and evolutionary rate σ^2 (Hansen, 1997). We fitted seven

TABLE 1 Description and interpretation of the fitted evolutionary models. The discrete character refers to lineages with different states of fruit colour (colourful or dull-coloured) and spinescence (with spines or spineless). The symbols σ^2 , θ and α represent, the evolutionary rate parameter, evolutionary optima and strength of selection, respectively

Evolutionary models	Description	Implications for trait association
BM1	Single-rate BM model	No association between fruit size and the discrete character
BMS	Multirate BM model, which allows σ^2 to differ among lineages with different states for each discrete character	Association between fruit size and the discrete character
OU1	OU model with a single optimum	No association between fruit size and the discrete character
OUM	OU model allowing distinct θ for each discrete character	Association between fruit size and the discrete character
OUMV	OU model allowing different σ^2 and θ for each discrete character	Association between fruit size and the discrete character
OUMA	OU model allowing different α and θ for each discrete character	Association between fruit size and the discrete character
OUMVA	OU model allowing different σ^2 , α and θ for each discrete character	Association between fruit size and the discrete character

evolutionary models derived from BM and OU models (Table 1) to estimate parameter values that best describe the evolution of fruit length given the phylogeny and each ancestral state reconstruction (Alencar, Martins, Burin, & Quental, 2017; Onstein & Linder, 2016). These models differ in whether the rate of evolution, evolutionary optima and strength of selection are allowed to vary, thus differing in their assumptions on how the evolution of fruit length is associated with the evolution of fruit colours or the presence/absence of spines.

For both the analyses on the evolutionary relationship between fruit length and colours or fruit length and spines, we considered 10 ancestral state reconstructions for each of the 100 phylogenies, that is 1,000 ancestral state reconstructions in total. We excluded reconstructions for which model fit resulted in negative eigenvalues of the Hessian matrix, which indicates that the model failed in obtaining reliable parameter estimates (Beaulieu & O'Meara, 2016), and those which returned very unrealistic fruit size optima ($\theta > 20$ cm or $\theta < 0.1$ cm). We also excluded statistical outliers for the remaining parameters, since these may also be produced when the model fails to converge (Alencar et al., 2017). All these cases are referred herein as analyses with convergence problems. Because OUMA and OUMVA models often failed to converge, we report the results for the remaining models and present the results for OUMA and OUMVA in the Supplementary Material (Figures S8-S11). For the remaining models (BM1, BMS, OU1, OUM, OUMV), only 20 of the 1,000 reconstructions (2%) showed convergence problems for at least one model and had to be discarded so that model fit comparisons were valid. Thus, we report model fit results for a total of 980 reconstructions.

We used the Akaike information criterion corrected for small sample sizes (AICc) to identify the model with the greatest fit to the trait distribution in the phylogenies (Burnham & Anderson, 2004).

We considered that a model was supported if the difference between the AICc of this and the other models was greater than two. We calculated the relative support for each model using Akaike weights (w_i); w_i varies from 0 (no support) to 1 (best fit).

If there is an evolutionary relationship between fruit length and fruit colour, as suggested by the seed-dispersal syndrome hypothesis, then we should expect the favoured models to be those with different evolutionary optima for fruit length on palm lineages differing in fruit colour, with a larger optimum in lineages with dull-coloured fruits. Similarly, if the evolution of fruit size and spines had any association, models with different optima for fruit length should be favoured for the analyses considering spinescence. Different rates associated with different colour or spinescence states could also indicate different evolutionary trends. One particular genus of spineless palms with very large seeds, *Attalea*, significantly raises the mean size of fruits in palms without spines. *Attalea* fruits are highly fibrous and known to be dispersed mainly by scatter-hoarding rodents and parrots (Baños-Villalba et al., 2017; Dracxler & Forget, 2017), therefore being often associated with a third particular seed-dispersal syndrome. To understand how this genus affects the obtained results, we performed a sensitivity analysis by removing *Attalea* from the phylogeny and rerunning the analyses (see Supplementary Material).

