UNCOUPLED EVOLUTION OF MALE AND FEMALE CONE SIZES IN AN ANCIENT CONIFER LINEAGE

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Premise of research. Sexual functions in gymnosperms are mostly performed by separate reproductive structures, which largely reduces sexual interference and sets the scene for morphological and functional sexual specialization. The evolutionary trajectories followed by traits related to the male and female functions are therefore expected to be uncoupled. Studies on the fossil record of the conifer family Araucariaceae revealed important morphological changes occurring in reproductive cones. Here, we explored the pattern of evolution of male and female cone sizes in Araucariaceae, with a special focus on *Araucaria*—the most variable and widespread genus in the family.

Methodology. We gathered data on male and female cone sizes from fossils and extant Araucariaceae species. Focusing on *Araucaria*, we analyzed whether cone sizes are phylogenetically structured. Furthermore, we compared the evolutionary trajectories of male and female cone sizes by evaluating the goodness of fit of different evolutionary models. Finally, we evaluated whether different selective regimes across the phylogeny could have shaped cone morphology.

Pivotal results. Size changes in Araucariaceae occurred in both male and female cones, with the largest cones appearing in extant *Araucaria*. In this genus, different evolutionary models best described cone size changes, with male cone evolution best described by a model not influenced by phylogeny and female cone evolution by a stabilizing selection model with two optima. This resulted in contrasting phylogenetic signals, with female cone size being more phylogenetically structured than male cone size.

Conclusions. Changes in cone size in Araucariaceae occurred in both male and female cones. The largest male and female cones appeared in *Araucaria* through uncoupled evolutionary pathways, both involving a natural selection component as a driver of evolutionary change. A contrasting pattern of phylogenetic signal in male and female cone size reflected the uncoupled evolutionary trajectories followed by these sexual structures.

Keywords: Araucariaceae, cone size, genome size, models of character evolution, phylogenetic signal, seed size.

Online enhancements: appendix tables and figures.

Introduction

An outstanding feature of seed plants is their enormous diversity in reproductive structures. Even though most angiosperms are hermaphrodites (Renner 2014), an exceptional variability in sexual strategies that reduces interference between both sexual functions has evolved in the flowering plants (Barrett 2002, 2010). Sexual reproduction in gymnosperms is comparably much simpler; sexual functions are performed by separate re-

productive structures in most species, which reduces sexual interference to a great extent (although sexual conflict due to geitonogamy in monoecious species or due to intralocus sexual conflict in dioecious species may still occur; e.g., Delph et al. 2011). The evolution of reproductive structures in gymnosperms is then expected to be mainly related to successful pollen export in male reproductive structures and to the reception of pollen, complete seed development, and effective seed dispersal in female reproductive structures (Leslie 2011*b*). Consequently, because of these disparate functions, the evolutionary trajectories followed by male and female reproductive structures in gymnosperms are expected to differ.

Different evolutionary histories have been described for male and female cones in conifers (Leslie 2011*a*), by far the largest gymnosperm lineage. In this group, female cones in-

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creased relative tissue investment during the Mesozoic (as evidenced by the appearance of longer and more tightly packaged seed scales), while the relative investment in male cones seems to have remained almost unchanged over their evolutionary history. This evolutionary pattern was interpreted as a result of increasing animal seed predation pressures during the Mesozoic and Early Cenozoic (Leslie 2011*a*), while the apparent evolutionary stasis of the male cones in terms of morphology was assumed to be reflected by the fact that their main function (i.e., pollen release) remained the same across time (Leslie 2011*b*). Interestingly, Leslie (2011*a*) noted that Araucariaceae, which was likely a major food source for many megaherbivores during the Mesozoic given the high-energy yield of their leaves and their global distribution during the Jurassic (Hummel et al. 2008), was the first conifer family developing large female cones. The appearance of an araucarian large male cone in the late Cretaceous, comparable in size to some of the large pollen cones of the extant Araucariaceae species and notably larger than fossil male cones from older geological periods, prompted other authors to propose an evolutionary transition from small to large male cones in Araucariaceae (Stults et al. 2012). Although no hypothesis was proposed to explain the appearance of these large male cones, this discovery suggests that specific patterns of cone size evolution may have occurred within different conifer lineages.

In this study, we explore the pattern of variation in male and female cone size in the conifer family Araucariaceae. By gathering information on fossil cone length and width, we compared the patterns of change across time (i.e., evolutionary trajectories) in male and female cone size in the family. Furthermore, we profited from the availability of a dated phylogeny for *Araucaria* to estimate the phylogenetic structure of male and female cone size and compared their evolutionary trajectories by evaluating the goodness of fit of different models of character evolution. We show that evolutionary changes in cone size within Araucariaceae occurred in both male and female cones and, at least in *Araucaria*, that particular natural selection events seem to have played a role in driving the evolution of male and female cone size.

