THE RADICAL STRUCTURAL EVOLUTION OF GENOMES IN FLOWERING PLANT MITOCHONDRIA

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Preface

This dissertation documents research focused on the structural evolution of genomes in flowering plant mitochondria. This work was performed over the course of several years by Logan W. Cole under the supervision of Jeffrey D. Palmer at Indiana University in Bloomington, Indiana. The focus of this dissertation is on recombination, rearrangement, and acquisition of foreign DNA. It has been known for many years that these processes are relatively common in flowering plant mitochondria, despite the conventional wisdom (mostly stemming from our understanding of animal mitochondria) that they are rare. This work greatly improves our understanding of these processes' rate and mode in flowering plant mitochondria. Two chapters of this dissertation were published elsewhere:

Cole LW, Guo W, Mower JP, Palmer JD. 2018. High and variable rates of repeat-mediated mitochondrial genome rearrangement in a genus of plants. *Mol Biol Evol.* 35(11): 2773–2785.

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Logan Wyatt Cole

THE RADICAL STRUCTURAL EVOLUTION OF GENOMES IN FLOWERING PLANT MITOCHONDRIA

Due to their propensity to undergo recombination, the mitochondrial genomes of flowering plants are subject to drastic structural changes, including frequent rearrangement and the integration of foreign DNA. This work focuses on this structural evolution with an emphasis on the genus *Monsonia* of the geranium family, Geraniaceae. The first chapter examines the rate and mechanism of rearrangement. The second describes a new mode of horizontal gene transfer in which existing foreign DNA facilitates further horizontal transfer. The final chapter reviews literature relevant to the evolution of per-cell organelle number (including mitochondria and chloroplasts).

To investigate the tempo of rearrangement, the rearrangement history of seven *Monsonia* mitochondrial genomes was reconstructed. This analysis revealed very high rates of rearrangement and also provides the first documentation of significant variation in mitochondrial rearrangement rates in plants. Further analyses of other species show even greater variation in rearrangement rates across the seed plants. It was also found that repeated sequences are preferentially located in close proximity to sites of rearrangement, which supports our understanding that rearrangement occurs through repeat-mediated recombination.

The work on *Monsonia* genomes has also led to the discovery of a new mode of horizontal gene transfer in plant mitochondria. In addition to six horizontally transferred genes in *M. emarginata*, there are also several horizontally transferred intergenic regions that are distributed across the seven *Monsonia* species. These sequences appear to be from the Solanales, including some from the parasitic dodder, *Cuscuta*, or a close relative. The phylogenetic distribution of these sequences and characteristic substitution patterns are strong evidence for a new mode of transfer in which existing foreign sequences have facilitated other horizontal transfer events via gene conversion.

Finally, literature relevant to the evolution of per-cell numbers of mitochondria and chloroplasts was reviewed. The transcriptional pathways involved in organelle biogenesis and the population genetic circumstances of those genes that may be involved in determining per-cell organelle numbers are discussed here. Furthermore, the possibility that two factors, metabolism and organelle-nuclear gene product stoichiometry, play a role in selection influencing per-cell organelle number is explored.

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CHAPTER 1

High and variable rates of repeat-mediated mitochondrial genome rearrangement in a genus of plants

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Abstract

For 30 years, it has been clear that angiosperm mitochondrial genomes evolve rapidly in sequence arrangement (i.e., synteny), yet absolute rates of rearrangement have not been measured in any plant group, nor is it known how much these rates vary. To investigate these issues, we sequenced and reconstructed the rearrangement history of seven mitochondrial genomes in *Monsonia* (Geraniaceae). We show that rearrangements (occurring mostly as inversions) take place at generally high rates in these genomes, yet also uncover significant variation in rearrangement rates. For example, the hyperactive mitochondrial genome of *Monsonia ciliata* has accumulated at least 30 rearrangements over the last million years, whereas the branch leading to *M. ciliata* and its sister species has sustained rearrangement at a rate that is at least ten times lower. Furthermore, our analysis of published data shows that rates of mitochondrial genome rearrangement in seed plants vary by at least 600-fold. We find that sites of rearrangement are highly preferentially located in very close proximity to

repeated sequences in *Monsonia*. This provides strong support for the hypothesis that rearrangement in angiosperm mitochondrial genomes occurs largely through repeat-mediated recombination. Because there is little variation in the amount of repeat sequence among *Monsonia* genomes, the variable rates of rearrangement in *Monsonia* probably reflect variable rates of mitochondrial recombination itself. Finally, we show that mitochondrial synonymous substitutions occur in a clock-like manner in *Monsonia*; rates of mitochondrial substitutions and rearrangements are therefore highly uncoupled in this group.

Introduction

The mitochondrial genomes of angiosperms evolve very rapidly in terms of genome rearrangement despite generally very low rates of sequence evolution. Most angiosperm mitochondrial genomes have synonymous substitution rates that are 3–9 and 15–20 times lower than those of plastid and nuclear genomes, respectively, from the same plants (Drouin et al. 2008; Richardson et al. 2013). In contrast, rearrangements are frequent even among closely related mitochondrial DNAs (mtDNAs), for example, within *Beta* (Kubo et al. 1999; Satoh et al. 2004; Darracq et al. 2011), *Brassica* (Palmer and Herbon 1988), *Olea* (Van de Paer et al. 2018), *Silene* (Sloan, Alverson, et al. 2012; Sloan, Müller, et al. 2012), and *Zea* (Fauron et al. 1987; Fauron and Havlik 1989; Allen et al. 2007). The frequent rearrangement in angiosperm mtDNA is thought to be the result of highly active recombination, for which the occurrence and genetic basis has been largely uncovered through studies of Arabidopsis (Shedge et al. 2007; Arrieta-Montiel et al. 2009; Davila et al. 2011). This

rearrangement has been attributed to recombination between repeat sequences. Most angiosperm mtDNAs have many repeat sequences, and repeats of intermediate length, those from 50–500 bp, are thought to be subject to not-infrequent recombination (Maréchal and Brisson 2010; Woloszynska 2010).

Although a high degree of mtDNA rearrangement has been shown to occur throughout angiosperms, absolute rates of rearrangement and the extent to which these rates vary over evolutionary time have yet to be explored. Extreme variation (100-fold or more) in rates of mitochondrial synonymous substitutions has been demonstrated in certain groups of closely related angiosperms (Cho et al. 2004; Parkinson et al. 2005; Mower et al. 2007; Sloan et al. 2009; Sloan, Alverson, et al. 2012). This raises important questions: Do rearrangement rates also vary substantially in angiosperm mtDNAs, and if so, do they covary with substitution rates? It is important to examine rearrangement rates on a relatively short timescale to avoid saturation effects resulting from overlapping rearrangements, direct reversals, or other types of reuse of rearrangement endpoints.

In order to measure mtDNA rearrangement rates and assess potential rate heterogeneity in a set of appropriately closely related angiosperms, as well as test a key prediction of the hypothesis that rearrangement is due to repeat-mediated recombination, we inferred the history of rearrangement for seven genomes in the genus *Monsonia* (Geraniaceae) We reconstructed high quality draft assemblies of these genomes, measured rearrangement and substitution rates, and investigated the

distribution of repeats and sites of rearrangement. Our analyses revealed extensive variation in rearrangement rates in *Monsonia*, even within relatively short time periods of ~1 My. In striking contrast, synonymous substitution rates are virtually invariant in *Monsonia*. There is also very little variation in repeat content among *Monsonia* mtDNAs. Therefore, the observed differences in rearrangement rate are probably the result of differences in mitochondrial recombination activity. Repeats and sites of rearrangement preferentially occur in close proximity to each other in *Monsonia*. This pattern provides strong evidence that repeat-mediated recombination is the primary mode of mtDNA rearrangement in angiosperms.

Results

Phylogenetic analyses

The phylogenetic tree inferred from a 39,277-nt plastid gene concatenate (fig. 1A) resolves clades within *Monsonia* with high support (>98% bootstrap support for all five bipartitions and 100% support for three of them). A chronogram inferred from the same plastid alignment and a single fossil calibration point estimates the split of *Monsonia* and *Geranium* at ~22.9 Ma and the most recent common ancestor of the seven examined *Monsonia* species at ~8.9 Ma (fig. S2). The phylogenetic tree inferred from a 29,070-nt mitochondrial gene concatenate (fig. 1B) has very poor support (all bootstrap values are <50%), which is not unexpected given the shorter length of the mitochondrial alignment compared with the plastid one, and, especially, that mitochondrial synonymous substitution rates in *Monsonia* are on average ~7.8 times lower than plastid rates.

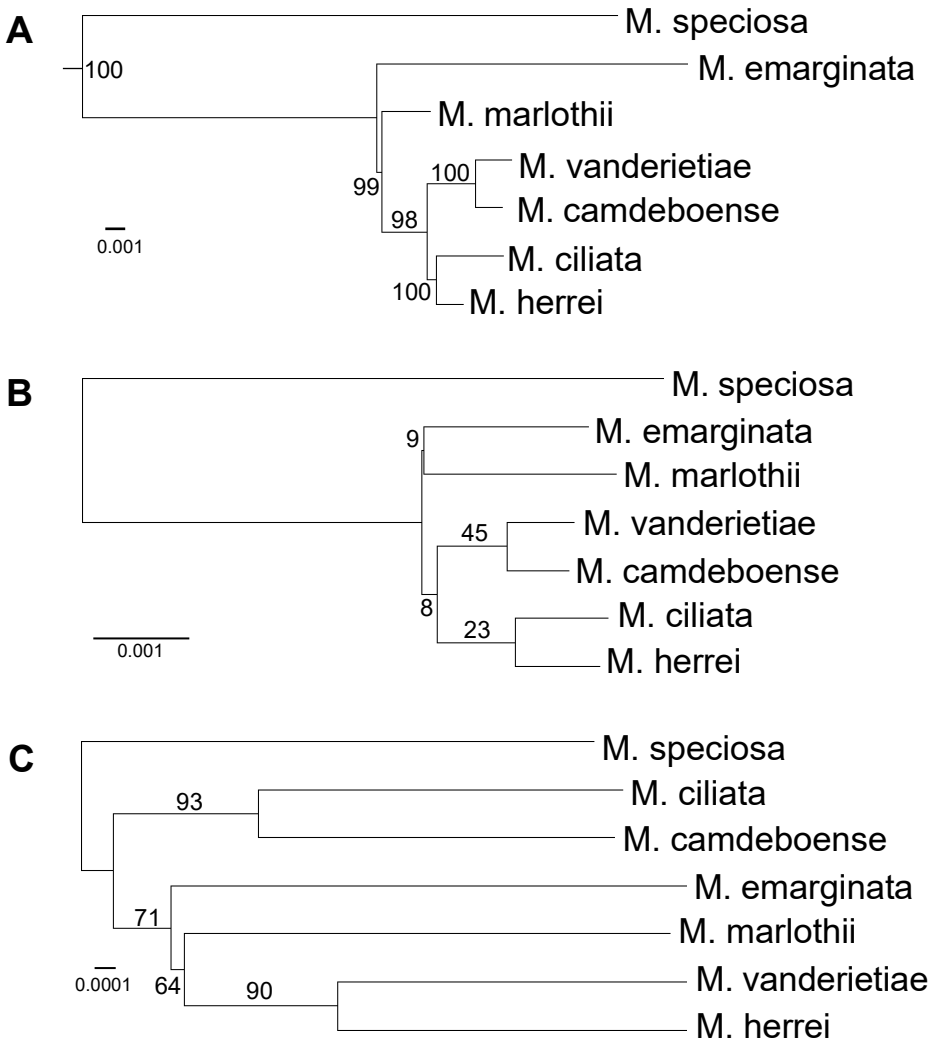

Figure 1: *Monsonia* phylograms based on (A) plastid gene sequences, (B) mitochondrial gene sequences, and (C) mitochondrial homologous-region adjacencies. The plastid tree was inferred with two unshown outgroups (*Pelargonium hortorum* and *Geranium palmatum*), and the mitochondrial gene tree with one unshown outgroup (*Geranium maderense*). No outgroups were used in the mitochondrial rearrangement analysis owing to very little shared sequence and synteny between *Monsonia* and the best potential outgroup (fig. S5), and so this tree was rooted according to the *Monsonia* root as determined in (A) and (B). Numbers on the trees are bootstrap support percentages. The scale bars for (A) and (B) are in units of number of nucleotide substitutions per site, while the bar for (C) is in units of adjacencies per site.

Nonetheless, the mitochondrial tree is congruent with the plastid tree with the exception of the placement of *Monsonia emarginata* and *M. marlothii*, which is due to a single nearest-neighbor interchange. In contrast, the tree inferred from mitochondrial rearrangements (fig. 1C), although relatively well supported (64–93% bootstrap), is entirely discordant from the other two trees, sharing no inferred bipartitions with either.

Genome assemblies and genome size variation

Draft assemblies of the seven examined *Monsonia* mitochondrial genomes (tables 1, S1, and S2) consist of 4–11 scaffolds, with minimum estimates of genome size that vary from 400 to 605 kb (table S1). To gain perspective on these estimates, we applied the same assembly protocol to the raw sequence data used by Park et al. (2015) to construct a complete assembly of the 736-kb mtDNA of the only fully assembled member of the Geraniaceae, *Geranium maderense*. The *G. maderense* sequence data are the same type used in the current study (i.e., 100-bp paired-end reads from an 800-bp insert library) and were generated using the same sequencing platform by the same sequencing facility. Our assembly of the *G. maderense* data recovered 95% (699 kb) of

Table 1: Salient features of the seven *Monsonia* mitochondrial genomes.

Species[1]	Genome size (bp)	Repeats					Rates		Scaffold number
		Totals			Size		Syn. subs.[2]	Rearrange-ments[3]	
		bp	%	Number	Median	Mean			
M. speciosa	605,487	33,728	5.6	415	72.0	81.3	5.4	5.4	9
M. emarginata	581,703	28,816	5.0	370	49.0	77.3	4.3	12.7	11
M. marlothii	571,776	27,956	4.9	409	46.0	68.4	4.4	13.2	6
M. vanderietiae	510,607	29,762	5.8	400	46.0	74.4	5.1	5.4	7
M. camdeboense	400,130	19,898	5.0	256	47.0	77.7	5.0	10.8	5
M. ciliata	412,246	15,992	3.9	240	44.0	66.6	4.7	35.2	4
M. herrei	604,880	41,808	6.9	544	42.0	76.9	4.6	16.7	9

[1]Species are ordered phylogenetically as per fig. 1A and fig. 2
[2]10^{-10} synonymous substitutions/site/year
[3]per million years

the complete published assembly and is comparable to the *Monsonia* assemblies in a number of ways (table S1). For example, it consists of nine scaffolds (compared with 4–11 for *Monsonia*), the largest of which comprises 62% of the assembly (compared with 59–61% for the three best *Monsonia* assemblies in this respect). Hence, the draft *Monsonia* assemblies probably contain the great majority of the genomes and are therefore appropriate for analyzing genome rearrangement and other global genomic properties.

The sequences that are most likely to be missing from these assemblies are perfect or near-perfect repeats and plastid-derived sequences, in both cases ones that are larger than the ~800-bp inserts used for sequencing. However, plastid-derived sequences typically constitute only 3–6% of angiosperm mtDNAs (Mower et al. 2012), and large repeats are generally quite rare (0–3 pairs per genome) among most sequenced eudicot mtDNAs (Alverson, Zhuo, et al. 2011). Indeed, inspection of the *Geranium* sequence that is not recovered in the assembly performed with our methodology shows that 52% of the missing sequence is repeated sequence (with all large repeats missing) and 19% of the missing sequence is of plastid origin.

The size estimates (400–605kb) for the seven *Monsonia* genomes fall within the size range of most fully sequenced angiosperm mtDNAs (Mower et al. 2012). Comparable or more limited levels of within-genus variation in genome size have been reported for *Oryza* (1.59-fold size range; Bentolila and Stefanov 2012), *Beta* (1.48-fold; Darracq et al. 2011), *Zea* (1.37-fold; Allen et al. 2007; Darracq et al. 2010), *Brassica* (1.24-fold;

Palmer and Herbon 1988; Tanaka et al. 2012), *Gossypium* (1.11-fold; Tang et al. 2015; Chen et al. 2017), and *Olea* (1.11-fold; Van de Paer et al. 2018). In striking contrast, the only two sequenced genomes from *Viscum* (Skippington et al. 2017) differ in size by a factor of 8.5, while there is an astonishing 45-fold range in genome size within *Silene*, which contains the largest known mitochondrial genome of 11.3Mb (Sloan, Alverson, et al. 2012).

Gene and intron content

The seven examined mitochondrial genomes of *Monsonia* have identical sets of protein and rRNA genes. The set of 27 protein genes (*atp1, atp4, atp6, atp8, atp9, ccmB, ccmC, ccmFc, ccmFn, cob, cox1, cox2, cox3, matR, mttB, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, nad7, nad9, rpl10, rps1, rps3*) is the same as that found in most other sequenced members of the Geraniaceae (Park et al. 2015). This gene complement reflects extensive gene loss (including functional transfer to the nucleus) that has occurred since the common ancestor of angiosperms, which had 41 genes (Richardson et al. 2013), with 9 of these 14 losses having occurred prior to the last common ancestor of extant Geraniaceae species (Park et al. 2015). The *Monsonia* mtDNAs contain the same set of rRNA genes (*rrn5*, *rrnS*, and *rrnL*) found in all other angiosperms (Skippington et al. 2017).

The seven *Monsonia* mtDNAs are also invariant with respect to intron content; they possess the same set of five cis-spliced and four trans-spliced group II introns that are present in the three other genera of the Geraniaceae with sequenced genomes (Park et

al. 2015). This is in contrast to the 25 introns present in the ancestral set of angiosperms and the 20–25 introns found in most angiosperms (Richardson et al. 2013). Fourteen of the 16 missing introns are the result of loss of the introns themselves, while two are the result of loss of the genes that normally house them (see table S3 of Park et al. 2015).

In contrast to the above features, the *Monsonia* assemblies vary extensively in their complement of tRNA genes, which ranges between 13 and 21 unique tRNA genes (fig. S3). Some of this variation could be the result of incomplete assembly. However, the complete recovery of the expected set of protein and rRNA genes in the assemblies and the failure to find additional tRNA genes in searches of the raw-read data indicate that most of it is probably real. The phylogenetic distribution of tRNA gene variation indicates a history of frequent gene loss with occasional gene gain (fig. S3). The best candidates for gain are the plastid-derived *trn*R(acg) and *trn*S(gcu) that are present in only *M. herrei*; in contrast to a single putative gain of these two genes, all-loss scenarios would require five losses of each gene within *Monsonia*.

Synonymous substitution rates

Synonymous substitution rates within *Monsonia* exhibit very little variation (fig. 2B) and fail to reject a test of the molecular clock (fig. 3B; P = 0.84). The group shows only 1.26-fold variation in synonymous substitution rates, ranging from 4.3 to 5.4 x 10^{-10} substitutions/site/year across the tree. These rates fall within the range observed among most angiosperm mtDNAs (Mower et al. 2007; Richardson et al. 2013). However, the

low level of variation within *Monsonia* is somewhat surprising, given the more extensive variation in synonymous substitution rates among mtDNAs of angiosperms in general (Mower et al. 2007) and within genera such as *Erodium* (2.0-fold; Parkinson et al. 2005), *Geranium* (2.6-fold; Park et al. 2017), and *Viscaria* (4.4-fold; Sloan et al. 2009), not to mention three extreme cases (*Pelargonium*, *Plantago*, and *Silene*) with ~100-fold

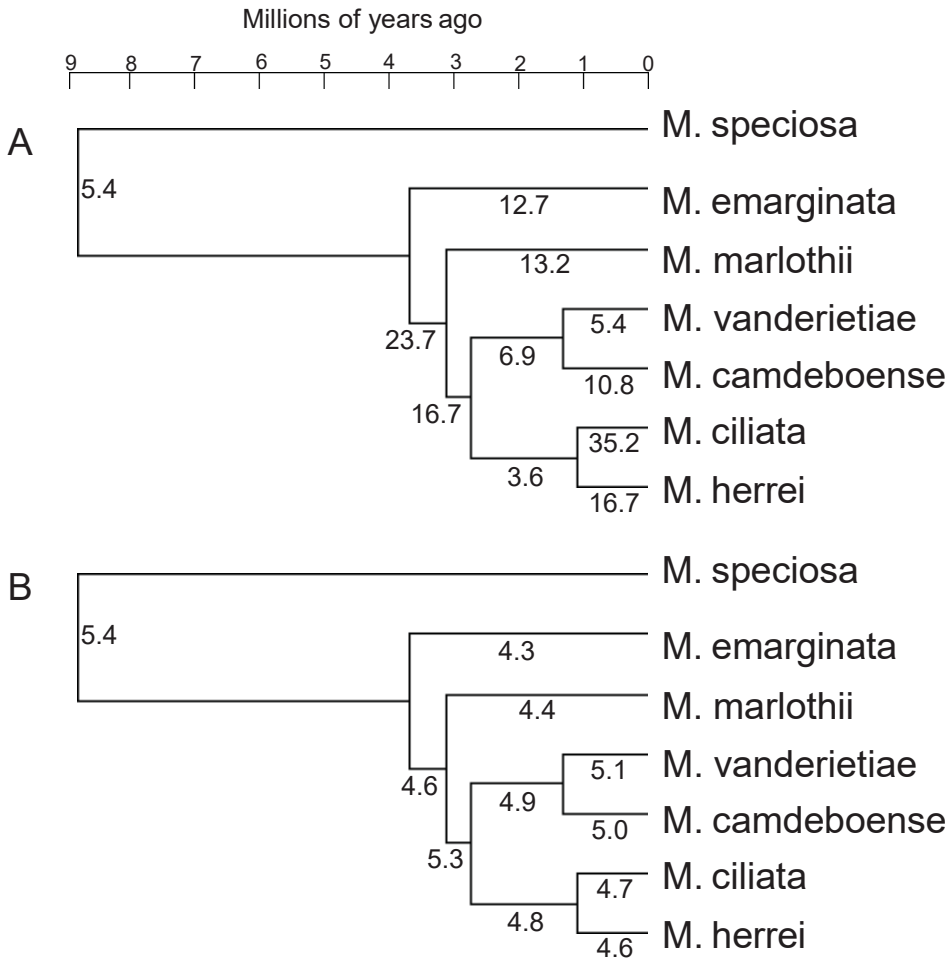

Figure 2: High variation in rearrangement rates (A) and low variation in synonymous substitution rates (B) in *Monsonia* mitochondrial genomes. Numbers of rearrangement events per million years and of synonymous substitutions in 10^{-10} substitutions/site/year are mapped on a *Monsonia* chronogram. As discussed in the text, the estimates of rearrangement-rate variation shown here are conservative.

intrageneric variation in synonymous substitution rates (Cho et al. 2004; Parkinson et al. 2005; Sloan, Alverson, et al. 2012).

Rearrangement rates

Rates of rearrangements vary 10-fold across *Monsonia* in the analysis summarized in figure 2A, with rates ranging from 3.6 to 35.2 rearrangements per million years. The hypothesis of a molecular clock explaining rates of rearrangement across the tree is rejected (fig. 3A; P = 0.009), and the hypothesis that these rates fit a Poisson distribution is also rejected (P < 0.001, mean = 9.5 rearrangements per million years, variance = 45.5 rearrangements per million years). The above estimate of the range of rearrangement rate variation is necessarily conservative because rate heterogeneity

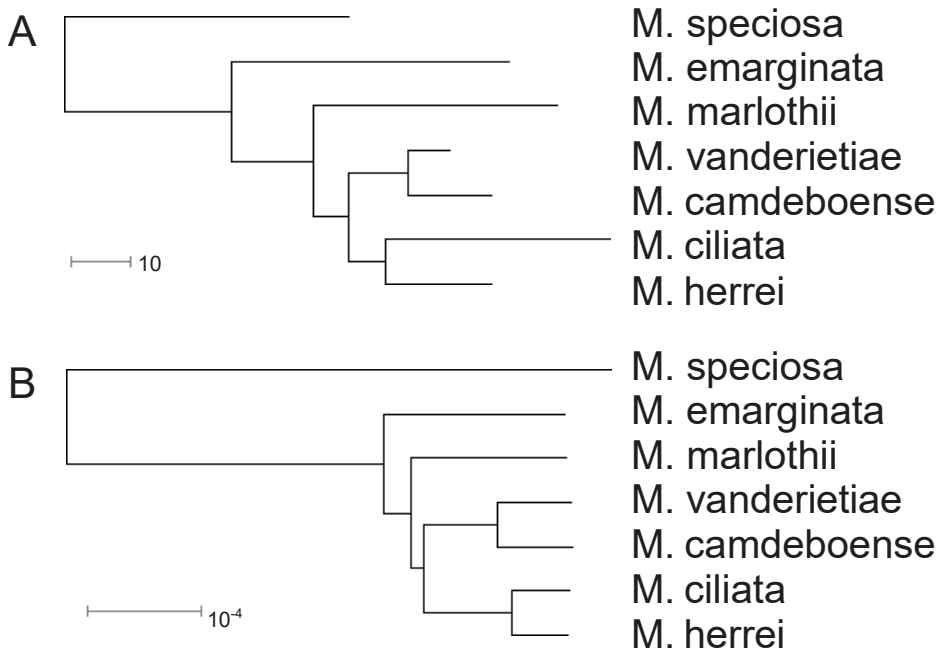

Figure 3: Highly variable rearrangement rates (A) and clock-like synonymous-substitution rates (B) in *Monsonia* mitochondrial genomes. Branch lengths are scaled to number of rearrangement events (A) and number of synonymous substitutions per site (B).

can be measured only between branches but not along them and because rates vary so much between branches in *Monsonia*. Therefore, at times, rearrangement rates must have been lower (perhaps substantially so) on the low-rate branches and higher (again, perhaps substantially so) on the high-rate branches.

The incomplete nature of the *Monsonia* assemblies (4–11 scaffolds; table S1) has inflated the inferred number of rearrangements, differentially so, across the tree. An adjustment (Muñoz and Sankoff 2010) was therefore applied to rearrangements inferred from pairwise comparisons (table 2) in order to estimate the minimum number of rearrangements given the incompleteness of the assemblies. This adjustment reduced the inferred number of rearrangements (by 7–18 depending on the

Table 2: Number of pairwise rearrangements inferred from the progressiveMauve alignments of fig. S4, either unadjusted (top), with half the combined number of scaffold endpoints included as rearrangements, or adjusted as per Muñoz and Sankoff (2010) (bottom), with scaffold endpoints effectively excluded.

	M. speciosa	M. emarginata	M. marlothii	M. vanderietiae	M. camdeboense	M. ciliata
M. speciosa						
M. emarginata	86					
M. marlothii	89	65				
M. vanderietiae	79	56	42			
M. camdeboense	81	59	43	14		
M. ciliata	89	64	54	38	41	
M. herrei	84	60	44	24	23	41
M. speciosa						
M. emarginata	69					
M. marlothii	76	50				
M. vanderietiae	65	40	31			
M. camdeboense	69	45	34	5		
M. ciliata	78	51	46	29	34	
M. herrei	68	42	31	10	11	30

pair). Note that the overestimation of rearrangement across trees arising from incomplete assemblies has been shown to be small in simulations on genomes that are much more fragmented and much less rearranged than these ones (Zheng and Sankoff 2016).

Substantial rearrangement (explained by as few as 30 and as many as 41 events) occurred between M. *ciliata* and M. *herrei*, which diverged ~1.1 Ma (fig. 4; table 2). Much less rearrangement (5 and 14 events) occurred between their close relatives *M. vanderietiae* and *M. camdeboense*, which diverged ~1.3 Ma. Pairwise alignment of

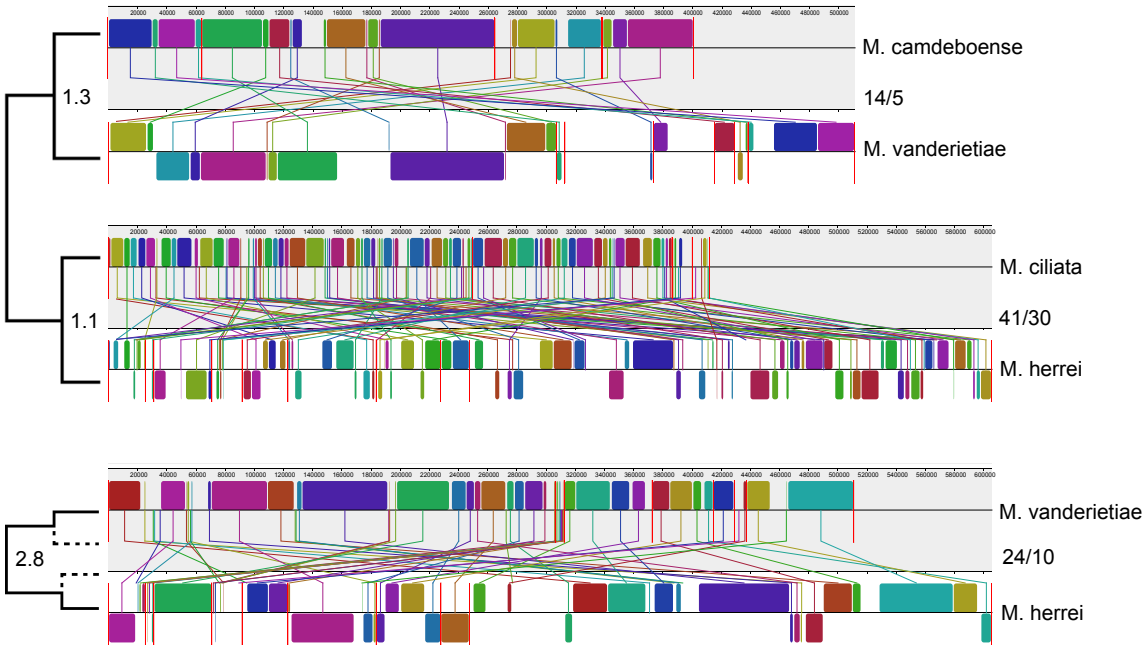

Figure 4: Evidence for an elevated rate of mitochondrial rearrangement in the *M. ciliata* lineage. The first number after each Mauve alignment is the number of rearrangements inferred, while the second is the minimum possible number of rearrangements given the incompleteness of the genomes (see text for fuller explanation). Numbers on the trees are in millions of years. Red lines represent "boundaries" between scaffolds, whose order was determined by Mauve.

these four genomes (fig. 4; fig. S4) and mapping of rearrangements on the *Monsonia* tree (figs. 2A and 3A) show that a disproportionate fraction of this rearrangement occurred in the *M. ciliata* lineage. For example, the alignments reveal that the *M. herrei* genome is actually more syntenic with the *M. vanderietiae* genome (an ~2.8-My divergence) than with its sister, *M. ciliata* genome (an ~1.1-My divergence). The rearrangement rate difference between *M. ciliata* and *M. herrei* shown in figure 3A is significant by the relative rates test ($P < 0.005$).

The different *Monsonia* tree topologies produce dramatically different estimates of rearrangement rate heterogeneity. Rearrangements inferred on the plastid-based topology rejected the hypothesis that mitochondrial rearrangement in *Monsonia* occurs in a clocklike fashion ($P = 0.009$; fig. 3A), whereas the rearrangement-based tree failed to reject this hypothesis ($P = 0.51$; fig. 1C). In other words, much lower rearrangement-rate heterogeneity is inferred when using the rearrangement-based tree. The *Monsonia* rearrangements are explained by a majority of inferred inversions in all pairwise comparisons (53–93%, mean = 71%, median = 71%), with on-average fewer than one-third as many inferred transpositions (6–41%, mean = 23%, median = 19%; table S3). This is despite the tendency of genome rearrangement reconstruction algorithms to resolve overlapping inversions (as would presumably occur in *Monsonia*-like situations with extensive rearrangement) as a single transposition event (Wang et al. 2017) and for transpositions to more parsimoniously resolve genome-rearrangement patterns, even in those weighting schemes that penalize them (Bernt, Wieseke, et al. 2013).