3 | RESULTS

Fruit length of Neotropical palms varies from 0.4 cm to 12.5 cm with the majority of fruits (63%) being smaller than or equal to 2 cm (Figure 2a). Regarding fruit colour, 177 palm species (41%) have dull-coloured fruits and 257 (59%) have colourful fruits, with both states being widely distributed in the phylogeny (Figure 3). Spines are

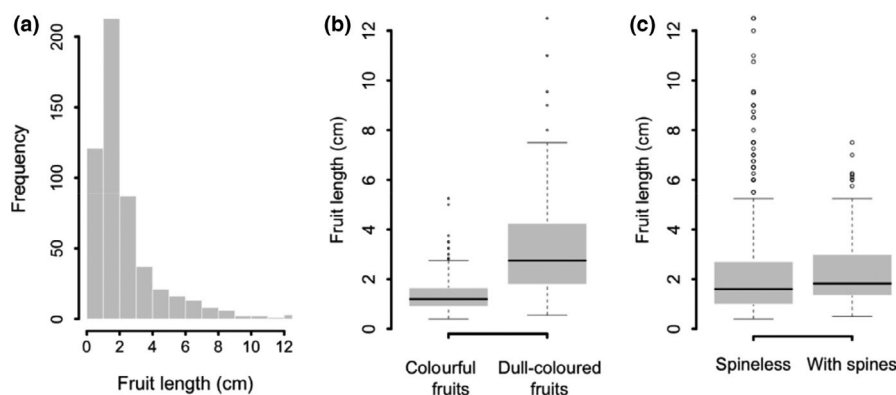


FIGURE 2 (a) Frequency distribution of fruit sizes in Neotropical palms ($n = 530$ species). (b) Length of fruits (cm) as a function of colourful versus dull-coloured fruits ($n = 434$ species). Mean fruit size was significantly smaller in colourful (mean = 1.36 cm) than in dull-coloured fruits (mean = 3.30 cm; Welch's $t = -12.07$, $p < .0001$) when ignoring the phylogenetic relationship between taxa. (c) Length of fruits (cm) as a function of the presence or absence of spines ($n = 530$ species). There was no statistically significant difference in mean fruit size between palms with spines (mean = 2.32 cm) and palms without spines (mean = 2.38 cm, Welch's $t = 0.38$; $p = .70$) when ignoring the phylogenetic relationship between taxa. In b and c, black horizontal lines indicate the medians. The whiskers extend to the data point which is no more than 1.5 times the interquartile range from the box. Outliers outside 1.5 times the interquartile range from the box are shown as dots

FIGURE 3 Reconstruction of ancestral states for fruit colour in Neotropical palms from stochastic character mapping (100 simulations for a given topology) shown for one representative maximum clade credibility tree (out of 100 randomly selected and analysed phylogenies). Yellow branches indicate dull-coloured fruits in a given lineage, and black branches indicate colourful fruits. Bars in the tips indicate the mean fruit length. The circles represent the posterior distribution of the marginal ancestral states of each node being dull-coloured fruits (yellow) and colourful fruits (black). Examples of representative palm genera are indicated at tips. See ladderized version in the Supplementary Material (Figure S1)