Methods

The Family Araucariaceae

Araucariaceae is a conifer family comprising monoecious and dioecious evergreen trees that occurs primarily in the Southern Hemisphere. As in most other conifers, its sexual functions are performed by separate reproductive cones. Both male and female cones are, on average, among the largest in conifers. Pollen grains are nonsaccate (i.e., without inflated air bladders and consequently with reduced floating capacity; Leslie 2010) and pollination drops are absent (Gelbart and Von Aderkas 2002); pollen tubes germinate outside the ovules (Tomlinson 1994). Seed scales in female cones are almost completely fused with bracts, with a single inverted seed per scale (Farjon 2017). Seed morphology is very variable in the family.

The family Araucariaceae may have originated in the Triassic; during the Jurassic it was a diverse family present in both Laurasia and Gondwana, while it disappeared from most parts of the Northern Hemisphere during the latest Cretaceous (Ker-

shaw and Wagstaff 2001; Kunzmann 2007). The family is nowadays probably less diverse than it used to be, occurring in both South America and the southwestern Pacific region (Farjon 2017). Living species are grouped into three genera: *Araucaria*, with 19 species; *Agathis*, with 17 species (Farjon 2017); and the quite recently discovered monotypic genus *Wollemia*, defined as the sister taxon of *Agathis* (Kranitz et al. 2014), with individuals growing only within protected areas in southeastern Australia (Jones et al. 1995 in Kershaw and Wagstaff 2001). *Araucaria* is more geographically spread than *Agathis*, occurring both in southern South America and the South Pacific, while the latter is absent from South America. Also, interspecific differences among living *Agathis* species are smaller compared with differences among living *Araucaria* species (Kunzmann 2007). In particular, there is large variation in reproductive cone sizes in *Araucaria* (Farjon 2017); for example, average male cone length ranges from 2 cm (in *Araucaria schmidii*) to 25 cm (in *Araucaria muelleri*), average male cone width ranges from 0.3 cm (in *Araucaria cunninghamii*) to 5 cm (in *Araucaria araucana*), and average female cone diameter ranges from 4.5 cm (in *Araucaria scopulorum*) to 30 cm (in *Araucaria bidwillii*).

Data Sources and Analyses

We gathered data on male and female cone sizes of fossil and extant species of Araucariaceae. Data on the length and width of male and female fossil cones were obtained mostly from the compilation of Leslie (2011*a*), which was completed with data from other sources (table A1; tables A1, A2 are available online). Data on the length and width of male and female cones of extant species of genus *Araucaria* were extracted from Farjon (2017); the midpoint of the reported variation in size was used. Cone volume was approximated by estimating the volume of a prolate ellipsoid,

$$volume = \frac{4}{3}\pi a^2 b,$$

with *a* and *b* being half the width and half the length of the cone, respectively. This approximation has been shown to be a good predictor of cone dry mass in both male and female cones in conifers (Leslie 2011*a*).

Phylogenetic relationships and times of divergence among the 19 extant *Araucaria* species were obtained from Kranitz et al. (2014). The extant species in this genus have been arranged into four monophyletic sections (Escapa and Catalano 2013; Kranitz et al. 2014): section *Araucaria*, which includes *A. araucana* and *Araucaria angustifolia*; section *Bunya*, including *A. bidwillii*; section *Intermedia*, including *Araucaria hunsteinii*; and section *Eutacta*, which includes *A. cunninghamii*, *Araucaria heterophylla*, and the 13 species from New Caledonia (*Araucaria bernieri*, *Araucaria biramulata*, *Araucaria columnaris*, *Araucaria humboldtensis*, *Araucaria laubenfelsii*, *Araucaria luxurians*, *Araucaria montana*, *A. muelleri*, *Araucaria nemorosa*, *Araucaria rulei*, *A. schmidii*, *A. scopulorum*, and *Araucaria subulata*). The presence of phylogenetic signal in male and female cone length, width, and volume was assessed by estimating four different indices to account for the differential sensibility of these indices to the size of phylogeny, the assumption of an underlying evolutionary model, or the accuracy of the branch lengths (Münkemüller et al. 2012). First, we estimated Blomberg's *K* (Blomberg

et al. 2003) and Pagel's λ (Pagel 1999), two indices formulated under an explicit Brownian motion (BM) model of trait evolution (see below) that have the advantage of providing a quantitative measure of the amount of phylogenetic signal relative to that expected under BM. A value of 0 indicates a random pattern of trait distribution, while a value of 1 reflects the amount of signal expected under BM evolution. Both indices were estimated using the function phylosig from the R package phytools (Revell 2012). In addition, we estimated Abouheif's C_{mean} and Moran's I, two autocorrelation indices that make no assumptions about underlying evolutionary models but do not allow quantification of the amount of phylogenetic signal. These last two indices were estimated running the function moran.abouheif from the R package adephylo (Jombart et al. 2010), using the oriAbouheif and patristic proximity matrices for C_{mean} and Moran's I, respectively. Significance of phylogenetic signal was assessed through randomization tests in all cases (i.e., with the null hypotheses being a lack of signal in all cases).

