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The evolution of haploid chromosome numbers in Meliponini

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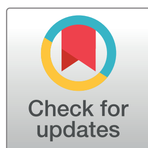

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Abstract

It is thought that two evolutionary mechanisms gave rise to chromosomal variation in bees: the first one points to polyploidy as the main cause of chromosomal evolution, while the second, Minimum Interaction Theory (MIT), is more frequently used to explain chromosomal changes in Meliponini and suggests that centric fission is responsible for variations in karyotype. However, differences in chromosome number between Meliponini and its sister taxa and in the karyotype patterns of the *Melipona* genus cannot be explained by MIT, suggesting that other events were involved in chromosomal evolution. Thus, we assembled cytogenetical and molecular information to reconstruct an ancestral chromosome number for Meliponini and its sister group, Bombini, and propose a hypothesis to explain the evolutionary pathways underpinning chromosomal changes in Meliponini. We hypothesize that the common ancestor shared by the Meliponini and Bombini tribes possessed a chromosome number of $n = 18$. The karyotype with $n = 17$ chromosomes was maintained in Meliponini, and variations of haploid numbers possibly originated through additional Robertsonian fissions and fusions. Thus, the low chromosome number would not be an ancestral condition, as predicted by MIT. We then conclude that Robertsonian fission and fusions are unlikely to be the cause of chromosomal rearrangements that originated the current karyotypes in Meliponini.

Introduction

Meliponini, Bombini, Apini, and Euglossini tribes comprise those bees known as "corbiculate", and their evolutionary history has been studied through morphological, phylogenetic, and cytogenetic analyses [1–10]. Cytogenetic analyses, in particular, are an important tool for understanding the macro-scale genomic organization of different any species. These analyses comprise descriptions of chromosome number [11], [2], [9], heterochromatin distribution patterns [12], characterization of AT and CG rich regions [13], [12], localization of 18S

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ribosomal genes [14], [12], mapping of repetitive DNA sequences [15], and inferences of karyotype evolution [10–11].

In bees, two main hypotheses have been proposed to explain changes related to chromosome number and structure. The first indicates that changes in ploidy, through whole-genome duplication, are the main mechanism involved in chromosome evolution [16]. On the other hand, a second hypothesis, known as Minimum Interaction Theory (MIT), suggests centric fission as the main mechanism responsible for chromosome variation [11] [17–21]. According to the MIT, modifications in karyotypes that occur through centric fission in different species evolve in order to minimize the deleterious effects of chromosomal interactions. However, they generate instability in the break regions of fictional chromosomes, which then tends to be minimized by the incorporation of heterochromatin [19], [20], [21]. This would generate chromosomes presenting one heterochromatic arm and one euchromatic arm, and we would expect to find this as a common pattern in the Meliponini [22], [23], [24].

Based on this theory, the ancestor of the living species of the Meliponini tribe would present a low chromosome number, and this number would increase through changes acquired by fission and a subsequent accumulation of heterochromatin. However, when we analyzed the karyotype of other corbiculate tribes phylogenetically close to Meliponini (which vary from $n = 08$, $n = 09$, $n = 15$, $n = 17$ and $n = 18$, predominating $n = 17$), such as Bombini ($n = 18–20$), Apini ($n = 17$) and Euglossini ($n = 20–21$), we observed that they have a high chromosome number [1], [2], [8]. In addition, the heterochromatin distribution patterns of several *Melipona* species [25] seem to have arisen from events different from those proposed by MIT.

Thus, the MIT, although widely used to explain the chromosomal evolution in Meliponini, does not seem to explain the chromosomal number observed across this tribe, nor the structural variations or heterochromatic patterns observed in *Melipona*. Thus, the objective of this study was to infer the ancestral chromosome number of the Meliponini tribe and its sister group Bombini in order to evaluate potential rearrangements that lead to the evolutionary karyotypic changes. Based on this phylogenetic approach, we propose a hypothesis alternative to MIT, which may have contributed to the evolutionary processes underpinning chromosomal changes in bees.

Material and methods

Phylogenetic analysis and molecular dating

A total of 67 species representing 28 genera with haploid chromosome numbers described in the literature, including 50 Meliponini and 17 Bombini species, were selected to compose our dataset (Table 1). As such, we essentially reconstructed the phylogenetic hypotheses from Rasmussen and Cameron [7]. To the phylogenetic analysis, the Meliponini and Bombini tribes were considered to be the in-groups, while the outgroups were *Apis dorsata* (Fabricius, 1793), *Euglossa imperialis* (Cockerell, 1922), *Eulaema boliviensis* (Friese, 1898), and *Exaerete smaragdina* (Guérin-Méneville, 1845). Partial sequences of the following nuclear genes were used to infer the phylogenetic tree: arginine kinase (ArgK), long-wavelength rhodopsin copy 1 (Opsin), elongation factor-1α F2 (EF1-α), 28S (28S rDNA), and the mitochondrial 16S rRNA [3], [4], [7], [9]. All sequences were retrieved from Genbank and the associated accession numbers are listed in S1 Table. Sequences were aligned using MAFFT [26] and visually verified in MEGA v7.0 [27]. The nuclear genes EF-1α, Opsin, and ArgK were partitioned into exons and introns [28], [29], while 28S and 16S were considered as a single partition. The final alignments were concatenated into a single matrix in the Sequence Matrix v.1.7.8 [30]. The analyses were performed on the CIPRES Science Gateway online server [31] using Bayesian inference by the MrBayes v3.2.2 software [32] with two independent runs with four Markov Chain Monte

Table 1. Species of bees, haploid number (*n*), karyotype formula, and references.