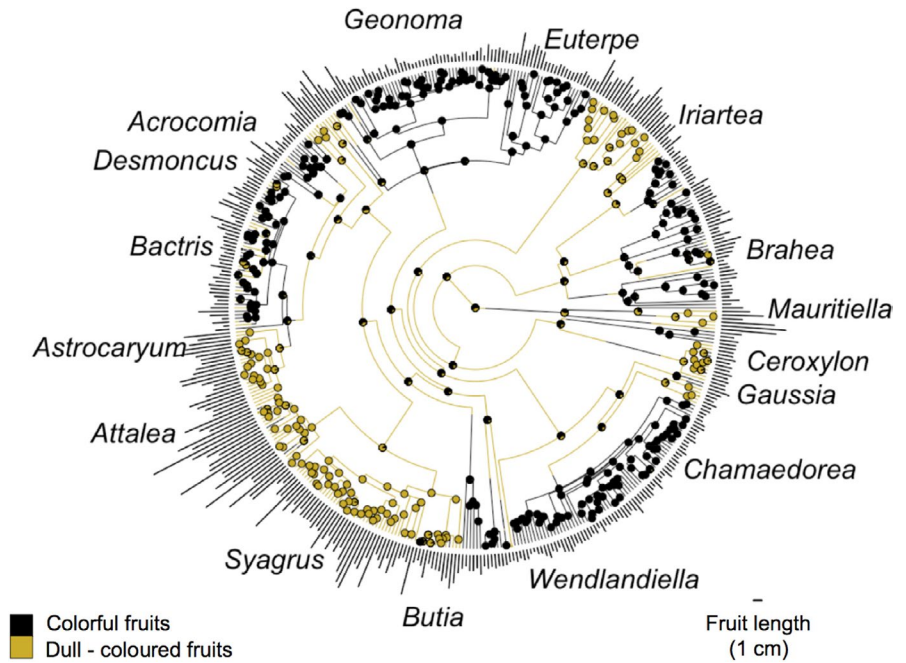
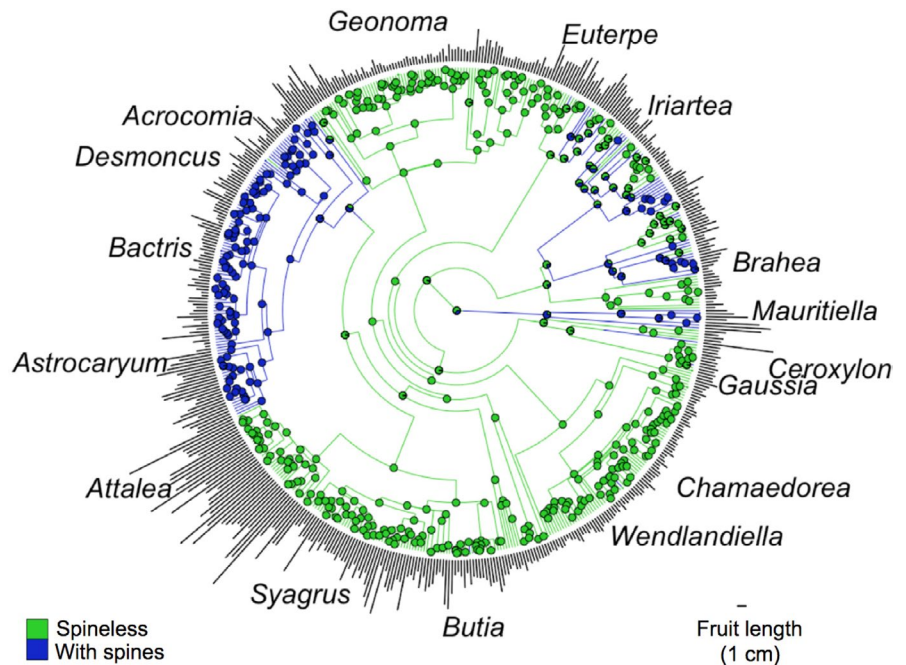


FIGURE 4 Reconstruction of ancestral states for spines in Neotropical palms from stochastic character mapping (100 simulations for a given topology) shown for one representative maximum clade credibility tree (out of 100 randomly selected and analysed phylogenies). Blue branches indicate the presence of spines in a given lineage, and green branches indicate the absence of spines. Bars in the tips indicate the mean fruit length. The circles represent the posterior distribution of the marginal ancestral states of each node being with spines (blue) and spineless (green). Examples of representative palm genera are indicated at tips. See ladderized version in the Supplementary Material (Figure S2)



present in 164 Neotropical palm species (31%), whereas 366 (69%) are spineless. Spines are mainly found in one large clade including the palm genera *Acrocomia*, *Astrocarium* and *Bactris*, and in an early-diverging clade containing *Mauritiella* and *Mauritia* (Figure 4). Of the 61 genera present in our data set, 26 have species with spines (43%), and in 35 genera, all species are spineless (57%), 38 have species with dull-coloured fruits (62%), and 33 have species with colourful fruits (54%).