The evolutionary trajectories of male and female cone sizes were compared by evaluating the fit of four macroevolutionary models to the phylogeny and the data: BM, Ornstein-Uhlenbeck (OU) process with a single optimum, early-burst (EB), and white noise (WN). Under BM, evolutionary changes are independent from one another and occur at a constant rate ($σ^2$ parameter) with no preferred direction; as a result, trait mean remains constant but variance increases with time. This model would represent neutral drift or directional selection on a quickly varying environment (Hansen and Martins 1996). The OU model is an extension of the BM model in which the phenotypic space of trait variation is bounded, as values are attracted to a phenotypic optimum (in this case, the ancestral trait value); the strength of attraction, represented by the α parameter, increases with the distance of the trait value from the phenotypic optima (Hansen 1997; Butler and King 2004). This model would represent stabilizing selection constraining trait variation. In an EB model, the evolutionary rate, represented by the a parameter, decreases or increases exponentially through time, such that trait variance is high toward the root of the phylogeny and low toward the tips, or vice versa. An EB model with decreasing evolutionary rates would mimic an adaptive radiation in which evolutionary change becomes increasingly constrained (Harmon et al. 2010). Finally, in a WN model the phylogenetic signal is erased and trait values are drawn from a single normal distribution. This model is an extension of the OU model in which the selection strength parameter α tends to infinity (Münkemüller et al. 2015). Models were run using the function fitContinuous from the R package geiger (Harmon et al. 2008), and their goodness of fit was compared by means of the small sample size corrected Akaike information criterion (AICc). We aimed to identify the single model that would best describe the evolution of a given trait across the phylogeny.

Given that the best-fitting evolutionary models describing female cone size evolution were the BM and OU models (see "Results"), we evaluated the possibility that different selective regimes could have shaped female cone morphology. For this, we compared the fit of a BM, OU with a single optimum (as the one described above) and an OU model with more than one optimum using the R package surface (Ingram and Mahler 2013). Importantly, this method has the advantage that it does not require an a priori adaptive hypothesis; instead, the position of the selective regimes is guided by the

data. As the method performs better with multitrait data, we ran the analyses using the data on female length, width, and volume. Model selection was performed by comparing overall AICc values as above (see Ingram and Mahler 2013). To detect possible cases of convergent phenotypic evolution, we conducted both the forward and the backward phases of the analysis using the functions surfaceForward and surfaceBackward, respectively. All analyses and plots have been performed in R (R Core Team 2018).

Results

Examination of the fossil record across geological periods revealed changes in male cone size over time in the family Araucariaceae (fig. 1). While the range of male cone size in extant *Agathis* overlapped the range of male cone size in extinct Araucariaceae, longer and wider male cones appeared in extant *Araucaria*. The same pattern was observed in female cones; size range of female cones in extant *Agathis* overlapped the size range observed in fossils, whereas larger female cones appeared in extant species of *Araucaria*. Male and female cone size in extant *Wollemia nobilis* were within the range observed in the fossil record in Araucariaceae.

Although changes in size occurred in both male and female cones, it seems these changes did not necessarily occur in parallel, as evidenced by relative cone size comparisons across sections within the genus *Araucaria* (fig. 2). Uncoupled size changes occurred in species belonging to all *Araucaria* sections, except in the two species from section *Eutacta* that do not occur in New Caledonia. Relative male cone size (in terms of both length and width) was greater than relative female cone size in *Araucaria muelleri* and *Araucaria rulei*, two species from New Caledonia. Similarly, relative male cone width was greater than relative female cone width in *Araucaria araucana*. The only species in which relative size was significantly greater in female cones than in male cones was *Araucaria bidwillii*, an Australian species with notably large female cones (up to 35 cm long and 25 cm wide; Farjon 2017).