Species	*n*	Karyotypic formula	References
Austroplebia australis	18	_	Unpublished data
Bombus (*Bombus*) *hypocrita*	18	$4A^{m}+2A+1A^{mi}+6\underline{M}^{c}+2\underline{M}+2\underline{M}^{Ci}+1M^{t}$	[11]
Bombus (*Bombus*) *ignitus*	18	$4A^{m}+1A^{c}+1A+1A^{i}+5\underline{M}^{c}+3\underline{M}+1\underline{M}^{i}+1\underline{M}^{C}+1M^{t}$	[11]
Bombus (*Bombus*) *terricola*	18		[2]
Bombus (*Cullumanobombus*) *griseocollis*	18		[2]
Bombus (*Cullumanobombus*) *rufocinctus*	18		[2]
Bombus (*Megabombus*) *diversus*	18	$1A^{m}+1A^{Mc}+5A^{c}+1A+1A^{i}+1A^{ci}+2\underline{M}^{C}+1\underline{M}+1\underline{M}^{CC}+1M^{i}+1\underline{M}^{Ci}+1\underline{M}^{t}$	[11]
Bombus (*Pyrobombus*) *ardens*	18	$3A^{M}+4A^{Mc}+1A^{c}+1A+5\underline{M}^{C}+1\underline{M}+2\underline{M}^{CC}+1\underline{M}^{CCT}$	[11]
Bombus (*Pyrobombus*) *huntii*	18		[2]
Bombus (*Pyrobombus*) *impatiens*	18		[2]
Bombus (*Pyrobombus*) *perplexus*	12		[2]
Bombus (*Subterraneo*) *appositus*	16		[2]
Bombus (*Subterraneo*) *borealis*	16		[2]
Bombus (*Thoracobombus*) *fervidus*	18		[2]
Bombus (*Thoracobombus*) *pauloensis*	20		Unpublished data
Bombus (*Thoracobombus*) *pensylvanicus*	18		[2]
Bombus (*Thoracobombus*) *pseudobaicalensis*	17	$1A^{Mc}+1A+1A^{i}+1A^{Mi}+1A^{Mt}+5\underline{M}^{C}+3\underline{M}^{CC}+2\underline{M}^{C}+2M^{Ci}$	[2]
Bombus (*Thoracobombus*) *schrencki*	17	$10\underline{M}^{c}+1\underline{M}+5\underline{M}^{i}+1\underline{M}^{Ci}$	[2]
Cephalotrigona capitata	17	$18A+16A^{m}$	[56]
Dactylurina staudingeri	17	_	[77], [78]
Duckeola ghilianii	15	_	[77], [78]
Friesella schrottkyi	17	_	[58], [79]
Frieseomelitta trichocerata	15	$4M+16A+10A^{m}$	[80]

Continuation

Species	*n*	Karyotypic formula	References
Frieseomelitta varia	15	$4M+4A+22A^{m}$	[56], [58], [78], [81]
Geotrigona mombuca	15	$2M+6A+7A^{m}$	[56], [58], [78]
Lestrimelitta limao	14	$6M+6A+16A^{m}$	[56], [81], [82]
Leurotrigona muelleri	08	_	[53], [58], [77], [81]
Leurotrigona pusilla	15	_	[53]
Meliplebeia becarii	17	_	[77], [80]
Melipona (*Eomelipona*) *bicolor*	09	_	[54], [55], [58]
Melipona (*Eomelipona*) *marginata*	09	_	[54], [55], [58], [77]
Melipona (*Melikerria*) *fasciculata*	09	_	[55], [75], [78], [83]
Melipona (*Melikerria*) *quinquefasciata*	09	_	[55], [58], [75], [76], [78]
Melipona (*Melipona*) *favosa*	09	_	[11], [84]
Melipona (*Melipona*) *mandacaia*	09	_	[25]
Melipona (*Melipona*) *quadrifasciata*	09	_	[54], [60]
Melipona (*Michmelia*) *crinita*	09	_	[55]
Melipona (*Michmelia*) *scutellaris*	09	_	[54], [55], [58]
Melipona (*Michmelia*) *seminigra*	11	_	[45]
Meliponula bocandei	18	_	[85]
Meliponula ferruginea	18	_	[85]
Mourella caerulea	17	$11^{a}+6A^{m}$	[56]
Nannotrigona testaceicornis	17	$18A+16A^{m}$	[11], [56], [58]
Oxytrigona tataira	17		[55], [75]
Paratrigona subnuda	17	$24A+10A^{m}$	[56], [58], [75], [78],

(*Continued*)

Table 1. (Continued)