The discovery of extensive variation in mitochondrial rearrangement rates in *Monsonia* prompted us to measure rates in two seed plant groups for which published data raised the possibility of either much slower rates than in *Monsonia* or even higher ones. Rearrangement between *Ginkgo* and *Cycas* mtDNAs was explained by 77 events (fig. S5), while rearrangement between pairs of four *Silene vulgaris* mtDNAs from different populations was explained by between 58 and 73 events (fig. S6). These data correspond to rates of only 0.11 rearrangements per million years in the *Ginkgo/Cycas* comparison given their common ancestry about 340 Ma (Guo et al. 2016) and at least 58–76 rearrangements per million years for different pairs of *Silene vulgaris* mtDNA given a common ancestry of the four populations of at most 490 ka (Rautenberg et al. 2012). Thus, even with such scant sampling, mtDNA rearrangement rates vary across seed plants by at least 600-fold.

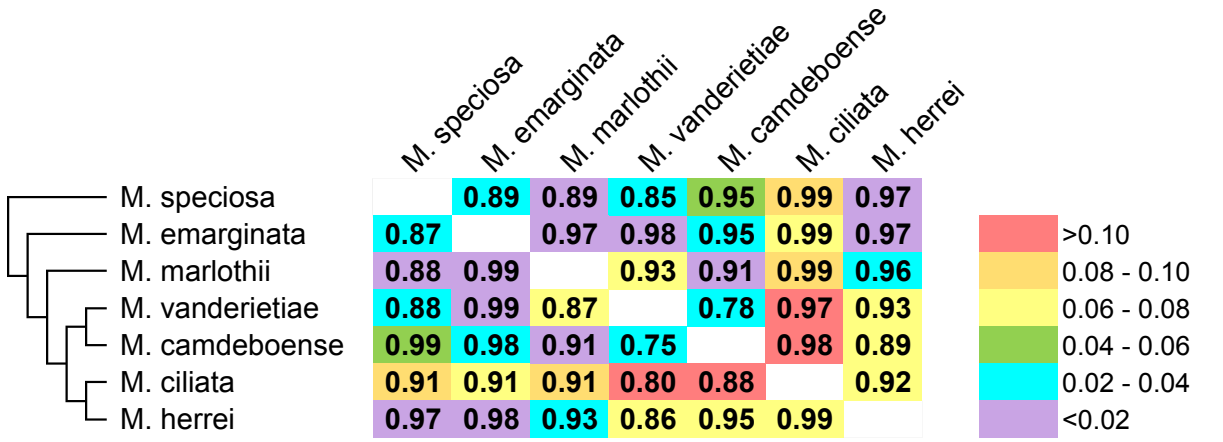

Figure 5: Most homologous-region boundaries (HRBs) are located very close to repeated sequences. Shown are the proportions of HRBs, defined through pairwise genome alignment (fig. S4), located within 50 bp of a repeat sequence. Most (39/42; 25/42 after Bonferroni correction) co-occurrence values are statistically significant ($p \leq 0.05$; $p \leq 0.012$ after Bonferroni correction). Colors indicate the difference between these proportions in reciprocal pairwise comparisons.

Co-occurrence of homologous region boundaries and repetitive sequence

If recombination between repeat sequences gives rise to genome rearrangements, then repeats should be overrepresented at rearrangement boundaries. Consistent with this hypothesis, although repeats comprise a relatively small fraction (4–7%) of these genomes (table 1), a high proportion (75–99%) of homologous-region boundaries (HRBs) are in close proximity (50 bp) to repeats in all 42 pairwise comparisons (fig. 5; table S4). Given the use of HRBs as proxies for rearrangement boundaries and the allowed separation (≤ 50 bp) of repeats from the locations of HRBs, these proportions may be slight overestimates of the actual proportion of rearrangement boundaries associated with repeats. The physical association between HRBs and repeats is significant ($P<0.05$; $P<0.0012$ after Bonferroni correction) in most pairwise comparisons (39/42; 25/42 after Bonferroni correction) based on the permutation test. Two of the three uncorrected cases that were not significant have relatively few HRBs (16 and 18), limiting the power of the test.

Recombination activity of repetitive sequences

The co-occurrence of HRBs and repeats suggests that repeat-mediated recombination has led to mitochondrial rearrangement within *Monsonia*. However, analysis of the frequency of alternative configurations (ACs) of the single-copy sequences that flank pairs of repeat reveals little or no evidence for ongoing recombination in the two sequenced DNA preparations, from *M. ciliata* and *M. herrei* (the terminal lineages with

the highest rates of rearrangement; fig. 2), that were examined in this regard. Only those repeats larger than 100 bp and smaller than the Illumina insert size of ~800 bp were examined for recombination. None of the five examined repeats from *M. ciliata* shows any evidence of ongoing recombination (i.e., not a single read pair supporting an AC was recovered against a background of about 500 read pairs supporting the predominant configurations, i.e., those present in the genome assembly) (table S6). Although ACs were recovered for 5 of the 13 examined repeats from M. herrei, their absolute number (1–6) and frequency (0.1–0.5%) are very low, so low that some could be artefactual. At best, then, there is AC evidence for only very low frequency recombination at a minority of repeats in *M. herrei*, while there is no AC evidence for ongoing recombination in *M. ciliata*.

Furthermore, the low level of sequence identity (83–84%; table S5) between repeat copies within a genome for the largest repeat pair (991–1,630 bp), shared by five *Monsonia* species, indicates that they have experienced very little if any concerted evolution, which in turn implies infrequent recombination. With rare exception (Sloan, Alverson, et al. 2012), repeats of this size in seed plant mtDNAs are 100% identical due to high frequency, homogenizing recombination between repeat copies within a genome. In addition, all repeat pairs over 300 bp, with the exception of a single repeat pair in *M. vanderietiae* (701 bp; 100% identity) have relatively low levels of sequence identity (81–93%; table S5), again suggesting reduced levels of concerted evolution and infrequent recombination.

Discussion

This first study of rates of mitochondrial rearrangement in plants has revealed both generally high rates of rearrangement (mostly occurring as inversions) and substantial (at least 10-fold) variation in rearrangement rate within a single genus. These findings, in combination with our analyses of two gymnosperms and *Silene*, indicate that mitochondrial rearrangement rates vary across seed plants by at least a factor of 600. As well, rearrangement and substitution rates are completely uncoupled temporally in *Monsonia*. Rearrangement breakpoints in *Monsonia* mtDNAs occur in close proximity to repeats; this provides strong evidence for the central role of repeat-mediated recombination in shaping these genomes through rearrangement. Puzzlingly, despite this clear historical signal of an association between repeat-mediated recombination and rearrangement, we found very little evidence of ongoing recombination in *Monsonia*. Below we discuss these findings in the context of the factors and mechanisms responsible for rearrangement in plant mtDNAs, the use of genome-arrangement data in inferring phylogenetic trees in plants, and comparison of genome rearrangement between plant mtDNA and the well-studied organelle genomes of animal mitochondria and angiosperm plastids.

Potential causes of extensive variation in rates of genome rearrangement

In contrast to findings on plastid DNA (see section below) and fungal mtDNA (Aguileta et al. 2014), we find no evidence that differences in rearrangement rate in *Monsonia* mtDNA arise from differences in repeat content. Contrary to intuition, which would suggest that differences in rearrangement rate arise from differences in number and

size of repeats—the presumed substrates for rearrangement—we observed the most rearrangement in the *M. ciliata* lineage (figs. 2 and 4), which has the least repetitive DNA by all measures (table 1). Likewise, though there is limited variation within *Monsonia* in absolute amount of repetitive DNA (~2.6-fold), relative amount (~1.8-fold), and number of repeat elements (~2.3-fold), none of these features is related to rearrangement rate (figs. 2A and S7, table 2). Though life history differences among *Monsonia* species are not well understood, their shared perennial habit and low variation in synonymous substitution rates (figs. 2 and 3; fig. S7) suggest that differences in rearrangement rates are not the result of differences in generation time (Li et al. 1996; Lehtonen and Lanfear 2014). Because repeat content clearly does not correlate with rearrangement rate, the observed variation is probably the result of lineage-specific differences in the rate of recombination.

Monsonia mtDNAs exhibit high variation in rearrangement rates and virtually no variation in synonymous substitution rates (figs. 2 and 3). These starkly contrasting patterns can be accommodated under a recently developed model in which different underlying mechanisms are proposed to control the rate of these two categories of mutation in angiosperm mtDNA. This model postulates that rearrangements are the consequence of highly active processes such as break-induced replication (BIR) and nonhomologous end-joining (NHEJ) that operate in intergenic regions and tend to produce structural changes (Christensen 2014). A second postulate is that the generally low genic substitution rates in angiosperm mtDNAs result from frequent and high-fidelity gene-conversional repair (Sloan, Alverson, et al. 2012; Christensen 2013). Under this

model, different lineages of *Monsonia* have utilized these two categories of mechanisms to different extents, with gene conversion-based mechanisms operating at a constant tempo across the genus, accounting for low variation in synonymous substitution rates, and with highly variable rates of BIR and possibly NHEJ accounting for high variation in rearrangement rates.

Uncoupled synonymous substitution and rearrangement rates may also be explained by substitution being influenced by base excision repair (BER) systems, which are wholly separate from recombination-based repair mechanisms such as BIR and NHEJ. The synonymous substitution rate is presumably negatively influenced by effective BER, which is now well established to occur in plant mitochondria (Boesch et al. 2009; Trasviña-Arenas et al. 2018; Ferrando et al. 2018). Rearrangement, however, would not be affected by the short-patch BER mechanisms known in plant mitochondria. Under this scheme, low variation in the (high) efficacy of BER within *Monsonia* would explain low variation in synonymous substitution rates, whereas, as described above, high variation in rates of BIR and possibly NHEJ would explain high variation in rearrangement rates.

The rearrangement rate variation in *Monsonia* may also, at least to some limited extent, be the result of lineage-specific differences in the persistence and fixation of new genome arrangements via neutral or selective forces. The great majority of rearrangements are probably neutral because all rearrangement breakpoints are located in noncoding regions. The power of genetic drift may have varied within

Monsonia owing to differences in effective population size differences resulting from, among others factors, variation in the germline mtDNA bottleneck. Unfortunately, to our knowledge, no relevant population-size data are available for *Monsonia*. On the other hand, plant mtDNA rearrangements have been shown to affect traits that could influence fitness, such as metabolism (Juszczuk et al. 2007; Szal et al. 2010), cytoplasmic male sterility (Hanson 1991; Schnable and Wise 1998; Delph et al. 2007), and thermotolerance (Shedge et al. 2010), so selection may play some role in the maintenance and fixation of a subset of new genome arrangements.

***Repeat-mediated recombination drives mitochondrial rearrangement in* Monsonia**

The co-occurrence of HRBs and repeat sequences (fig. 5) is strong evidence that repeat-mediated recombination is a major force driving the rearrangement of *Monsonia* mtDNAs. To our knowledge, this is the first time that such an analysis has been conducted on plant mitochondrial genomes. Furthermore, the greater number of rearrangements inferred when using phylogenetic (fig. 2) over pairwise comparisons (table 2) and the saturation apparent in the rearrangement-based tree topology indicate the occurrence of parallel or convergent rearrangements across *Monsonia* evolution. This would be expected if repeats were being reused in genome rearrangement. These findings, consistent with mechanistic studies (Shedge et al. 2007; Arrieta-Montiel et al. 2009; Davila et al. 2011) and intraspecific comparisons (Davila et al. 2011), provide important evidence at the interspecific level for the central role of repeat-mediated recombination in driving genome rearrangement in angiosperm mtDNAs.

Under the repeat-mediated rearrangement model, the co-occurrence of HRBs and repeat sequences should be most apparent in those lineages in which rearrangement has occurred recently and at high rates. This is because the high rates of sequence turnover in plant mtDNAs (Sloan, Alverson, et al. 2012; Liu et al. 2013; Guo et al. 2016) will cause the co-occurrence of repeats and HRBs to decay over time. This co-occurrence should be stronger when repeat-mediated rearrangement is creating these physical associations at a greater pace than sequence turnover is breaking them up. This prediction is met where most expected, in the genome of *M. ciliata*. The *M. ciliata* lineage has experienced the highest measured rate of rearrangement in *Monsonia*, with these events having occurred in the last 1.1 My (figs. 2 and 4). For the 12 total pairwise comparisons involving *M. ciliata*, the proportion of HRBs located near repeats present in the *M. ciliata* genome is consistently higher than in the six reciprocal comparisons in which HRB co-occurrence is measured with respect to repeats present in the other *Monsonia* genomes in the comparisons (fig. 5). The difference in these two sets of reciprocal proportions is significantly higher for *M. ciliata* than for the other six *Monsonia* genomes ($P = 0.004$ by the paired t-test).

Low ongoing recombination between repeats despite high levels of repeat-mediated rearrangement over evolutionary time

We found little evidence of recombination activity in DNA samples of *M. ciliata* and *M. herrei* (tables S5 and S6) despite their historically frequent and recent rearrangement. *Monsonia ciliata*, for example, exhibits the highest rearrangement rate of any *Monsonia* lineage, yet none of its repeats displays evidence of ongoing recombination as

measured by either AC frequencies or repeat homogenization. Observed rates of rearrangement in *M. ciliata* and *M. herrei* result from the differential generation, persistence, and fixation of new genome arrangements over the course of evolution; this cannot be measured by simply observing the recombination activity of contemporary plants. This is somewhat analogous to the relationship between the generation and fixation of single-nucleotide mutations and the measurement of substitutions from comparative data. Nonetheless, this disparity poses a fascinating quandary: How do we reconcile high levels of repeat-mediated rearrangement in the recent past with little-to-no evidence for the recombination of repeats in the present?

This quandary may in part be explained by a methodological limitation: our approach to measure recombination between repeats could not be applied to repeats smaller than the 100-bp Illumina read sizes, yet such short repeats also mediate rearrangements in plant mtDNA, through mechanisms such as microhomology-mediated BIR (Kato et al. 1998; Maréchal and Brisson 2010; Taylor et al. 2015). On the other hand, the clear evidence that *Monsonia* mtDNAs have aberrantly low levels of homogenizing recombination between repeats stands on its own, not subject to this obvious methodological caveat.

Tree inference using genome-arrangement data and its consequences for estimates of genome-rearrangement rates

Mitochondrial rearrangement-based and plastid sequence based topologies for *Monsonia* are entirely discordant (fig. 1), indicating that, in the case of *Monsonia*, using

rearrangement-based characters for reconstructing phylogeny is most likely misleading. Indeed, the plastid sequence-based topology is highly preferred over the rearrangement-based topology by the approximately unbiased test ($P < 0.001$). This discordance is probably not the consequence of differing phylogenetic histories of mitochondrial and plastid genomes in *Monsonia*, as the sequence-based trees for the mitochondrial and plastid genomes are largely concordant (fig. 1). This discordance contrasts with results from the less rearranged mtDNAs of *Beta* (Darracq et al. 2011) and *Zea* (Darracq et al. 2010), in which rearrangement-based trees were congruent with sequence-based trees. Rearrangements in mtDNA are generally considered “rare genomic changes” useful for low-homoplasy resolution of phylogenetic relationships (Rokas and Holland 2000) and can accurately resolve phylogenetic relationships in animals (Cameron 2014; Eberhard and Wright 2016) and in *Beta* and *Zea*. The high level of rearrangement observed in *Monsonia* mtDNA, however, would appear to make rearrangement-based characters susceptible to saturation effects that are known to lead to erroneous estimates of phylogeny (Hillis and Huelsenbeck 1992). Though past studies show that mitochondrial rearrangement-based characters can lead to accurate inferences of phylogenetic relationships, our analyses suggest that these inferences may be problematic when mtDNAs are very highly rearranged, which could be the case with other angiosperms (Sloan, Müller, et al. 2012) and with fungi (Beaudet et al. 2013; Aguileta et al. 2014).

Diverse tempos and mechanisms of organelle genome rearrangement in eukaryotes

Our findings provide an opportunity to compare the tempo and mechanism of rearrangement in angiosperm mtDNAs with those in two of the best studied groups of organelle genomes, those of angiosperm plastids and animal mitochondria. In utter contrast to angiosperm mitochondria, rearrangement is in general very rare in both groups of genomes. In fact, most of the >2,000 sequenced angiosperm plastid genomes are entirely collinear, with this gene order tracing back to the common ancestor of euphyllophytes, some 400 Ma (Mower and Vickrey 2018). Likewise, many arthropod mtDNAs have undergone no detectable rearrangements over an ~550 My period (Shao et al. 2003; Xu et al. 2006; dos Reis et al. 2015), while rearrangement is also extremely rare in most vertebrates (Saccone et al. 2002; Mueller and Boore 2005; Lavrov and Pett 2016).

A number of exceptional groups with moderately to highly rearranged genomes have been found in both plastids (Cosner et al. 2004; Jansen et al. 2007; Wicke et al. 2011; Knox 2014; Weng et al. 2014) and animal mitochondria (Lavrov et al. 2002; Rubinstein et al. 2013; Figueroa and Baco 2015; Lavrov and Pett 2016). However, the extent and rate of rearrangement in these groups do not approach those in angiosperm mitochondria. For example, in the “highly” (for plastids) rearranged Geraniaceae (Weng et al. 2014), 10 sampled branches have 0 or 1 rearrangements, and the five branches with multiple rearrangements have rates of only 0.25–1.3 rearrangements per million years. This compares with the 3.6–35.2 range of rates for *Monsonia* mtDNAs.

The comparatively infrequent occurrence of inversions in plastids and animal mitochondria is in part due to the paucity of short and intermediate-sized repeats in their genomes. When plastid genomes have abundant repeats, inversions are more common, although never as frequent as in most angiosperm mitochondrial genomes (Haberle et al. 2008; Guisinger et al. 2011; Guo et al. 2014; Knox 2014; Mower and Vickrey 2018). Similarly, in the few cases of animal mtDNAs where repeats of this size do exist, repeat-mediated inversion appears to have occurred (Dowton et al. 2003; Brockman and McFadden 2012; Mao, Austin, et al. 2014; Mao, Gibson, et al. 2014). An additional obstacle to rearrangement in plastid and especially animal mitochondrial genomes is their relatively compact, gene-dense organization, as well as the inclusion of many plastid genes in operons.

In angiosperms, repeat-mediated inversion is the predominant mechanism of rearrangement in both the mitochondrial (table S3; Makaroff and Palmer 1988; Palmer and Herbon 1988; Siculella and Palmer 1988; Bafna and Pevzner 1995; Darracq et al. 2010) and plastid genomes (Palmer et al. 1987; Chumley et al. 2006; Cai et al. 2008; Haberle et al. 2008; Knox 2014; Weng et al. 2014; Mower and Vickrey 2018). In contrast, mtDNA rearrangement in animals is thought to mostly occur through the generation of large tandem duplications followed by differential loss of gene duplicates (Moritz et al. 1987; Boore 2000). The outcome of this mechanism, and therefore the most common form of rearrangement in animal mtDNA, is transposition (Bernt, Braband, et al. 2013; Cameron 2014).

Conclusion

Mitochondrial genomes of seed plants are already known to vary dramatically in rates of synonymous substitutions (Mower et al. 2007; Richardson et al. 2013), gene loss accompanied by functional transfer to the nucleus (Adams et al. 2002; Adams and Palmer 2003; Guo et al. 2016), and sequence turnover (Guo et al. 2016). We now add rearrangement to this growing list of genomic traits that vary enormously in rate across seed plants, and even among closely related species. Seed plant mtDNAs exhibit moderate-to-high variation in other genomic features, including genome size (Ward et al. 1981; Sloan, Alverson, et al. 2012; Skippington et al. 2015), amount of plastid-derived DNA (Mower et al. 2012; Rice et al. 2013), intron content (Park et al. 2015; Guo et al. 2016), and edit-site content (Sloan, Alverson, et al. 2012, Richardson et al. 2013; Park et al. 2015; Guo et al. 2016). This trait variation has not yet been put in the framework of absolute evolutionary rates. Going forward, such efforts may require improved taxon sampling, both broadly and also densely within closely related groups for those traits that, like rearrangement, are prone to saturation.

Materials and Methods

Genome sequencing, assembly, and annotation

Total genomic DNA was isolated from fresh leaf tissue from seven species of *Monsonia*. Approximately 4–5Gb of 100-bp paired-end readswere generated froman 800-bp insert library for each species using the Illumina HiSeq2000 sequencing platform at the Genome and Sequence Analysis Facility at the University of Texas at Austin.

Genome sequences were assembled de novo using Velvet 1.2.08 (Zerbino and Birney 2008) using k-mer values ranging from 31 to 99 and expected coverage values ranging from 50 to 500 with minimum coverage set at 5. Mitochondrial scaffolds selected postassembly were 1) those containing mitochondrial genes and 2) those nongenic scaffolds with a comparable k-mer coverage as the genic scaffolds (~5 to ~55 depending on the assembly). Genome assemblies were annotated using MITOFY (Alverson et al. 2010). BLAST 2.2.30 (Altschul et al. 1990) was used to search raw reads for evidence of any tRNA genes of mitochondrial origin that were not recovered in the assembly. Ungapped BlastN with a raw score cutoff of 30 was used for these searches.

Inference of Monsonia *phylogeny and divergence times*

A concatenated alignment of 39,277 nucleotides from 27 plastid protein genes for the seven species of *Monsonia* (sequences provided by J. Zhang and R. Jansen) and two outgroups, *Pelargonium hortorum* (Chumley et al. 2006) and *Geranium palmatum* (Guisinger et al. 2011), was generated using MUSCLE 3.8.29 (Edgar 2004) with default parameters. A concatenated alignment of 27,090 nucleotides from all 27 protein-coding mitochondrial genes for the seven species of *Monsonia* and an outgroup, *Geranium maderense* (Park et al. 2015), was also generated using MUSCLE 3.8.29 (Edgar 2004) with default parameters. Phylogenetic trees were inferred from each alignment using PhyML 3.1.2 (Guindon and Gascuel 2003) with the GTR+G substitution model employed on the basis of the Bayesian information criterion in jModeltest 2.1.6 (Darriba et al. 2012). Bootstrap values were calculated using 1,000 pseudoreplicates. Tree

topologies were compared using the approximately unbiased test (Shimodaira 2002) as implemented in CONSEL 0.20 (Shimodaira and Hasegawa 2001). Divergence times were inferred in RelTime (Tamura et al. 2012) using the plastid sequence tree and a fossil calibration (Palazzesi et al. 2012) that constrained the common ancestor of *Pelargonium* and *Monsonia* to be at least 28.4 My old.

Estimation of mitochondrial substitution rates

Synonymous substitution rates were calculated in HyPhy version 2.2 (Pond et al. 2005) using the concatenated alignment of 27mitochondrial genes and constraining the tree topology to the inferred plastid-based estimate of *Monsonia* phylogeny. Analyses were performed using theMG94xREV codon model (Pond andMuse 2005) with local variation in rate parameters. This model allows for the estimation of all parameters in the GTR substitution matrix as recommended by jModeltest. Absolute rates of mitochondrial synonymous substitutions were obtained by dividing branch lengths in terms of synonymous substitutions per site by branch lengths in absolute time from the inference of divergence times.

Analysis of rearrangement

Alignments of all pairwise combinations of the seven *Monsonia* genomes and a multiple alignment of all seven genomes were generated using the progressiveMauve algorithm (Darling et al. 2010) as implemented in Mauve Build 10 (Darling et al. 2004). Homologous regions in each alignment were inferred using the minimum LCB size setting. Pairwise rearrangement distances in terms of minimum number of

rearrangements were inferred using GRIMM (Tesler 2002). The minimum possible numbers of rearrangements given the incompleteness of these genomes were calculated using the rationale in Muñoz and Sankoff (2010) under the assumption that these genomes should assemble as a single circular chromosome.

To infer the rate of rearrangement across different branches of the tree, the order and orientation of homologous regions as determined by the seven-species alignment produced by Mauve were used in MLGO (Hu et al. 2014) to infer the ancestral genome arrangement at each internal node. Subsequently, the rearrangement distance between each of these nodes and their neighboring nodes was inferred with GRIMM. The resulting numbers of rearrangements on each branch of the tree were divided by the lengths of the branches in absolute time to calculate branch-wise rearrangement rates. The relative-rates-of-gene rearrangement test (Dowton 2004) was performed to compare the rearrangement rates of the *M. ciliata* and *M. herrei* lineages and to address rate variation in a manner that is independent of divergence time estimates. Pairwise rearrangement distances between species were used, and the test was performed with both *M. vanderietiae* and *M. camdeboense* as outgroups.

A rearrangement-based tree was inferred using homologous-region adjacencies as binary characters using MLGO without an outgroup because the closest sequenced mitochondrial genome, *Geranium maderense*, shares very little sequence content and synteny with *Monsonia* (fig. S1). A phylogenetic test of the molecular clock (Takezaki et al. 1995) as implemented in MEGA 6 (Tamura et al. 2013) was performed for both the

plastid tree topology with branch lengths scaled by numbers of rearrangements and the mitochondrial tree inferred using MLGO as another way of addressing rate heterogeneity in a way that is independent of estimates of divergence time. A chi-square test for goodness of fit was also performed to determine whether rates of rearrangement could be explained by a Poisson distribution, which is another expectation of the molecular clock.

A multiple alignment of four *Silene vulgaris* genomes (from Sloan, Müller, et al. [2012]) and pairwise alignments of *Ginkgo biloba* and *Cycas taitungensis* (from Guo et al. [2016] and Chaw et al. [2008], respectively) and of *Geranium maderense* (from Park et al. [2015]) and *Monsonia emarginata* were generated using the progressiveMauve algorithm (Darling et al. 2010) as implemented in Mauve Build 10 (Darling et al. 2004). Homologous regions in each alignment were inferred using the minimum LCB size setting. Pairwise rearrangement distances in terms of minimum number of rearrangements were inferred between all combinations of *S. vulgaris* genomes as well as the *Cycas*–*Ginkgo* pair using GRIMM (Tesler 2002).

Repeats and recombination

Repeats were detected using BLAST 2.2.30 (Altschul et al. 1990) to search each of the seven *Monsonia* genomes against themselves. Ungapped BlastN with a raw score cutoff of 30 was used for these searches. Further searches for tandem duplications too divergent to be detected through BLAST were performed using Tandem Repeats Finder with the most permissive possible settings (Benson 1999).

To examine recombinational activity of repeats in *M. ciliata* and *M. herrei*, AC frequencies were assessed using paired-end data. Read pairs were classified as consistently mapping if they mapped to the genome sequence in the predominant orientation at the expected distance (± 50% of the 800-bp insert size). To determine the number of read pairs supporting each arrangement of each repeat pair, we first counted the number of consistently mapping pairs, that is, those that corresponded to the predominant configuration of single copy sequences flanking each repeat as present in the genome assemblies. Those read pairs that did not map consistently to the predominant genome sequence were classified as inconsistently mapping read pairs. These were subsequently checked to determine whether they map to a recombinant form of the genome produced by recombination at a repeat. Read pairs supporting recombinant forms were counted for each repeat. Recombination frequency was calculated by dividing the number of recombinant read pairs by the total number of read pairs spanning each repeat.

Proximity of repeats and rearrangement breakpoints

HRBs from the pairwise Mauve alignments were used as proxies for sites of rearrangement in order to assess whether such sites are preferentially found in close proximity to the repeated sequences detected using BLAST. HRBs on the ends of scaffolds were excluded from these analyses. The number of HRBs within 50 bp of each repeat was counted and divided by the total number of HRBs to give the proportion of HRBs found in close proximity to repeats. This 50-bp separation is

introduced to account for small-scale structural changes that may occur near rearrangement boundaries, including the potential loss or replacement of some portion of the repeated sequences involved in recombination (Davila et al. 2011). To compare this proportion to a null expectation, replicates were simulated in which the positions of HRBs were randomized, with genome size, repeat sizes, and repeat positions held constant and the same as those in the genome of interest. A total of 10,000 of these replicates were generated for each comparison. P-values were calculated by counting the number of replicates with a larger proportion of HRBs in close proximity of repeats than the genome in question and dividing by the number of replicates.

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Research contributions

Logan Cole performed all but one set of analyses and prepared all tables and figures. Wenhu Guo performed the alternative configuration analyses.

References

Adams KL, Palmer JD. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol Phylogenet Evol.* 29(3): 380–395.

Adams KL, Qiu YL, Stoutemyer M, Palmer JD. 2002. Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. *Proc Natl Acad Sci USA.* 99(1515): 9905–9912.

Aguileta G, de Vienne DM, Ross ON, Hood ME, Giraud T, Petit E, Gabaldon T. 2014. High variability of mitochondrial gene order among fungi. *Genome Biol Evol.* 6(2): 451–465.

Allen JO, Fauron CM, Minx P, Roark L, Oddiraju S, Lin GN, Meyer L, Sun H, Kim K, Wang C, et al. 2007. Comparison among two fertile and three male-sterile mitochondrial genomes of maize. *Genetics* 177(2): 1173–1192.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol.* 215(3): 403–410.

Alverson AJ, Wei X, Rice DW, Stern DB, Barry K, Palmer JD. 2010. Insights into the evolution of plant mitochondrial genome size from complete sequences of *Citrullus lanatus* and *Cucurbita pepo* (Cucurbitaceae). *Mol Biol Evol.* 27(6): 1436–1448.

Alverson AJ, Zhuo S, Rice DW, Sloan DB, Palmer JD. 2011. The mitochondrial genome of the legume *Vigna radiata* and the analysis of recombination across short mitochondrial repeats. *PLoS One* 6(1): e16404.

Arrieta-Montiel MP, Shedge V, Davila J, Christensen AC, Mackenzie SA. 2009. Diversity of Arabidopsis mitochondrial genome occurs via nuclear-controlled recombination activity. *Genetics* 183(4): 1261–1268.

Bafna V, Pevzner PA. 1995. Sorting by reversals: genome rearrangements in plant organelles and evolutionary history of X chromosome. *Mol Biol Evol.* 12(2): 239–246.

Beaudet D, Terrat Y, Halary S, de la Providencia IE, Hijri M. 2013. Mitochondrial genome rearrangements in *Glomus* species triggered by homologous recombination between distinct mtDNA haplotypes. *Genome Biol Evol.* 5(9): 1628–1643.

Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.* 27(2): 573–580.

Bentolila S, Stefanov S. 2012. A reevaluation of mitochondrial evolution based on the complete sequence of male-fertile and male-sterile mitochondrial genomes. *Plant Physiol.* 158(2): 996–1017.

Bernt M, Braband B, Schierwater B, Stadler PF. 2013. Genetic aspects of mitochondrial genome evolution. *Mol Phylogenet Evol.* 69(2): 328–338.

Bernt M, Wieseke N, Middendorf M. 2013. On weight schemes for gene order analysis. In: Beißbarth T, Kollmar M, Leha A, Morgenstern B, Schultz A-K, Waack S, Wingender E, editors. *German Conference on Bioinformatics* 2013 (GCB ’13). Gottingen, Germany: Dagstuhl Publishing. p. 14–23.

Boesch P, Ibrahim N, Paulus F, Cosset A, Tarasenko V, Dietrich A. 2009. Plant mitochondria possess a short-patch base excision DNA repair pathway. *Nucleic Acids Res.* 37(17): 5690–5700.

Boore JL. 2000. The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animals. In: Sankoff D, Nadeau J, editors. *Comparative Genomics*. Dordrecht, Germany: Kluwer. p. 133–147.

Brockman SA, McFadden CS. 2012. The mitochondrial genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. *Genome Biol Evol*. 4(9): 994–1006.

Cai Z, Guisinger M, Kim H-G, Ruck E, Blazier JC, McMurtry V, Kuehl JV, Boore J, Jansen RK. 2008. Extensive reorganization of the plastid genome of *Trifolium*

subterraneum (Fabaceae) is associated with numerous repeated sequences and novel DNA insertions. *J Mol Evol.* 67(6): 696–704.

Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annu Rev Entomol*. 59(1): 95–117.

Chaw SM, Shih AC, Wang D, Wu YW, Liu SM, Chou TY. 2008. The mitochondrial genome of the gymnosperm *Cycas taitungensis* contains a novel family of short interspersed elements, Bpu sequences, and abundant RNA editing sites. *Mol Biol Evol.* 25(3): 603–615.

Chen Z, Nie H, Wang Y, Pei H, Li S, Zhang L, Hua J. 2017. Rapid evolutionary divergence of diploid and allotetraploid *Gossypium* mitochondrial genomes. *BMC Genomics* 18(1): 876.