Phylogenetic signal in cone length, width, and volume differed between male and female cones (fig. 3; table 1). While significant phylogenetic signal was found in all size measures in female cones, the distribution of male cone sizes across the phylogeny followed a random pattern; this pattern was consistent for the four estimates of phylogenetic signal (table 1). Selection of the best evolutionary models describing cone size evolution was consistent for the three size measures in both sexual functions. The best model representing the evolutionary trajectory followed by male cone size was the WN model, while both the BM and the single-optimum OU models had substantial empirical support (as defined by Burnham and Anderson 2002) in describing the evolutionary trajectory followed by female cone size (table 2). Moreover, the analyses conducted with the R package surface showed that an OU model with two optima outperformed both the single-optimum OU and the BM models (AICc for BM = 79.66; AICc for OU = 78.01; AICc for OU with two optima = 36.65) and identified one selective regime for the clade including sections *Araucaria*, *Intermedia*, and *Bunya* (the large-seeded clade; see "Discussion") and one selective regime for the clade including the rest of the species in the genus (table 3; fig. A1; figs. A1–A3 are available online). No instances of shared
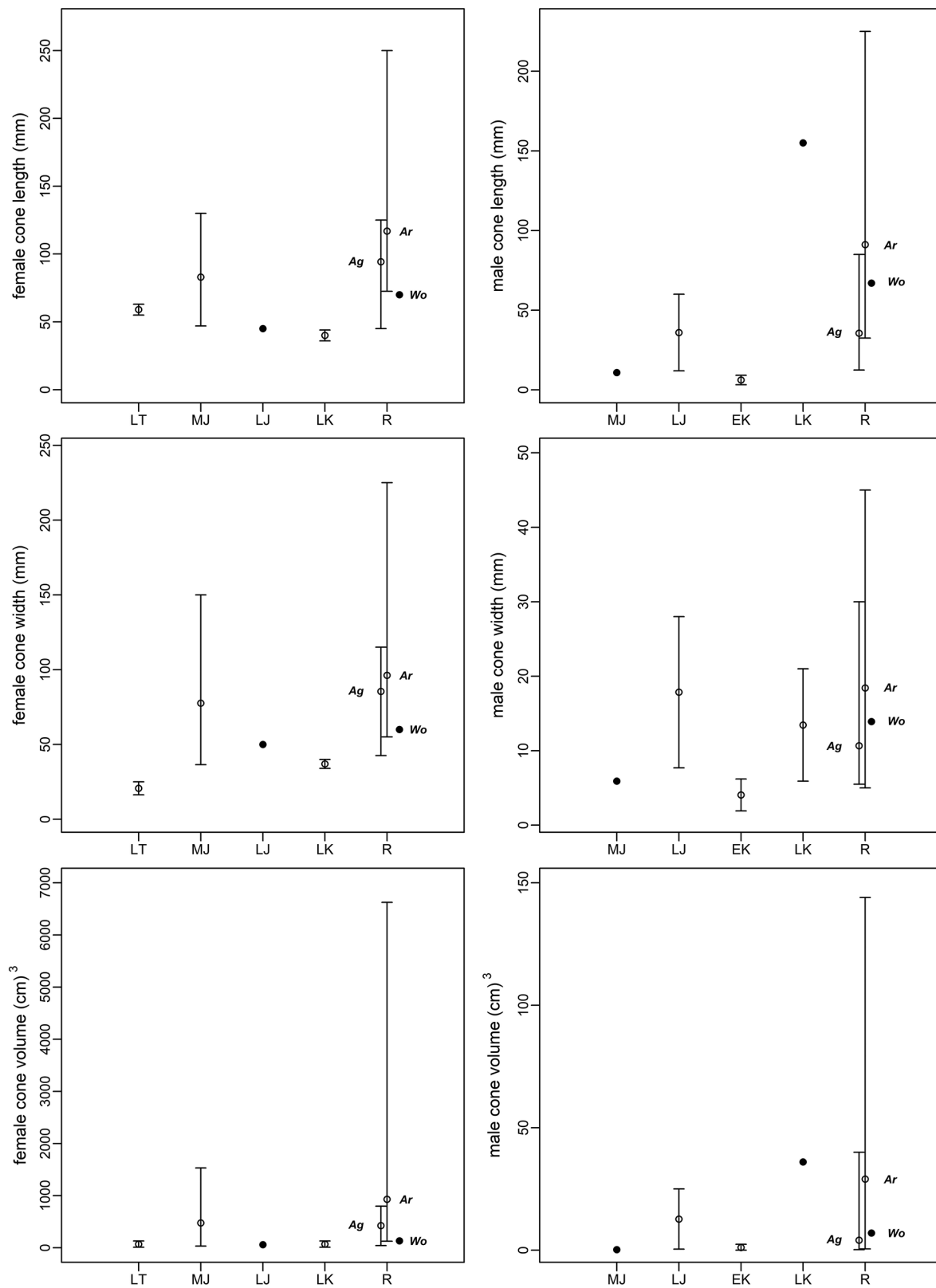