Species	*n*	Karyotypic formula	References
Partamona auripennis	17	_	Unpublished data
Partamona testacea	18	_	[78]
Plebeia droryana	17	_	[11], [58], [75], [78], [86]
Plebeina hildebrandti	18	_	[77], [78]
Continuation			
Species	***n***	**Karyotypic formula**	**References**
Ptilotrigona lurida	11	$6M+3A+2A^{m}$	[56]
Scaptotrigona bipunctata	17	$2A+30A^{m}+2M$	[87]
Scaptotrigona depilis	17	$26A+8A^{m}$	[56], [81]
Scaura latitarsis	17	$1M+2A+14A^{m}$	[25], [56], [81], [82], [88]
Scaura longula	17	$3A+14A^{m}$	[88]
Schwarziana quadripunctata	17	$18A+16A^{m}$	[56], [58], [81]
Tetragona clavipes	17	$6A+28A^{m}$	[56], [81]
Tetragonisca angustula	17	$34A^{m}$	[11], [56], [57], [58], [81]
Trigona chanchamayoensis	17	$18^{a}+2A^{e}+12A^{m}$	[22]
Trigona cilipes	18	_	[77]
Trigona fuscipennis	17	_	[81], [89]
Trigona hyalinata	17	$4A+2A^{e}+30A^{m}$	[22], [88]
Trigona hypogea	17	$2A+24A^{m}+8A^{mc}$	[90]
Trigona pallens	17	$2A^{e}+32A^{m}$	[90]
Trigona recursa	17	$12A+2A^{e}+30A^{m}$	[56], [81], [90]
Trigona spinipes	17	$6A+28A^{m}$	[56], [81], [90]
Trigona truculenta	17	$4A^{e}+24A^{m}+6A^{mc}$	[90]
Trigona williana	17	$2A^{e}+2A+30A^{m}$	[90]

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Carlo (MCMC) in each. The mixed model [33]) was implemented for all partitions with a proportion of invariable sites and a Gamma correction. We used 50,000,000 generations of MCMC with trees sampled every 1000 generations. The convergence of the Markov chains was verified in Tracer v.1.5 [34]. Twenty-five percent of the initial trees were discarded and those that remained were used to generate the consensus tree. The trees were viewed and edited in FigTree v.1.3.1 [35].

The same data matrix from phylogenetic analyses was used for molecular dating according to methods previously described by Rasmussen and Cameron [7]. Briefly, the divergence times were estimated using the Bayesian relaxed clock uncorrelated lognormal method implemented in BEAST 2.0 [36] on the CIPRES server [31]. This is the most suitable model for Hymenoptera since it allows evolutionary rates to vary between trees branches [37]. The nucleotide substitution model was GTR+G+I for all partitions and the Yule process was used as *a priori* probability for the trees [38]. We used 300,000,000 generations of MCMC and the convergence was checked in Tracer v.1.7 [34]. A maximum clade credibility tree was created in the program TreeAnnotator v2.4.1 (implemented in BEAST) using 25% burn-in, and was visualized and edited in FigTree v.1.3.1 [35]. Calibration points were based on previous work by Rasmussen and Cameron [7] and Martins *et al.* [39].

Reconstruction of the ancestral state

In order to evaluate the ancestral chromosome number of Meliponini and further test the fission, fusion, or duplication hypothesis of karyotype evolution in this group of bees, we used

three phylogenetic approaches to ancestral reconstruction to estimate the potential ancestral chromosome number.

First, the ancestral chromosome number was reconstructed using Maximum Parsimony (MP) and Maximum Likelihood (MLm) analyses performed with Mesquite software v.3.04 [40]. For these analyses, either the last 1000 trees from the Bayesian MCMC analyses, or the dated phylogeny, were used as the input. In both analyses, the different haploid numbers (n) of each species were considered as character states (S2 Table), and the values of the ancestral chromosome number, the most parsimonious state(s) in MP, were represented by percentages (%) in the MLm analysis.

Second, we performed additional analysis with a different methodology to evaluate the consistency of the recovered data. We estimated the ancestral haploid chromosome number of the Meliponini and sister group in three independent analyses using Chromevol 2.0 [41], which on the basis of molecular phylogeny estimates the haploid ancestral chromosome number by using two probabilistic methods, maximum likelihood (ML) and Bayesian inference (BI), with the latter providing a posterior probability. Chromevol 2.0 can evaluate ten chromosome evolution models and different transitions between chromosome numbers. The models evaluate dysploidy (under constant or linear rates), polyploidy (duplication), and demi-polyploidy (demi-duplication), thus testing the possibility of changes in the karyotype that result from changes in ploidy, and also the null model in each case for no duplication. All parameters were adjusted for the data, as described by Glick and Mayrose [41], Cristiano et al. [42] and Cardoso et al. [43]. The model that fits best was analyzed with 10,000 simulations under the AIC.

Results

Chromosome number, phylogenetic analyses, and molecular dating

Meliponini species showed variation of haploid number ranging from $n = 8$ to $n = 18$ chromosomes, with $n = 17$ being the predominant chromosome number. The Old World species presented only $n = 17$ and $n = 18$ chromosomes, and in the New World species the number of chromosomes ranged from $n = 8$ to $n = 18$. In Bombini species, on the other hand, the haploid number varied from $n = 12$ to $n = 20$ chromosomes, with $n = 18$ predominating (Table 1).

The concatenated dataset resulted in 3,263 aligned base pairs and the phylogenetic tree obtained from Bayesian inference analysis recovered the phylogeny proposed by Rasmussen and Cameron [7] (S1 Fig). According to this phylogeny, the Old World clade is formed by the Meliponini of the Afrotropical, Australasian, and Indo-Malayan regions, and the New World clade is formed by the species of the Neotropical region. The Neotropical Meliponini initially diverged into two clades, separating *Trigonisca* sensu lato (clade *Trigonisca* s.l.) which includes the genera *Dolichotrigona* (Moure, 1950), *Trigonisca* (Moure, 1950), *Celetrigona* (Moure, 1950), and *Leurotrigona* (Moure, 1950) from the remaining species. Subsequently, there was a second split between *Melipona* sensu lato (*Melipona* s.l.) and the other Meliponini (also see Rasmussen and Cameron [7]).