Cho Y, Mower JP, Qiu YL, Palmer JD. 2004. Mitochondrial substitution rates are extraordinarily high and elevated within a genus of flowering plants. *Proc Natl Acad Sci USA.* 101(51): 17741–17746.

Christensen AC. 2013. Plant mitochondrial genome evolution can be explained by DNA repair mechanisms. *Genome Biol Evol.* 5(6): 1079–1086.

Christensen AC. 2014. Genes and junk in plant mitochondria—repair mechanisms and selection. *Genome Biol Evol.* 6(6): 1448–1453.

Chumley TW, Palmer JD, Mower JP, Fourcade HM, Calie PJ, Boore JL, Jansen RK. 2006. The complete chloroplast genome sequence of *Pelargonium* X *hortorum*: organization and evolution of the largest and most highly rearranged chloroplast genome of land plants. *Mol Biol Evol*. 23(11): 2175–2190.

Cosner ME, Raubeson LA, Jansen RK. 2004. Chloroplast DNA rearrangements in Campanulaceae: phylogenetic utility of highly rearranged genomes. *BMC Evol Biol.* 4:27.

Darling AE, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* 14(7): 1394–1403.

Darling AE, Mau B, Perna NT. 2010. progressiveMauve:multiple genome alignment with gene gain, loss, and rearrangement. *PLoS One* 5(6): e11147.

Darracq A, Varré JS, Maréchal-Drouard L, Courseaux A, Castric V, Saumitou-Laprade P, Oztas S, Lenoble P, Vacherie B, Barbe V, et al. 2011. Structural and content diversity of mitochondrial genome in beet: a comparative genomic analysis. *Genome Biol Evol*. 8(3): 723–726.

Darracq A, Varré JS, Touzet P. 2010. A scenario of mitochondrial genome evolution in maize based on rearrangement events. *BMC Genomics* 11:233.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModeltest 2: more models, new heuristics and parallel computing. *Nat Methods*. 9(8): 772.

Davila JI, Arrieta-Montiel MP, Wamboldt Y, Cao J, Hagmann J, Shedge V, Xu YZ, Weigel D, Mackenzie SA. 2011. Double-strand break repair processes drive evolution of the mitochondrial genome in Arabidopsis. *BMC Biol.* 9(1): 64.

Delph LF, Touzet P, Bailey MF. 2007. Merging theory and mechanism in studies of gynodioecy. *Trends Ecol Evol.* 22(1): 17–24.

dos Reis M, Thawornwattana Y, Angelis K, Telford MJ, Donoghue PC, Yang Z. 2015. Uncertainty in the timing of the origin of animals and the limits of precision in molecular timescales. *Curr Biol.* 25(22): 2939–2950.

Dowton M. 2004. Assessing the relative rate of (mitochondrial) genomic change. *Genetics* 167(2): 1027–1030.

Dowton M, Castro LR, Campbell SL, Bargon SD, Austin AD. 2003. Frequent mitochondrial gene rearrangements at the Hymenopteran *nad3-nad5* junction. *J Mol Evol.* 56(5): 517–526.

Drouin G, Daoud H, Xia J. 2008. Relative rates of synonymous substitution in the mitochondrial, chloroplast, and nuclear genomes of seed plants. *Mol Phylogenet Evol.* 49(3): 827–831.

Eberhard JR, Wright TF. 2016. Rearrangement and evolution of mitochondrial genomes in parrots. *Mol Phylogenet Evol.* 94:34–46.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32(5): 1792–1797.

Fauron C-M, Abbott AG, Brettell R, Gesteland R. 1987. Maize mitochondrial DNA rearrangements between the normal type, the Texas male sterile cytoplasm and a fertile revertant cms-T regenerated plant. *Curr Genet.* 11(5): 339–346.

Fauron C-M, Havlik M. 1989. The maize mitochondrial genomes of the normal type and the cytoplasmic male sterile type T have very different organization. *Curr Genet*. 15(2): 149–154.

Ferrando B, A.L.D.MF, Gredilla R, Havelund JF, Hebelstrup KH,Møller IM, Stevnsner T. 2019. DNA repair in plant mitochondria—a complete base excision repair pathway in potato tuber mitochondria. *Physiol Plant.* 166(2): 494–512.

Figueroa DF, Baco AR. 2015. Octocoral mitochondrial genomes provide insights into the phylogenetic history of gene order rearrangements, order reversals, and cnidarian phylogenetics. *Genome Biol Evol.* 7(1): 391–409.

Guindon S, Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst Biol.* 52(5): 696–704.

Guisinger MM, Kuehl JV, Boore JL, Jansen RK. 2011. Extreme reconfiguration of plastid genomes in the angiosperm family Geraniacaeae: rearrangements, repeats, and codon usage. *Mol Biol Evol.* 28(1): 583–600.

Guo W, Grewe F, Cobo-Clark A, Fan W, Duan Z, Adams RP, Schwarzbach AE, Mower JP. 2014. Predominant and substoichiometric isomers of the plastid genome coexist within *Juniperus* plants and have shifted multiple times during cupressophyte evolution. *Genome Biol Evol.* 6(3): 580–590.

Guo W, Grewe F, Fan W, Young GJ, Knoop V, Palmer JD, Mower JP. 2016. *Ginkgo* and *Welwitschia* mitogenomes reveal extreme contrasts in gymnosperm mitochondrial evolution. *Mol Biol Evol.* 33(6): 1448–1460.

Haberle RC, Fourcade HM, Boore JL, Jansen RK. 2008. Extensive rearrangements in the chloroplast genome of *Trachelium caeruleum* are associated with repeats and tRNA genes. *J Mol Evol.* 66(4): 350–361.

Hanson MR. 1991. Plant mitochondrial mutations and male sterility. *Annu Rev Genet.* 25(1): 461–486.

Hillis DM, Huelsenbeck JP. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Heredity* 83(3): 189–195.

Hu F, Zhou J, Zhou L, Tang J. 2014. Probabilistic reconstruction of ancestral genomes with gene insertions and deletions. *IEEE/ACM Transactions on Computational Biology and Bioinformatics.* 11(4): 667–672.

Jansen RK, Cai Z, Raubeson LA, Daniell H, Leebens-Mack J, Muller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, et al. 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. *Proc Natl Acad Sci U S A.* 104(49): 19369–19374.

Juszczuk IM, Flexas J, Szal B, Da˛browska Z, Ribas-Carbo M, Rychter AM. 2007. Effect of mitochondrial genome rearrangement on respiratory activity, photosynthesis, photorespiration, and energy status of MSC16 cucumber (Juszczuk) mutant. *Physiol Plant.* 131(4): 527–541.

Kato S, Kanazawa A, Mikami T, Shimamoto Y. 1998. Evolutionary changes in the structures of the *cox2* and *atp6* loci in the mitochondrial genome of soybean involving recombination across small interspersed sequences. *Curr Genet*. 34(4): 303–312.

Knox EB. 2014. The dynamic history of plastid genomes in the Campanulaceae *sensu lato* is unique among angiosperms. *Proc Natl Acad Sci U S A.* 111(3030): 11097–11102.

Kubo T, Nishizawa S, Mikami T. 1999. Alterations in organization and transcription of the mitochondrial genome of cytoplasmic male sterile sugar beet (*Beta vulgaris* L.). *Mol Genomics Genet.* 262(2): 283–290.

Lavrov DV, Boore JL, Brown WM. 2002. Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: duplication and nonrandom loss. *Mol Biol Evol.* 19(2): 163–169.

Lavrov DV, Pett W. 2016. Animal mitochondrial DNA as we do not know it: mt-genome organization and evolution and nonbilaterian lineages. *Genome Biol Evol.* 8(9): 2896–2913.

Lehtonen J, Lanfear R. 2014. Generation time, life history and the substitution rate of neutral mutations. *Biol Lett.* 10(11): 20140801.

Li W-H, Ellsworth DL, Krushkal J, Chang BH-J, Hewett-Emmett D. 1996. Rates of nucleotide substitution in primates and rodents and the generation-time effect hypothesis. *Mol Phylogenet Evol.* 5(1): 182–187.

Liu G, Cao D, Li S, Su A, Geng J, Grover CE, Hu S, Hua J. 2013. The complete mitochondrial genome of *Gossypium hirsutum* and evolutionary analysis of higher plant mitochondrial genomes. *PLoS One* 8(8): e69476.

Makaroff CA, Palmer JD. 1988. Mitochondrial DNA rearrangements and transcriptional alterations in the male-sterile cytoplasm of Ogura radish. Mol Cell Biol. 8(4): 1474–1480.

Mao M, Austin AD, Johnson NF, Dowton M. 2014. Coexistence of minicircular and a highly rearranged mtDNA molecule suggests that recombination shapes mitochondrial genome organization. *Mol Biol Evol.* 31(3): 636–644.

Mao M, Gibson T, Dowton M. 2014. Evolutionary dynamics of the mitochondrial genome in the Evaniomorpha (Hymenoptera)—a group with an intermediate rate of gene rearrangement. *Genome Biol Evol.* 6(7): 1862–1874.

Maréchal A, Brisson N. 2010. Recombination and the maintenance of plant organelle genome stability. *New Phytol.* 186(2): 299–317.

Moritz C, Dowling TE, Brown WM. 1987. Evolution of animal mitochondrial DNA, relevance for population biology and systematics. *Annu Rev Ecol Syst.* 18(1): 269–292.

Mower JP, Sloan DB, Alverson AJ. 2012. Plant mitochondrial genome diversity: the genomics revolution. In: Wendel J, Greilhuber J, Dolezel J, Leitch I, editors. *Plant Genome Diversity.* Volume 1: Plant Genomes, Their Residents, and Their Evolutionary Dynamics. Dordrecht (The Netherlands): Springer. p. 123–144.

Mower JP, Touzet P, Gummow JS, Delph LF, Palmer JD. 2007. Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. *BMC Biol.* 7(1): 135.

Mower JP, Vickrey TL. 2018. Structural diversity among plastid genomes of land plants. In: Chaw S-M, Jansen RK, editors. *Advances in Botanical Research*. Vol. 85. Amsterdam, Netherlands: Elsevier. p. 263–292.

Mueller RL, Boore, J. 2005. Molecular mechanisms of extensive mitochondrial gene rearrangement in plethodontid salamanders. *Mol Biol. Evol.* 22(10): 2104–2112.

Muñoz A, Sankoff D. 2010. Rearrangement phylogeny of genomes in contig form. *Trans Comput Biol Bioinform.* 7(4): 579–587.

Palazzesi L, Gottschling M, Barreda V, Weigend M. 2012. First Miocene fossils of Vivianiaceae shed new light on phylogeny, divergence times, and historical biogeography of Geraniales. *Biol J Linn Soc.* 107(1): 67–85.

Palmer JD, Herbon LA. 1988. Plantmitochondrial DNA evolves rapidly in structure, but slowly in sequence. *J Mol Evol.* 28(1–2): 87–97.

Palmer JD, Nugent JM, Herbon LA. 1987. Unusual structure of geranium chloroplast DNA: a triple-sized inverted repeat, extensive gene duplications, multiple inversions, and two repeat families. *Proc Natl Acad Sci U S A.* 84(3): 769–773.

Park S, Grewe F, Zhu A, Ruhlman TA, Sabir J,Mower JP, Jansen RK. 2015. Dynamic evolution of *Geranium* mitochondrial genomes through multiple horizontal and intracellular gene transfers. *New Phytol.* 208(2): 570–583.

Park S, Ruhlman TA, Weng M-L, Hajrah NH, Sabir J, Jansen RK. 2017. Contrasting patterns of nucleotides substitution rates provide insight into dynamic evolution of plastid and mitochondrial genomes of *Geranium*. *Genome Biol Evol.* 9(6): 1766–1780.

Parkinson CL, Mower JP, Qiu YL, Shirk YL, Song K, Young ND, dePamphilis CW, Palmer JD. 2005. Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. *BMC Evol Biol.* 5(1): 73.

Pond SLK, Frost SDW, Muse SV. 2005. HyPhy: hypothesis testing using phylogenies. *Bioinformatics* 21(5): 676–679.

Pond SLK, Muse SV. 2005. Site-to-site variation of synonymous substitution rates. *Mol Biol Evol.* 22(12): 2375–2385.

Rautenberg A, Sloan DB, Alden V, Oxelman B. 2012. Phylogenetic relationships of *Silene multinervia* and *Silene* section Conoimorpha (Caryophyllaceae). *Syst Bot.* 37(1): 226–237.

Rice DW, Alverson AJ, Richardson AO, Young GJ, Sanchez-Puerta MV, Munzinger J, Barry K, Boore JL, Zhang Y, dePamphilis CW, et al. 2013. Horizontal transfer of entire genomes via mitochondrial fusion in the angiosperm *Amborella. Science* 342(6165): 1468–1473.

Richardson AO, Rice DW, Young GJ, Alverson AJ, Palmer JD. 2013. The “fossilized” mitochondrial genome of *Liriodendron tulipifera*: ancestral gene content and order, ancestral editing sites, and extraordinarily low mutation rate. *BMC Biol.* 11(1): 29.

Rokas A, Holland PWH. 2000. Rare genomic changes as a tool for phylogenetics. *Trends Ecol Evol.* 15(11): 454–459.

Rubinstein ND, Feldstein T, Shenkar N, Botero-Castro F, Griggio F, Mastrototaro F, Delsuc F, Douzery EJP, Gissi C, Huchon D. 2013. Deep sequencing of mixed total DNA without barcodes allows efficient assembly of highly plastic ascidian mitochondrial genomes. *Genome Biol Evol.* 5(6): 1185–1190.

Saccone C, Gissi C, Reyes A, Larizza A, Sbisa E, Pesole G. 2002. Mitochondrial DNA in metazoan: degree of freedom in a frozen event. *Gene* 286(1): 3–12.

Satoh M, Kubo T, Nishizawa S, Estiati A, Itchoda N,Mikami T. 2004. The cytoplasmic-sterile type and normal type mitochondrial genomes of sugar beet share the same complement of genes of known function but differ in the content of expressed ORFs. *Mol Genet Genomics.* 272(3): 247–256.

Schnable PS, Wise RP. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends Plant Sci.* 3(5): 175–180.

Shao R, Dowton M, Murrell A, Barker SC. 2003. Rates of gene rearrangement and nucleotide substitution are correlated in the mitochondrial genomes of insects. *Mol Biol Evol.* 20(10): 1612–1619.

Shedge V, Arrieta-Montiel MP, Christensen AC, Mackenzie SA. 2007. Plant mitochondrial recombination surveillance requires unusual *RecA* and *MutS* homologs. *Plant Cell* 19(4): 1251–1264.

Shedge V, Davila J, Arrieta-Montiel MP, Mohammed S, Mackenzie SA. 2010. Extensive rearrangement of the Arabidopsis mitochondrial genome elicits cellular conditions for thermotolerance. *Plant Physiol*. 152(4): 1960–1970.

Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst Biol.* 51(3): 492–508.

Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17(12): 1246–1247.

Siculella L, Palmer JD. 1988. Physical and gene organization of mitochondrial DNA in fertile and male sterile sunflower. CMS-associated alterations in structure and transcription of the *atpA* gene. *Nucleic Acids Res.* 16(9): 3787–3799.

Skippington E, Barkman TJ, Rice DW, Palmer JD. 2015. Miniaturized mitogenome of the parasitic plant *Viscum scurruloideum* is extremely divergent and dynamic and has lost all nad genes. *Proc Natl Acad Sci U S A.* 112(27): E3515–E3524.

Skippington E, Barkman TJ, Rice DW, Palmer JD. 2017. Comparative mitogenomics indicates respiratory competence in parasitic *Viscum* despite loss of complex I and extreme sequence divergence, and reveals horizontal gene transfer and remarkable variation in genome size. *BMC Plant Biol.* 17(1): 49.

Sloan DB, Alverson AJ, Chuckalovcak JP,WuM,McCauley DE, Palmer JD, Taylor DR. 2012. Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. *PLoS Biol.* 10(1): e1001241. e1991241.

Sloan DB, Müller K, McCauley DE, Taylor DR, Štorchová H. 2012. Intraspecific variation in mitochondrial genome sequence, structure, and gene content in *Silene vulgaris*, an angiosperm with pervasive cytoplasmic male sterility. *New Phytol*. 196(4): 1228–1239.

Sloan DB, Oxelman B, Rautenberg A, Taylor DR. 2009. Phylogenetic analysis of mitochondrial substitution rate variation in the angiosperm tribe Sileneae. *BMC Evol Biol.* 9(1): 260.

Szal B, Jastrzębska A, Kulka M, Leśniak K, Podgórska A, Pärnik T, Ivanova H, Keerberg O, Gardeström P, Rychter AM. 2010. Influence of mitochondrial genome rearrangement on cucumber leaf carbon and nitrogen metabolism. *Planta* 232(6): 1371–1382.

Takezaki N, Rzhetsky A, Nei M. 1995. Phylogenetic test of the molecular clock and linearized trees. *Mol Biol Evol.* 12(5): 823–825.

Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S. 2012. Estimating divergence times in large molecular phylogenies. *Proc Natl Acad Sci U S A*. 109(47): 19333–19338.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis. Version 6.0. *Mol Biol Evol.* 30(12): 2725–2729.

Tanaka T, Tsuda Y, Yasumoto K, Yamagishi H, Terachi T. 2012. A complete mitochondrial genome sequence of Ogura-type male-sterile cytoplasm and its comparative analysis with that of normal cytoplasm in radish (*Raphanus sativus* L.). *BMC Genomics* 13(1): 352.

Tang M, Chen Z, Grover CE,Wang Y, Li S, Liu G,Ma Z, Wendel JF, Hua J. 2015. Rapid evolutionary divergence of *Gossypium barbadense* and *G. hirsutum* mitochondrial genomes. *BMC Genomics* 16(1): 770.

Taylor ZN, Rice DW, Palmer JD. 2015.The complete moss mitochondrial genome in the angiosperm *Amborella* is a chimera derived from two moss whole-genome transfers. *PLoS One* 10(11): e0137532.

Tesler G. 2002. GRIMM: genome rearrangements web server. *Bioinformatics* 18(3): 492–493.

Trasviña-Arenas CH, Baruch-Torres N, Cordoba-Andrade FJ, Ayala-Garcia VM, Garcia-Medel PL, Diaz-Quezada C, Peralta-Castro A, Ordaz-Ortiz JJ, Brieba LG. 2018. Identification of a unique insertion in plant organellar DNA polymerases responsible for 50-dRP lyase and strand-displacement activities: implications for base excision repair. *DNA Repair* 65:1–10.

Van de Paer C, Bouchez O, Besnard G. 2018. Prospects on the evolutionary mitogenomics of plants: a case study on the olive family (Oleaceae). *Mol Ecol Resour.* 18(3): 407–423.

Wang D, Li S, Guo F, Ning K, Wang L. 2017. Core-genome scaffold comparison reveals that inversion events are associated with pairs of inverted repeats. *BMC Genomics* 18(1): 268.

Ward B, Anderson R, Bendich A. 1981. The size of the mitochondrial genomes is large and variable in a family of plants (Cucurbitaceae). *Cell* 25(3): 793–803.

Weng M-L, Blazier JC, Govindu M, Jansen RK. 2014. Reconstruction of the ancestral plastid genome in Geraniaceae reveals a correlation between genome rearrangements, repeats, and nucleotide substitution rates. *Mol Biol Evol.* 31(3): 645–659.

Wicke S, Schneeweiss GM, dePamphilis CW, Muller KF, Quandt D. 2011. The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. *Plant Mol Biol.* 76(3–5): 273–297.

Woloszynska M. 2010. Heteroplasmy and stoichiometric complexity of plant mitochondrial genomes—though this be madness, yet there's method in't. *J Exp Bot.* 61(3): 657–671.

Xu W, Jameson D, Tang B, Higgs PG. 2006. The relationship between the rate of molecular evolution and the rate of genome rearrangement in animal mitochondrial genomes. *J Mol Evol.* 63(3): 375–392.

Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18(5): 821–829.

Zheng C, Sankoff D. 2016. Locating rearrangement events in a phylogeny based on highly fragmented assemblies. *BMC Genomics* 17(1 Suppl): 1.

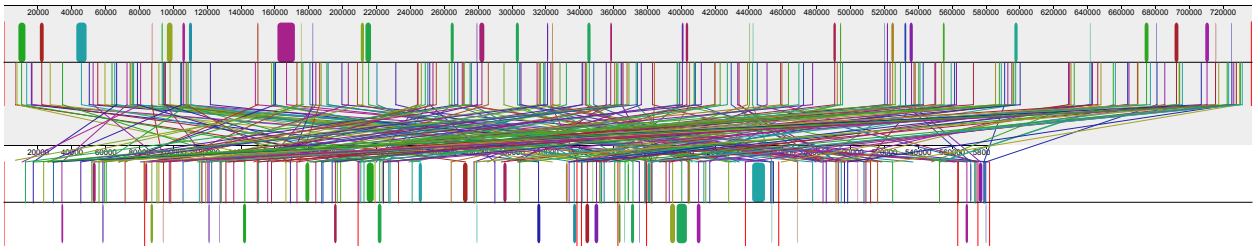


Figure S1. Evidence of very little shared mitochondrial sequence or synteny between *Geranium* and *Monsonia* as shown through a pairwise alignment of the *Geranium maderense* mitochondrial genome (top) (Park et al. 2015) and the *Monsonia emarginata* mitochondrial genome (bottom). Red lines represent “boundaries” between scaffolds, whose order was determined by Mauve.

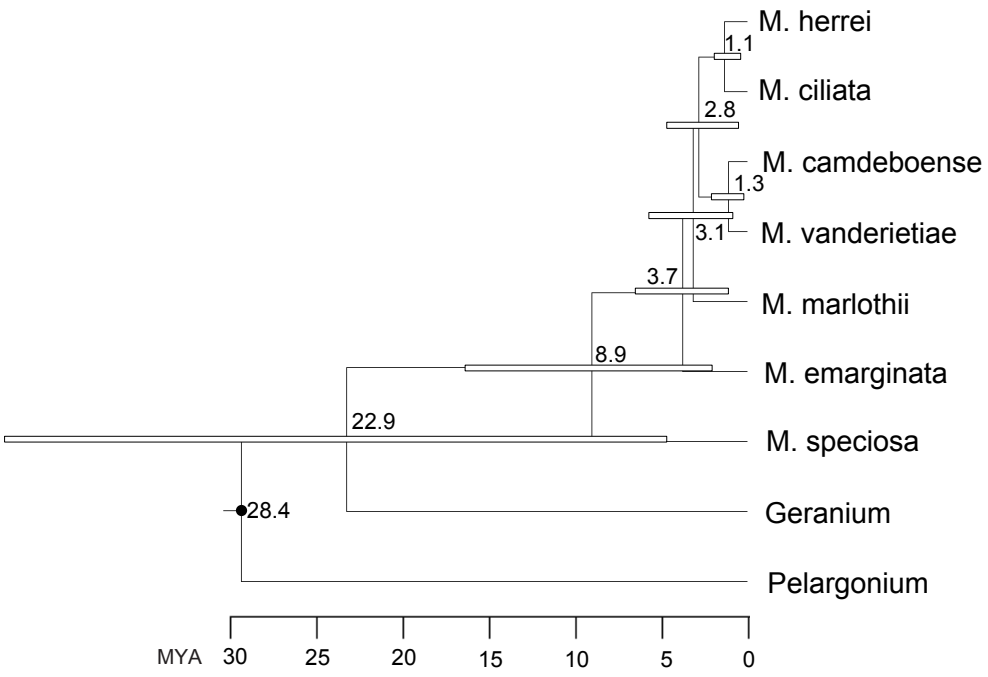


Figure S2. Fossil-calibrated *Monsonia* tree with estimates of divergence time in millions of years. This chronogram is based on plastid gene sequences. Open bars represent 95% confidence intervals. The black circle indicates that this value is the fossil calibration point used in this analysis.

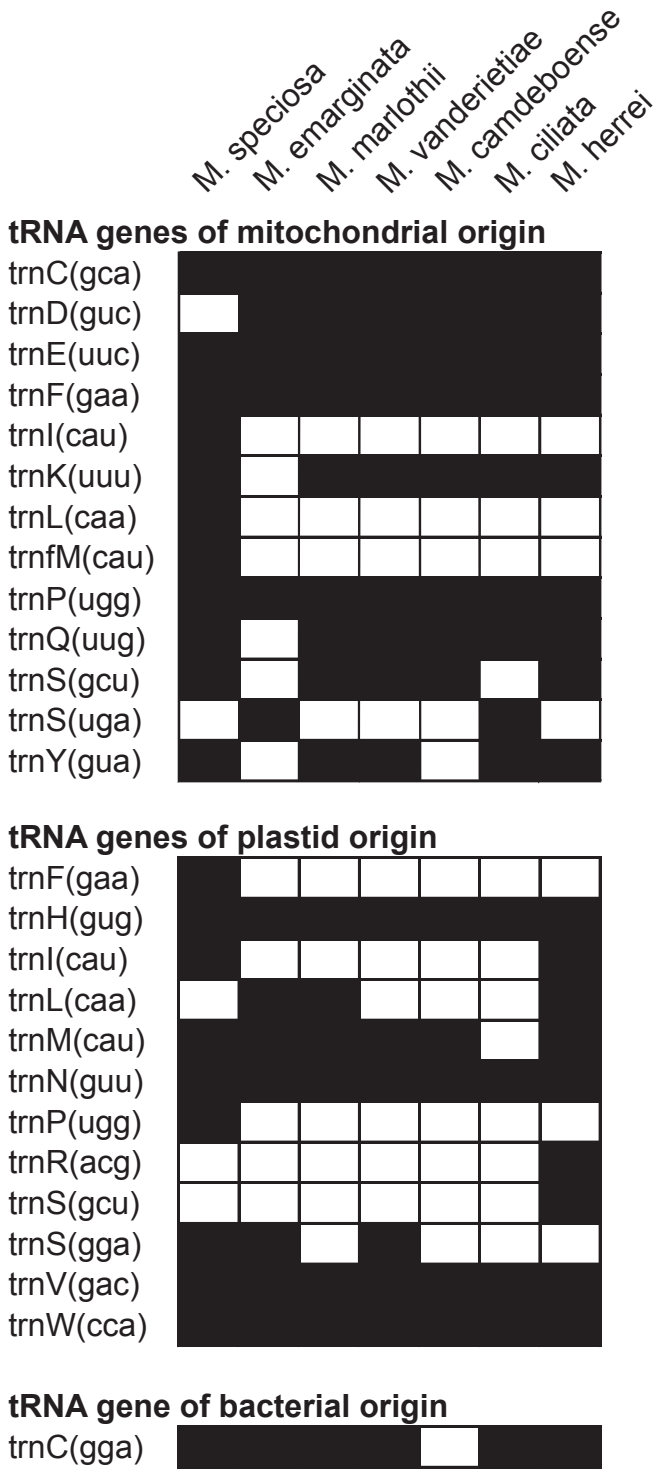
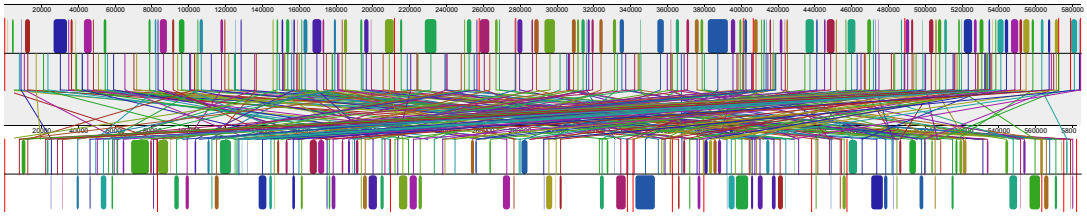
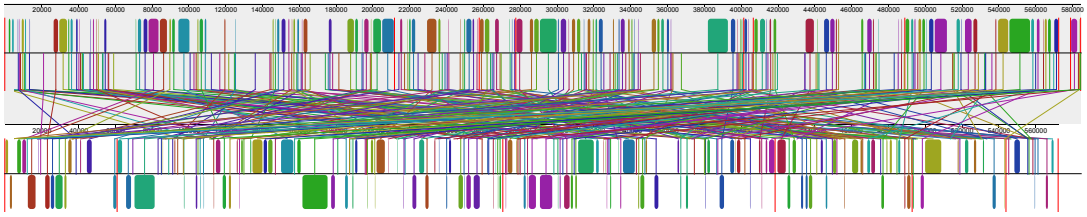


Figure S3. Variable tRNA-gene content in *Monsonia* mitochondrial assemblies. Black indicates gene presence, and white indicates gene absence.

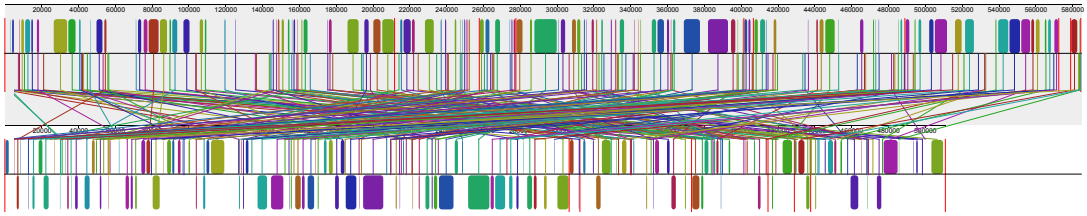
M. speciosa and M. emarginata



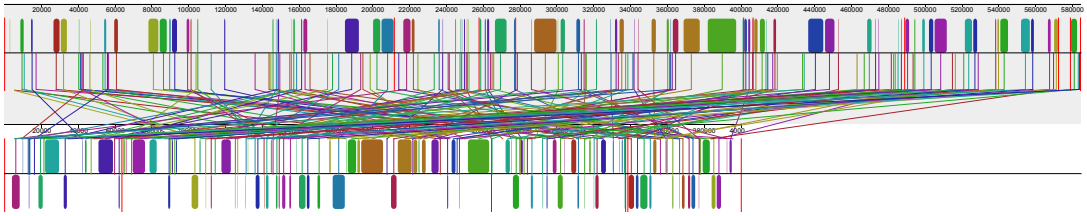
M. speciosa and M. marlothii



M. speciosa and M. vanderietiae



M. speciosa and M. camdeboense



M. speciosa and M. ciliata

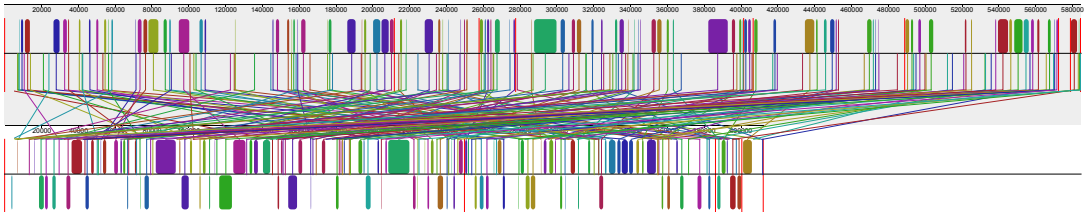
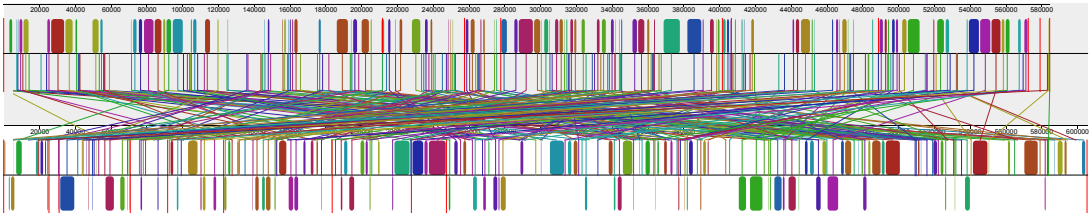
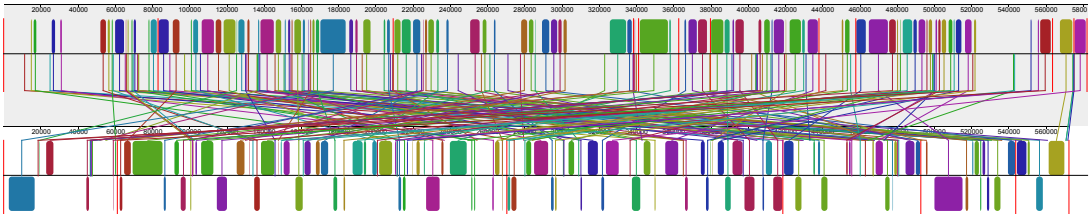


Figure S4. All 21 pairwise alignments of *Monsonia* mitochondrial genomes. These alignments were used for the HRB analyses of fig. 5 and table 1. Three of the alignments are also shown in fig. 4. Red lines represent "boundaries" between scaffolds, whose order was determined by Mauve.