Fig. 1 Variation in female and male cone size across geological age, including both extinct and extant Araucariaceae species. Bars represent size ranges, open circles indicate mean values, and filled circles indicate cases in which only one fossil was found for a given geological age. Values from extant species were plotted separately for *Araucaria*, *Agathis*, and *Wollemia* and were slightly displaced for visual purposes. *Ag* = *Agathis*, *Ar* = *Araucaria*, *Wo* = *Wollemia*. Age abbreviations: EK = early Cretaceous, LJ = late Jurassic, LK = late Cretaceous, LT = late Triassic, MJ = middle Jurassic, R = recent.
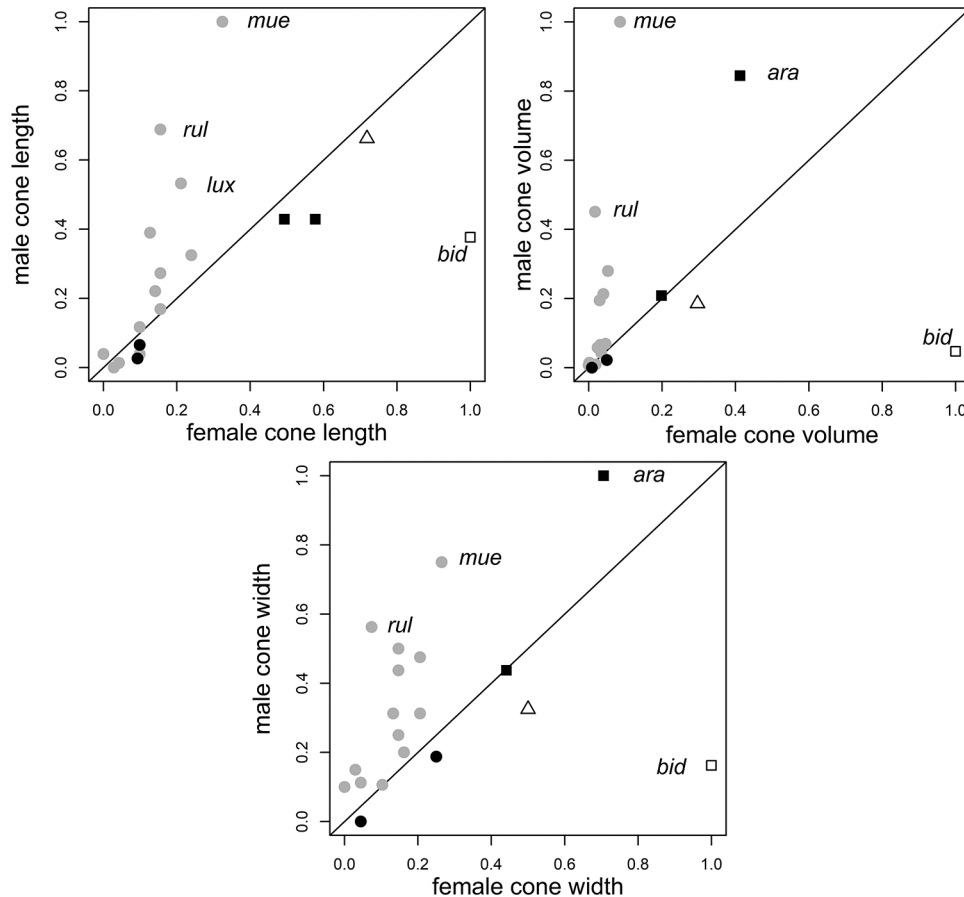
Fig. 2   Comparison between female and male relative cone sizes for *Araucaria* species. Cone sizes have been scaled to range linearly between 1 (highest) and 0 (lowest) by computing $z_i = [x_i - \min(x)]/[\max(x) - \min(x)]$. Departures from the dashed line (with slope = 1 and y-intercept = 0) indicate nonparallel changes in size. Species from section *Araucaria* are indicated with black squares, species from section *Bunya* with white squares, species from section *Intermedia* with white triangles, species from section *Eutacta* (excluding the New Caledonian species) with black circles, and species from section *Eutacta* from New Caledonia with gray circles. Ara = *Araucaria araucana*, bid = *Araucaria bidwillii*, lux = *Araucaria luxurians*, mue = *Araucaria muelleri*, rul = *Araucaria rulei*.