According to molecular dating, the most recent common ancestor between Bombini and Meliponini is dated to about 79.1 (95% HPD = 74–83.3) million years ago (mya) in the upper Cretaceous. Among the Meliponini, the common ancestor dates to about 65.5 (95% HPD = 65–66.6) mya, corresponding to the Paleocene, and, between species of the genus *Melipona*, to about 18.1 (95% HPD = 12–26) mya, corresponding to the Miocene (Fig 1; S2 Fig).

Reconstruction of the ancestral chromosome number

The ancestral reconstruction performed in Mesquite, which considered both the phylogram and the chronogram using both MP and MLm, indicated $n = 18$ as the ancestral chromosome

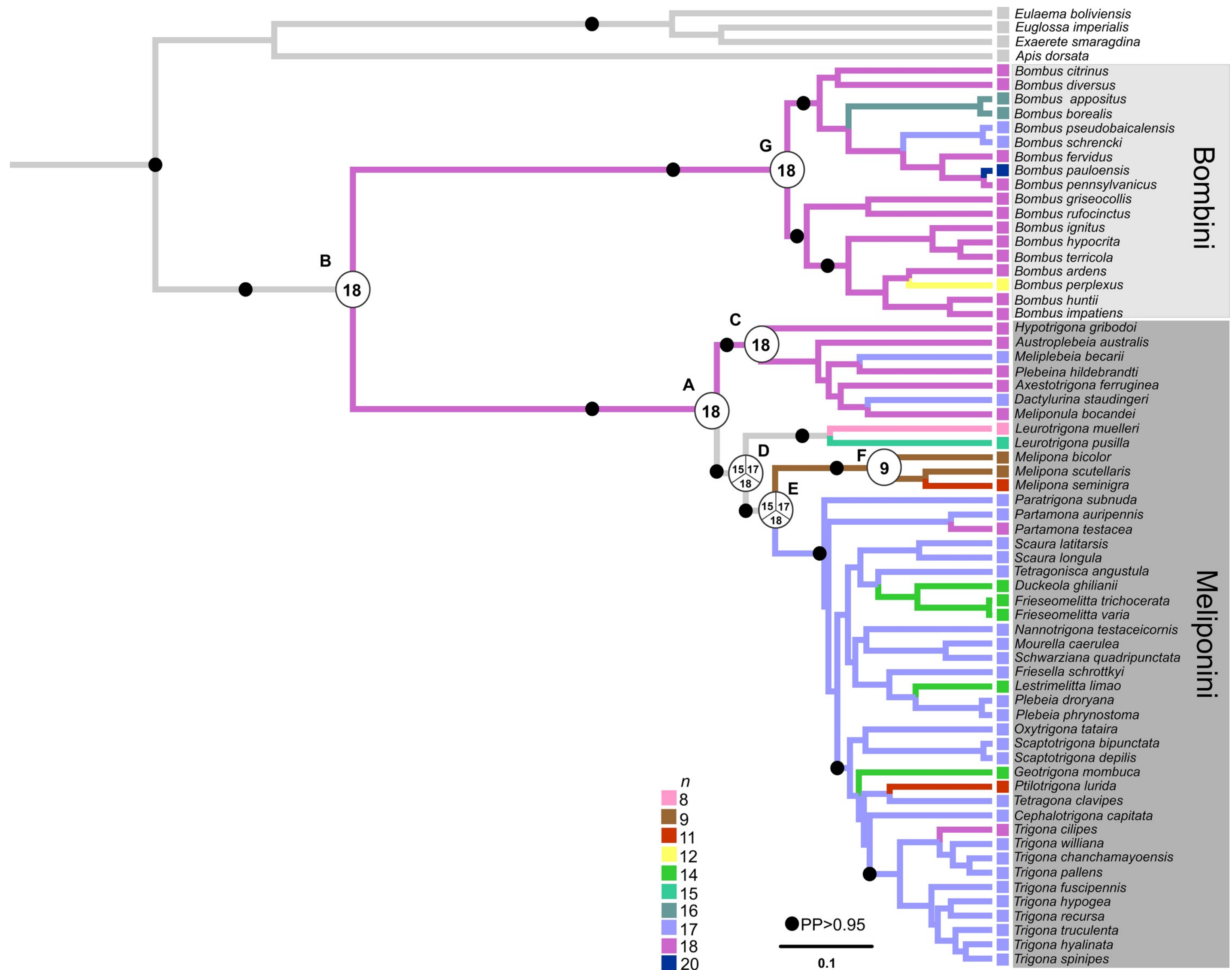

Fig 1. Consensus tree obtained from the Bayesian analysis of concatenated data based on partial sequences of the Arg-K, Opsin, EF1-α, 28S and 16S genes from Meliponini and Bombini species, and ancestral chromosome number inference as implemented in Mesquite by MP analysis. The squares in the terminal branches and the color of the branches represent the different haploid numbers, and the ancestor nodes indicate the ancestral states estimated to be the most parsimonious.