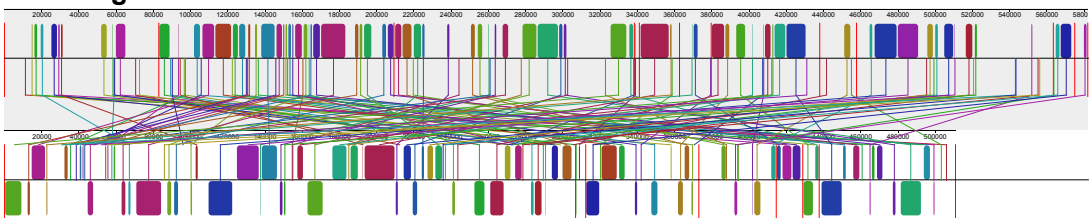
M. speciosa and M. herrei



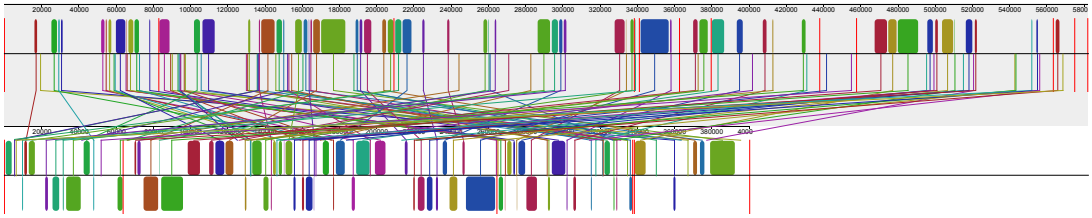
M. emarginata and M. marlothii



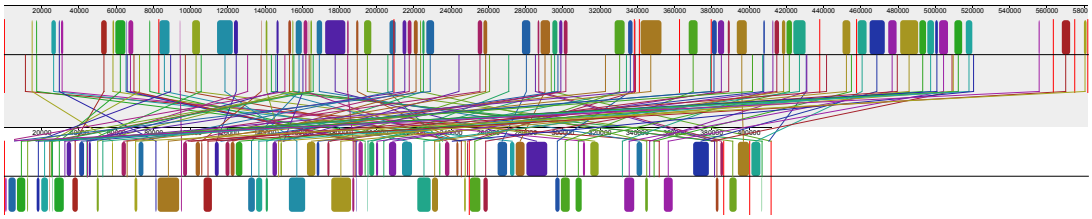
M. emarginata and M. vanderietiae



M. emarginata and M. camdeboense



M. emarginata and M. ciliata



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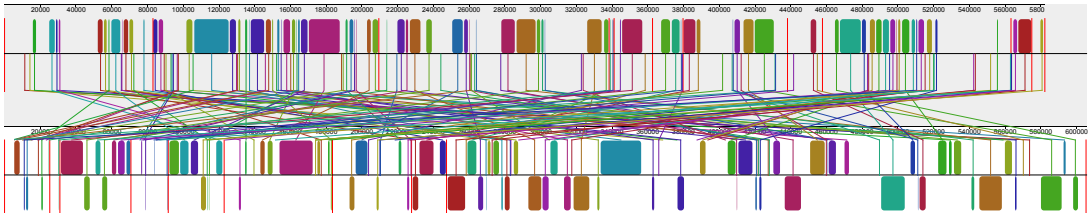
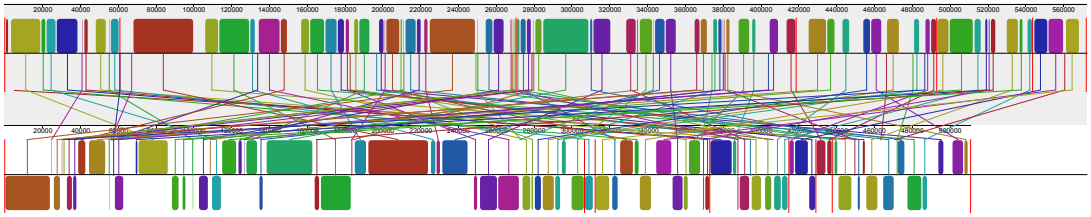
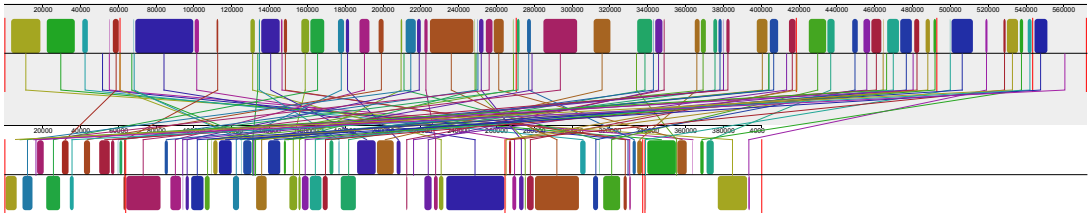
M. emarginata and M. herrei



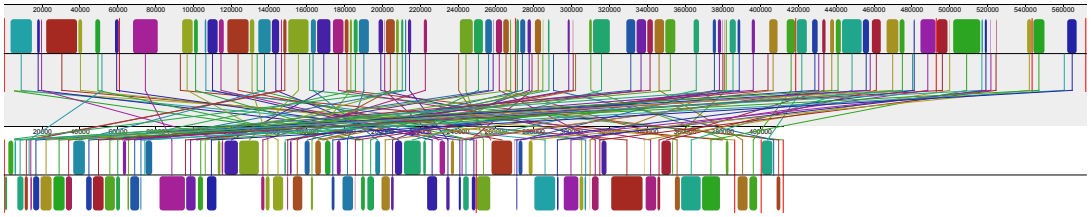
M. marlothii and M. vanderietiae



M. marlothii and M. camdeboense



M. marlothii and M. ciliata



M. marlothii and M. herrei

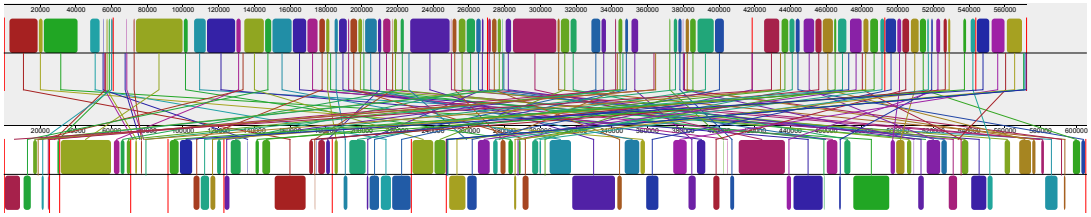
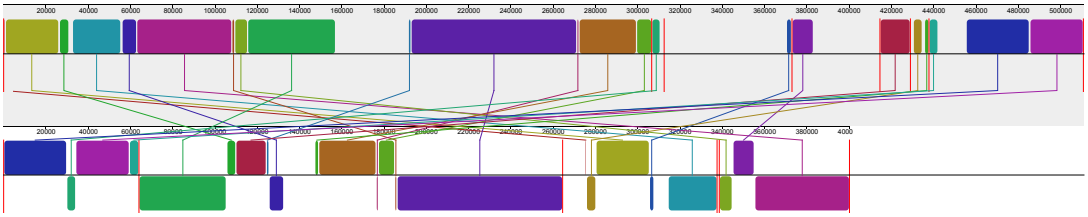
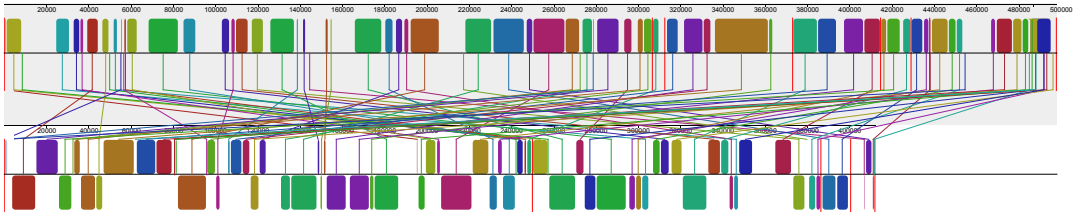


Figure S4. All 21 pairwise alignments of *Monsonia* mitochondrial genomes. These alignments were used for the HRB analyses of fig. 5 and table 1. Three of the alignments are also shown in fig. 4. Red lines represent "boundaries" between scaffolds, whose order was determined by Mauve.

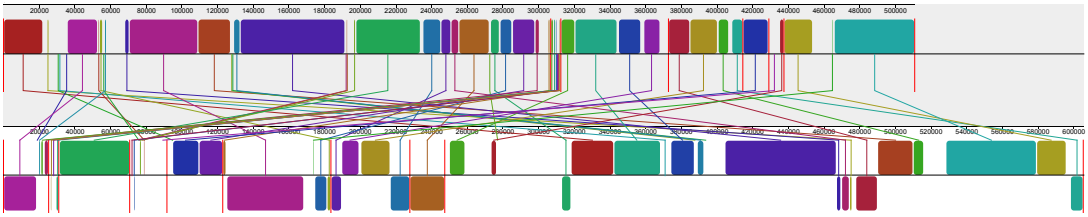
M. vanderietiae and M. camdeboense



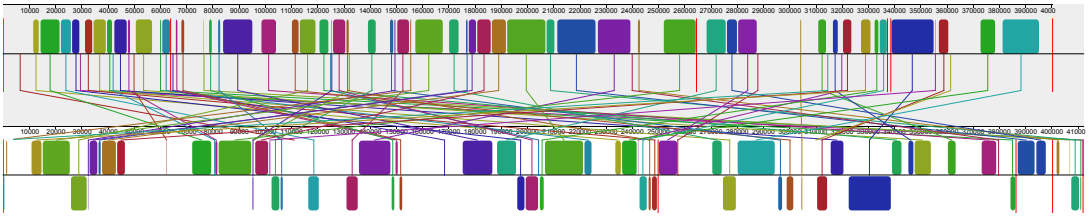
M. vanderietiae and M. ciliata



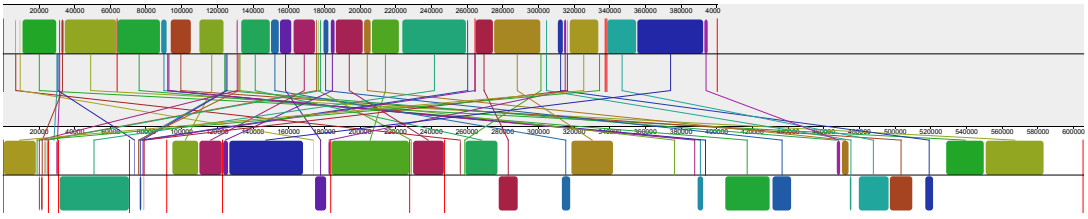
M. vanderietiae and M. herrei



M. camdeboense and M. ciliata



M. camdeboense and M. herrei



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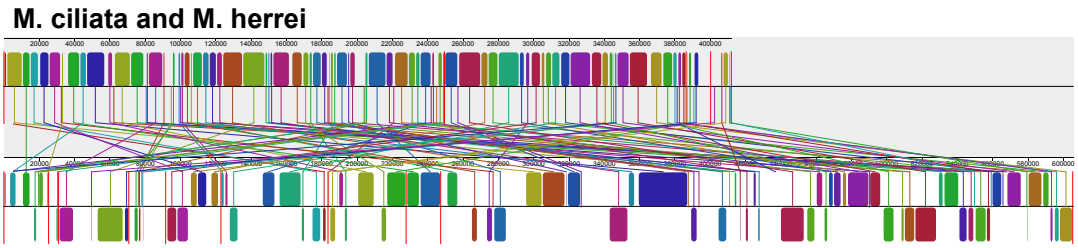


Figure S4. All 21 pairwise alignments of *Monsonia* mitochondrial genomes. These alignments were used for the HRB analyses of fig. 5 and table 1. Three of the alignments are also shown in fig. 4. Red lines represent "boundaries" between scaffolds, whose order was determined by Mauve.

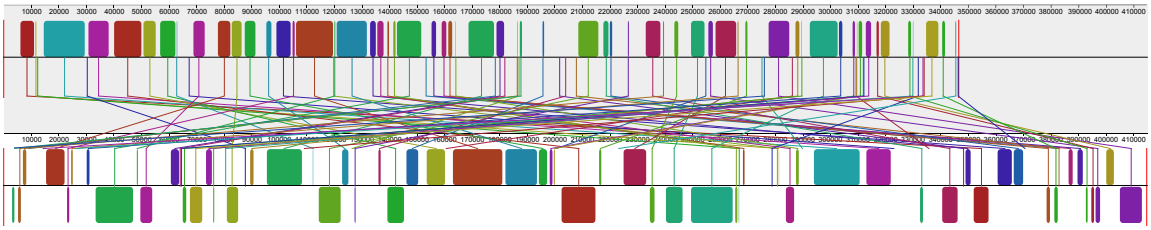


Figure S5. Relatively little rearrangement between the mitochondrial genomes of *Ginkgo biloba* (top; Guo et al. 2016) and *Cycas taitungensis* (bottom; Chaw et al. 2008). Despite having diverged roughly 343 million years ago (Guo et al. 2016), these two genomes are separated by a rearrangement distance of only 77, as inferred by GRIMM.

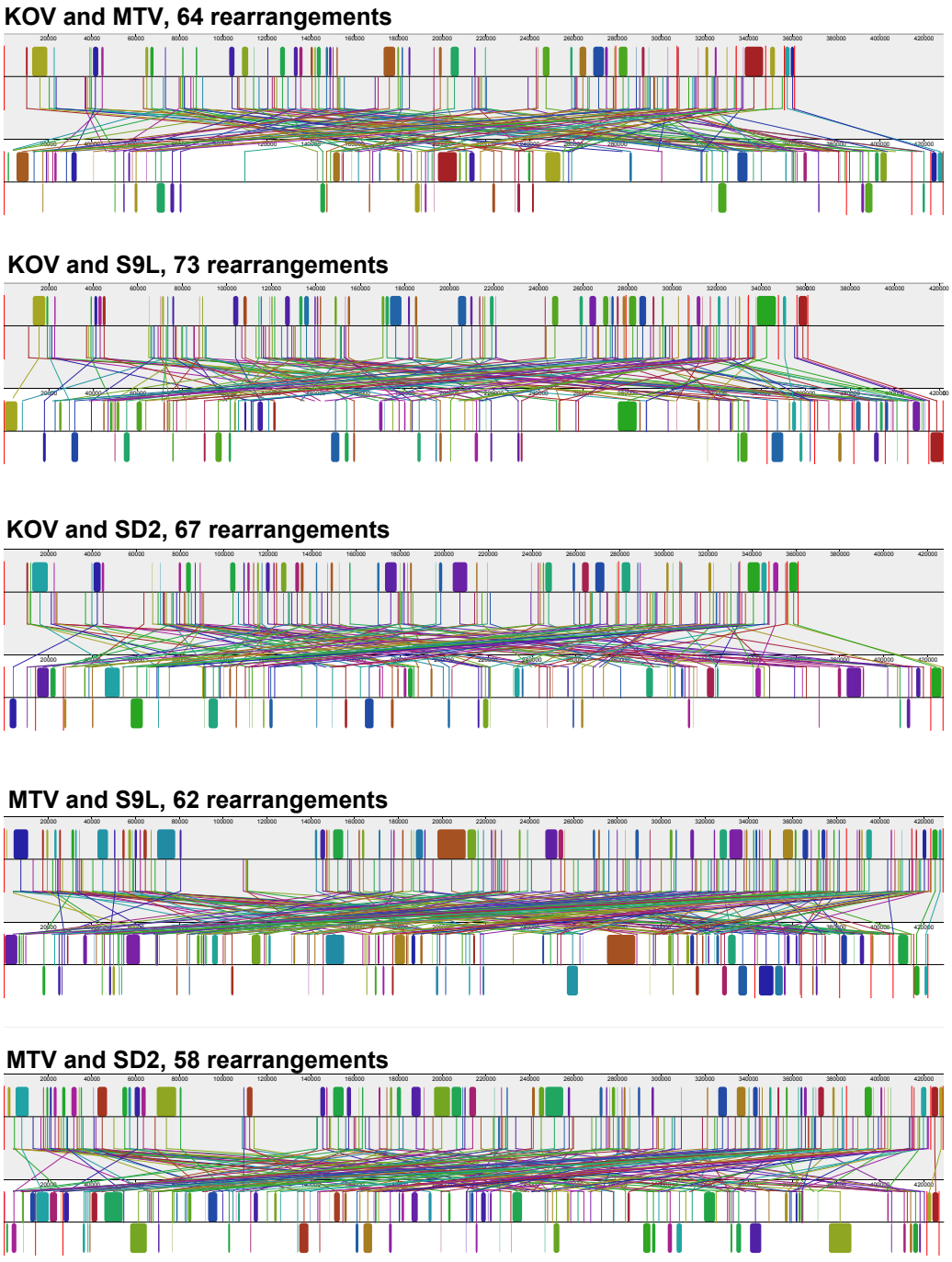


Figure S6. Extensive rearrangement among the mitochondrial genomes of four populations of *Silene vulgaris* (genomes from Sloan et al. 2012b). The four genomes are from populations collected near Kovary Meadows, Czech Republic (KOV); Mountain View, VA, USA (MTV); Simmonsville, VA, USA (S9L); and Stuarts Draft, VA, USA (SD2). Pairwise rearrangement distances were calculated using GRIMM.

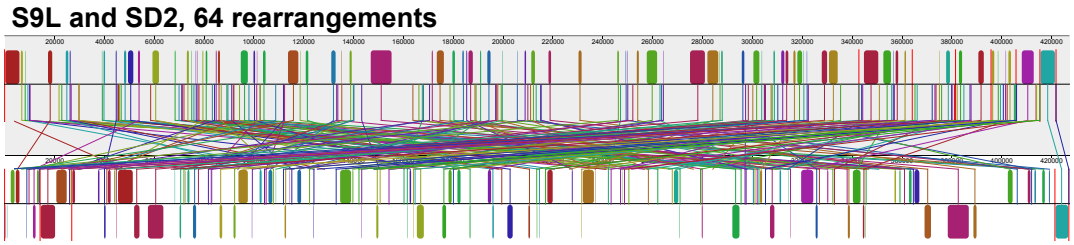


Figure S6. Extensive rearrangement among the mitochondrial genomes of four populations of *Silene vulgaris* (genomes from Sloan et al. 2012b). The four genomes are from populations collected near Kovary Meadows, Czech Republic (KOV); Mountain View, VA, USA (MTV); Simmonsville, VA, USA (S9L); and Stuarts Draft, VA, USA (SD2). Pairwise rearrangement distances were calculated using GRIMM.

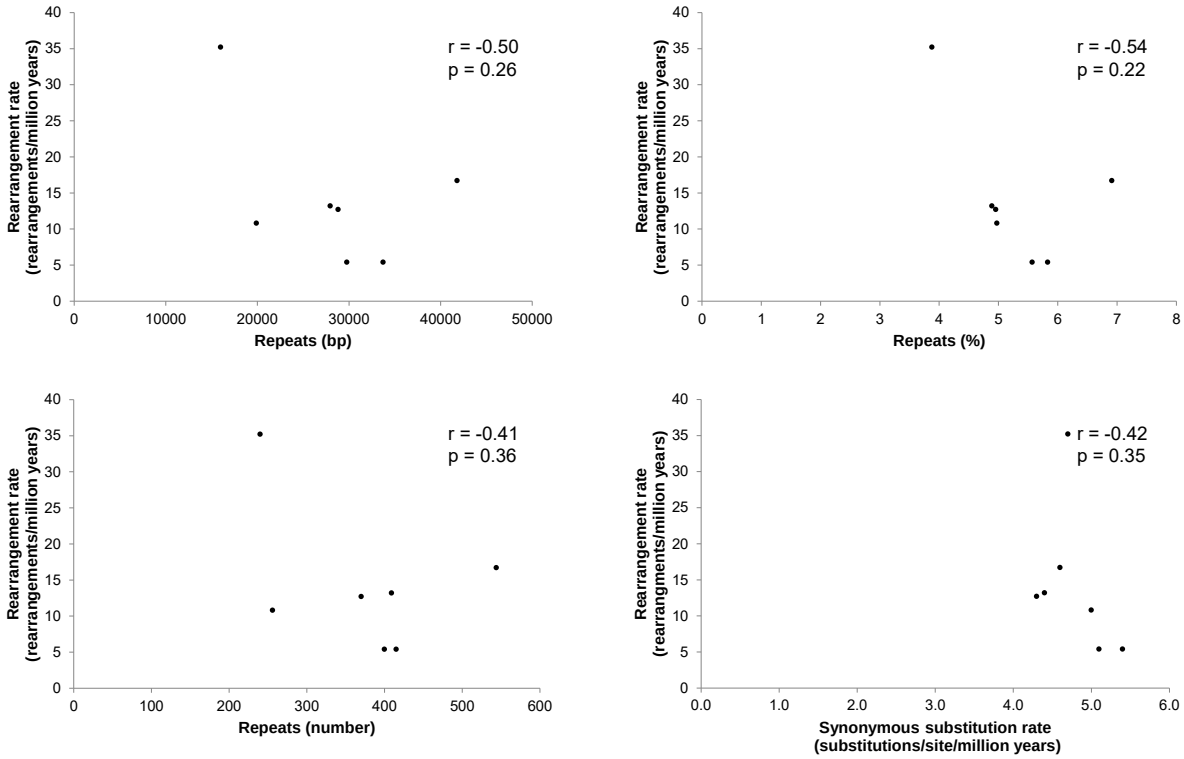


Figure S7. Scatterplots of terminal branch rearrangement rate vs. repeats (in terms of bp, %, and number of elements) and synonymous substitution rates.

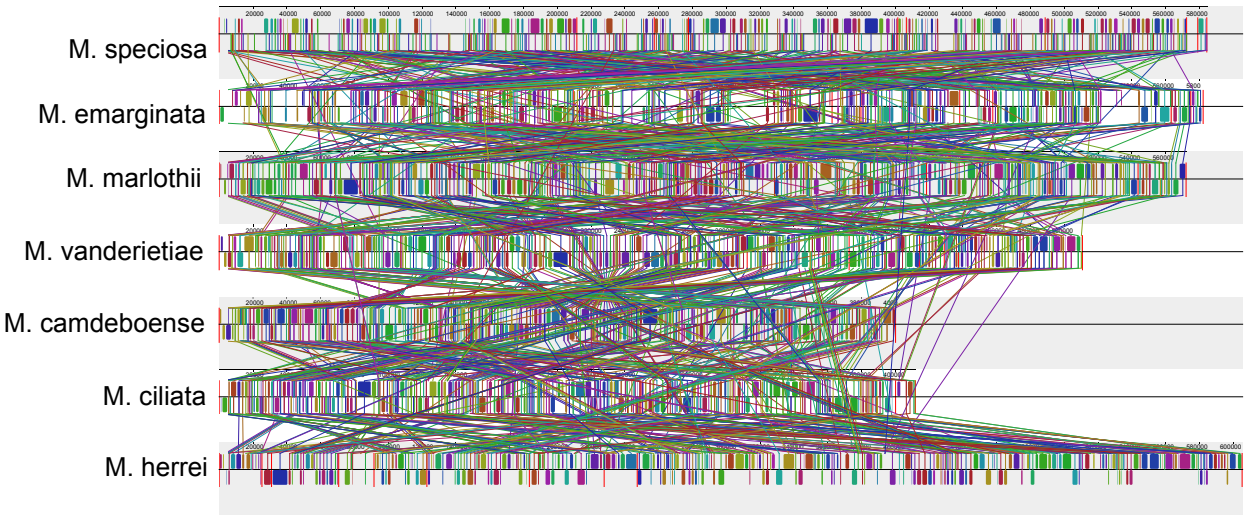
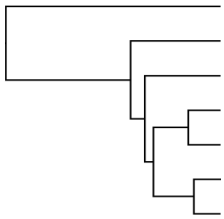


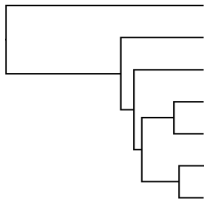
Figure S8. Seven-way alignment of *Monsonia* mitochondrial genomes constructed with progressiveMauve. This alignment was used to estimate rearrangement rates (results shown in fig. 2, fig.3, and table 1) and to infer a rearrangement-based tree (fig. S1C).

Table S1. Features of draft assemblies for the seven *Monsonia* mitochondrial genomes and the control genome of *Geranium maderense*, for which a complete assembly made independently is available (Park et al. 2015).



Species	Genome size (bp)	Scaffold number	Scaffold sizes (bp)		
			Largest	Smallest	Median
M. speciosa	605,487	9	211,778	5,130	45,824
M. emarginata	581,703	11	128,834	2,881	21,315
M. marlothii	571,776	6	209,355	28,334	55,916
M. vanderietiae	510,607	7	306,738	5,631	60,476
M. camdeboense	400,130	5	200,534	1,028	73,826
M. ciliata	412,246	4	250,108	11,589	75,275
M. herrei	604,880	9	359,098	5,689	28,212
G. maderense	699,001	9	436,802	1,777	21,431

Table S2. Content of native genes, introns, GC base pairs, and plastid-derived DNA in *Monsonia* mitochondrial genomes.



Species	Genome size (bp)	GC content (%)	# genes			# introns		Plastid-derived DNA (bp)
			protein	rRNA	tRNA	cis	trans	
M. speciosa	605,487	42.6	27	3	21	5	4	44,945
M. emarginata	581,703	42.2	27	3	14	5	4	28,795
M. marlothii	571,776	42.5	27	3	16	5	4	48,670
M. vanderietiae	510,607	42.8	27	3	16	5	4	23,748
M. camdeboense	400,130	42.9	27	3	13	5	4	17,441
M. ciliata	412,246	42.7	27	3	14	5	4	16,447
M. herrei	604,880	42.7	27	3	19	5	4	49,199

Table S3. Numbers of inversions (I), transpositions (T), and fissions plus fusions (F) inferred by GRIMM from the pairwise rearrangement alignments shown in fig. S4.

	M. speciosa			M. emarginata			M. marlothii			M. vanderietiae			M. camdeboense			M. ciliata		
	I	T	F	I	T	F	I	T	F	I	T	F	I	T	F	I	T	F
M. speciosa																		
M. emarginata	50	35	1															
M. marlothii	56	31	2	52	10	3												
M. vanderietiae	56	21	2	35	18	3	39	3	0									
M. camdeboense	72	5	4	44	10	5	38	3	2	9	4	1						
M. ciliata	78	7	4	47	12	5	44	8	2	29	7	2	35	6	0			
M. herrei	50	33	1	34	26	0	23	18	3	14	7	3	14	4	5	26	10	5

Table S4. Number of permutations out of 10,000 from the HRB/repeat proximity test (top) and the resulting p-values (bottom) (cf. fig 5).

	M. speciosa	M. emarginata	M. marlothii	M. vanderietiae	M. camdeboense	M. ciliata	M. herrei
M. speciosa		10,000	9,910	9,990	10,000	10,000	10,000
M. emarginata	9,821		10,000	10,000	10,000	10,000	10,000
M. marlothii	9,931	10,000		9,823	9,491	10,000	9,751
M. vanderietiae	9,929	10,000	9,876		9,401	10,000	9,927
M. camdeboense	10,000	10,000	9,562	9,454		10,000	9,881
M. ciliata	10,000	10,000	10,000	9,998	10,000		10,000
M. herrei	9,845	9,941	9,837	9,541	9,845	10,000	
M. speciosa		< 0.0001	0.0090	0.0010	< 0.0001	< 0.0001	< 0.0001
M. emarginata	0.0179		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
M. marlothii	0.0069	< 0.0001		0.0177	0.0509	< 0.0001	0.0249
M. vanderietiae	0.0071	< 0.0001	0.0124		0.0599	< 0.0001	0.0073
M. camdeboense	< 0.0001	< 0.0001	0.0438	0.0546		< 0.0001	0.0119
M. ciliata	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001		< 0.0001
M. herrei	0.0155	0.0059	0.0163	0.0459	0.0155	< 0.0001	

Table S5: Repeats ≥30 bp in size in the seven *Monsonia* mitochondrial genome assemblies.