According to the ancestral state reconstructions for all analysed trees, colourful fruits and spineless palms were the most likely root ancestral states (Figures 3-4). All reconstructions indicated that dull-coloured fruits evolved relatively late in Neotropical palms as

seen in the clade encompassing *Syagrus*, *Astrocarium* and *Attalea*, all of which present relatively large fruits (Figure 3). After emerging though, reversals to colourful fruits were infrequent. There was also an early emergence of spines in lineages of *Mauritiella*, but spines evolved again in the clade that comprises the genera *Acrocomia*, *Desmoncus*, *Bactris* and *Astrocarium* and multiple times in other lineages (Figure 4). Reconstructions suggest that transitions from the colourful state to the dull-coloured state (40.8 ± 5.67) were almost twice as frequent as transitions in the other direction (26.70 ± 5.78 ; $t = 174.26$, $p < .001$). Similarly, the acquisition of spines was much more frequent (25.98 ± 2.95) across the phylogeny than the loss of spines (9.2 ± 3.03 ; $t = 393.96$, $p < .001$).

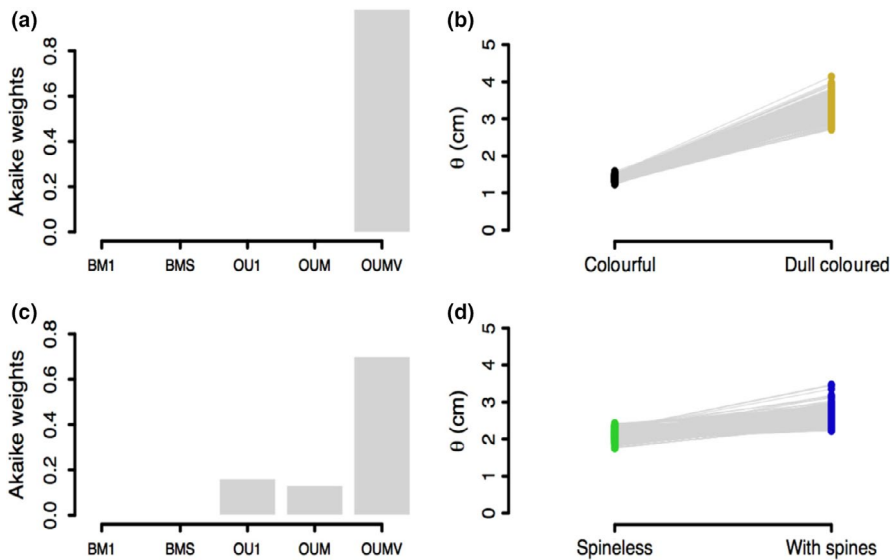


FIGURE 5 The evolution of fruit traits and spines in Neotropical palms. Akaike weights (a) and the estimated evolutionary optimum of fruit length (θ) in lineages with colourful fruits and dull-coloured fruits (b). Akaike weights (c) and evolutionary optimum of fruit length (θ) in lineages with spines and without spines (d). Each line in b and d represents one phylogeny after ancestral state reconstruction out of 980, and points represent estimated θ for the OUMV model

For all analysed phylogenies, a model where fruit size had different optima and evolutionary rates (OUMV) for lineages with different fruit colours had stronger support than any model assuming no association between fruit colour and size (Figure 5a). This suggests that evolutionary trends in fruit size differ for palms with colourful and dull-coloured fruits. The estimated optimum values (θ) consistently indicated that lineages with dull-coloured fruits tended to have larger fruits (mean = 3.38 cm) than lineages with colourful fruits (mean = 1.39 cm; Figure 5b). The evolutionary rate parameter (σ^2) was lower for lineages with colourful fruits than for lineages with dull-coloured fruits (Figure S3), suggesting higher stochasticity in fruit size evolution in lineages with dull-coloured fruits.

We also found greater support for models assuming different evolutionary trends of fruit size for lineages with and without spines (OUMV had the best fit for 758 out of 980 phylogenies; Figure 5c). The estimated optimum values (θ) consistently indicated that fruits tended to evolve towards larger sizes in lineages with spines (mean = 2.51 cm) compared to lineages without spines (mean = 2.12 cm; Figure 5d). Differences in the rate parameter (σ^2) between the lineages with and without spines did not show a consistent trend across replicates (Figure S4). The sensitivity analysis where we removed the genus *Attalea* from the phylogeny and re-ran the analysis shows that the difference in the mean fruit size optima between lineages with and without spines increases considerably without considering *Attalea* (2.52 cm versus 1.81 cm, respectively; Figures S5–S7).