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number for the Meliponini tribe (73%, node A), and $n = 18$ (75%, node B) as the ancestral chromosome number for Meliponini and Bombini (Fig 1 and Fig 2). In Meliponini species belonging to the Old World clade, $n = 18$ chromosomes remained in most of the lineages (97%, node C), whereas there was a reduction from $n = 18$ (37%, node D) to $n = 17$ chromosomes (50%, node E) in the New World clade. One exception was *Melipona*, which experienced a reduction to half the number of chromosomes (from $n = 18$ to $n = 9$) (100%, node F). In Bombini, $n = 18$ chromosomes remained the most common number (100%, node G), with a reduction to $n = 17$ and $n = 16$ chromosomes in the subgenera *Subterraneobombus* and *Thoracobombus*, respectively. All values referring to the probabilities of each character found in the ancestor nodes of the Meliponini and Bombini species are indicated in the Appendix (S2 Table).

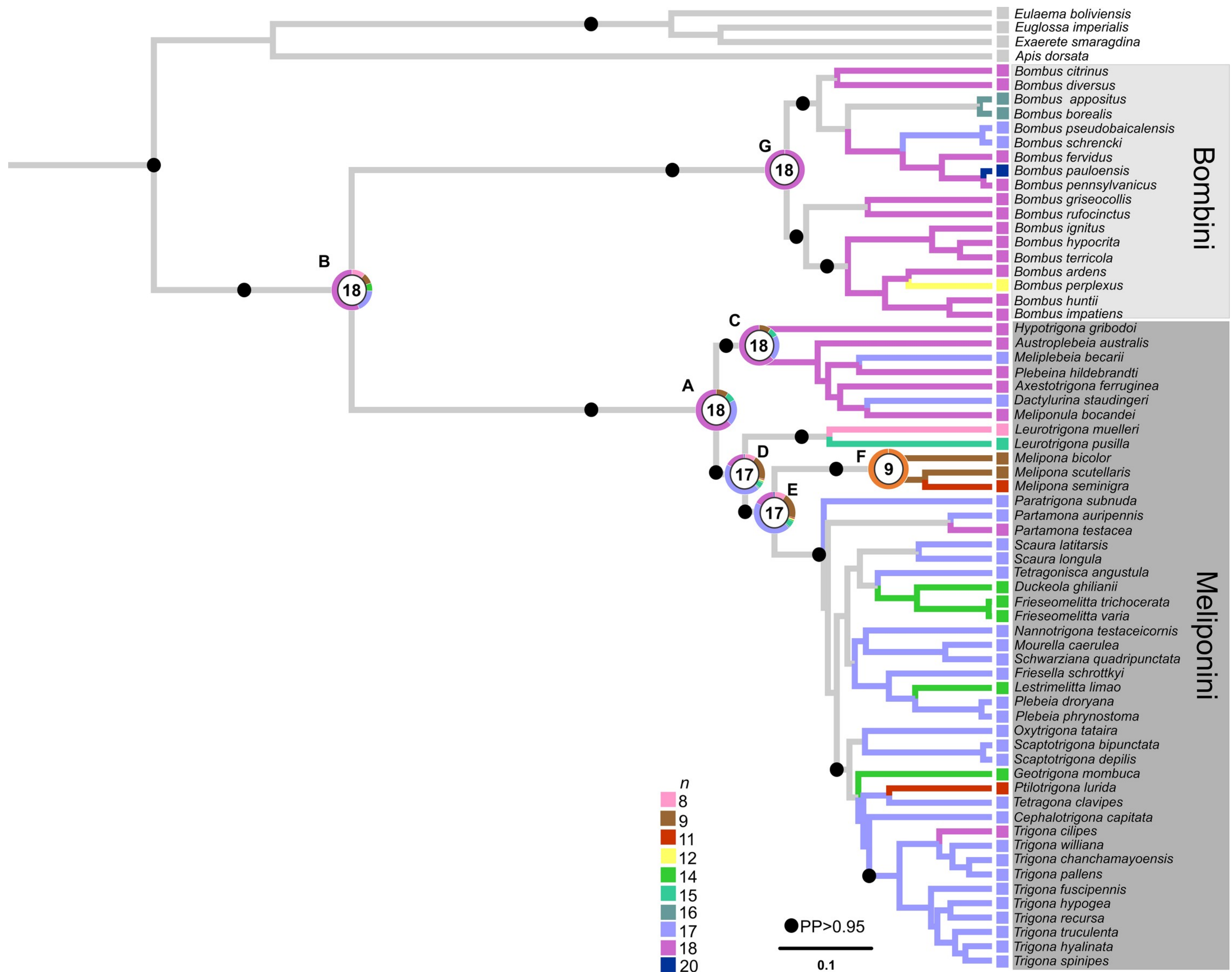

Fig 2. Consensus tree obtained from the Bayesian analysis of concatenated data based on partial sequences of the Arg-K, Opsin, EF1-α, 28S and 16S genes from Meliponini and Bombini species, and ancestral chromosome number inference as implemented in Mesquite by ML analysis. The squares in the terminal branches and the color of the branches represent the different haploid numbers, and the ancestor nodes indicate the most likely ancestral state. Pie charts indicate the probabilities of each ancestral state.