M. speciosa

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
454	69	84.8	6	14178	14631	2.00E-96	353.0
454	69	84.8	4	69116	69569	3.00E-96	353.0
262	23	90.8	8	1	261	6.00E-88	321.0
262	23	90.8	8	2983	3244	6.00E-88	321.0
255	8	96.5	8	1	254	1.00E-119	426.0
255	8	96.5	8	3291	3545	1.00E-119	426.0
209	23	88.0	8	2305	2512	2.00E-57	220.0
209	23	88.0	8	6014	6221	2.00E-57	220.0
201	36	82.1	1	143499	143699	9.00E-24	113.0
201	36	82.1	1	149069	149269	9.00E-24	113.0
191	35	81.7	4	3381	3571	2.00E-20	101.0
191	35	81.7	1	160784	160974	3.00E-20	101.0
178	2	96.1	9	3874	4051	7.00E-81	297.0
178	2	96.1	3	2969	3141	3.00E-80	297.0
178	9	94.9	5	4732	4909	4.00E-76	281.0
178	9	94.9	7	50049	50226	6.00E-75	281.0
174	5	93.7	7	68147	68314	2.00E-68	260.0
174	5	93.7	4	56045	56218	3.00E-68	260.0
174	15	91.4	4	56081	56254	5.00E-58	226.0
174	15	91.4	1	146666	146839	8.00E-58	226.0
164	3	96.3	4	15286	15449	2.00E-72	274.0
164	3	96.3	1	105022	105182	4.00E-72	274.0
156	4	96.2	4	64949	65103	5.00E-64	246.0
156	4	96.2	1	111824	111978	9.00E-64	246.0
156	23	85.3	8	106	261	2.00E-29	127.0
156	23	85.3	8	2883	3038	2.00E-29	127.0
155	11	91.0	6	4787	4939	4.00E-45	182.0
155	11	91.0	7	42465	42618	4.00E-45	182.0
152	8	94.7	4	46290	46441	1.00E-61	238.0
152	8	94.7	1	178975	179126	2.00E-61	238.0
150	2	86.7	4	33202	33341	1.00E-33	145.0
150	2	86.7	4	51997	52138	1.00E-33	145.0
145	8	94.5	8	998	1142	1.00E-58	224.0
145	8	94.5	8	2897	3041	1.00E-58	224.0
145	11	89.7	1	90638	90779	6.00E-34	147.0
145	11	89.7	1	130272	130415	6.00E-34	147.0
144	20	86.1	8	998	1141	2.00E-29	127.0
144	20	86.1	8	2923	3066	2.00E-29	127.0
143	10	93.0	7	54817	54959	1.00E-51	204.0

143	10	93.0	1	109280	109422	3.00E-51	204.0
141	16	88.7	8	999	1139	3.00E-37	153.0
141	16	88.7	8	2950	3090	3.00E-37	153.0
140	8	91.4	4	46430	46568	2.00E-41	170.0
140	8	91.4	1	178806	178942	4.00E-41	170.0
140	10	86.4	7	25335	25465	9.00E-31	135.0
140	10	86.4	4	13392	13531	1.00E-30	135.0
140	24	82.9	8	998	1137	1.00E-17	87.7
140	24	82.9	8	2877	3016	1.00E-17	87.7
139	22	84.2	8	1003	1141	1.00E-21	101.0
139	22	84.2	8	2973	3111	1.00E-21	101.0
137	21	84.7	8	108	244	6.00E-23	105.0
137	21	84.7	8	2950	3086	6.00E-23	105.0
131	9	93.1	1	77169	77299	2.00E-46	188.0
131	9	93.1	1	128629	128759	2.00E-46	188.0
127	7	94.5	2	12752	12878	2.00E-49	196.0
127	7	94.5	4	21559	21685	4.00E-49	196.0
127	10	92.1	7	50622	50748	4.00E-42	172.0
127	10	92.1	1	211694	211820	1.00E-41	172.0
120	4	96.7	8	2393	2512	2.00E-53	206.0
120	4	96.7	8	4938	5057	2.00E-53	206.0
114	9	91.2	8	1914	2026	4.00E-33	139.0
114	9	91.2	8	2523	2636	4.00E-33	139.0
113	1	99.1	2	29810	29922	2.00E-55	216.0
113	1	99.1	4	15162	15274	5.00E-55	216.0
113	3	97.4	8	1604	1716	1.00E-51	200.0
113	3	97.4	8	2098	2210	1.00E-51	200.0
112	6	94.6	8	476	587	8.00E-44	174.0
112	6	94.6	8	3165	3276	8.00E-44	174.0
111	10	91.0	8	698	808	1.00E-33	141.0
111	10	91.0	8	3718	3828	1.00E-33	141.0
110	0	100.0	4	104357	104466	1.00E-55	218.0
110	0	100.0	1	53705	53814	2.00E-55	218.0
110	9	91.8	8	700	809	2.00E-35	147.0
110	9	91.8	8	3745	3854	2.00E-35	147.0
110	12	89.1	6	1158	1267	3.00E-27	123.0
110	12	89.1	4	21889	21998	5.00E-27	123.0
110	14	87.3	8	699	808	2.00E-23	107.0
110	14	87.3	8	3690	3799	2.00E-23	107.0
109	5	95.4	8	502	610	2.00E-44	176.0
109	5	95.4	8	3172	3280	2.00E-44	176.0
108	1	99.1	8	1160	1267	2.00E-53	206.0
108	1	99.1	8	2894	3001	2.00E-53	206.0
108	17	84.3	8	154	261	4.00E-15	79.8

108	17	84.3	8	2958	3065	4.00E-15	79.8
107	1	98.1	7	42369	42475	7.00E-47	188.0
107	1	98.1	4	46268	46373	1.00E-46	188.0
102	0	100.0	4	49193	49294	7.00E-51	202.0
102	0	100.0	1	189291	189392	1.00E-50	202.0
102	3	95.1	4	57394	57493	4.00E-37	157.0
102	3	95.1	1	24208	24309	6.00E-37	157.0
101	5	95.1	4	122788	122888	2.00E-38	161.0
101	5	95.1	7	18093	18193	2.00E-38	161.0
99	0	95.0	2	28140	28238	1.00E-37	157.0
99	0	95.0	6	5111	5204	2.00E-37	157.0
99	6	93.9	8	1410	1508	5.00E-36	149.0
99	6	93.9	8	2972	3070	5.00E-36	149.0
98	0	98.0	3	3932	4029	1.00E-42	172.0
98	0	98.0	7	67750	67845	4.00E-42	172.0
98	15	84.7	8	1166	1263	6.00E-14	75.8
98	15	84.7	8	2973	3070	6.00E-14	75.8
97	5	94.9	8	728	824	3.00E-37	153.0
97	5	94.9	8	3758	3854	3.00E-37	153.0
97	14	85.6	8	1171	1267	9.00E-16	81.8
97	14	85.6	8	3295	3391	9.00E-16	81.8
95	13	86.3	8	1411	1505	6.00E-17	85.7
95	13	86.3	8	2950	3044	6.00E-17	85.7
93	3	96.8	6	69614	69706	2.00E-38	161.0
93	3	96.8	7	53383	53475	2.00E-38	161.0
93	9	90.3	8	1415	1507	3.00E-25	113.0
93	9	90.3	8	2998	3090	3.00E-25	113.0
93	15	83.9	8	1171	1263	5.00E-11	65.9
93	15	83.9	8	2923	3015	5.00E-11	65.9
92	12	87.0	8	1412	1503	1.00E-17	87.7
92	12	87.0	8	2877	2968	1.00E-17	87.7
91	10	89.0	8	1049	1139	1.00E-21	101.0
91	10	89.0	8	3301	3391	1.00E-21	101.0
91	11	87.9	8	1024	1114	2.00E-19	93.7
91	11	87.9	8	3301	3391	2.00E-19	93.7
90	10	88.9	8	1419	1508	4.00E-21	99.6
90	10	88.9	8	3348	3437	4.00E-21	99.6
90	14	84.4	8	1419	1508	1.00E-11	67.9
90	14	84.4	8	3022	3111	1.00E-11	67.9
88	4	95.5	8	470	557	3.00E-34	143.0
88	4	95.5	8	3165	3252	3.00E-34	143.0
88	5	94.3	1	110119	110206	2.00E-30	135.0
88	5	94.3	1	159534	159621	2.00E-30	135.0
88	7	92.1	2	29511	29598	3.00E-26	119.0

88	7	92.1	6	27646	27733	5.00E-26	119.0
87	12	86.2	8	106	192	1.00E-14	77.8
87	12	86.2	8	2877	2963	1.00E-14	77.8
87	13	85.1	8	1419	1505	3.00E-12	69.9
87	13	85.1	8	3301	3387	3.00E-12	69.9
86	1	98.8	7	68153	68238	4.00E-39	163.0
86	1	98.8	1	146712	146797	1.00E-38	163.0
86	12	86.1	8	1423	1508	6.00E-14	75.8
86	12	86.1	8	3047	3132	6.00E-14	75.8
85	0	100.0	2	2732	2816	4.00E-41	168.0
85	0	100.0	4	2422	2506	1.00E-40	168.0
85	6	91.8	7	42391	42475	8.00E-22	105.0
85	6	91.8	1	179043	179126	2.00E-21	105.0
84	2	97.6	5	4352	4435	1.00E-36	151.0
84	2	97.6	4	10912	10995	2.00E-35	151.0
82	0	100.0	5	1	82	3.00E-40	163.0
82	0	100.0	8	1728	1809	3.00E-40	163.0
82	0	100.0	8	2084	2165	3.00E-40	163.0
82	0	100.0	2	2045	2126	2.00E-39	163.0
82	0	100.0	2	11580	11661	2.00E-39	163.0
82	0	100.0	2	14994	15075	2.00E-39	163.0
82	0	100.0	2	45825	45906	2.00E-39	163.0
82	0	100.0	6	27260	27341	4.00E-39	163.0
82	0	100.0	6	23719	23800	4.00E-39	163.0
82	0	100.0	6	82162	82243	4.00E-39	163.0
82	0	100.0	6	1	82	4.00E-39	163.0
82	0	100.0	7	83632	83713	4.00E-39	163.0
82	0	100.0	7	43018	43099	4.00E-39	163.0
82	0	100.0	7	83178	83259	4.00E-39	163.0
82	0	100.0	4	80780	80861	6.00E-39	163.0
82	0	100.0	4	10592	10673	6.00E-39	163.0
82	0	100.0	4	1	82	6.00E-39	163.0
82	0	100.0	4	7914	7995	6.00E-39	163.0
82	0	100.0	4	65318	65399	6.00E-39	163.0
82	0	100.0	1	119854	119935	1.00E-38	163.0
82	0	100.0	1	53635	53716	1.00E-38	163.0
82	0	100.0	1	204619	204700	1.00E-38	163.0
82	1	98.8	8	536	617	7.00E-38	155.0
82	1	98.8	8	3466	3547	7.00E-38	155.0
82	4	93.9	8	1820	1900	6.00E-26	115.0
82	4	93.9	8	6031	6112	6.00E-26	115.0
82	11	86.6	8	180	261	6.00E-14	75.8
82	11	86.6	8	3309	3390	6.00E-14	75.8
81	3	96.3	2	7537	7617	1.00E-31	137.0

81	3	96.3	7	26109	26189	2.00E-31	137.0
79	1	98.7	6	23825	23903	6.00E-35	149.0
79	1	98.7	7	44257	44335	6.00E-35	149.0
79	5	93.7	4	105004	105082	3.00E-25	117.0
79	5	93.7	1	77215	77293	5.00E-25	117.0
78	1	98.7	1	16068	16145	6.00E-34	147.0
78	1	98.7	1	182494	182571	6.00E-34	147.0
78	2	97.4	4	79441	79518	9.00E-32	139.0
78	2	97.4	1	10370	10447	1.00E-31	139.0
77	2	96.1	7	42299	42375	1.00E-26	121.0
77	2	96.1	1	179130	179205	3.00E-26	121.0
76	0	100.0	2	23144	23219	8.00E-36	151.0
76	0	100.0	7	48734	48809	2.00E-35	151.0
76	1	98.7	4	105004	105079	6.00E-33	143.0
76	1	98.7	1	128635	128710	1.00E-32	143.0
76	2	97.4	8	1822	1897	7.00E-32	135.0
76	2	97.4	8	2325	2400	7.00E-32	135.0
75	1	98.7	8	506	580	1.00E-33	141.0
75	1	98.7	8	3473	3547	1.00E-33	141.0
75	1	98.7	3	4518	4592	4.00E-33	141.0
75	1	98.7	7	73803	73877	1.00E-32	141.0
75	1	98.7	4	17129	17203	2.00E-32	141.0
75	1	98.7	4	113789	113863	2.00E-32	141.0
75	2	97.3	8	476	550	3.00E-31	133.0
75	2	97.3	8	3473	3547	3.00E-31	133.0
75	4	94.7	1	2121	2195	5.00E-25	117.0
75	4	94.7	1	161830	161904	5.00E-25	117.0
75	9	88.0	8	1193	1267	1.00E-14	77.8
75	9	88.0	8	2994	3068	1.00E-14	77.8
73	4	94.5	8	1	73	3.00E-25	113.0
73	4	94.5	8	3202	3274	3.00E-25	113.0
73	7	90.4	8	990	1062	4.00E-18	89.7
73	7	90.4	8	2877	2949	4.00E-18	89.7
72	6	91.7	8	1071	1142	6.00E-20	95.6
72	6	91.7	8	2997	3068	6.00E-20	95.6
72	10	86.1	8	1409	1480	2.00E-10	63.9
72	10	86.1	8	3301	3372	2.00E-10	63.9
72	10	86.1	8	3361	3432	2.00E-10	63.9
71	1	98.6	3	2881	2951	9.00E-31	133.0
71	1	98.6	2	21386	21456	2.00E-30	133.0
71	6	91.6	8	1279	1349	2.00E-19	93.7
71	6	91.6	8	2997	3067	2.00E-19	93.7
70	8	88.6	3	4044	4113	2.00E-13	75.8
70	8	88.6	1	121717	121786	2.00E-12	75.8

70	9	87.1	8	1279	1348	1.00E-11	67.9
70	9	87.1	8	2973	3042	1.00E-11	67.9
69	0	100.0	1	79612	79680	6.00E-31	137.0
69	0	100.0	1	82805	82873	6.00E-31	137.0
69	5	91.3	8	1906	1973	9.00E-16	81.8
69	5	91.3	8	5941	6009	9.00E-16	81.8
68	0	100.0	8	1279	1346	7.00E-32	135.0
68	0	100.0	8	2950	3017	7.00E-32	135.0
68	7	89.7	8	1281	1348	4.00E-15	79.8
68	7	89.7	8	3324	3391	4.00E-15	79.8
68	9	86.8	8	1074	1141	2.00E-10	63.9
68	9	86.8	8	1279	1346	2.00E-10	63.9
68	9	86.8	8	3301	3368	2.00E-10	63.9
68	9	86.8	8	3324	3391	2.00E-10	63.9
67	1	98.5	5	4216	4282	5.00E-29	125.0
67	1	98.5	2	29766	29832	5.00E-28	125.0
67	1	98.5	6	60834	60900	9.00E-28	125.0
67	1	98.5	4	3874	3940	1.00E-27	125.0
67	7	89.6	6	1396	1462	2.00E-13	77.8
67	7	89.6	1	143783	143849	5.00E-13	77.8
66	4	92.4	7	71259	71323	3.00E-15	83.8
66	4	92.4	1	29416	29481	8.00E-15	83.8
65	0	100.0	8	2576	2640	4.00E-30	129.0
65	0	100.0	8	1528	1592	4.00E-30	129.0
65	0	100.0	8	5941	6005	4.00E-30	129.0
65	0	100.0	8	2753	2817	4.00E-30	129.0
65	3	89.2	7	14700	14761	1.00E-08	61.9
65	3	89.2	1	101886	101949	3.00E-08	61.9
64	0	100.0	3	17226	17289	5.00E-29	127.0
64	0	100.0	2	18943	19006	1.00E-28	127.0
64	0	100.0	6	361	424	2.00E-28	127.0
64	0	100.0	7	68253	68316	2.00E-28	127.0
64	0	100.0	1	24363	24426	6.00E-28	127.0
64	0	100.0	1	75215	75278	6.00E-28	127.0
64	1	98.4	2	18835	18898	3.00E-26	119.0
64	1	98.4	2	22735	22798	3.00E-26	119.0
64	2	96.9	2	12683	12746	7.00E-24	111.0
64	2	96.9	1	204290	204353	3.00E-23	111.0
64	7	89.1	7	49935	49998	1.00E-11	71.9
64	7	89.1	4	105819	105882	2.00E-11	71.9
62	2	88.7	3	9682	9738	4.00E-11	67.9
62	2	88.7	7	45164	45225	2.00E-10	67.9
62	2	87.1	2	2441	2496	6.00E-09	61.9
62	2	87.1	1	126984	127045	3.00E-08	61.9

60	2	96.7	5	80	139	2.00E-22	103.0
60	2	96.7	4	439	498	5.00E-21	103.0
59	3	94.9	2	29768	29826	2.00E-18	93.7
59	3	94.9	4	19746	19804	5.00E-18	93.7
59	4	93.2	5	4222	4280	5.00E-17	85.7
59	4	93.2	4	19746	19804	1.00E-15	85.7
58	7	87.9	8	1083	1140	3.00E-09	60.0
58	7	87.9	8	3375	3432	3.00E-09	60.0
58	7	87.9	6	38549	38606	4.00E-08	60.0
58	7	87.9	1	133448	133505	1.00E-07	60.0
57	4	93.0	3	4148	4204	3.00E-15	81.8
57	4	93.0	1	121634	121690	3.00E-14	81.8
57	6	89.5	9	3246	3302	5.00E-11	65.9
57	6	89.5	4	105114	105170	1.00E-09	65.9
56	0	100.0	2	18949	19004	7.00E-24	111.0
56	0	100.0	7	50203	50258	1.00E-23	111.0
56	0	100.0	4	56045	56100	2.00E-23	111.0
56	0	100.0	1	211805	211860	3.00E-23	111.0
55	0	100.0	2	29849	29903	3.00E-23	109.0
55	0	100.0	4	15181	15235	8.00E-23	109.0
55	0	100.0	4	19674	19728	8.00E-23	109.0
55	0	100.0	4	19674	19728	8.00E-23	109.0
53	0	100.0	7	4703	4755	8.00E-22	105.0
53	0	100.0	1	41000	41052	2.00E-21	105.0
53	1	98.1	7	15851	15903	2.00E-19	97.6
53	1	98.1	1	165797	165849	5.00E-19	97.6
53	2	96.2	4	2113	2165	7.00E-17	89.7
53	2	96.2	4	17289	17341	7.00E-17	89.7
53	2	96.2	4	82914	82966	7.00E-17	89.7
53	2	96.2	1	119488	119540	1.00E-16	89.7
52	2	96.2	3	4037	4088	5.00E-17	87.7
52	2	96.2	1	99800	99851	5.00E-16	87.7
51	3	94.1	8	470	520	1.00E-14	77.8
51	3	94.1	8	3473	3523	1.00E-14	77.8
50	0	100.0	3	12333	12382	1.00E-20	99.6
50	0	100.0	4	122695	122744	7.00E-20	99.6
50	1	98.0	4	1096	1145	2.00E-17	91.7
50	1	98.0	1	149258	149307	3.00E-17	91.7
50	4	92.0	5	795	844	1.00E-11	67.9
50	4	92.0	6	9623	9672	2.00E-10	67.9
50	4	92.0	7	24199	24248	2.00E-10	67.9
50	4	92.0	1	24074	24123	5.00E-10	67.9
49	0	100.0	9	2979	3027	1.00E-20	97.6
49	0	100.0	3	2009	2057	5.00E-20	97.6

49	0	100.0	4	38024	38072	3.00E-19	97.6
49	0	100.0	1	80707	80755	5.00E-19	97.6
49	4	91.8	8	25	73	5.00E-11	65.9
49	4	91.8	8	3232	3280	5.00E-11	65.9
49	4	91.8	6	2356	2404	7.00E-10	65.9
49	4	91.8	4	88777	88825	1.00E-09	65.9
48	0	100.0	3	2996	3043	2.00E-19	95.6
48	0	100.0	6	12491	12538	8.00E-19	95.6
48	0	100.0	4	43065	43112	1.00E-18	95.6
48	0	100.0	4	106943	106990	1.00E-18	95.6
48	2	95.8	8	1301	1348	4.00E-15	79.8
48	2	95.8	8	3023	3070	4.00E-15	79.8
48	3	93.8	7	68727	68774	1.00E-11	71.9
48	3	93.8	4	49457	49504	2.00E-11	71.9
47	0	100.0	2	18266	18312	2.00E-18	93.7
47	0	100.0	4	22077	22123	5.00E-18	93.7
47	0	100.0	1	7522	7568	8.00E-18	93.7
47	0	100.0	1	200830	200876	8.00E-18	93.7
47	1	97.9	6	65570	65616	7.00E-16	85.7
47	1	97.9	7	68399	68445	8.00E-16	85.7
46	0	97.8	3	76	120	2.00E-13	75.8
46	0	97.8	3	1930	1975	2.00E-13	75.8
45	2	95.6	2	37915	37959	2.00E-12	73.8
45	2	95.6	1	98724	98768	7.00E-12	73.8
45	3	93.3	1	20866	20910	2.00E-09	65.9
45	3	93.3	1	111580	111624	2.00E-09	65.9
44	0	100.0	6	490	533	2.00E-16	87.7
44	0	100.0	7	14313	14356	2.00E-16	87.7
44	0	100.0	4	12853	12896	3.00E-16	87.7
44	0	100.0	4	41287	41330	3.00E-16	87.7
43	0	100.0	4	48701	48743	1.00E-15	85.7
43	0	100.0	1	100709	100751	2.00E-15	85.7
43	1	97.7	4	50700	50742	3.00E-13	77.8
43	1	97.7	1	203663	203705	5.00E-13	77.8
42	1	97.6	2	19020	19061	4.00E-13	75.8
42	1	97.6	4	64873	64914	1.00E-12	75.8
42	1	97.6	4	19707	19748	1.00E-12	75.8
42	1	97.6	1	111997	112038	2.00E-12	75.8
42	3	92.9	6	27188	27229	4.00E-08	60.0
42	3	92.9	4	10729	10770	6.00E-08	60.0
41	0	100.0	5	5172	5212	7.00E-16	81.8
41	0	100.0	9	3970	4010	8.00E-16	81.8
41	0	100.0	3	1	41	3.00E-15	81.8
41	0	100.0	7	68321	68361	1.00E-14	81.8

41	0	100.0	4	40181	40221	2.00E-14	81.8
41	0	100.0	1	90575	90615	3.00E-14	81.8
41	1	97.6	3	12306	12346	7.00E-13	73.8
41	1	97.6	2	13811	13851	2.00E-12	73.8
41	1	97.6	7	18019	18059	3.00E-12	73.8
41	1	97.6	1	123959	123999	7.00E-12	73.8
41	2	95.1	7	54689	54729	7.00E-10	65.9
41	2	95.1	1	205411	205451	2.00E-09	65.9
40	1	97.5	2	29936	29975	6.00E-12	71.9
40	1	97.5	7	62233	62272	1.00E-11	71.9
40	1	97.5	1	112166	112205	3.00E-11	71.9
40	1	97.5	1	115787	115826	3.00E-11	71.9
40	2	95.0	8	1280	1319	2.00E-10	63.9
40	2	95.0	8	2877	2916	2.00E-10	63.9
39	0	100.0	7	48832	48870	2.00E-13	77.8
39	0	100.0	4	56034	56072	3.00E-13	77.8
39	1	97.4	8	1311	1349	3.00E-12	69.9
39	1	97.4	8	2897	2935	3.00E-12	69.9
39	2	94.9	4	881	919	2.00E-08	61.9
39	2	94.9	1	204928	204966	3.00E-08	61.9
38	1	97.4	7	15814	15851	2.00E-10	67.9
38	1	97.4	7	66137	66174	2.00E-10	67.9
38	1	97.4	1	75493	75530	5.00E-10	67.9
38	1	97.4	1	172531	172568	5.00E-10	67.9
38	2	94.7	4	10641	10678	6.00E-08	60.0
38	2	94.7	4	115426	115463	6.00E-08	60.0
38	2	94.7	4	16902	16939	6.00E-08	60.0
38	2	94.7	4	16113	16150	6.00E-08	60.0
38	2	94.7	1	74253	74290	1.00E-07	60.0
38	2	94.7	1	4931	4968	1.00E-07	60.0
37	0	100.0	6	3726	3762	3.00E-12	73.8
37	0	100.0	1	75672	75708	7.00E-12	73.8
37	1	97.3	6	4804	4840	7.00E-10	65.9
37	1	97.3	6	8062	8098	7.00E-10	65.9
37	1	97.3	6	4959	4995	7.00E-10	65.9
37	1	97.3	1	92264	92300	2.00E-09	65.9
36	0	100.0	2	29900	29935	6.00E-12	71.9
36	0	100.0	7	48766	48801	1.00E-11	71.9
36	0	100.0	1	195049	195084	3.00E-11	71.9
36	0	100.0	1	205497	205532	3.00E-11	71.9
36	1	97.2	4	110416	110451	4.00E-09	63.9
36	1	97.2	1	75244	75279	7.00E-09	63.9
35	0	100.0	2	22827	22861	2.00E-11	69.9
35	0	100.0	7	2947	2981	5.00E-11	69.9

35	0	100.0	4	2659	2693	7.00E-11	69.9
35	0	100.0	4	21860	21894	7.00E-11	69.9
35	0	100.0	4	115411	115445	7.00E-11	69.9
35	0	100.0	1	205980	206014	1.00E-10	69.9
35	1	97.1	3	17226	17260	3.00E-09	61.9
35	1	97.1	6	8062	8096	1.00E-08	61.9
35	1	97.1	7	42484	42518	1.00E-08	61.9
35	1	97.1	4	110416	110450	2.00E-08	61.9
34	0	100.0	6	27438	27471	2.00E-10	67.9
34	0	100.0	7	28930	28963	2.00E-10	67.9
34	0	100.0	7	72530	72563	2.00E-10	67.9
34	0	100.0	1	75707	75740	5.00E-10	67.9
33	0	100.0	4	42370	42402	1.00E-09	65.9
33	0	100.0	1	26378	26410	2.00E-09	65.9
31	0	100.0	8	928	958	8.00E-10	61.9
31	0	100.0	8	3291	3321	8.00E-10	61.9
31	0	100.0	1	40014	40044	3.00E-08	61.9
31	0	100.0	1	204473	204503	3.00E-08	61.9
30	0	100.0	3	10083	10112	1.00E-08	60.0
30	0	100.0	6	40013	40042	4.00E-08	60.0
30	0	100.0	7	54767	54796	4.00E-08	60.0
30	0	100.0	4	40366	40395	6.00E-08	60.0
30	0	100.0	1	75294	75323	1.00E-07	60.0
30	0	100.0	1	146538	146567	1.00E-07	60.0

[1]Identity calculations do not include sites that contain indels.

M. emarginata

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
715	46	93.6	1	43166	43880	0	1053.0
715	46	93.6	3	127355	128069	0	1053.0
571	50	91.2	8	4908	5478	0	735.0
571	50	91.2	3	128314	128884	0	735.0
446	62	85.0	7	57340	57780	1.00E-96	353.0
446	62	85.0	2	12705	13150	3.00E-96	353.0
416	24	94.2	1	45272	45687	0	634.0
416	24	94.2	3	126513	126928	0	634.0
395	23	94.2	1	44866	45260	5.00E-171	601.0
395	23	94.2	3	126948	127342	8.00E-171	601.0
322	0	100.0	11	6497	6818	0	638.0
322	0	100.0	2	47657	47978	0	638.0
322	42	87.0	1	12489	12810	4.00E-82	305.0
322	42	87.0	2	36196	36517	7.00E-82	305.0
267	11	95.9	3	52631	52897	4.00E-123	442.0
267	11	95.9	9	64186	64452	4.00E-123	442.0

192	9	88.0	1	1329	1519	3.00E-49	196.0
192	9	88.0	9	78809	78987	4.00E-49	196.0
186	6	92.5	1	3365	3543	1.00E-66	254.0
186	6	92.5	3	12797	12981	2.00E-66	254.0
185	18	90.3	1	13025	13209	1.00E-57	224.0
185	18	90.3	3	84231	84415	2.00E-57	224.0
174	7	93.7	3	107361	107533	5.00E-64	246.0
174	7	93.7	3	109069	109239	5.00E-64	246.0
164	5	95.1	1	56676	56836	9.00E-68	258.0
164	5	95.1	2	103	266	1.00E-67	258.0
164	7	95.1	3	51872	52034	2.00E-66	254.0
164	7	95.1	3	53707	53870	2.00E-66	254.0
155	16	82.6	5	17147	17299	2.00E-16	85.7
155	16	82.6	1	11129	11274	8.00E-16	85.7
154	14	82.5	8	3144	3296	4.00E-11	67.9
154	14	82.5	9	12267	12408	2.00E-10	67.9
152	9	94.1	1	40196	40347	2.00E-59	230.0
152	9	94.1	3	44746	44897	3.00E-59	230.0
143	13	90.9	8	4758	4900	4.00E-45	180.0
143	13	90.9	1	59404	59546	2.00E-44	180.0
140	9	90.7	1	40379	40515	4.00E-39	163.0
140	9	90.7	3	44886	45024	6.00E-39	163.0
127	8	93.7	7	17119	17245	5.00E-47	188.0
127	8	93.7	2	5813	5939	1.00E-46	188.0
120	22	81.7	1	60837	60956	3.00E-09	63.9
120	22	81.7	2	24220	24339	4.00E-09	63.9
119	5	95.8	1	47743	47861	3.00E-49	196.0
119	5	95.8	2	83200	83318	4.00E-49	196.0
119	13	87.4	1	37000	37116	1.00E-23	111.0
119	13	87.4	3	105351	105469	2.00E-23	111.0
118	7	91.5	1	1822	1936	2.00E-35	151.0
118	7	91.5	3	27282	27399	2.00E-35	151.0
115	0	100.0	6	10015	10129	2.00E-59	228.0
115	0	100.0	9	41761	41875	1.00E-58	228.0
112	7	92.9	7	31774	31884	1.00E-35	151.0
112	7	92.9	2	109874	109985	2.00E-35	151.0
108	1	94.4	7	13123	13225	2.00E-40	167.0
108	1	94.4	2	109733	109840	4.00E-40	167.0
107	3	96.3	1	11033	11139	4.00E-42	172.0
107	3	96.3	3	44724	44829	6.00E-42	172.0
106	3	97.2	3	53511	53616	4.00E-46	186.0
106	3	97.2	9	64988	65093	4.00E-46	186.0
106	8	92.5	7	1407	1512	2.00E-34	147.0
106	8	92.5	1	12127	12232	2.00E-34	147.0

105	0	100.0	9	41753	41857	1.00E-52	208.0
105	0	100.0	3	83919	84023	1.00E-52	208.0
103	7	89.3	3	31356	31458	1.00E-24	115.0
103	7	89.3	2	89529	89627	1.00E-24	115.0
103	7	88.4	1	3498	3596	5.00E-20	99.6
103	7	88.4	3	12679	12780	8.00E-20	99.6
101	0	100.0	3	94020	94120	3.00E-50	200.0
101	1	99.0	9	74619	74719	6.00E-48	192.0
101	1	99.0	9	103505	103605	6.00E-48	192.0
100	2	90.0	3	28809	28907	1.00E-24	115.0
100	2	90.0	2	61768	61860	1.00E-24	115.0
97	0	100.0	6	10015	10111	9.00E-49	192.0
97	0	100.0	3	83919	84015	7.00E-48	192.0
93	10	89.3	1	13246	13338	8.00E-22	105.0
93	10	89.3	3	84017	84109	1.00E-21	105.0
90	1	98.9	4	1100	1189	6.00E-43	170.0
90	1	98.9	1	57595	57684	2.00E-41	170.0
87	5	94.3	8	255	341	8.00E-31	133.0
87	5	94.3	1	72464	72550	4.00E-30	133.0
87	5	88.5	9	100615	100696	4.00E-18	93.7
87	5	88.5	2	45051	45137	5.00E-18	93.7
83	1	98.8	3	15010	15092	4.00E-37	157.0
83	1	98.8	3	90806	90888	4.00E-37	157.0
82	8	90.2	1	59662	59743	5.00E-20	99.6
82	8	90.2	2	109762	109843	8.00E-20	99.6
80	5	93.8	6	12692	12771	1.00E-26	119.0
80	5	93.8	1	48537	48616	5.00E-26	119.0
80	8	90.0	5	2845	2924	2.00E-19	95.6
80	8	90.0	1	18235	18314	8.00E-19	95.6
80	8	90.0	9	68563	68642	1.00E-18	95.6
80	8	90.0	9	93136	93215	1.00E-18	95.6
79	0	100.0	3	84533	84611	4.00E-37	157.0
79	0	100.0	3	124177	124255	4.00E-37	157.0
79	8	89.9	7	13123	13201	2.00E-18	93.7
79	8	89.9	1	59662	59740	3.00E-18	93.7
78	11	85.9	10	5043	5120	2.00E-11	67.9
78	11	85.9	9	4338	4415	2.00E-10	67.9
76	4	94.7	9	63483	63558	7.00E-26	119.0
76	4	94.7	3	52046	52121	8.00E-26	119.0
75	1	98.7	5	11933	12007	4.00E-33	141.0
75	1	98.7	1	28861	28935	1.00E-32	141.0
75	2	97.3	5	2757	2831	9.00E-31	133.0
75	2	97.3	3	123597	123671	6.00E-30	133.0
72	4	94.4	1	47768	47839	1.00E-23	111.0

72	4	94.4	1	52435	52506	1.00E-23	111.0
72	4	94.4	1	82481	82552	1.00E-23	111.0
72	4	94.4	1	82481	82552	1.00E-23	111.0
72	4	94.4	3	32429	32500	2.00E-23	111.0
72	4	94.4	2	83222	83293	2.00E-23	111.0
70	3	95.7	1	21817	21886	8.00E-25	115.0
70	3	95.7	2	6323	6392	1.00E-24	115.0
68	4	94.1	9	78303	78370	4.00E-21	103.0
68	4	94.1	3	39553	39620	5.00E-21	103.0
67	1	98.5	7	11719	11785	6.00E-28	125.0
67	1	98.5	1	69779	69845	9.00E-28	125.0
66	6	90.9	9	103410	103475	4.00E-15	83.8
66	6	90.9	2	37500	37565	5.00E-15	83.8
65	0	100.0	7	58441	58505	4.00E-29	129.0
65	2	95.4	5	11063	11126	5.00E-20	97.6
65	2	95.4	2	99493	99557	3.00E-19	97.6
64	0	93.8	1	55590	55649	3.00E-18	93.7
64	0	93.8	2	53436	53499	5.00E-18	93.7
63	3	90.5	2	44901	44963	4.00E-12	73.8
63	3	90.5	9	100480	100539	4.00E-12	73.8
62	0	100.0	3	10250	10311	5.00E-27	123.0
62	0	100.0	2	36594	36655	5.00E-27	123.0
62	0	100.0	3	83975	84036	5.00E-27	123.0
62	0	100.0	3	93465	93526	5.00E-27	123.0
62	2	96.8	1	55517	55578	2.00E-22	107.0
62	2	96.8	3	128439	128500	3.00E-22	107.0
60	1	98.3	9	19631	19690	2.00E-23	111.0
60	1	98.3	2	70702	70761	2.00E-23	111.0
60	6	90.0	4	1055	1114	4.00E-13	71.9
60	6	90.0	1	57684	57743	1.00E-11	71.9
58	1	98.3	2	72511	72568	3.00E-22	107.0
58	1	98.3	3	75760	75817	3.00E-22	107.0
58	4	93.1	9	63586	63643	4.00E-15	83.8
58	4	93.1	3	52125	52182	5.00E-15	83.8
58	2	87.9	7	30773	30825	3.00E-08	60.0
58	2	87.9	2	1892	1949	7.00E-08	60.0
57	0	100.0	11	3320	3376	3.00E-25	113.0
57	0	100.0	9	57194	57250	4.00E-24	113.0
57	0	100.0	3	18791	18847	5.00E-24	113.0
57	0	100.0	2	109717	109773	5.00E-24	113.0
57	4	93.0	8	5292	5348	3.00E-15	81.8
57	4	93.0	1	55522	55578	1.00E-14	81.8
56	0	100.0	7	5770	5825	9.00E-24	111.0
56	0	100.0	2	72740	72795	2.00E-23	111.0