selective regimes resulting in convergent phenotypic evolution were found with this analysis.

Discussion

When plant sexual functions are performed by separate structures, the evolutionary trajectories of traits related to the male and the female functions can follow independent paths. Examination of data from the fossil record and from extant species of the conifer family Araucariaceae revealed changes in both male and female cone size, with the largest male and female cones in the family appearing in extant *Araucaria*. Although both male and female cone size increased, the evolutionary trajectories followed by the two types of cones differed—at least within the genus *Araucaria*.

The uncoupled evolutionary trajectories in size followed by male and female cones in *Araucaria* resulted in contrasting phylogenetic patterns, with female cone size being more phylogenetically structured than male cone size. Male cone size was randomly distributed along the phylogeny, and its evolution was best described by a WN model. Although the biological interpretation of this model is not clear (Münkemüller et al. 2015),

the evolution of male cone size could have resulted from strong selective pressures acting even within lineages toward different optima, driving fast evolutionary changes that left no traces of shared evolutionary history among species. An association between male cone size and branch size was found in conifers globally (Leslie 2012), and it has been suggested that male cone size partly reflects the pattern of evolution of branch size. We think that it is more likely that selection would act primarily on reproductive cone size and that branch morphology would evolve in response to size changes in reproductive tissues for mechanical reasons (e.g., thick branches evolving to bear large cones). Eventually, studies on the role of possible abiotic or biotic drivers of such evolutionary changes (see below), and whether these drivers would act primarily on vegetative or reproductive structures, are clearly needed to understand the phylogenetically random pattern of variation in male cone size.

Female cones in *Araucaria* were noticeably larger in the clade including sections *Araucaria*, *Intermedia*, and *Bunya*, suggesting the evolution of an ancestor with large female cones for this clade. Leslie (2011*a*) proposed that the increase in female cone size in conifers reflected more investment in protective cone tissues (i.e., more robust cone scales), as average seed size remained similar
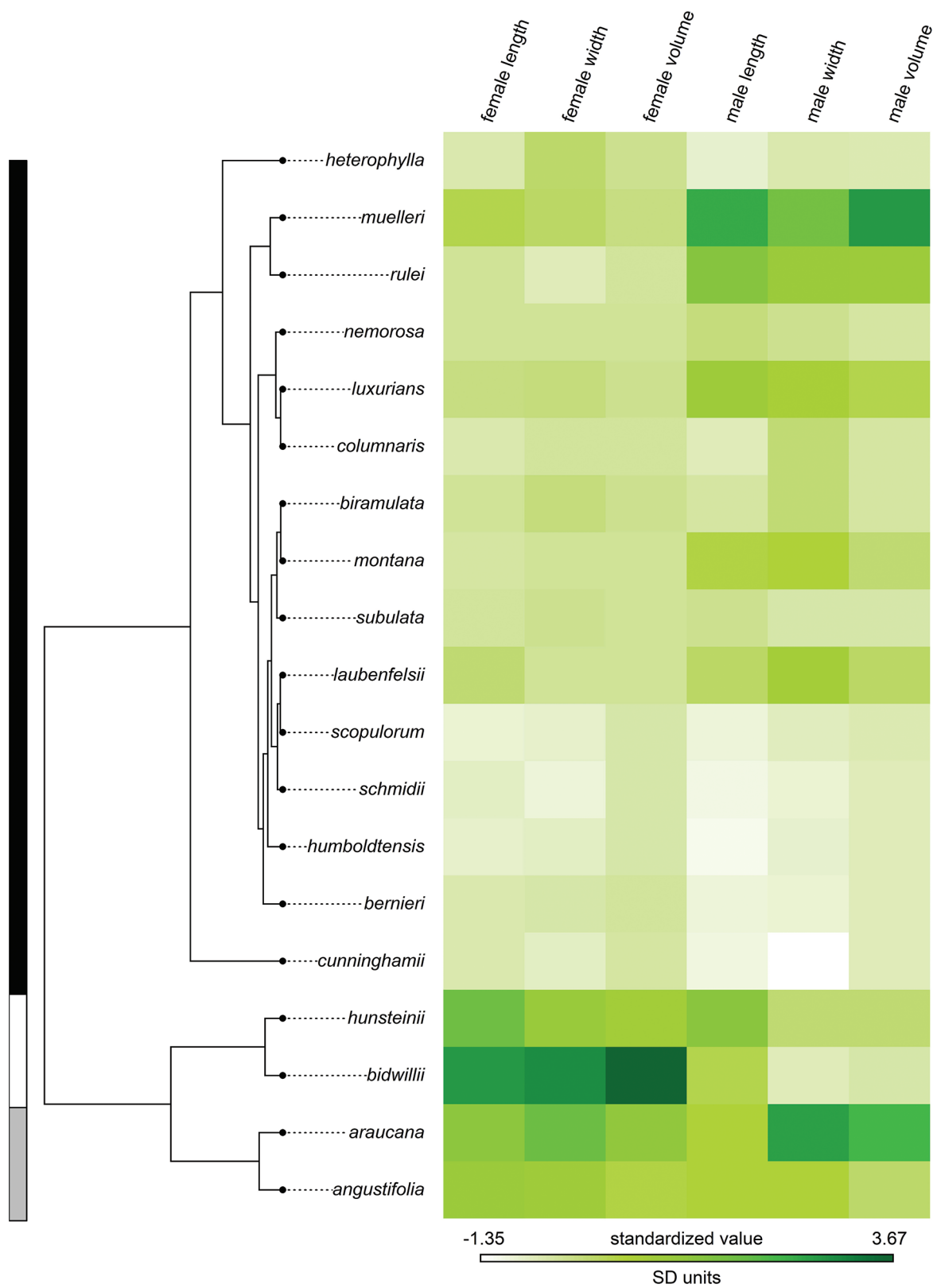
Fig. 3 Heat map showing standardized tip values for length, width, and volume of female and male reproductive cones of extant *Araucaria* species. Cells in the heat map represent species' standardized cone sizes and are colored with a gradient reflecting standard deviation units. Sections are indicated by the vertical bars on the left (in gray, section *Araucaria*; in white, sections *Bunya* and *Intermedia*; in black, section *Eutacta*). The heat map was drawn using the R package phytools (Revell 2012).