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The reconstruction using ML and BI optimization in Chromevol 2.0, performed using the same trees, also recovered ancestral haploid numbers around 17, 18, and 19 chromosomes (Fig 3), considering the linear rate with no duplication model (AIC = 254, Likelihood = -123). As with ML analysis implemented in Mesquite, ML optimization on Chromevol 2.0 also found n = 18 to be the ancestral chromosome number for the Meliponini tribe (node A), but determined n = 19 (node B) to be the ancestral chromosome number for Meliponini and Bombini. Meliponini species belonging to the Old World clade were found to have n = 18 chromosomes in node C, whereas n = 17 chromosomes was determined for in the New World clade in nodes D and E. Yet for the *Melipona* genus, n = 11 was recovered instead of n = 9 (node F), while n = 18 chromosomes was identified for Bombini. Results from Bayesian optimization in Chromevol 2.0 were very similar to those generated by ML optimization, recovering the same

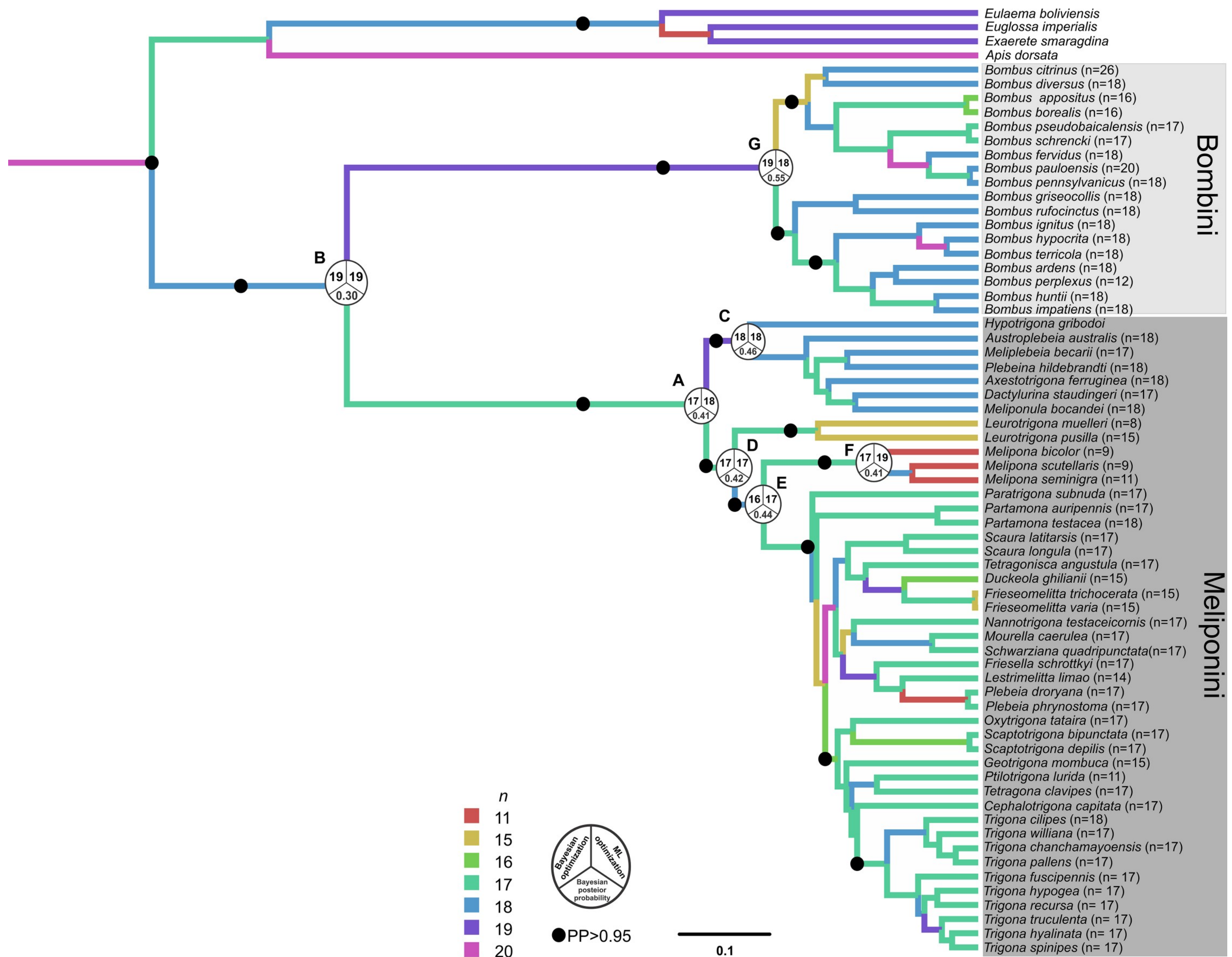

Fig 3. Consensus tree obtained from the Bayesian analysis of concatenated data based on partial sequences of the Arg-K, Opsin, EF1-α, 28S and 16S genes from Meliponini and Bombini species, including ancestral haploid chromosome state reconstruction inferred under Bayesian and Maximum Likelihood optimizations in Chromevol 2.0 software. Pie charts at nodes represent the inferred chromosome number in both Maximum Likelihood optimization and the first data for Bayesian optimization and its Bayesian posterior probabilities.

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ancestral chromosome number in one out of the two estimates with the highest posterior probability (Table 2).

Discussion

This is the first study reconstructing the ancestral chromosome number in Meliponini based on cytogenetic and molecular data by means of distinct and complementary approaches. Our results indicate that the most likely common ancestor of the Meliponini tribe had n = 18 chromosomes and that, in the Neotropical species, this chromosome number decreased to n = 17. According to karyotype descriptions, Meliponini can be separated into three groups based on the most frequent number of chromosomes in the species (reviewed in Tavares *et al.* [10]). The first group consists of Meliponini species with n = 17 chromosomes. Although different

Table 2. Haploid ancestral chromosome number recovered by the different methods implemented in Mesquite 3.04 and Chromevol 2.0.