55	6	89.1	7	24206	24260	8.00E-09	61.9
55	6	89.1	2	94071	94125	2.00E-08	61.9
54	2	96.3	2	6816	6869	2.00E-17	91.7
54	2	96.3	3	64363	64416	2.00E-17	91.7
54	5	90.7	5	10998	11051	5.00E-11	67.9
54	5	90.7	7	13869	13922	1.00E-10	67.9
53	2	96.2	1	12302	12354	5.00E-17	89.7
53	2	96.2	2	36009	36061	7.00E-17	89.7
53	5	90.6	1	23428	23480	7.00E-10	65.9
53	5	90.6	9	1571	1623	9.00E-10	65.9
52	4	92.3	1	67146	67197	1.00E-11	71.9
52	4	92.3	2	109792	109843	2.00E-11	71.9
51	0	100.0	9	54255	54305	2.00E-20	101.0
51	0	100.0	2	80946	80996	2.00E-20	101.0
50	0	100.0	4	1	50	2.00E-21	99.6
50	0	100.0	8	1	50	1.00E-20	99.6
50	0	100.0	6	12887	12936	1.00E-20	99.6
50	0	100.0	5	16064	16113	1.00E-20	99.6
50	0	100.0	8	19439	19488	1.00E-20	99.6
50	0	100.0	7	1	50	4.00E-20	99.6
50	0	100.0	7	13272	13321	4.00E-20	99.6
50	0	100.0	1	1	50	5.00E-20	99.6
50	0	100.0	1	12115	12164	5.00E-20	99.6
50	0	100.0	1	12166	12215	5.00E-20	99.6
50	0	100.0	1	48303	48352	5.00E-20	99.6
50	0	100.0	1	51847	51896	5.00E-20	99.6
50	0	100.0	2	2915	2964	8.00E-20	99.6
50	0	100.0	3	29998	30047	8.00E-20	99.6
50	0	100.0	3	38600	38649	8.00E-20	99.6
50	0	100.0	2	39318	39367	8.00E-20	99.6
50	0	100.0	2	53725	53774	8.00E-20	99.6
50	0	100.0	2	58541	58590	8.00E-20	99.6
50	0	100.0	3	120111	120160	8.00E-20	99.6
50	0	100.0	3	125050	125099	8.00E-20	99.6
50	0	100.0	3	125682	125731	8.00E-20	99.6
50	0	100.0	2	126324	126373	8.00E-20	99.6
49	0	100.0	7	14161	14209	1.00E-19	97.6
49	0	100.0	2	36607	36655	3.00E-19	97.6
49	0	100.0	9	41753	41801	3.00E-19	97.6
49	0	100.0	3	126250	126298	3.00E-19	97.6
49	3	93.9	8	5263	5311	7.00E-13	73.8
49	3	93.9	5	14964	15012	7.00E-13	73.8
49	4	91.8	7	13123	13171	5.00E-10	65.9
49	4	91.8	1	67146	67194	7.00E-10	65.9

49	4	91.8	2	111667	111715	1.00E-09	65.9
49	4	91.8	3	114009	114057	1.00E-09	65.9
48	0	100.0	2	44997	45044	1.00E-18	95.6
48	0	100.0	9	100543	100590	1.00E-18	95.6
47	0	100.0	5	14964	15010	8.00E-19	93.7
47	0	100.0	7	32300	32346	2.00E-18	93.7
47	0	100.0	2	113912	113958	5.00E-18	93.7
47	0	100.0	3	128481	128527	5.00E-18	93.7
47	2	95.7	8	19441	19487	4.00E-14	77.8
47	2	95.7	7	1426	1472	1.00E-13	77.8
47	2	95.7	9	74903	74949	2.00E-13	77.8
47	2	95.7	2	58969	59015	3.00E-13	77.8
46	0	100.0	6	10021	10066	2.00E-18	91.7
46	0	100.0	3	18747	18792	2.00E-17	91.7
46	0	100.0	3	18747	18792	2.00E-17	91.7
46	0	100.0	3	18747	18792	2.00E-17	91.7
46	0	100.0	2	41690	41735	2.00E-17	91.7
46	0	100.0	9	41767	41812	2.00E-17	91.7
46	0	100.0	9	67051	67096	2.00E-17	91.7
46	0	100.0	3	83964	84009	2.00E-17	91.7
46	1	97.8	6	15910	15955	6.00E-16	83.8
46	1	97.8	7	31577	31622	2.00E-15	83.8
46	3	93.5	1	67139	67184	2.00E-10	67.9
46	3	93.5	2	4040	4085	3.00E-10	67.9
45	3	93.3	7	52397	52441	5.00E-10	65.9
45	3	93.3	1	36973	37017	7.00E-10	65.9
45	3	93.3	9	8977	9021	9.00E-10	65.9
45	3	93.3	3	97970	98014	1.00E-09	65.9
44	0	100.0	9	28499	28542	2.00E-16	87.7
44	0	100.0	2	113430	113473	3.00E-16	87.7
44	3	93.2	8	6162	6205	6.00E-10	63.9
44	3	93.2	2	120954	120997	4.00E-09	63.9
43	0	100.0	1	32613	32655	8.00E-16	85.7
43	0	100.0	2	72477	72519	1.00E-15	85.7
42	1	97.6	4	596	637	3.00E-14	75.8
42	1	97.6	7	31645	31686	5.00E-13	75.8
42	1	97.6	1	13482	13523	7.00E-13	75.8
42	1	97.6	1	71909	71950	7.00E-13	75.8
41	0	100.0	6	10015	10055	2.00E-15	81.8
41	0	100.0	2	36615	36655	2.00E-14	81.8
41	1	97.6	3	54047	54087	4.00E-12	73.8
41	1	97.6	2	105499	105539	4.00E-12	73.8
41	2	95.1	8	4620	4660	2.00E-10	65.9
41	2	95.1	1	38757	38797	7.00E-10	65.9

41	2	95.1	1	43047	43087	7.00E-10	65.9
41	2	95.1	3	103459	103499	1.00E-09	65.9
40	0	100.0	1	36134	36173	5.00E-14	79.8
40	0	100.0	3	97755	97794	7.00E-14	79.8
40	1	97.5	10	6712	6751	2.00E-12	71.9
40	1	97.5	1	30103	30142	1.00E-11	71.9
40	1	97.5	2	21771	21810	2.00E-11	71.9
40	1	97.5	3	128369	128408	2.00E-11	71.9
40	2	95.0	10	6712	6751	4.00E-10	63.9
40	2	95.0	8	5384	5423	6.00E-10	63.9
40	2	95.0	2	50783	50822	4.00E-09	63.9
40	2	95.0	9	61949	61988	4.00E-09	63.9
39	0	100.0	7	34386	34424	1.00E-13	77.8
39	0	100.0	2	33516	33554	3.00E-13	77.8
39	1	97.4	2	72734	72772	7.00E-11	69.9
39	1	97.4	3	75989	76027	7.00E-11	69.9
39	2	94.9	10	461	499	2.00E-09	61.9
39	2	94.9	2	10118	10156	2.00E-08	61.9
39	2	94.9	2	10118	10156	2.00E-08	61.9
39	2	94.9	2	52385	52423	2.00E-08	61.9
38	0	100.0	9	93341	93378	9.00E-13	75.8
38	0	100.0	2	18399	18436	1.00E-12	75.8
38	0	100.0	3	29828	29865	1.00E-12	75.8
38	0	100.0	2	37482	37519	1.00E-12	75.8
38	0	100.0	3	75431	75468	1.00E-12	75.8
38	0	100.0	3	125308	125345	1.00E-12	75.8
38	1	97.4	9	67055	67092	2.00E-10	67.9
38	1	97.4	3	29826	29863	3.00E-10	67.9
38	1	97.4	3	29826	29863	3.00E-10	67.9
38	1	97.4	2	41694	41731	3.00E-10	67.9
38	2	94.7	1	33871	33908	4.00E-08	60.0
38	2	94.7	1	48095	48132	4.00E-08	60.0
38	2	94.7	9	63126	63163	5.00E-08	60.0
38	2	94.7	3	13109	13146	7.00E-08	60.0
38	2	94.7	3	75641	75678	7.00E-08	60.0
38	2	94.7	3	102270	102307	7.00E-08	60.0
37	0	100.0	5	17319	17355	7.00E-13	73.8
37	0	100.0	7	5860	5896	2.00E-12	73.8
37	0	100.0	7	5860	5896	2.00E-12	73.8
37	0	100.0	2	66071	66107	4.00E-12	73.8
37	0	100.0	2	71285	71321	4.00E-12	73.8
37	0	100.0	2	72573	72609	4.00E-12	73.8
37	1	97.3	5	15033	15069	2.00E-10	65.9
37	1	97.3	7	32568	32604	5.00E-10	65.9

37	1	97.3	7	47678	47714	5.00E-10	65.9
37	1	97.3	1	59577	59613	7.00E-10	65.9
37	1	97.3	9	61847	61883	9.00E-10	65.9
37	1	97.3	9	67057	67093	9.00E-10	65.9
37	1	97.3	2	4132	4168	1.00E-09	65.9
37	1	97.3	3	7704	7740	1.00E-09	65.9
37	1	97.3	3	7704	7740	1.00E-09	65.9
37	1	97.3	2	41696	41732	1.00E-09	65.9
37	1	97.3	2	113455	113491	1.00E-09	65.9
37	1	97.3	2	114176	114212	1.00E-09	65.9
36	0	100.0	7	20134	20169	8.00E-12	71.9
36	0	100.0	1	30103	30138	1.00E-11	71.9
36	0	100.0	9	93341	93376	1.00E-11	71.9
36	0	100.0	3	7704	7739	2.00E-11	71.9
36	0	100.0	3	7704	7739	2.00E-11	71.9
36	0	100.0	3	29828	29863	2.00E-11	71.9
36	1	97.2	7	20134	20169	2.00E-09	63.9
36	1	97.2	1	32174	32209	3.00E-09	63.9
36	1	97.2	1	67211	67246	3.00E-09	63.9
36	1	97.2	9	2915	2950	4.00E-09	63.9
36	1	97.2	2	21775	21810	4.00E-09	63.9
36	1	97.2	2	35930	35965	4.00E-09	63.9
36	1	97.2	2	41696	41731	4.00E-09	63.9
36	1	97.2	3	93175	93210	4.00E-09	63.9
36	1	97.2	9	93341	93376	4.00E-09	63.9
36	1	97.2	3	98334	98369	4.00E-09	63.9
35	0	100.0	4	276	310	2.00E-12	69.9
35	0	100.0	7	39729	39763	3.00E-11	69.9
35	0	100.0	7	43675	43709	3.00E-11	69.9
35	0	100.0	1	57830	57864	4.00E-11	69.9
35	0	100.0	9	74721	74755	6.00E-11	69.9
35	0	100.0	9	103505	103539	6.00E-11	69.9
35	0	100.0	3	18747	18781	7.00E-11	69.9
35	0	100.0	2	21774	21808	7.00E-11	69.9
35	0	100.0	3	29645	29679	7.00E-11	69.9
35	0	100.0	2	36621	36655	7.00E-11	69.9
35	0	100.0	3	49679	49713	7.00E-11	69.9
35	0	100.0	2	126206	126240	7.00E-11	69.9
35	1	97.1	7	5779	5813	8.00E-09	61.9
35	1	97.1	7	43675	43709	8.00E-09	61.9
35	1	97.1	1	30105	30139	1.00E-08	61.9
35	1	97.1	3	44372	44406	2.00E-08	61.9
35	1	97.1	2	57889	57923	2.00E-08	61.9
35	1	97.1	2	72752	72786	2.00E-08	61.9

35	1	97.1	3	75936	75970	2.00E-08	61.9
35	1	97.1	3	75936	75970	2.00E-08	61.9
35	1	97.1	3	86735	86769	2.00E-08	61.9
35	1	97.1	2	101545	101579	2.00E-08	61.9
35	1	97.1	3	102544	102578	2.00E-08	61.9
35	1	97.1	2	114173	114207	2.00E-08	61.9
34	0	100.0	7	5984	6017	1.00E-10	67.9
34	0	100.0	1	59467	59500	2.00E-10	67.9
34	1	97.1	7	20136	20169	3.00E-08	60.0
34	1	97.1	7	43675	43708	3.00E-08	60.0
33	0	100.0	5	5838	5870	2.00E-10	65.9
33	0	100.0	5	8664	8696	2.00E-10	65.9
33	0	100.0	7	24231	24263	5.00E-10	65.9
33	0	100.0	7	28335	28367	5.00E-10	65.9
33	0	100.0	7	40021	40053	5.00E-10	65.9
33	0	100.0	9	71322	71354	9.00E-10	65.9
33	0	100.0	2	21776	21808	1.00E-09	65.9
33	0	100.0	2	72666	72698	1.00E-09	65.9
33	0	100.0	3	75920	75952	1.00E-09	65.9
33	0	100.0	3	128198	128230	1.00E-09	65.9
32	0	100.0	6	7981	8012	6.00E-10	63.9
32	0	100.0	7	30548	30579	2.00E-09	63.9
32	0	100.0	7	32169	32200	2.00E-09	63.9
32	0	100.0	7	32384	32415	2.00E-09	63.9
32	0	100.0	7	32384	32415	2.00E-09	63.9
32	0	100.0	7	32568	32599	2.00E-09	63.9
32	0	100.0	9	6603	6634	4.00E-09	63.9
32	0	100.0	3	9416	9447	4.00E-09	63.9
32	0	100.0	3	102547	102578	4.00E-09	63.9
32	0	100.0	2	109595	109626	4.00E-09	63.9
32	0	100.0	2	113946	113977	4.00E-09	63.9
32	0	100.0	3	126458	126489	4.00E-09	63.9
31	0	100.0	5	1410	1440	3.00E-09	61.9
31	0	100.0	5	1410	1440	3.00E-09	61.9
31	0	100.0	7	5793	5823	8.00E-09	61.9
31	0	100.0	1	47372	47402	1.00E-08	61.9
31	0	100.0	9	73381	73411	1.00E-08	61.9
31	0	100.0	2	72742	72772	2.00E-08	61.9
30	0	100.0	7	873	902	3.00E-08	60.0
30	0	100.0	9	62329	62358	5.00E-08	60.0
30	0	100.0	2	39857	39886	7.00E-08	60.0
30	0	100.0	2	40487	40516	7.00E-08	60.0
30	0	100.0	2	82820	82849	7.00E-08	60.0
30	0	100.0	2	104847	104876	7.00E-08	60.0

30	0	100.0	2	113544	113573	7.00E-08	60.0
30	0	100.0	2	114138	114167	7.00E-08	60.0

[1]Identity calculations do not include sites that contain indels.

M. marlothii

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
1630	271	83.0	2	37289	38912	0	1037.0
1630	271	83.0	2	104771	106400	0	1037.0
473	85	82.0	6	1397	1869	5.00E-70	264.0
473	85	82.0	2	29494	29966	4.00E-69	264.0
221	45	79.6	2	29153	29373	3.00E-14	81.8
221	45	79.6	2	171670	171890	3.00E-14	81.8
197	34	82.7	2	37007	37203	3.00E-26	121.0
197	34	82.7	2	104489	104685	3.00E-26	121.0
174	2	98.9	6	288	461	1.00E-89	329.0
174	2	98.9	1	41683	41856	2.00E-89	329.0
171	8	95.3	6	285	455	1.00E-73	276.0
171	8	95.3	3	72995	73165	7.00E-73	276.0
168	10	94.1	1	41683	41850	1.00E-66	254.0
168	10	94.1	3	72998	73165	2.00E-66	254.0
164	3	96.3	4	60649	60809	1.00E-72	274.0
164	3	96.3	3	43137	43300	3.00E-72	274.0
157	1	99.4	2	71946	72102	4.00E-81	303.0
157	1	99.4	2	188588	188744	4.00E-81	303.0
143	10	93.0	4	27465	27607	1.00E-51	204.0
143	10	93.0	3	133704	133846	2.00E-51	204.0
143	10	93.0	2	77758	77900	3.00E-51	204.0
143	10	93.0	2	6902	7044	3.00E-51	204.0
143	11	83.9	3	133557	133691	1.00E-21	105.0
143	11	83.9	2	6761	6899	2.00E-21	105.0
141	7	94.3	2	94646	94785	2.00E-52	208.0
141	7	94.3	2	187015	187155	2.00E-52	208.0
138	5	92.0	6	1	138	2.00E-47	188.0
138	5	92.0	3	72432	72563	1.00E-46	188.0
134	25	81.3	2	29637	29770	4.00E-10	67.9
134	25	81.3	2	172144	172277	4.00E-10	67.9
133	4	93.2	3	133330	133457	8.00E-48	192.0
133	4	93.2	2	6425	6557	1.00E-47	192.0
129	23	82.2	6	1540	1668	1.00E-12	73.8
129	23	82.2	2	172144	172272	7.00E-12	73.8
127	6	95.3	3	48853	48979	2.00E-51	204.0
127	6	95.3	3	144180	144306	2.00E-51	204.0
118	2	91.5	5	50760	50869	1.00E-38	161.0
118	2	91.5	2	71884	72001	4.00E-38	161.0

112	7	92.9	3	35739	35849	3.00E-35	151.0
112	7	92.9	3	73196	73307	3.00E-35	151.0
112	19	83.0	6	1158	1269	4.00E-12	71.9
112	19	83.0	2	29255	29366	3.00E-11	71.9
110	15	86.4	3	49301	49410	9.00E-20	99.6
110	15	86.4	3	76021	76130	9.00E-20	99.6
104	8	92.3	3	71915	72018	7.00E-33	143.0
104	8	92.3	2	73551	73654	9.00E-33	143.0
103	0	99.0	1	27297	27398	5.00E-47	188.0
103	0	99.0	3	64730	64832	1.00E-46	188.0
100	2	90.0	3	21511	21603	1.00E-24	115.0
100	2	90.0	2	25751	25849	2.00E-24	115.0
96	0	100.0	2	16318	16413	4.00E-47	190.0
96	0	100.0	2	134378	134473	4.00E-47	190.0
94	0	98.9	2	94696	94788	4.00E-41	170.0
94	0	98.9	2	100743	100836	4.00E-41	170.0
94	4	95.7	3	25006	25099	2.00E-36	155.0
94	4	95.7	3	97197	97290	2.00E-36	155.0
93	7	90.3	1	9926	10018	1.00E-22	107.0
93	7	90.3	3	133878	133968	4.00E-22	107.0
92	0	100.0	2	100338	100429	1.00E-44	182.0
92	0	100.0	2	188573	188664	1.00E-44	182.0
87	13	85.1	2	38977	39063	1.00E-10	69.9
87	13	85.1	2	106465	106551	1.00E-10	69.9
86	7	90.7	4	15727	15812	4.00E-20	99.6
86	7	90.7	3	76531	76615	9.00E-20	99.6
84	2	97.6	2	100731	100814	4.00E-35	151.0
84	2	97.6	2	187087	187170	4.00E-35	151.0
79	2	97.5	3	72845	72923	3.00E-32	141.0
79	2	97.5	2	82883	82961	4.00E-32	141.0
79	5	93.7	3	82711	82789	4.00E-25	117.0
79	5	93.7	2	19626	19704	5.00E-25	117.0
78	1	98.7	2	118838	118915	6.00E-34	147.0
78	1	98.7	2	189490	189567	6.00E-34	147.0
78	2	97.4	5	29247	29324	3.00E-32	139.0
78	2	97.4	4	63201	63278	5.00E-32	139.0
77	4	94.8	3	1453	1529	2.00E-26	121.0
77	4	94.8	2	208798	208874	3.00E-26	121.0
76	1	98.7	6	10655	10730	1.00E-33	143.0
76	1	98.7	2	6107	6182	9.00E-33	143.0
75	4	94.7	2	10929	11003	5.00E-25	117.0
75	4	94.7	2	186512	186586	5.00E-25	117.0
74	1	98.7	1	27560	27633	4.00E-32	139.0
74	1	98.7	1	44293	44366	4.00E-32	139.0

73	0	100.0	2	17911	17983	2.00E-33	145.0
73	0	100.0	3	100768	100840	2.00E-33	145.0
72	0	100.0	5	50818	50889	2.00E-33	143.0
72	0	100.0	1	61015	61086	3.00E-33	143.0
72	0	100.0	4	74156	74227	3.00E-33	143.0
72	0	100.0	3	72367	72438	7.00E-33	143.0
72	0	100.0	3	148102	148173	7.00E-33	143.0
72	0	100.0	3	1	72	7.00E-33	143.0
72	0	100.0	2	187961	188032	9.00E-33	143.0
72	0	100.0	2	118921	118992	9.00E-33	143.0
72	0	100.0	2	100259	100330	9.00E-33	143.0
72	0	100.0	2	73069	73140	9.00E-33	143.0
72	4	94.4	6	6992	7063	4.00E-24	111.0
72	4	94.4	3	142473	142544	2.00E-23	111.0
68	0	100.0	6	142	209	3.00E-31	135.0
68	0	100.0	3	72807	72874	2.00E-30	135.0
68	4	94.1	3	40639	40706	6.00E-21	103.0
68	4	94.1	3	120607	120674	6.00E-21	103.0
68	3	86.8	3	96192	96259	1.00E-09	65.9
68	3	86.8	2	85565	85626	2.00E-09	65.9
67	6	91.0	1	27107	27173	5.00E-16	85.7
67	1	91.0	4	70944	71010	7.00E-16	85.7
67	6	91.0	3	36249	36315	1.00E-15	85.7
67	1	91.0	2	188057	188118	2.00E-15	85.7
66	1	87.9	4	141	199	1.00E-11	71.9
66	1	87.9	4	45447	45512	1.00E-11	71.9
65	5	90.8	1	4068	4132	2.00E-12	73.8
65	5	90.8	2	19623	19686	7.00E-12	73.8
64	6	90.6	4	45412	45475	4.00E-14	79.8
64	7	89.1	5	16731	16794	7.00E-12	71.9
64	7	89.1	4	12980	13043	1.00E-11	71.9
63	4	87.3	3	43013	43075	8.00E-08	60.0
63	4	87.3	2	39457	39515	1.00E-07	60.0
62	0	100.0	5	50821	50882	2.00E-27	123.0
62	0	100.0	3	8331	8392	6.00E-27	123.0
62	0	100.0	3	93693	93754	6.00E-27	123.0
62	0	100.0	2	100262	100323	9.00E-27	123.0
62	0	100.0	2	142290	142351	9.00E-27	123.0
62	0	100.0	2	142290	142351	9.00E-27	123.0
60	1	98.3	5	50810	50869	8.00E-24	111.0
60	1	98.3	1	24742	24801	9.00E-24	111.0
60	1	98.3	3	39546	39605	2.00E-23	111.0
60	1	98.3	2	188689	188748	3.00E-23	111.0
60	2	96.7	2	7847	7906	8.00E-21	103.0

60	2	96.7	2	127395	127454	8.00E-21	103.0
60	6	90.0	4	1803	1862	1.00E-11	71.9
60	6	90.0	2	172430	172489	3.00E-11	71.9
59	1	98.3	3	42977	43035	9.00E-23	109.0
59	1	98.3	2	145774	145832	1.00E-22	109.0
59	3	94.9	6	290	348	1.00E-18	93.7
59	3	94.9	1	41685	41743	2.00E-18	93.7
59	3	94.9	3	47150	47208	5.00E-18	93.7
59	3	94.9	3	73000	73058	5.00E-18	93.7
59	3	94.9	3	47150	47208	5.00E-18	93.7
59	3	94.9	3	47150	47208	5.00E-18	93.7
58	7	87.9	1	47826	47883	3.00E-08	60.0
58	7	87.9	4	73881	73938	4.00E-08	60.0
57	0	100.0	4	23761	23817	3.00E-24	113.0
57	0	100.0	4	70928	70984	3.00E-24	113.0
54	2	96.3	4	74170	74223	1.00E-17	91.7
54	2	96.3	3	72381	72434	2.00E-17	91.7
54	2	96.3	2	73141	73194	3.00E-17	91.7
54	2	96.3	2	73141	73194	3.00E-17	91.7
54	5	90.7	6	19770	19823	6.00E-11	67.9
54	5	90.7	1	4103	4156	1.00E-10	67.9
54	5	90.7	5	36932	36985	1.00E-10	67.9
54	5	90.7	4	13098	13151	2.00E-10	67.9
54	5	90.7	3	114046	114099	3.00E-10	67.9
54	5	90.7	3	82705	82758	3.00E-10	67.9
54	6	88.9	4	13098	13151	4.00E-08	60.0
54	6	88.9	4	13098	13151	4.00E-08	60.0
54	6	88.9	3	148104	148157	8.00E-08	60.0
54	6	88.9	2	187963	188016	1.00E-07	60.0
53	0	100.0	4	68120	68172	7.00E-22	105.0
53	0	100.0	4	23806	23858	7.00E-22	105.0
53	0	100.0	3	63737	63789	1.00E-21	105.0
53	0	100.0	2	188706	188758	2.00E-21	105.0
53	1	98.1	4	17822	17874	2.00E-19	97.6
53	1	98.1	4	14001	14053	2.00E-19	97.6
53	1	98.1	2	152047	152099	5.00E-19	97.6
53	1	98.1	2	31158	31210	5.00E-19	97.6
53	2	96.2	3	34738	34790	8.00E-17	89.7
53	2	96.2	3	44933	44985	8.00E-17	89.7
52	0	100.0	4	70973	71024	3.00E-21	103.0
52	0	100.0	3	73084	73135	6.00E-21	103.0
52	1	98.1	2	100259	100310	2.00E-18	95.6
52	1	98.1	2	188689	188740	2.00E-18	95.6
52	4	92.3	3	73114	73165	2.00E-11	71.9

52	4	92.3	2	39457	39508	3.00E-11	71.9
51	2	94.1	3	22562	22612	8.00E-11	69.9
51	2	94.1	3	100608	100657	8.00E-11	69.9
50	1	98.0	3	93705	93754	2.00E-17	91.7
50	1	98.0	3	8331	8380	2.00E-17	91.7
50	1	98.0	3	133618	133667	2.00E-17	91.7
50	1	98.0	3	133618	133667	2.00E-17	91.7
49	0	100.0	4	71893	71941	2.00E-19	97.6
49	0	100.0	3	147215	147263	3.00E-19	97.6
49	1	98.0	2	71953	72001	1.00E-16	89.7
49	1	98.0	2	142303	142351	1.00E-16	89.7
49	2	95.9	6	18052	18100	4.00E-15	81.8
49	2	95.9	1	40677	40725	8.00E-15	81.8
49	2	95.9	3	76022	76070	2.00E-14	81.8
49	2	95.9	2	163548	163596	3.00E-14	81.8
48	0	100.0	6	18026	18073	3.00E-19	95.6
48	0	100.0	6	10465	10512	3.00E-19	95.6
48	0	100.0	3	42972	43019	1.00E-18	95.6
48	0	100.0	2	14374	14421	2.00E-18	95.6
48	1	97.9	4	11064	11111	2.00E-16	87.7
48	1	97.9	2	188059	188106	5.00E-16	87.7
47	0	100.0	1	31012	31058	2.00E-18	93.7
47	0	100.0	2	141613	141659	8.00E-18	93.7
47	2	95.7	1	38962	39008	1.00E-13	77.8
47	2	95.7	3	49488	49534	3.00E-13	77.8
47	2	95.7	3	72416	72462	3.00E-13	77.8
47	2	95.7	2	23519	23565	5.00E-13	77.8
46	0	100.0	1	38119	38164	9.00E-18	91.7
46	0	100.0	3	56114	56159	2.00E-17	91.7
46	1	97.8	1	48349	48394	2.00E-15	83.8
46	1	97.8	3	43035	43080	5.00E-15	83.8
46	3	93.5	4	12966	13011	2.00E-10	67.9
46	3	93.5	3	74708	74753	3.00E-10	67.9
46	3	93.5	3	47076	47121	3.00E-10	67.9
46	3	93.5	2	39470	39515	4.00E-10	67.9
45	0	100.0	1	31001	31045	3.00E-17	89.7
45	0	100.0	4	3180	3224	4.00E-17	89.7
45	0	100.0	2	93675	93719	1.00E-16	89.7
45	0	100.0	2	128164	128208	1.00E-16	89.7
45	3	93.3	5	19910	19954	4.00E-10	65.9
45	3	93.3	3	85127	85171	1.00E-09	65.9
44	0	100.0	4	3	46	2.00E-16	87.7
44	0	100.0	4	45469	45512	2.00E-16	87.7
44	0	100.0	3	76755	76798	3.00E-16	87.7

44	0	100.0	2	129661	129704	5.00E-16	87.7
43	0	100.0	6	18026	18068	3.00E-16	85.7
43	0	100.0	6	374	416	3.00E-16	85.7
43	0	100.0	5	50810	50852	5.00E-16	85.7
43	0	100.0	4	23816	23858	7.00E-16	85.7
43	0	100.0	4	70973	71015	7.00E-16	85.7
43	0	100.0	2	145790	145832	2.00E-15	85.7
43	2	95.4	1	41769	41811	3.00E-11	69.9
43	2	95.4	4	70897	70939	4.00E-11	69.9
43	2	95.4	4	70973	71015	4.00E-11	69.9
43	2	95.4	2	142311	142353	1.00E-10	69.9
42	0	100.0	4	71038	71079	3.00E-15	83.8
42	0	100.0	3	32338	32379	5.00E-15	83.8
42	0	100.0	2	177441	177482	7.00E-15	83.8
42	0	100.0	2	182791	182832	7.00E-15	83.8
42	0	100.0	2	143007	143048	7.00E-15	83.8
42	0	100.0	2	141501	141542	7.00E-15	83.8
42	1	97.6	6	9895	9936	2.00E-13	75.8
42	1	97.6	1	59339	59380	5.00E-13	75.8
42	1	97.6	3	94019	94060	1.00E-12	75.8
42	1	97.6	3	35610	35651	1.00E-12	75.8
42	1	97.6	3	47111	47152	1.00E-12	75.8
42	1	97.6	2	142367	142408	2.00E-12	75.8
41	0	100.0	4	15447	15487	1.00E-14	81.8
41	0	100.0	2	112371	112411	3.00E-14	81.8
41	2	95.1	5	50821	50861	4.00E-10	65.9
41	2	95.1	4	70897	70937	6.00E-10	65.9
41	2	95.1	4	70897	70937	6.00E-10	65.9
41	2	95.1	3	65790	65830	1.00E-09	65.9
41	2	95.1	2	100262	100302	2.00E-09	65.9
41	2	95.1	2	77630	77670	2.00E-09	65.9
40	0	100.0	2	100227	100266	1.00E-13	79.8
40	0	100.0	2	188782	188821	1.00E-13	79.8
40	1	97.5	6	21287	21326	4.00E-12	71.9
40	1	97.5	4	6276	6315	1.00E-11	71.9
40	1	97.5	4	3	42	1.00E-11	71.9
40	1	97.5	4	45469	45508	1.00E-11	71.9
40	1	97.5	4	6276	6315	1.00E-11	71.9
40	1	97.5	4	74093	74132	1.00E-11	71.9
40	1	97.5	2	39404	39443	3.00E-11	71.9
40	1	97.5	2	108885	108924	3.00E-11	71.9
39	0	100.0	5	47797	47835	1.00E-13	77.8
39	0	100.0	5	66	104	1.00E-13	77.8
39	0	100.0	4	129	167	2.00E-13	77.8