Table 1

Phylogenetic Signal in Length, Width, and Volume of Male and Female Cones of the 19 Extant *Araucaria* Species

Cone type and measurement	Blomberg's *K*		Pagel's *λ*		Abouheif's C_{mean}			Moran's *I*		
	K	*P*	*λ*	*P*	C_{mean}	SO	*P*	Moran's *I*	SO	*P*
Female cone:										
Length	.518	.002	.896	<.001	.681	5.122	.002	.402	5.305	.001
Width	.570	.001	.854	<.001	.593	4.503	.001	.327	4.584	.002
Volume	.508	.013	.780	<.001	.460	4.239	.001	.236	4.631	.002
Male cone:										
Length	.092	.196	<.001	1.000	.259	1.841	.050	.031	.991	.157
Width	.114	.124	.700	1.000	.160	1.129	.140	<−.001	.661	.253
Volume	.155	.114	<.001	1.000	.180	1.306	.093	.005	.771	.146

Note.   Both Blomberg's *K* and Pagel's *λ* are model-based indexes that take a value of 0 for a random trait pattern and a value of 1 for Brownian-motion evolution. For Abouheif's C_{mean} and Moran's *I*, SO indicates deviation from random expectation. Statistical significance in all cases was evaluated with randomization tests in which the null distributions represented random trait variation.

across geological periods. In Araucariaceae, seed size increased through time, with some large-seeded species appearing in genus *Araucaria* (fig. A2). Interestingly, female cone volume in *Araucaria* increases with increasing seed volume (phylogenetic generalized least squares [PGLS] regression coefficient ± SE: 0.685 ± 0.075, *P* ≪ 0.001; for a description of this type of regression, see Paradis 2012). Again, large-seeded species appear mostly in the clade composed by sections *Araucaria*, *Bunya*, and *Intermedia*. Although the drivers of the evolution of large seeds in gymnosperms are still unknown, an association between large seeds

and animal seed dispersal has recently been suggested (Leslie et al. 2017). Indeed, seed dispersal by different mammal and bird species was reported for the large-seeded species *Araucaria araucana* (Tella et al. 2016b), *Araucaria angustifolia* (Vieira and Iob 2009; Tella et al. 2016a; Dénes et al. 2018), and *Araucaria bidwillii* (Tella et al., forthcoming). Moreover, the recent findings that partial seed predation by seed-dispersing parrots enhances the germination of large-seeded *Araucaria* species (Tella et al. 2016a; Speziale et al. 2018) suggest that large seed sizes might have evolved to satiate parrots and other predators, thus facilitat-

Table 2

Goodness of Fit and Model Parameters of Alternative Evolutionary Models Describing the Evolution of the Male and Female Cone Length, Width, and Volumes in *Araucaria*

Cone type and measurement	Model	Δ_i	w_i	Parameters
Female cone:				
Length	BM	2.606	.185	
	OU	0	.681	σ^2: .019; α: .065; z_0: 12.83
	EB	5.457	.044	
	WN	4.051	.090	
Width	BM	.575	.386	σ^2: .011; z_0: 11.13
	OU	0	.515	σ^2: .015; α: .041; z_0: 10.86
	EB	3.425	.093	
	WN	8.848	.006	
Volume	BM	1.063	.336	σ^2: .097; z_0: 873.93
	OU	0	.571	σ^2: .135; α: .045; z_0: 801.11
	EB	3.913	.081	
	WN	7.761	.012	
Male cone:				
Length	BM	17.047	0	
	OU	2.850	.194	
	EB	19.897	0	
	WN	0	.806	σ^2: .333; z_0: 7.752
Width	BM	10.433	.004	
	OU	2.850	.193	
	EB	13.283	.001	
	WN	0	.802	σ^2: .274; z_0: 1.613
Volume	BM	13.408	.001	
	OU	2.850	.194	
	EB	16.258	0	
	WN	0	.805	σ^2: 2.410; z_0: 10.570

Note.   Model parameters are shown only for the models with strongest support. BM = Brownian motion, EB = early-burst, OU = Ornstein-Ulhlenbeck with a single optimum, WN = white noise. σ^2 = evolutionary rate, α = selection strength, z_0 = trait value at the root of the phylogeny. Δ_i is the difference in the corrected Akaike information criterion (AICc) values estimated between the AICc of a given model and the AICc of the best-ranked one (Δ_i = $AICc_i$ − AICc min). w_i = Akaike weights.