Nodes	Estimated Haploid Ancestral Chromosome Number				
	Maximum Parsimony in Mesquite	Maximum Likelihood in Mesquite (%)	Maximum likelihood optimization in Chromevol 2.0	Bayesian optimization in Chromevol 2.0	
				1st highest P.P. estimate (P.P.)	2nd highest P.P. estimate (P.P.)
A–Meliponini	18	18 (52)	18	17 (0.41)	18 (0.34)
B–Meliponini plus Bombini	18	18 (42)	19	19 (0.30)	20 (0.25)
C–Old World Meliponini	18	18 (99)	18	18 (0.46)	17 (0.32)
D–New World Meliponini	15/ 17/18	18 (31)	17	17 (0.42)	16 (0.39)
E–*Melipona* plus remaining Meliponini	15/ 17/ 18	17 (45)	17	16 (0.44)	17 (0.41)
F–*Melipona*	9	9 (100)	11	11 (0.40)	12 (0.39)
G–Bombini	18	18 (100)	18	19 (0.55)	18 (0.31)

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species have the same chromosome number (n = 17), the morphological variation observed in the karyotypes (Table 1) indicates that rearrangements such as inversions and translocations were responsible for variations in chromosome structure [16], [25], [41]. A variation in the number of chromosomes was observed in *Trigona* sp., possibly *Trigona braueri* (Friese, 1900) (described as *Trigona fulviventris* Guérin, 1844 in Domingues *et al.*[44]) with $2n$ = 32 chromosomes, unlike the other *Trigona* species with $2n$ = 34. This reduction of the chromosome number is the result of centric fusion of two pseudoacrocentric chromosomes, which generated a larger metacentric chromosome with heterochromatin restricted to the pericentromeric region [44].

The second group is formed by species with n = 15 chromosomes, a chromosomal number which would have appeared independently several times during the evolution of Meliponini. The third group is composed of species of the genus *Melipona* that typically have n = 9 chromosomes. This low chromosome number is apomorphic for this group, and departures from this basic number are known variations particular to this genus. *Melipona seminigra* Friese, 1903 (n = 11) is one exception whose chromosome number could have arisen by fission from an ancestor with n = 9 [45]. Yet, *Melipona quinquefasciata* (Lepeletier, 1836) and *Melipona rufiventris* (Lepeletier, 1836) sometimes demonstrate a karyotype with more than 9 chromosomes due to the presence of chromosomes B, which are not part of complement A [46–47]. B chromosomes are expendable elements found together with the chromosome set (complement A) in some specimens belonging to different taxa [48–49]. These chromosomes are characterized by a non-Mendelian inheritance pattern, as they do not undergo recombination due to their lack of homology with complement A chromosomes. Repetitive DNA sequences are generally enriched in B chromosomes, especially those associated with satellite DNA, ribosomal DNA (rDNA) and transposable elements [48–52].

Initial studies in bees revealed that some species have a low chromosome number, between n = 8 and n = 9 [11], [53–54], and that the pattern of heterochromatin distribution within chromosomes is similar to that observed in ant species of the genus *Myrmecia* (Fabricius, 1804) [22], [55–57],. Using cytogenetic data collected from the *Myrmecia pilosula* complex, Imai *et al.* [18], [19], [20] observed that the ancestor of this group had a lower chromosome number when compared to species that had recently diverged. They also observed that there was an increase in heterochromatin in one of the chromosome arms in the species with the highest diploid number. Thus, considering the cytogenetic information and phylogenetic relationships between these species, they proposed that the ancestral karyotype of this group should have a low chromosome number (*i.e.* n = 3) and that centric fissions would be the main

rearrangement responsible for the increase in chromosome number [18–20]. Such cytogenetic patterns led the researchers to suggest that the same mechanism would be involved in chromosome evolution in bees, and that the ancestral species would have a chromosome number smaller than that found in species that diverged more recently [11], [22–25], [54–55], [58–59]. However, our analysis indicates that the ancestral karyotype of Meliponini had a high chromosomal number ($n = 18$), which was maintained in many species, and that, possibly as a result of fusion events, this number decreased from $n = 18$ to $n = 17$ in the Neotropical Meliponini, contrary to the expected pattern indicated by the MIT for chromosome evolution in bees. According to the theory, modifications in the karyotypes that occur through centric fission in different species occur in order to minimize the deleterious effects of chromosomal interactions [19–21].

In addition to a decrease from the ancestral chromosome number in the Meliponini, some structural characteristics of the chromosomes of from *Melipona* species also suggest that this group does not follow the evolutionary model proposed by MIT. Species of *Melipona* have unique characteristics that distinguish them from other Meliponini species, such as a caste differentiation system that is based on genetic characteristics shaped by the environment rather than the amount of food received [60], [61], and phylogenetically, the genus is monophyletic in relation to the other Neotropical Meliponini [7], [62], [63]. Furthermore, cytogenetically the species present a haploid number of nine chromosomes and the genus is subdivided into two groups characterized by the spatial distribution of heterochromatin along the chromosome arms. In Group I, heterochromatin is observed in the pericentromeric region, whereas in Group II, it is dispersed evenly along most chromosomes [54–56].