39	0	100.0	4	45414	45452	2.00E-13	77.8
39	0	100.0	4	23820	23858	2.00E-13	77.8
39	0	100.0	3	78863	78901	3.00E-13	77.8
39	0	100.0	3	2701	2739	3.00E-13	77.8
39	0	100.0	2	71946	71984	5.00E-13	77.8
39	0	100.0	2	126146	126184	5.00E-13	77.8
39	0	100.0	2	58691	58729	5.00E-13	77.8
39	0	100.0	2	128947	128985	5.00E-13	77.8
39	0	100.0	2	17216	17254	5.00E-13	77.8
39	1	97.4	2	24794	24832	1.00E-10	69.9
39	1	97.4	2	147871	147909	1.00E-10	69.9
39	2	94.9	3	35828	35866	2.00E-08	61.9
39	2	94.9	3	65305	65343	2.00E-08	61.9
38	0	100.0	6	374	411	2.00E-13	75.8
38	0	100.0	1	23917	23954	5.00E-13	75.8
38	0	100.0	1	39901	39938	5.00E-13	75.8
38	0	100.0	1	25654	25691	5.00E-13	75.8
38	0	100.0	3	73084	73121	1.00E-12	75.8
38	0	100.0	3	66359	66396	1.00E-12	75.8
38	0	100.0	2	188081	188118	2.00E-12	75.8
38	0	100.0	2	188081	188118	2.00E-12	75.8
38	0	100.0	2	145431	145468	2.00E-12	75.8
38	0	100.0	2	197355	197392	2.00E-12	75.8
38	1	97.4	1	32623	32660	1.00E-10	67.9
38	1	97.4	1	39062	39099	1.00E-10	67.9
38	1	97.4	3	62696	62733	3.00E-10	67.9
38	1	97.4	3	84501	84538	3.00E-10	67.9
38	1	97.4	2	102175	102212	4.00E-10	67.9
38	1	97.4	2	156766	156803	4.00E-10	67.9
38	2	94.7	6	25962	25999	1.00E-08	60.0
38	2	94.7	1	9306	9343	3.00E-08	60.0
38	2	94.7	1	41769	41806	3.00E-08	60.0
38	2	94.7	1	25655	25692	3.00E-08	60.0
38	2	94.7	5	22255	22292	3.00E-08	60.0
38	2	94.7	3	43855	43892	8.00E-08	60.0
38	2	94.7	3	63578	63615	8.00E-08	60.0
38	2	94.7	3	44547	44584	8.00E-08	60.0
38	2	94.7	3	71577	71614	8.00E-08	60.0
38	2	94.7	2	9334	9371	1.00E-07	60.0
38	2	94.7	2	82607	82644	1.00E-07	60.0
38	2	94.7	2	123834	123871	1.00E-07	60.0
38	2	94.7	2	188081	188118	1.00E-07	60.0
38	2	94.7	2	14006	14043	1.00E-07	60.0
37	1	97.3	4	6846	6882	6.00E-10	65.9

37	1	97.3	4	53187	53223	6.00E-10	65.9
37	1	97.3	3	124545	124581	1.00E-09	65.9
37	1	97.3	3	76780	76816	1.00E-09	65.9
37	1	97.3	3	147493	147529	1.00E-09	65.9
37	1	97.3	2	50259	50295	2.00E-09	65.9
37	1	97.3	2	165529	165565	2.00E-09	65.9
37	1	97.3	2	19558	19594	2.00E-09	65.9
37	1	97.3	2	189487	189523	2.00E-09	65.9
37	1	97.3	2	23588	23624	2.00E-09	65.9
36	0	100.0	5	861	896	7.00E-12	71.9
36	0	100.0	4	45467	45502	1.00E-11	71.9
36	0	100.0	4	6280	6315	1.00E-11	71.9
36	0	100.0	3	65876	65911	2.00E-11	71.9
36	0	100.0	3	68475	68510	2.00E-11	71.9
36	0	100.0	3	42420	42455	2.00E-11	71.9
36	0	100.0	2	46322	46357	3.00E-11	71.9
36	0	100.0	2	139730	139765	3.00E-11	71.9
36	0	100.0	2	137436	137471	3.00E-11	71.9
36	0	100.0	2	137434	137469	3.00E-11	71.9
36	0	100.0	2	24795	24830	3.00E-11	71.9
36	0	100.0	2	53563	53598	3.00E-11	71.9
36	1	97.2	4	34047	34082	2.00E-09	63.9
36	1	97.2	4	33081	33116	2.00E-09	63.9
36	1	97.2	5	31266	31301	2.00E-09	63.9
36	1	97.2	3	68475	68510	5.00E-09	63.9
36	1	97.2	3	17615	17650	5.00E-09	63.9
36	1	97.2	3	100901	100936	5.00E-09	63.9
36	1	97.2	2	188089	188124	7.00E-09	63.9
36	1	97.2	2	159252	159287	7.00E-09	63.9
36	1	97.2	2	147873	147908	7.00E-09	63.9
36	1	97.2	2	102982	103017	7.00E-09	63.9
35	0	100.0	6	10477	10511	1.00E-11	69.9
35	0	100.0	6	7956	7990	1.00E-11	69.9
35	0	100.0	1	47818	47852	3.00E-11	69.9
35	0	100.0	1	28546	28580	3.00E-11	69.9
35	0	100.0	1	47818	47852	3.00E-11	69.9
35	0	100.0	4	23824	23858	4.00E-11	69.9
35	0	100.0	3	34211	34245	8.00E-11	69.9
35	0	100.0	3	49272	49306	8.00E-11	69.9
35	0	100.0	3	53286	53320	8.00E-11	69.9
35	0	100.0	2	139731	139765	1.00E-10	69.9
35	0	100.0	2	46322	46356	1.00E-10	69.9
35	0	100.0	2	156765	156799	1.00E-10	69.9
35	0	100.0	2	79408	79442	1.00E-10	69.9

35	0	100.0	2	114674	114708	1.00E-10	69.9
35	0	100.0	2	39444	39478	1.00E-10	69.9
35	0	100.0	2	156765	156799	1.00E-10	69.9
35	0	100.0	2	71868	71902	1.00E-10	69.9
35	0	100.0	2	100259	100293	1.00E-10	69.9
35	0	100.0	2	14008	14042	1.00E-10	69.9
35	0	100.0	2	14386	14420	1.00E-10	69.9
35	1	97.1	5	35689	35723	7.00E-09	61.9
35	1	97.1	4	23827	23861	1.00E-08	61.9
35	1	97.1	4	70903	70937	1.00E-08	61.9
35	1	97.1	2	27316	27350	3.00E-08	61.9
34	0	100.0	5	5215	5248	1.00E-10	67.9
34	0	100.0	4	55158	55191	2.00E-10	67.9
34	0	100.0	4	3180	3213	2.00E-10	67.9
34	0	100.0	4	3	36	2.00E-10	67.9
34	0	100.0	3	109020	109053	3.00E-10	67.9
34	0	100.0	2	141626	141659	4.00E-10	67.9
34	0	100.0	2	137436	137469	4.00E-10	67.9
34	0	100.0	2	1261	1294	4.00E-10	67.9
34	1	97.1	1	39062	39095	3.00E-08	60.0
34	1	97.1	1	39062	39095	3.00E-08	60.0
34	1	97.1	4	12976	13009	4.00E-08	60.0
34	1	97.1	3	8769	8802	8.00E-08	60.0
34	1	97.1	3	8769	8802	8.00E-08	60.0
34	1	97.1	3	8769	8802	8.00E-08	60.0
34	1	97.1	2	188089	188122	1.00E-07	60.0
34	1	97.1	2	156766	156799	1.00E-07	60.0
34	1	97.1	2	139731	139764	1.00E-07	60.0
34	1	97.1	2	46323	46356	1.00E-07	60.0
34	1	97.1	2	139731	139764	1.00E-07	60.0
34	1	97.1	2	46323	46356	1.00E-07	60.0
33	0	100.0	5	31372	31404	4.00E-10	65.9
33	0	100.0	2	136772	136804	2.00E-09	65.9
32	0	100.0	4	68863	68894	2.00E-09	63.9
32	0	100.0	4	23827	23858	2.00E-09	63.9
32	0	100.0	3	36134	36165	5.00E-09	63.9
32	0	100.0	2	142320	142351	7.00E-09	63.9
31	0	100.0	1	40071	40101	8.00E-09	61.9
31	0	100.0	2	111055	111085	3.00E-08	61.9
31	0	100.0	2	176833	176863	3.00E-08	61.9
31	0	100.0	2	205446	205476	3.00E-08	61.9
30	0	100.0	6	299	328	1.00E-08	60.0
30	0	100.0	6	2	31	1.00E-08	60.0
30	0	100.0	6	6595	6624	1.00E-08	60.0

30	0	100.0	1	41694	41723	3.00E-08	60.0
30	0	100.0	5	34728	34757	3.00E-08	60.0
30	0	100.0	4	53673	53702	4.00E-08	60.0
30	0	100.0	3	73009	73038	8.00E-08	60.0
30	0	100.0	3	47170	47199	8.00E-08	60.0
30	0	100.0	3	98730	98759	8.00E-08	60.0
30	0	100.0	3	98730	98759	8.00E-08	60.0
30	0	100.0	3	98730	98759	8.00E-08	60.0
30	0	100.0	3	98730	98759	8.00E-08	60.0
30	0	100.0	3	84323	84352	8.00E-08	60.0
30	0	100.0	3	76869	76898	8.00E-08	60.0
30	0	100.0	3	8330	8359	8.00E-08	60.0
30	0	100.0	2	14394	14423	1.00E-07	60.0
30	0	100.0	2	65905	65934	1.00E-07	60.0
30	0	100.0	2	23536	23565	1.00E-07	60.0
30	0	100.0	2	164299	164328	1.00E-07	60.0
30	0	100.0	2	146239	146268	1.00E-07	60.0
30	0	100.0	2	6817	6846	1.00E-07	60.0
30	0	100.0	2	77708	77737	1.00E-07	60.0

[1]Identity calculations do not include sites that contain indels.

M. vanderietiae

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
1630	268	83.2	1	222491	224114	0	1061.0
1630	268	83.2	7	41375	43004	0	1061.0
701	0	100.0	1	25803	26503	0	1390.0
701	0	100.0	3	59834	60534	0	1390.0
473	85	82.0	5	4015	4487	2.00E-70	264.0
473	85	82.0	1	214720	215192	5.00E-69	264.0
346	48	86.1	7	40965	41310	3.00E-82	305.0
346	48	86.1	1	224179	224524	1.00E-81	305.0
281	20	90.0	1	272258	272533	2.00E-89	331.0
281	20	90.0	1	299270	299547	2.00E-89	331.0
267	4	98.5	7	14761	15027	4.00E-140	498.0
267	4	98.5	1	163135	163401	2.00E-139	498.0
253	7	97.2	1	62946	63198	6.00E-124	446.0
253	7	97.2	1	192032	192284	6.00E-124	446.0
246	41	83.3	7	43090	43335	3.00E-39	163.0
246	41	83.3	1	222160	222405	1.00E-38	163.0
221	45	79.6	1	16122	16342	4.00E-14	81.8
221	45	79.6	1	214379	214599	4.00E-14	81.8
198	11	91.9	6	5707	5904	3.00E-71	266.0
198	11	91.9	3	16849	17041	2.00E-70	266.0
173	7	94.2	7	47352	47521	2.00E-68	260.0

173	7	94.2	1	231021	231193	7.00E-68	260.0
158	11	93.0	5	2912	3069	5.00E-59	226.0
158	11	93.0	1	231151	231308	1.00E-57	226.0
143	10	93.0	4	34436	34578	5.00E-52	204.0
143	10	93.0	1	267640	267782	4.00E-51	204.0
138	17	87.7	1	53710	53847	2.00E-31	139.0
138	17	87.7	1	234086	234223	2.00E-31	139.0
134	25	81.3	1	15735	15868	6.00E-10	67.9
134	25	81.3	1	214863	214996	6.00E-10	67.9
129	23	82.2	5	4158	4286	4.00E-13	73.8
129	23	82.2	1	15740	15868	9.00E-12	73.8
127	7	94.5	6	1596	1722	3.00E-50	196.0
127	7	94.5	1	252454	252580	9.00E-49	196.0
115	0	95.7	3	7933	8047	4.00E-47	188.0
115	0	95.7	1	22211	22320	2.00E-46	188.0
112	9	87.5	4	23304	23410	6.00E-24	111.0
112	9	87.5	1	40372	40483	4.00E-23	111.0
112	19	83.0	5	3776	3887	2.00E-12	71.9
112	19	83.0	1	214481	214592	4.00E-11	71.9
107	13	87.9	1	271891	271997	2.00E-22	109.0
107	13	87.9	1	299811	299917	2.00E-22	109.0
101	0	100.0	2	937	1037	1.00E-51	200.0
101	0	100.0	7	47701	47801	1.00E-50	200.0
96	0	100.0	7	47561	47656	1.00E-47	190.0
96	0	100.0	1	298934	299029	6.00E-47	190.0
93	2	96.8	1	145952	146044	1.00E-35	153.0
93	2	96.8	1	210984	211075	1.00E-35	153.0
93	7	90.3	1	62824	62914	7.00E-22	107.0
93	7	90.3	1	77432	77524	7.00E-22	107.0
92	2	97.8	7	47352	47443	2.00E-40	167.0
92	2	97.8	1	273510	273601	8.00E-40	167.0
91	5	94.5	1	32638	32728	5.00E-32	141.0
91	5	94.5	1	149447	149537	5.00E-32	141.0
89	0	98.9	4	1	88	7.00E-39	161.0
89	0	98.9	1	109173	109261	5.00E-38	161.0
86	7	90.7	1	53168	53253	2.00E-19	99.6
86	7	90.7	1	234671	234755	2.00E-19	99.6
82	1	98.8	6	8643	8724	9.00E-38	155.0
82	1	98.8	6	8643	8724	9.00E-38	155.0
82	1	98.8	1	272349	272430	3.00E-36	155.0
82	1	98.8	1	299378	299459	3.00E-36	155.0
80	2	97.5	5	2996	3075	6.00E-34	143.0
80	2	97.5	1	25782	25861	1.00E-32	143.0
79	1	98.7	7	46973	47051	5.00E-35	149.0

79	1	98.7	1	849	927	2.00E-34	149.0
79	5	93.7	3	42541	42619	1.00E-25	117.0
79	5	93.7	1	204867	204945	7.00E-25	117.0
78	1	98.7	1	191316	191393	8.00E-34	147.0
78	1	98.7	1	266014	266091	8.00E-34	147.0
78	2	97.4	1	165729	165806	2.00E-31	139.0
78	2	97.4	1	293019	293096	2.00E-31	139.0
77	4	94.8	7	2347	2423	1.00E-26	121.0
77	4	94.8	1	31414	31490	4.00E-26	121.0
77	1	92.2	2	4823	4899	5.00E-23	105.0
77	1	92.2	1	99150	99221	3.00E-21	105.0
76	0	100.0	3	34637	34712	1.00E-35	151.0
76	0	100.0	1	56110	56185	5.00E-35	151.0
76	0	93.4	1	272965	273035	4.00E-23	111.0
76	0	93.4	1	299048	299123	4.00E-23	111.0
75	1	98.7	7	62226	62300	1.00E-32	141.0
75	1	98.7	1	205726	205800	5.00E-32	141.0
75	4	94.7	1	1433	1507	7.00E-25	117.0
75	4	94.7	1	196169	196243	7.00E-25	117.0
75	9	88.0	1	53894	53968	6.00E-13	77.8
75	9	88.0	1	233966	234040	6.00E-13	77.8
74	1	98.7	4	250	323	3.00E-32	139.0
74	1	98.7	3	57991	58064	4.00E-32	139.0
72	9	87.5	1	53350	53421	4.00E-11	71.9
72	9	87.5	1	234503	234574	4.00E-11	71.9
71	5	93.0	2	1087	1157	8.00E-22	101.0
71	5	93.0	1	231235	231305	4.00E-20	101.0
71	3	87.3	3	23184	23248	7.00E-09	61.9
71	3	87.3	1	114726	114796	4.00E-08	61.9
70	0	98.6	2	1036	1105	2.00E-28	123.0
70	0	98.6	2	1036	1105	2.00E-28	123.0
70	0	98.6	7	47562	47630	3.00E-27	123.0
70	0	98.6	1	298960	299028	1.00E-26	123.0
68	0	100.0	1	272777	272844	3.00E-30	135.0
68	0	100.0	1	299199	299266	3.00E-30	135.0
68	1	98.5	7	73117	73184	2.00E-28	127.0
68	1	98.5	1	231036	231103	7.00E-28	127.0
68	2	97.1	7	73117	73184	4.00E-26	119.0
68	2	97.1	1	273525	273592	2.00E-25	119.0
68	2	88.2	1	33666	33733	9.00E-12	73.8
68	2	88.2	1	39531	39592	9.00E-12	73.8
66	0	100.0	5	43	108	2.00E-30	131.0
66	0	100.0	7	897	962	1.00E-29	131.0
66	0	100.0	1	250182	250247	5.00E-29	131.0

66	0	100.0	1	30940	31005	5.00E-29	131.0
66	0	92.4	6	5619	5679	1.00E-18	91.7
66	0	92.4	6	5619	5679	1.00E-18	91.7
66	0	92.4	5	2959	3024	2.00E-18	91.7
66	0	92.4	1	231198	231263	4.00E-17	91.7
64	2	96.9	3	52570	52633	8.00E-24	111.0
64	2	96.9	1	260718	260781	4.00E-23	111.0
64	3	87.5	1	254225	254283	9.00E-09	63.9
64	3	87.5	1	299055	299118	9.00E-09	63.9
62	0	100.0	3	14534	14595	2.00E-27	123.0
62	0	100.0	7	47458	47519	3.00E-27	123.0
62	3	95.2	3	52566	52627	3.00E-20	99.6
62	3	95.2	7	13005	13066	4.00E-20	99.6
60	1	98.3	7	14100	14159	1.00E-23	111.0
60	1	98.3	1	111771	111830	4.00E-23	111.0
59	0	100.0	5	3017	3075	3.00E-26	117.0
59	0	100.0	3	60476	60534	1.00E-25	117.0
59	1	98.3	3	18178	18236	3.00E-23	109.0
59	1	98.3	1	258403	258461	2.00E-22	109.0
58	0	100.0	2	1	58	5.00E-26	115.0
58	0	100.0	6	5523	5580	8.00E-26	115.0
58	0	100.0	5	14323	14380	1.00E-25	115.0
58	0	100.0	5	1	58	1.00E-25	115.0
58	0	100.0	4	41555	41612	4.00E-25	115.0
58	0	100.0	7	22032	22089	7.00E-25	115.0
58	0	100.0	7	10890	10947	7.00E-25	115.0
58	0	100.0	1	109344	109401	3.00E-24	115.0
58	0	100.0	1	306739	306796	3.00E-24	115.0
58	0	100.0	1	191472	191529	3.00E-24	115.0
58	0	100.0	1	1	58	3.00E-24	115.0
58	0	100.0	1	57964	58021	3.00E-24	115.0
58	1	98.3	7	13004	13061	2.00E-22	107.0
58	1	98.3	7	73220	73277	2.00E-22	107.0
58	1	98.3	1	231136	231193	7.00E-22	107.0
58	1	98.3	1	260723	260780	7.00E-22	107.0
57	1	98.3	7	1	57	6.00E-22	105.0
57	1	98.3	1	231137	231193	3.00E-21	105.0
55	0	100.0	3	14541	14595	3.00E-23	109.0
55	0	100.0	3	14541	14595	3.00E-23	109.0
55	0	100.0	7	73221	73275	4.00E-23	109.0
55	0	100.0	7	1	55	4.00E-23	109.0
55	1	98.2	3	14541	14595	8.00E-21	101.0
55	1	98.2	1	231137	231191	4.00E-20	101.0
55	5	90.9	2	4869	4923	3.00E-12	69.9

55	5	90.9	4	11218	11272	2.00E-11	69.9
54	1	98.2	5	14039	14092	8.00E-21	99.6
54	1	98.2	1	285429	285482	2.00E-19	99.6
54	5	90.7	3	42572	42625	1.00E-10	67.9
54	5	90.7	3	4848	4901	1.00E-10	67.9
54	5	90.7	7	24094	24147	1.00E-10	67.9
54	5	90.7	1	71609	71662	6.00E-10	67.9
54	6	88.9	6	5525	5578	4.00E-09	60.0
54	6	88.9	1	50946	50999	1.00E-07	60.0
53	0	100.0	5	135	187	1.00E-22	105.0
53	0	100.0	7	14997	15049	6.00E-22	105.0
53	0	100.0	1	194758	194810	3.00E-21	105.0
53	0	100.0	1	108180	108232	3.00E-21	105.0
53	1	98.1	7	31345	31397	2.00E-19	97.6
53	1	98.1	1	51849	51901	6.00E-19	97.6
53	1	98.1	1	216384	216436	6.00E-19	97.6
53	1	98.1	1	90784	90836	6.00E-19	97.6
52	1	98.1	7	73227	73278	6.00E-19	95.6
52	1	98.1	7	47471	47522	6.00E-19	95.6
52	1	98.1	7	7	58	6.00E-19	95.6
52	1	98.1	1	273626	273677	3.00E-18	95.6
52	1	98.1	1	273626	273677	3.00E-18	95.6
52	1	98.1	1	273626	273677	3.00E-18	95.6
49	0	100.0	4	28369	28417	9.00E-20	97.6
49	0	100.0	1	156194	156242	6.00E-19	97.6
49	1	98.0	3	14547	14595	3.00E-17	89.7
49	1	98.0	1	273626	273674	2.00E-16	89.7
49	2	95.9	3	6566	6614	8.00E-15	81.8
49	2	95.9	1	101077	101125	4.00E-14	81.8
49	2	95.9	1	159120	159168	4.00E-14	81.8
49	2	95.9	1	234162	234210	4.00E-14	81.8
48	0	100.0	3	6593	6640	5.00E-19	95.6
48	0	100.0	3	34447	34494	5.00E-19	95.6
48	0	100.0	1	199615	199662	3.00E-18	95.6
48	0	100.0	1	258419	258466	3.00E-18	95.6
48	1	97.9	6	5621	5668	2.00E-17	87.7
48	1	97.9	6	2163	2210	2.00E-17	87.7
48	1	97.9	7	50788	50835	1.00E-16	87.7
48	1	97.9	1	48903	48950	6.00E-16	87.7
47	0	100.0	4	3702	3748	1.00E-18	93.7
47	0	100.0	3	13857	13903	2.00E-18	93.7
47	2	95.7	4	11652	11698	8.00E-14	77.8
47	2	95.7	1	251899	251945	6.00E-13	77.8
47	4	91.5	4	16397	16443	5.00E-09	61.9

47	4	91.5	7	62175	62221	8.00E-09	61.9
46	3	93.5	1	50814	50859	6.00E-10	67.9
46	3	93.5	1	232852	232897	6.00E-10	67.9
46	4	91.3	4	26911	26956	2.00E-08	60.0
46	4	91.3	1	258358	258403	1.00E-07	60.0
45	1	97.8	7	47164	47208	9.00E-15	81.8
45	1	97.8	1	87029	87073	4.00E-14	81.8
45	2	95.6	6	8680	8724	3.00E-13	73.8
45	2	95.6	1	208752	208796	9.00E-12	73.8
45	2	95.6	1	299378	299422	9.00E-12	73.8
45	2	95.6	1	208752	208796	9.00E-12	73.8
44	3	93.2	7	61007	61050	2.00E-09	63.9
44	3	93.2	1	68849	68892	9.00E-09	63.9
43	0	100.0	3	6598	6640	5.00E-16	85.7
43	0	100.0	3	18194	18236	5.00E-16	85.7
43	2	95.4	5	2912	2954	7.00E-12	69.9
43	2	95.4	5	2912	2954	7.00E-12	69.9
43	2	95.4	5	2912	2954	7.00E-12	69.9
43	2	95.4	7	73235	73277	3.00E-11	69.9
43	2	95.4	7	47479	47521	3.00E-11	69.9
43	2	95.4	7	15	57	3.00E-11	69.9
43	3	93.0	5	2912	2954	2.00E-09	61.9
43	3	93.0	1	273634	273676	4.00E-08	61.9
42	0	100.0	1	5184	5225	1.00E-14	83.8
42	0	100.0	1	10530	10571	1.00E-14	83.8
42	1	97.6	2	1940	1981	4.00E-14	75.8
42	1	97.6	3	14611	14652	5.00E-13	75.8
42	1	97.6	1	254281	254322	2.00E-12	75.8
42	1	97.6	1	160074	160115	2.00E-12	75.8
42	2	95.2	7	26641	26682	1.00E-10	67.9
42	2	95.2	1	102637	102678	6.00E-10	67.9
41	0	100.0	1	182231	182271	4.00E-14	81.8
41	0	100.0	1	210206	210246	4.00E-14	81.8
41	2	95.1	5	2912	2952	1.00E-10	65.9
41	2	95.1	3	13745	13785	4.00E-10	65.9
41	2	95.1	3	14555	14595	4.00E-10	65.9
41	2	95.1	1	267870	267910	2.00E-09	65.9
41	2	95.1	1	305986	306026	2.00E-09	65.9
41	2	95.1	1	156779	156819	2.00E-09	65.9
40	0	100.0	7	100	139	4.00E-14	79.8
40	0	100.0	1	273670	273709	2.00E-13	79.8
40	1	97.5	6	8568	8607	1.00E-12	71.9
40	1	97.5	3	3345	3384	7.00E-12	71.9
40	2	95.0	4	11745	11784	1.00E-09	63.9

40	2	95.0	3	13747	13786	2.00E-09	63.9
40	2	95.0	7	59150	59189	2.00E-09	63.9
40	2	95.0	1	156811	156850	9.00E-09	63.9
39	0	100.0	4	1237	1275	8.00E-14	77.8
39	0	100.0	3	11457	11495	1.00E-13	77.8
39	0	100.0	3	31910	31948	1.00E-13	77.8
39	0	100.0	7	59145	59183	1.00E-13	77.8
39	0	100.0	1	176770	176808	6.00E-13	77.8
39	0	100.0	1	68849	68887	6.00E-13	77.8
39	1	97.4	6	496	534	4.00E-12	69.9
39	1	97.4	1	241180	241218	1.00E-10	69.9
39	2	94.9	6	5676	5714	1.00E-09	61.9
39	2	94.9	3	6565	6603	7.00E-09	61.9
39	2	94.9	1	160292	160330	4.00E-08	61.9
39	2	94.9	1	46970	47008	4.00E-08	61.9
39	2	94.9	1	306473	306511	4.00E-08	61.9
39	2	94.9	1	183868	183906	4.00E-08	61.9
38	0	100.0	5	2988	3025	1.00E-13	75.8
38	0	100.0	3	17835	17872	5.00E-13	75.8
38	0	100.0	3	20275	20312	5.00E-13	75.8
38	0	100.0	1	210028	210065	2.00E-12	75.8
38	0	100.0	1	305420	305457	2.00E-12	75.8
38	0	100.0	1	273054	273091	2.00E-12	75.8
38	1	97.4	6	8379	8416	2.00E-11	67.9
38	1	97.4	4	5313	5350	8.00E-11	67.9
38	1	97.4	4	16394	16431	8.00E-11	67.9
38	1	97.4	3	40793	40830	1.00E-10	67.9
38	1	97.4	7	59145	59182	1.00E-10	67.9
38	1	97.4	7	26641	26678	1.00E-10	67.9
38	1	97.4	7	45570	45607	1.00E-10	67.9
38	1	97.4	1	102641	102678	6.00E-10	67.9
38	1	97.4	1	68850	68887	6.00E-10	67.9
38	1	97.4	1	169694	169731	6.00E-10	67.9
38	2	94.7	4	39156	39193	2.00E-08	60.0
38	2	94.7	3	48782	48819	3.00E-08	60.0
38	2	94.7	1	163483	163520	1.00E-07	60.0
38	2	94.7	1	188589	188626	1.00E-07	60.0
38	2	94.7	1	256854	256891	1.00E-07	60.0
38	2	94.7	1	300221	300258	1.00E-07	60.0
37	0	100.0	6	5643	5679	3.00E-13	73.8
37	0	100.0	3	44938	44974	2.00E-12	73.8
37	0	100.0	3	46428	46464	2.00E-12	73.8
37	0	100.0	3	20277	20313	2.00E-12	73.8
37	0	100.0	3	29842	29878	2.00E-12	73.8

37	0	100.0	3	44938	44974	2.00E-12	73.8
37	0	100.0	3	46428	46464	2.00E-12	73.8
37	0	100.0	7	38547	38583	2.00E-12	73.8
37	0	100.0	7	38547	38583	2.00E-12	73.8
37	0	100.0	1	43585	43621	9.00E-12	73.8
37	0	100.0	1	184160	184196	9.00E-12	73.8
37	0	100.0	1	202458	202494	9.00E-12	73.8
37	0	100.0	1	303306	303342	9.00E-12	73.8
37	0	100.0	1	273054	273090	9.00E-12	73.8
37	1	97.3	4	23430	23466	3.00E-10	65.9
37	1	97.3	4	11752	11788	3.00E-10	65.9
37	1	97.3	7	4901	4937	5.00E-10	65.9
37	1	97.3	7	55488	55524	5.00E-10	65.9
37	1	97.3	7	26642	26678	5.00E-10	65.9
37	1	97.3	1	43583	43619	2.00E-09	65.9
37	1	97.3	1	22436	22472	2.00E-09	65.9
37	1	97.3	1	102640	102676	2.00E-09	65.9
37	1	97.3	1	44869	44905	2.00E-09	65.9
37	1	97.3	1	102638	102674	2.00E-09	65.9
37	1	97.3	1	285503	285539	2.00E-09	65.9
37	1	97.3	1	40323	40359	2.00E-09	65.9
36	0	100.0	4	11749	11784	5.00E-12	71.9
36	0	100.0	3	29050	29085	7.00E-12	71.9
36	0	100.0	7	55487	55522	9.00E-12	71.9
36	0	100.0	7	59147	59182	9.00E-12	71.9
36	0	100.0	7	59316	59351	9.00E-12	71.9
36	0	100.0	7	61017	61052	9.00E-12	71.9
36	0	100.0	1	210028	210063	4.00E-11	71.9
36	0	100.0	1	303306	303341	4.00E-11	71.9
36	0	100.0	1	43583	43618	4.00E-11	71.9
36	0	100.0	1	68850	68885	4.00E-11	71.9
36	0	100.0	1	43583	43618	4.00E-11	71.9
36	0	100.0	1	184162	184197	4.00E-11	71.9
36	1	97.2	3	20277	20312	2.00E-09	63.9
36	1	97.2	3	29049	29084	2.00E-09	63.9
36	1	97.2	3	20277	20312	2.00E-09	63.9
36	1	97.2	7	44751	44786	2.00E-09	63.9
36	1	97.2	7	8843	8878	2.00E-09	63.9
36	1	97.2	1	87067	87102	9.00E-09	63.9
36	1	97.2	1	279893	279928	9.00E-09	63.9
36	1	97.2	1	303306	303341	9.00E-09	63.9
36	1	97.2	1	303306	303341	9.00E-09	63.9
36	1	97.2	1	258976	259011	9.00E-09	63.9
36	1	97.2	1	87067	87102	9.00E-09	63.9

36	1	97.2	1	279893	279928	9.00E-09	63.9
36	1	97.2	1	142059	142094	9.00E-09	63.9
36	1	97.2	1	43584	43619	9.00E-09	63.9
35	0	100.0	7	55488	55522	3.00E-11	69.9
35	0	100.0	1	43585	43619	1.00E-10	69.9
35	1	97.1	2	1123	1157	7.00E-10	61.9
35	1	97.1	5	2996	3030	2.00E-09	61.9
35	1	97.1	7	59316	59350	8.00E-09	61.9
35	1	97.1	7	8844	8878	8.00E-09	61.9
35	1	97.1	7	8843	8877	8.00E-09	61.9
35	1	97.1	1	258977	259011	4.00E-08	61.9
35	1	97.1	1	184162	184196	4.00E-08	61.9
35	1	97.1	1	68850	68884	4.00E-08	61.9
34	0	100.0	4	1237	1270	8.00E-11	67.9
34	0	100.0	3	31939	31972	1.00E-10	67.9
34	0	100.0	7	59149	59182	1.00E-10	67.9
34	0	100.0	7	26646	26679	1.00E-10	67.9
34	0	100.0	7	55488	55521	1.00E-10	67.9
34	0	100.0	7	26646	26679	1.00E-10	67.9
34	0	100.0	1	183872	183905	6.00E-10	67.9
34	0	100.0	1	210212	210245	6.00E-10	67.9
34	0	100.0	1	184163	184196	6.00E-10	67.9
34	0	100.0	1	68850	68883	6.00E-10	67.9
34	0	100.0	1	184162	184195	6.00E-10	67.9
34	0	100.0	1	43586	43619	6.00E-10	67.9
34	1	97.1	4	11751	11784	2.00E-08	60.0
34	1	97.1	4	11752	11785	2.00E-08	60.0
34	1	97.1	3	1435	1468	3.00E-08	60.0
34	1	97.1	7	12546	12579	3.00E-08	60.0
34	1	97.1	1	50824	50857	1.00E-07	60.0
34	1	97.1	1	273062	273095	1.00E-07	60.0
34	1	97.1	1	43655	43688	1.00E-07	60.0
34	1	97.1	1	68849	68882	1.00E-07	60.0
33	0	100.0	4	11752	11784	3.00E-10	65.9
33	0	100.0	7	12547	12579	5.00E-10	65.9
33	0	100.0	7	55488	55520	5.00E-10	65.9
33	0	100.0	7	59149	59181	5.00E-10	65.9
33	0	100.0	7	61017	61049	5.00E-10	65.9
33	0	100.0	7	12547	12579	5.00E-10	65.9
33	0	100.0	7	12547	12579	5.00E-10	65.9
33	0	100.0	1	43585	43617	2.00E-09	65.9
33	0	100.0	1	123251	123283	2.00E-09	65.9
33	0	100.0	1	102641	102673	2.00E-09	65.9
33	0	100.0	1	123251	123283	2.00E-09	65.9

33	0	100.0	1	123251	123283	2.00E-09	65.9
33	0	100.0	1	68850	68882	2.00E-09	65.9
33	0	100.0	1	184163	184195	2.00E-09	65.9
33	0	100.0	1	43586	43618	2.00E-09	65.9
33	0	100.0	1	102641	102673	2.00E-09	65.9
32	0	100.0	5	878	909	4.00E-10	63.9
32	0	100.0	7	25645	25676	2.00E-09	63.9
32	0	100.0	7	50960	50991	2.00E-09	63.9
32	0	100.0	7	12547	12578	2.00E-09	63.9
32	0	100.0	7	26646	26677	2.00E-09	63.9
32	0	100.0	1	47184	47215	9.00E-09	63.9
32	0	100.0	1	76809	76840	9.00E-09	63.9
32	0	100.0	1	123251	123282	9.00E-09	63.9
32	0	100.0	1	123251	123282	9.00E-09	63.9
32	0	100.0	1	160598	160629	9.00E-09	63.9
31	0	100.0	3	31495	31525	7.00E-09	61.9
31	0	100.0	1	11149	11179	4.00E-08	61.9
31	0	100.0	1	187664	187694	4.00E-08	61.9
31	0	100.0	1	125744	125774	4.00E-08	61.9
30	0	100.0	3	18643	18672	3.00E-08	60.0
30	0	100.0	3	29820	29849	3.00E-08	60.0
30	0	100.0	3	40979	41008	3.00E-08	60.0
30	0	100.0	1	105533	105562	1.00E-07	60.0
30	0	100.0	1	132800	132829	1.00E-07	60.0
30	0	100.0	1	23668	23697	1.00E-07	60.0
30	0	100.0	1	108020	108049	1.00E-07	60.0
30	0	100.0	1	235009	235038	1.00E-07	60.0

[1]Identity calculations do not include sites that contain indels.