4 | DISCUSSION

We used species-level phylogenies and trait evolution models to investigate the evolutionary history of fruit and defensive traits of Neotropical palms. We found that the most recent ancestor of all Neotropical palms most likely had colourful fruits and no spines and transitions from colourful to dull-coloured fruits and the acquisition

of spines were more frequent than from dull to colourful fruits or losing spines, respectively, across the clade evolutionary history. Our results indicate that fruit size evolved towards values that were larger for palms that also evolved dull-coloured fruits and spines. The evolution towards optimal values, as implied by an OU model, can be interpreted as the result of evolutionary constraints on fruit size (Arnold, 1992; Futuyma, 2010). Smaller-sized seed dispersers are limited by gape size in what they can consume, imposing selective pressures that may constrain the evolution of large fruits (Galetti et al., 2013). Conversely, small seeds have less reserves and are less resistant to desiccation (Galetti et al., 2013; Parolin, 2000). Thus, abiotic selective pressures that favour larger sizes may act in the opposite direction of selection against large sizes imposed by small frugivores.

Our analyses suggest that fruit size optimum in palms with dull-coloured fruits was more than twice as high as the optimum of palms with colourful fruits. Because birds preferentially consume colourful fruits, small-gaped birds impose selection towards smaller fruit size (Galetti et al., 2013), whereas dull-coloured fruits chiefly consumed by mammals can attain larger sizes. These different evolutionary trends for palm lineages with colourful and dull-coloured fruits support the adaptive interpretation of seed-dispersal syndromes (Gautier-Hion et al., 1985). Recent results for other plant clades also found that multiple fruit traits, including colour, evolved in response to selective pressures exerted by seed dispersers (Lomáscolo, Levey, Kimball, Bolker, & Alborn, 2010; Nevo, Valenta, et al., 2018). Our results indicate that, at least in Neotropical palms, fruit size and fruit colours evolved together over long time scales. One possibility for the mixed conclusions about the evolutionary basis of seed-dispersal syndromes for other plant clades is that the relationship among fruit traits is not consistent across lineages within a clade. Certain lineages whose species are more dependent on interactions with seed dispersers and establish interactions with a wide range of frugivores should have more pronounced associations between fruit traits related to seed dispersal.

Besides the relationship between fruit size and colour, our analysis also indicates that fruits which have evolved towards larger sizes also evolved spines, suggesting that there is no signature of trade-offs in resource allocation related to defence and reproduction in Neotropical palms at the macroevolutionary scale. Interactions with large mammals could be the underlying reason for such a relationship. Although large mammals can disperse large amounts of seeds and promote long-distance seed dispersal (Jordano, 2017; Pires, Guimarães, Galetti, & Jordano, 2018), they may also damage the plant when scratching, browsing or reaching for fruits (Owen-Smith, 1992). Moreover, although mammals often consume ripe fruits that have fallen from the plant, large mammals may reach and consume fruits before they have ripened. This behaviour, which has been frequently observed among primates (Dunham, 1990; Yeager, 1989), may reduce recruitment since the embryo may not be ready for germination when dispersed. Spines have been shown to be efficient in reducing the damage done by browsers (Milewski, Young, & Madden, 1991) and the rates of biomass ingestion by herbivores (Belovsky, Schmitz, Slade, & Dawson, 1991) and may discourage early consumption of fruits and seeds (Smythe, 1989). If interactions with large mammals can be beneficial but can also result in negative impacts on fitness, the interplay between selective pressures resulting from mutualistic and antagonistic outcomes of interactions with the same partners could generate the evolutionary relationship between large fruits and mechanical defences as observed in this study.

Even though most large mammals are now extinct in the neotropics, Neotropical palms evolved in an ecological context where mammalian assemblages were rich in large-bodied species, before the Late Quaternary Extinction episode (Guimarães, Galetti, & Jordano, 2008; Janzen & Martin, 1982). Therefore, certain observed characteristics of fruits and defences could be 'anachronic', that is, traits shaped by extinct interactions (Janzen & Martin, 1982). Illustrative examples of palms with fruit and defensive traits that have been considered anachronic are *Astrocaryum aculeatisimum* from the Brazilian Atlantic forest and *Acrocomia aculeata* from the Brazilian Cerrado and other open habitats, both species with long spines and large fruits (≥ 4 cm), and whose seeds are currently dispersed by scatter-hoarding rodents or domestic cattle (Galetti, Donatti, Pires, Guimarães, & Jordano, 2006; Gödel et al., 2016). Interactions with large browsing mammals from the past like *Ereotherium*, *Notiomastodon*, *Palaeolama* and *Macrauchenia*, for example, would have allowed large fruits to evolve, but could also have favoured the evolution of defences, thus generating the observed association between spines and very large ('megafaunal') fruits for some of the studied lineages.