Phylogenetic reconstructions and the time of divergence suggest that the *Melipona* species diverged more recently (± 20 Ma) than those Meliponini with a higher number of chromosomes (± 54 Ma) [7]. Thus, the unique characteristics of the genus in relation to its divergence time suggest that *Melipona* followed a "different" pattern from the other Meliponini, and underwent different evolutionary processes that were different from the remaining species of this tribe. Thus, given there has been about 20 million years of divergence from the time of the common *Melipona* ancestor, we believe that repetitive centric fusions were responsible for the decreasing the chromosome number. Further changes in karyotypic structure may be the outcome of inversions, translocations, and the repositioning of transposable elements.

Centric fusion is considered one of the major chromosomal rearrangements in animal karyotype evolution [64]. Rearrangements of this type were used to explain the karyotype evolution in wasps of the Epiponini tribe [65], parasitic wasps (*Minotetrastichus frontalis* (Nees, 1834) and *Chrysocharis laomedon* (Walker, 1839) [66], and ants (*Mycetophylax morschi* (Emery, 1888)) [43]. In other taxonomic groups, fusions have also been suggested as the main mechanism responsible for changes in chromosome numbers, as in locusts of the Ephippigerini tribe [67], and in several species of mammals (*Elaphodus cephalophus* (Milne-Edwards, 1873), *Muntiacus reevesi* (Ogilby, 1839) and *Muntiacus muntjak vaginalis* (Boddaert, 1785)) [68–71].

On the other hand, in different taxa such as ants, fish, mammals, and frogs, fissions are also important events in chromosome rearrangement throughout evolutionary time [21], [72–74]. In Meliponini, an example of chromosome fission was observed in *Melipona seminigra* (Friese, 1903), which has $n = 11$ chromosomes [12], [45]. According to our findings, this chromosome number observed today likely originated by fission events from an ancestor with $n = 9$. Similar events may have shaped chromosome number evolution in *Trigona cilipes* (Fabricius, 1804), so that fission in an ancestor with $n = 17$ led to the karyotype with $n = 18$ chromosomes. However, chromosome fission requires the formation of new centromeres and telomeres for the

new chromosomes [75], and therefore may not be the most common mechanism in karyotype evolution in different groups.

The results of this study, with cytogenetic evidence and ancestral states, also suggest that the ancestor between Meliponini and Bombini had $n = 18$ chromosomes. Cytogenetic descriptions found for the other corbiculate tribes show a range in chromosome number between $n = 8$ and $n = 21$. For example, Apini ($n = 17$) ([1]), Euglossini ($n = 20–21$), Bombini ($n = 18–20$) [11], [2], [8], and Meliponini ($n = 8–18$, with the most common being $n = 17$) [10], [76]. Owen *et al.* [2] considered the ancestral number to be $n = 18$ for *Bombus*, and that variations of $n = 16$ (*Bombus* (*Subterraneo*) *appositus* (Cresson, 1878) and *Bombus* (*Subterraneo*) *borealis* (Kirby, 1837), $n = 17$ (*Bombus* (*Thoracobombus*) *pseudobaicalensis* (Vogt, 1911) and *Bombus* (*Thoracobombus*) *schrenck* (Morawitz, 1881) and $n = 20$ (*Bombus* (*Thoracobombus*) *pauloensis* (Friese, 1913)) would be the result of chromosomal fusions and fissions. Although the Meliponini and Bombini species have similar ancestral chromosome numbers, the Meliponini have diploid numbers, chromosome morphologies, and heterochromatin distribution patterns conserved among species, differently from Bombini, which show variations in these cytogenetic patterns. Our results suggest that the ancestor of the Bombini tribe had a high chromosomal number ($n = 18$), and that this chromosome number was maintained throughout evolution in several species, which contradicts what was expected from MIT [11].

Based on the cytogenetic information, as well as on insights into chromosome evolution using a phylogenetic approach in Meliponini, we propose here that the ancestral chromosome number between the Meliponini and Bombini tribes is $n = 18$ chromosomes. This chromosome number remained in the common ancestor of Meliponini, and by Robertsonian chromosomal fusion, decreased from $n = 18$ to $n = 17$ in the Neotropical Meliponini. Yet, the low number of chromosomes found in *Melipona* is an apomorphy of that clade likely due chromosomal fusions. We also conclude that chromosome fissions, as predicted by MIT, are not the main mechanism in karyotype evolution of Meliponini and Bombini. It was more likely that the ancestral chromosome number (i.e. $n = 18$) was maintained across bee lineages, and that it is equally possible for the variation in haploid chromosome number to have arisen by chromosomal fusion and fission.

Supporting information

S1 Fig. Consensus tree of Bayesian analysis, based on partial sequences of the Arg-K, Opsin, EF1-α, 28S and 16S concatenated genes of the Meliponini and Bombini. The numbers after the nodes represent the later probabilities, blue branches represent the tribe Meliponini, while green branches indicate Bombini. The outgroups were represented by *Exaerete smaragdina*, *Eulaema boliviensis* and *Euglossa imperialis*.
(TIFF)

S2 Fig. Consensus tree of Bayesian analysis based on partial sequences of the Arg-K, Opsin, EF1-α, 28S and 16S concatenated genes of the Meliponini and Bombini species including the times of divergence estimated in the Beast program. The bars indicate 95% confidence. Outgroups were represented by *Exaerete smaragdina*, *Eulaema boliviensis* and *Euglossa imperialis*.
(TIF)

S1 Table. Species of bees and the external group analyzed, collection site, gene access number in GenBank (http://www.ncbi.nlm.nih.gov) and references.
(DOCX)

S2 Table. Probabilities (in percentages) of the haploid numbers in the reconstruction of the ancestral state between the clades.
(DOCX)

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