Table 3

Parameters from the Ornstein-Uhlenbeck Model with Two Optima, as Estimated Using the R Package surface, Describing the Evolution of Female Cone Length, Width, and Volume in *Araucaria*

	Length	Width	Volume
σ^2	.655	.049	8.027
α	14.303	.700	16.486
θ^1	9.473	7.577	286.526
θ^2	19.343	16.361	2711.301

Note.   Analyses were run using log-transformed data; the values at the optima (θ) shown have been back-transformed. σ^2 = evolutionary rate, α = selection strength.

ing the germination of partially eaten dispersed seeds (Tella et al. 2016*a*). However, more research is needed for testing this hypothesis. In contrast, Leslie et al. (2017) found that the ancestral seed dispersal strategy of the clade composed by sections *Araucana*, *Bunya*, and *Intermedia* was animal dispersal and that there was a reversal to wind dispersal in *Araucaria hunsteini*. In this species, seeds are flattened and the seed scales are relatively large in comparison with seed scales from species in the other sections (Farjon 2017; van der Ham et al. 2010); however, to our knowledge, seed dispersal ecology has not yet been explored in this species. Estimation of phylogenetic signal in seed volume (table A2) shows a lack of phylogenetic structure in this trait; nonetheless, when *A. hunsteini* is removed from the analyses, phylogenetic signal significantly increases, lying well above the BM expected value of $K = 1$. Furthermore, it is interesting to note that genome size (2C value), another key correlate of seed mass (for a general association in seed plants, see Beaulieu et al. 2007; for the association in pines, see Grotkopp et al. 2004), is phylogenetically structured in the genus *Araucaria* (table A2) and that the large-seeded clade also has the highest genome sizes (fig. A3). In addition, seed volume in *Araucaria* varies positively with genome size (PGLS coefficient $\pm$ SE: 2.47 $\pm$ 0.66; $P = 0.002$). We propose that the increase in genome size in the ancestor of the large-seeded clade could have allowed an increase in seed size, which was in turn also probably favored by the presence of animal dispersers preferring large seeds, and that these traits were inherited by the descendant species, with a reversal to wind dispersal in one of the species (Leslie et al. 2017). The observed changes in female cone sizes would then mostly reflect changes in seed size, with *A. hunsteini* retaining large female cones in spite of a derived reduction in seed volume.

According to our results, we can discard the fact that the evolution of male and female cone sizes would have occurred solely by neutral genetic drift, as both the WN model and the OU models include a natural selection component constraining phenotypic variation. It has been largely assumed that pollen dispersal in extant conifers is completely anemophilous, although recent research on *A. araucana* suggests that biotic pollination by parakeets may occur in this species, in which pollen cones are remarkably wider than in other *Araucaria* species (Gleiser et al. 2017). In this species, parakeets feed intensively on male cones and, having their bodies covered with pollen grains, perch on female cones without damaging the ovules contained in the cones. Wide male cones, probably associated with greater pollen production, could have evolved to offset pollen predation pressure. Although measures of trait variability and fitness-associated measures are still lacking in *A. araucana*, these observations open the possibility that selection driven by pollinators could shape cone morphology in this species. Intriguingly, the pollen cones of the extinct conifer family Cheirolepidiaceae, although small, are also abnormally wide as compared with pollen cones of other conifers (Rothwell et al. 2007; Leslie 2011*a*), and insect pollination has been suggested to have occurred in this family (Labandeira et al. 2007). Finally, animal seed dispersal has recently been reported in species from the *Araucaria* large-seeded clade (see Vieira and Iob 2009; Tella et al. 2016*a*, 2016*b*, forthcoming; Dénes et al. 2018), suggesting that natural selection favoring large seeds could be shaping female cone morphology, at least in some species. Empirical studies like selection gradient analyses are required, however, to evaluate the hypothesis that natural selection could be influencing the evolution of male and female cone sizes in *Araucaria*.

In conclusion, there were changes in cone sizes over time in the conifer family Araucariaceae, where both male and female cone sizes increased—especially in *Araucaria*. In addition, changes in female cone size in this genus were probably related to changes in seed volume. Female cone sizes were more phylogenetically structured than male cone sizes. This pattern resulted from different evolutionary trajectories shaping cone sizes. In both pathways, natural selection played a role, at least in part, in modeling cone sizes.

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