Palms in genus *Attalea* clearly do not show the association between spines and fruit size. The *Attalea* palms are spineless, and fruits and seeds are very large. When we performed the same analyses without this clade, the estimated difference in evolutionary optima of fruit size between lineages with and without spines was much more pronounced. *Attalea* fruits are dull-coloured and fibrous, and seeds have a thick and hard protective coat (Silvius,

2005) related to dispersal by scatter-hoarding rodents and parrots rather than large mammals (Baños-Villalba et al., 2017; Dracxler & Forget, 2017). Thus, trait evolution in *Attalea* may have been strongly influenced by interactions with rodents and parrots and less influenced by interactions with other groups such as large-bodied frugivorous, which would explain no association between spinescence and large fruits. Moreover, *Attalea*, and other palms, may rely on other defensive strategies against seed predators and herbivores, including chemical defences and resprouting (Liesenfeld & Vieira, 2018). We focused on spines, which have a well-established function as a defensive trait, to examine the possible effects of antagonisms on patterns of trait evolution. Although information on other types of defences including chemical defences in palms is currently limited for a several species, future work may enable to investigate the relationship between fruit size and other defensive strategies besides spines in Neotropical palms and other plant groups.

Another hypothesis that could explain the evolution of large fruits in plants with spines is that the evolution of both large fruits and spines responded to seed predators. Producing large fruits and seeds is costly for the plant (Goheen et al., 2007), but large seeds often have a greater chance of germination (Galetti et al., 2013; Gómez, 2004). Seed predation can significantly impact fitness (Gómez, 2004; Orrock, Levey, Danielson, & Damschen, 2006) and plants that invest on producing large seeds also invest in defending them against predation (Bodmer, 1991). The evolutionary relationship between fruit size and presence of spines could be a response to seed predation pressure by vertebrates, especially rodents such as squirrels, which occasionally act as seed dispersers (Muñoz, Schaefer, Böhning-Gaese, & Schleuning, 2017) but often consume palm seeds (Alves, Mendes, & Ribeiro, 2018; Mendes, Koprowski, & Galetti, 2019). However, even though the effectiveness of spines in preventing seed predation has not been systematically investigated, the spiny trunks of *Astrocaryum* spp. do not seem to be enough to prevent the access of squirrels to fruits and seeds (Palmer & Koprowski, 2014). If spines are not effective in preventing seed predation by rodents in the present, then the hypothesis that these interactions are the underlying cause for the evolutionary association between fruit size and spines is less plausible.

Interactions between frugivores and angiosperms have a long history, of at least 80 million years (Eriksson, 2016). Phylogenetic and fossil evidence suggest that these interactions had a major influence on the evolution and diversification of several plant and animal clades (Burin, Kissling, Guimarães, Şekercioglu, & Quental, 2016; Gómez & Verdú, 2012), including palms (Onstein et al., 2017, 2018). Phylogenetic comparative methods can help us to understand how this long history of plant-vertebrate interactions affected trait evolution (Cornwell & Nakagawa, 2017). We used different macroevolutionary models to shed light on the evolution of reproductive and defensive plant traits. By exploring the relationship between fruit size, fruit colour and defensive traits, our analyses suggest that the interplay between mutualisms and antagonisms (Hanley, Lamont, & Armbruster, 2009) affected the

macroevolutionary dynamics of Neotropical palm traits. Future studies could investigate whether similar patterns occur in other clades, and whether these patterns are replicated in different geographic regions (e.g. Afrotropics, South-East Asia) and across spatial and temporal scales.

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AUTHOR CONTRIBUTIONS

L.F.N. and M.M.P. designed the study; L.F.N. performed the analyses; P.R.G., R.E.O. and W.D.K. helped interpreting the results; L.F.N. wrote the manuscript; all authors reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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