M. camdeboense

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
1630	267	83.3	1	23800	25429	0	1068.0
1630	267	83.3	2	169340	170963	0	1068.0
473	85	82.0	2	49985	50457	2.00E-69	264.0
473	85	82.0	2	178262	178734	2.00E-69	264.0
411	22	93.2	2	85934	86338	5.00E-169	595.0
411	22	93.2	2	162378	162788	5.00E-169	595.0
346	48	86.1	1	23390	23735	2.00E-82	305.0
346	48	86.1	2	168930	169275	7.00E-82	305.0
246	42	82.9	1	25515	25760	5.00E-37	155.0
246	42	82.9	2	171049	171294	2.00E-36	155.0
221	45	79.6	3	30886	31106	7.00E-15	81.8
221	45	79.6	2	178855	179075	2.00E-14	81.8
190	4	95.3	2	85086	85270	7.00E-82	305.0

190	4	95.3	2	112098	112287	7.00E-82	305.0
164	4	95.7	2	81026	81186	6.00E-70	266.0
164	4	95.7	2	135049	135212	6.00E-70	266.0
138	16	88.4	3	51496	51633	1.00E-34	147.0
138	16	88.4	2	159123	159260	4.00E-34	147.0
134	25	81.3	3	30499	30632	1.00E-10	67.9
134	25	81.3	2	178458	178591	3.00E-10	67.9
129	23	82.2	3	30504	30632	2.00E-12	73.8
129	23	82.2	2	50128	50256	5.00E-12	73.8
127	7	94.5	3	15329	15455	2.00E-49	196.0
127	7	94.5	2	140770	140896	5.00E-49	196.0
112	19	83.0	2	49746	49857	2.00E-11	71.9
112	19	83.0	2	178862	178973	2.00E-11	71.9
102	2	94.1	3	10204	10301	2.00E-36	153.0
102	2	94.1	2	85791	85892	6.00E-36	153.0
93	2	96.8	2	11099	11191	6.00E-36	153.0
93	2	96.8	2	182379	182470	6.00E-36	153.0
93	7	90.3	4	47432	47524	1.00E-22	107.0
93	7	90.3	2	61835	61925	3.00E-22	107.0
91	5	94.5	3	72745	72835	9.00E-33	141.0
91	5	94.5	2	7606	7696	2.00E-32	141.0
89	8	91.0	2	48951	49039	6.00E-24	113.0
89	8	91.0	2	162038	162126	6.00E-24	113.0
86	7	90.7	3	52096	52181	3.00E-20	99.6
86	7	90.7	2	158591	158675	8.00E-20	99.6
80	2	97.5	3	40679	40758	2.00E-33	143.0
80	2	97.5	2	48966	49045	6.00E-33	143.0
79	2	97.5	2	85552	85630	2.00E-32	141.0
79	2	97.5	2	130656	130734	2.00E-32	141.0
75	1	98.7	1	48640	48714	8.00E-33	141.0
75	1	98.7	2	187654	187728	2.00E-32	141.0
75	4	94.7	3	16078	16152	1.00E-25	117.0
75	4	94.7	2	197211	197285	4.00E-25	117.0
75	9	88.0	3	51375	51449	1.00E-13	77.8
75	9	88.0	2	159306	159380	3.00E-13	77.8
74	1	98.7	4	6776	6849	3.00E-32	139.0
74	1	98.7	3	42921	42994	4.00E-32	139.0
74	10	86.5	3	40679	40752	1.00E-10	67.9
74	10	86.5	2	162038	162111	3.00E-10	67.9
72	9	87.5	3	51928	51999	7.00E-12	71.9
72	9	87.5	2	158772	158843	2.00E-11	71.9
71	5	93.0	1	32953	33023	7.00E-21	101.0
71	5	93.0	2	162041	162111	2.00E-20	101.0
70	0	98.6	1	33005	33074	2.00E-27	123.0

70	0	98.6	2	111947	112015	6.00E-27	123.0
68	1	98.5	1	59535	59602	1.00E-28	127.0
68	1	98.5	2	162387	162454	4.00E-28	127.0
68	2	97.1	1	59535	59602	3.00E-26	119.0
68	2	97.1	2	86262	86329	9.00E-26	119.0
68	2	88.2	3	65881	65942	2.00E-12	73.8
68	2	88.2	3	71740	71807	2.00E-12	73.8
67	1	91.0	3	10264	10325	5.00E-16	85.7
67	1	91.0	2	48929	48995	1.00E-15	85.7
66	0	100.0	1	29936	30001	7.00E-30	131.0
66	0	100.0	1	59639	59704	7.00E-30	131.0
66	0	100.0	1	60535	60600	7.00E-30	131.0
66	0	100.0	2	110885	110950	2.00E-29	131.0
66	0	100.0	2	124126	124191	2.00E-29	131.0
66	0	100.0	2	143103	143168	2.00E-29	131.0
65	5	90.8	4	53318	53382	1.00E-12	73.8
65	5	90.8	2	188527	188590	5.00E-12	73.8
64	7	89.1	3	54581	54644	7.00E-12	71.9
64	7	89.1	2	36931	36994	2.00E-11	71.9
62	1	96.8	1	31706	31766	3.00E-20	99.6
62	1	96.8	2	82105	82166	8.00E-20	99.6
60	2	96.7	2	28053	28112	5.00E-21	103.0
60	2	96.7	2	200308	200367	5.00E-21	103.0
59	4	93.2	2	85707	85765	1.00E-15	85.7
59	4	93.2	2	139067	139125	1.00E-15	85.7
54	1	98.2	2	60009	60062	8.00E-20	99.6
54	1	98.2	2	98294	98347	8.00E-20	99.6
54	6	88.9	3	10366	10419	3.00E-08	60.0
54	6	88.9	3	54473	54526	3.00E-08	60.0
54	6	88.9	3	11827	11880	3.00E-08	60.0
54	6	88.9	2	188602	188655	7.00E-08	60.0
53	1	98.1	1	13770	13822	1.00E-19	97.6
53	1	98.1	3	53571	53623	1.00E-19	97.6
53	1	98.1	4	34120	34172	1.00E-19	97.6
53	1	98.1	2	177018	177070	3.00E-19	97.6
52	1	98.1	1	59645	59696	4.00E-19	95.6
52	1	98.1	1	29942	29993	4.00E-19	95.6
52	1	98.1	2	86363	86414	1.00E-18	95.6
52	1	98.1	2	86363	86414	1.00E-18	95.6
52	4	92.3	3	5983	6034	7.00E-12	71.9
52	4	92.3	2	162038	162089	2.00E-11	71.9
50	0	100.0	1	29777	29826	3.00E-20	99.6
50	0	100.0	1	30032	30081	3.00E-20	99.6
50	0	100.0	1	29777	29826	3.00E-20	99.6

50	0	100.0	1	31414	31463	3.00E-20	99.6
50	0	100.0	1	34156	34205	3.00E-20	99.6
50	0	100.0	1	29427	29476	3.00E-20	99.6
50	0	100.0	3	11212	11261	3.00E-20	99.6
50	0	100.0	4	6269	6318	3.00E-20	99.6
50	0	100.0	4	61764	61813	3.00E-20	99.6
50	0	100.0	2	66720	66769	8.00E-20	99.6
50	0	100.0	2	81944	81993	8.00E-20	99.6
50	0	100.0	2	111967	112016	8.00E-20	99.6
50	0	100.0	2	86247	86296	8.00E-20	99.6
50	0	100.0	2	162420	162469	8.00E-20	99.6
50	0	100.0	2	60853	60902	8.00E-20	99.6
50	0	100.0	2	120926	120975	8.00E-20	99.6
50	0	98.0	1	30033	30081	2.00E-15	83.8
50	0	98.0	1	33005	33054	2.00E-15	83.8
49	0	100.0	1	32975	33023	1.00E-19	97.6
49	0	100.0	3	5983	6031	1.00E-19	97.6
49	0	100.0	4	1	49	1.00E-19	97.6
49	0	100.0	2	41537	41585	3.00E-19	97.6
48	0	100.0	3	10276	10323	5.00E-19	95.6
48	0	100.0	3	56522	56569	5.00E-19	95.6
48	1	97.9	1	37202	37249	1.00E-16	87.7
48	1	97.9	3	14841	14888	1.00E-16	87.7
47	0	100.0	2	46101	46147	5.00E-18	93.7
47	0	100.0	2	122098	122144	5.00E-18	93.7
47	1	97.9	3	22294	22340	5.00E-16	85.7
47	1	97.9	3	22413	22459	5.00E-16	85.7
47	2	95.7	2	85123	85169	3.00E-13	77.8
47	2	95.7	2	184656	184702	3.00E-13	77.8
46	0	100.0	2	85727	85772	2.00E-17	91.7
46	0	100.0	2	112035	112080	2.00E-17	91.7
46	3	93.5	3	54613	54658	1.00E-10	67.9
46	3	93.5	2	160449	160494	3.00E-10	67.9
46	4	91.3	3	5976	6021	3.00E-08	60.0
46	4	91.3	2	138993	139038	7.00E-08	60.0
45	1	97.8	1	29589	29633	6.00E-15	81.8
45	1	97.8	4	37883	37927	6.00E-15	81.8
45	2	95.6	2	112206	112250	5.00E-12	73.8
45	2	95.6	2	184658	184702	5.00E-12	73.8
44	0	100.0	2	25791	25834	3.00E-16	87.7
44	0	100.0	2	67948	67991	3.00E-16	87.7
44	0	100.0	2	111991	112034	3.00E-16	87.7
44	0	100.0	2	158408	158451	3.00E-16	87.7
44	3	93.2	1	47421	47464	1.00E-09	63.9

44	3	93.2	4	56064	56107	1.00E-09	63.9
43	2	95.4	1	59653	59695	2.00E-11	69.9
43	2	95.4	1	29950	29992	2.00E-11	69.9
43	2	95.4	2	48882	48924	7.00E-11	69.9
43	2	95.4	2	48882	48924	7.00E-11	69.9
43	3	93.0	2	48882	48924	2.00E-08	61.9
43	3	93.0	2	86371	86413	2.00E-08	61.9
42	0	100.0	3	19829	19870	2.00E-15	83.8
42	0	100.0	3	25294	25335	2.00E-15	83.8
42	2	95.2	1	9066	9107	9.00E-11	67.9
42	2	95.2	4	22281	22322	9.00E-11	67.9
42	3	92.9	2	112042	112083	7.00E-08	60.0
42	3	92.9	2	139067	139108	7.00E-08	60.0
40	0	100.0	1	59738	59777	2.00E-14	79.8
40	0	100.0	2	86407	86446	8.00E-14	79.8
39	0	100.0	4	12	50	9.00E-14	77.8
39	0	100.0	4	56069	56107	9.00E-14	77.8
39	0	100.0	1	45559	45597	1.00E-13	77.8
39	0	100.0	2	41518	41556	3.00E-13	77.8
39	1	97.4	1	9065	9103	2.00E-11	69.9
39	1	97.4	1	45558	45596	2.00E-11	69.9
38	0	100.0	3	10264	10301	4.00E-13	75.8
38	0	100.0	4	37853	37890	4.00E-13	75.8
38	0	100.0	2	85791	85828	1.00E-12	75.8
38	0	100.0	2	29386	29423	1.00E-12	75.8
38	0	100.0	2	48958	48995	1.00E-12	75.8
38	0	100.0	2	162082	162119	1.00E-12	75.8
38	0	100.0	2	85791	85828	1.00E-12	75.8
38	0	100.0	2	148032	148069	1.00E-12	75.8
38	0	100.0	2	162082	162119	1.00E-12	75.8
38	0	100.0	2	92753	92790	1.00E-12	75.8
38	1	97.4	1	9066	9103	9.00E-11	67.9
38	1	97.4	1	45559	45596	9.00E-11	67.9
38	1	97.4	4	22281	22318	9.00E-11	67.9
38	1	97.4	4	56069	56106	9.00E-11	67.9
37	1	97.3	1	41902	41938	4.00E-10	65.9
37	1	97.3	3	37200	37236	4.00E-10	65.9
37	1	97.3	3	60567	60603	4.00E-10	65.9
37	1	97.3	3	61853	61889	4.00E-10	65.9
37	1	97.3	4	22283	22319	4.00E-10	65.9
37	1	97.3	4	37854	37890	4.00E-10	65.9
37	1	97.3	4	22285	22321	4.00E-10	65.9
37	1	97.3	2	158390	158426	1.00E-09	65.9
37	1	97.3	2	92753	92789	1.00E-09	65.9

37	1	97.3	2	184597	184633	1.00E-09	65.9
37	1	97.3	2	183391	183427	1.00E-09	65.9
37	1	97.3	2	183391	183427	1.00E-09	65.9
36	0	100.0	1	9071	9106	6.00E-12	71.9
36	0	100.0	1	41903	41938	6.00E-12	71.9
36	0	100.0	1	45561	45596	6.00E-12	71.9
36	0	100.0	4	56071	56106	6.00E-12	71.9
36	0	100.0	3	61854	61889	7.00E-12	71.9
36	0	100.0	3	61854	61889	7.00E-12	71.9
36	0	100.0	2	117359	117394	2.00E-11	71.9
36	0	100.0	2	183391	183426	2.00E-11	71.9
36	1	97.2	1	27176	27211	1.00E-09	63.9
36	1	97.2	4	37854	37889	1.00E-09	63.9
36	1	97.2	3	45933	45968	2.00E-09	63.9
36	1	97.2	2	92754	92789	5.00E-09	63.9
36	1	97.2	2	117359	117394	5.00E-09	63.9
36	1	97.2	2	15049	15084	5.00E-09	63.9
36	1	97.2	2	74200	74235	5.00E-09	63.9
36	1	97.2	2	117359	117394	5.00E-09	63.9
35	0	100.0	1	16780	16814	2.00E-11	69.9
35	0	100.0	1	41902	41936	2.00E-11	69.9
35	0	100.0	3	61853	61887	3.00E-11	69.9
35	0	100.0	2	75504	75538	7.00E-11	69.9
35	1	97.1	1	29792	29826	6.00E-09	61.9
35	1	97.1	1	59535	59569	6.00E-09	61.9
35	1	97.1	1	45730	45764	6.00E-09	61.9
35	1	97.1	1	32953	32987	6.00E-09	61.9
35	1	97.1	3	2380	2414	7.00E-09	61.9
35	1	97.1	2	134339	134373	2.00E-08	61.9
35	1	97.1	2	48966	49000	2.00E-08	61.9
35	1	97.1	2	180878	180912	2.00E-08	61.9
34	0	100.0	1	9071	9104	9.00E-11	67.9
34	0	100.0	1	41902	41935	9.00E-11	67.9
34	0	100.0	4	56073	56106	9.00E-11	67.9
34	0	100.0	3	61853	61886	1.00E-10	67.9
34	0	100.0	2	22755	22788	3.00E-10	67.9
34	0	100.0	2	75539	75572	3.00E-10	67.9
34	1	97.1	3	10260	10293	3.00E-08	60.0
34	1	97.1	3	54615	54648	3.00E-08	60.0
34	1	97.1	3	54615	54648	3.00E-08	60.0
34	1	97.1	2	85799	85832	7.00E-08	60.0
33	0	100.0	1	45563	45595	4.00E-10	65.9
33	0	100.0	1	41902	41934	4.00E-10	65.9
33	0	100.0	1	47431	47463	4.00E-10	65.9

33	0	100.0	3	61855	61887	4.00E-10	65.9
33	0	100.0	4	56073	56105	4.00E-10	65.9
33	0	100.0	4	22286	22318	4.00E-10	65.9
33	0	100.0	2	33860	33892	1.00E-09	65.9
33	0	100.0	2	33860	33892	1.00E-09	65.9
33	0	100.0	2	33860	33892	1.00E-09	65.9
33	0	100.0	2	33860	33892	1.00E-09	65.9
32	0	100.0	1	8070	8101	1.00E-09	63.9
32	0	100.0	1	37374	37405	1.00E-09	63.9
32	0	100.0	1	9071	9102	1.00E-09	63.9
32	0	100.0	4	48116	48147	1.00E-09	63.9
32	0	100.0	3	58257	58288	2.00E-09	63.9
32	0	100.0	2	18933	18964	5.00E-09	63.9
32	0	100.0	2	74843	74874	5.00E-09	63.9
32	0	100.0	2	33861	33892	5.00E-09	63.9
30	0	100.0	4	6585	6614	2.00E-08	60.0
30	0	100.0	4	19397	19426	2.00E-08	60.0
30	0	100.0	2	55064	55093	7.00E-08	60.0
30	0	100.0	2	43404	43433	7.00E-08	60.0
30	0	100.0	2	97919	97948	7.00E-08	60.0
30	0	100.0	2	85716	85745	7.00E-08	60.0
30	0	100.0	2	24314	24343	7.00E-08	60.0
30	0	100.0	2	121125	121154	7.00E-08	60.0

[1]Identity calculations do not include sites that contain indels.

M. ciliata

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
1309	210	84.0	1	116980	118288	0	930.0
1309	210	84.0	1	142267	143575	0	930.0
346	48	86.1	1	118668	119013	9.00E-82	305.0
346	48	86.1	1	141549	141894	9.00E-82	305.0
291	54	81.4	1	118313	118603	1.00E-34	149.0
291	54	81.4	1	141959	142249	1.00E-34	149.0
246	41	83.3	1	116649	116894	9.00E-39	163.0
246	41	83.3	1	143661	143906	9.00E-39	163.0
143	10	93.0	2	115635	115777	1.00E-51	204.0
143	10	93.0	1	40797	40939	3.00E-51	204.0
127	7	94.5	3	11248	11374	3.00E-50	196.0
127	7	94.5	1	219187	219313	6.00E-49	196.0
107	10	90.7	1	33235	33341	8.00E-30	133.0
107	10	90.7	1	36585	36691	8.00E-30	133.0
95	6	93.7	1	95599	95693	3.00E-32	141.0
95	6	93.7	1	204162	204256	3.00E-32	141.0
93	7	90.3	2	35239	35331	2.00E-22	107.0

93	7	90.3	1	203992	204082	4.00E-22	107.0
92	2	97.8	4	11193	11284	3.00E-41	167.0
92	2	97.8	1	135190	135281	5.00E-40	167.0
86	7	90.7	3	3620	3704	6.00E-21	99.6
86	7	90.7	2	95878	95963	6.00E-20	99.6
82	1	89.0	3	7160	7233	2.00E-17	87.7
82	1	89.0	2	44909	44990	2.00E-16	87.7
81	0	98.8	2	82594	82673	1.00E-33	145.0
81	0	98.8	1	100364	100444	2.00E-33	145.0
79	5	93.7	1	55058	55136	5.00E-25	117.0
79	5	93.7	1	82823	82901	5.00E-25	117.0
78	1	98.7	2	93207	93284	3.00E-34	147.0
78	1	98.7	1	42488	42565	5.00E-34	147.0
77	8	89.6	3	7157	7233	6.00E-18	89.7
77	8	89.6	1	32155	32231	1.00E-16	89.7
75	5	93.3	2	529	603	6.00E-23	109.0
75	5	93.3	1	95696	95770	1.00E-22	109.0
75	5	93.3	1	204269	204343	1.00E-22	109.0
75	5	93.3	1	91519	91593	1.00E-22	109.0
72	9	87.5	3	3835	3906	1.00E-12	71.9
72	9	87.5	2	95710	95781	1.00E-11	71.9
68	1	98.5	1	79587	79654	5.00E-28	127.0
68	1	98.5	1	120565	120632	5.00E-28	127.0
68	1	97.1	1	129930	129996	3.00E-23	111.0
68	1	97.1	1	195836	195903	3.00E-23	111.0
68	3	95.6	1	32704	32771	3.00E-23	111.0
68	3	95.6	1	35457	35524	3.00E-23	111.0
68	3	86.8	1	14313	14374	1.00E-09	65.9
68	3	86.8	1	243505	243572	1.00E-09	65.9
67	1	91.0	2	48881	48947	9.00E-16	85.7
67	1	91.0	1	194877	194938	2.00E-15	85.7
66	1	98.5	2	35	100	4.00E-27	123.0
66	1	98.5	1	129853	129918	7.00E-27	123.0
65	5	90.8	2	41121	41185	3.00E-12	73.8
65	5	90.8	1	82841	82904	6.00E-12	73.8
63	4	87.3	1	190594	190652	9.00E-08	60.0
63	4	87.3	1	213342	213404	9.00E-08	60.0
62	0	100.0	4	11117	11178	3.00E-28	123.0
62	0	100.0	1	156119	156180	7.00E-27	123.0
60	2	96.7	1	94616	94675	7.00E-21	103.0
60	2	96.7	1	183448	183507	7.00E-21	103.0
59	3	94.9	1	32262	32320	7.00E-18	93.7
59	3	94.9	1	217484	217542	7.00E-18	93.7
56	0	100.0	4	11096	11151	1.00E-24	111.0

56	0	100.0	1	4875	4930	3.00E-23	111.0
56	1	98.2	2	133607	133662	4.00E-21	103.0
56	1	98.2	1	210980	211035	7.00E-21	103.0
54	5	90.7	2	41097	41150	2.00E-10	67.9
54	5	90.7	1	55089	55142	4.00E-10	67.9
54	6	88.9	3	7997	8050	5.00E-09	60.0
54	6	88.9	1	82916	82969	9.00E-08	60.0
53	0	100.0	2	51715	51767	9.00E-22	105.0
53	0	100.0	1	101385	101437	2.00E-21	105.0
53	1	98.1	2	131619	131671	2.00E-19	97.6
53	1	98.1	1	92952	93004	4.00E-19	97.6
52	1	98.1	4	11114	11165	8.00E-20	95.6
52	1	98.1	1	135114	135165	2.00E-18	95.6
52	4	92.3	3	7157	7208	1.00E-12	71.9
52	4	92.3	1	190601	190652	2.00E-11	71.9
50	0	100.0	4	10226	10275	5.00E-21	99.6
50	0	100.0	4	11590	11639	5.00E-21	99.6
50	0	100.0	3	7184	7233	6.00E-21	99.6
50	0	100.0	2	24630	24679	6.00E-20	99.6
50	0	100.0	2	136541	136590	6.00E-20	99.6
50	0	100.0	2	1	50	6.00E-20	99.6
50	0	100.0	2	30208	30257	6.00E-20	99.6
50	0	100.0	2	44951	45000	6.00E-20	99.6
50	0	100.0	2	48864	48913	6.00E-20	99.6
50	0	100.0	1	35394	35443	1.00E-19	99.6
50	0	100.0	1	209113	209162	1.00E-19	99.6
50	0	100.0	1	32192	32241	1.00E-19	99.6
50	0	100.0	1	32255	32304	1.00E-19	99.6
50	0	100.0	1	195881	195930	1.00E-19	99.6
50	0	100.0	1	195825	195874	1.00E-19	99.6
50	0	100.0	1	221364	221413	1.00E-19	99.6
50	1	98.0	1	204293	204342	3.00E-17	91.7
50	1	98.0	1	246006	246055	3.00E-17	91.7
48	0	100.0	1	44110	44157	2.00E-18	95.6
48	0	100.0	1	194889	194936	2.00E-18	95.6
48	1	97.9	3	10754	10801	2.00E-17	87.7
48	1	97.9	2	26921	26968	2.00E-16	87.7
47	0	100.0	3	8289	8335	4.00E-19	93.7
47	0	100.0	2	78934	78980	4.00E-18	93.7
47	0	100.0	2	47950	47996	4.00E-18	93.7
47	0	100.0	1	156811	156857	7.00E-18	93.7
47	2	95.7	2	70984	71030	2.00E-13	77.8
47	2	95.7	1	219822	219868	4.00E-13	77.8
47	4	91.5	1	67277	67323	2.00E-08	61.9

47	4	91.5	1	81909	81955	2.00E-08	61.9
46	4	91.3	1	190594	190639	9.00E-08	60.0
46	4	91.3	1	217410	217455	9.00E-08	60.0
45	0	100.0	4	3127	3171	5.00E-18	89.7
45	0	100.0	2	78947	78991	6.00E-17	89.7
45	0	100.0	1	95725	95769	1.00E-16	89.7
45	0	100.0	1	246006	246050	1.00E-16	89.7
45	1	97.8	4	11428	11472	1.00E-15	81.8
45	1	97.8	1	239698	239742	2.00E-14	81.8
45	3	93.3	2	35914	35958	8.00E-10	65.9
45	3	93.3	1	66385	66429	1.00E-09	65.9
44	0	100.0	3	3437	3480	2.00E-17	87.7
44	0	100.0	2	30164	30207	2.00E-16	87.7
44	0	100.0	1	246042	246085	4.00E-16	87.7
44	0	100.0	1	181194	181237	4.00E-16	87.7
44	3	93.2	2	43867	43910	3.00E-09	63.9
44	3	93.2	2	70888	70931	3.00E-09	63.9
43	0	100.0	2	44953	44995	9.00E-16	85.7
43	0	100.0	2	48876	48918	9.00E-16	85.7
43	0	100.0	2	48876	48918	9.00E-16	85.7
43	0	100.0	1	32194	32236	2.00E-15	85.7
43	2	95.4	2	48952	48994	5.00E-11	69.9
43	2	95.4	1	156117	156159	1.00E-10	69.9
43	3	93.0	2	30215	30257	1.00E-08	61.9
43	3	93.0	1	217484	217526	2.00E-08	61.9
42	0	100.0	2	48812	48853	3.00E-15	83.8
42	0	100.0	2	48907	48948	3.00E-15	83.8
42	0	100.0	1	4835	4876	6.00E-15	83.8
42	0	100.0	1	155253	155294	6.00E-15	83.8
42	1	97.6	1	169939	169980	2.00E-12	75.8
42	1	97.6	1	213368	213409	2.00E-12	75.8
42	2	95.2	1	32942	32983	4.00E-10	67.9
42	2	95.2	1	36260	36301	4.00E-10	67.9
41	0	100.0	1	169404	169444	2.00E-14	81.8
41	0	100.0	1	184781	184821	2.00E-14	81.8
41	2	95.1	4	11117	11157	7.00E-11	65.9
41	2	95.1	2	48954	48994	8.00E-10	65.9
41	2	95.1	1	40669	40709	1.00E-09	65.9
41	2	95.1	1	99463	99503	1.00E-09	65.9
40	0	100.0	4	11033	11072	5.00E-15	79.8
40	0	100.0	3	1322	1361	6.00E-15	79.8
40	0	100.0	1	169407	169446	1.00E-13	79.8
40	0	100.0	1	135082	135121	1.00E-13	79.8
40	1	97.5	2	45784	45823	1.00E-11	71.9

40	1	97.5	1	121088	121127	2.00E-11	71.9
40	1	97.5	1	190666	190705	2.00E-11	71.9
40	1	97.5	1	109448	109487	2.00E-11	71.9
40	2	95.0	3	4262	4301	3.00E-10	63.9
40	2	95.0	1	219705	219744	6.00E-09	63.9
39	0	100.0	4	3	41	2.00E-14	77.8
39	0	100.0	4	6192	6230	2.00E-14	77.8
39	0	100.0	2	43872	43910	2.00E-13	77.8
39	0	100.0	2	69039	69077	2.00E-13	77.8
39	0	100.0	2	9672	9710	2.00E-13	77.8
39	0	100.0	1	159219	159257	4.00E-13	77.8
39	2	94.9	3	1326	1364	1.00E-09	61.9
39	0	97.4	4	11601	11639	1.00E-09	61.9
39	2	94.9	1	58819	58857	2.00E-08	61.9
39	0	97.4	1	129959	129996	2.00E-08	61.9
38	0	100.0	3	1324	1361	9.00E-14	75.8
38	0	100.0	2	56069	56106	8.00E-13	75.8
38	0	100.0	2	86112	86149	8.00E-13	75.8
38	0	100.0	2	44958	44995	8.00E-13	75.8
38	0	100.0	1	32199	32236	2.00E-12	75.8
38	0	100.0	1	4893	4930	2.00E-12	75.8
38	0	100.0	1	85274	85311	2.00E-12	75.8
38	0	100.0	1	88105	88142	2.00E-12	75.8
38	0	100.0	1	194877	194914	2.00E-12	75.8
38	0	100.0	1	135114	135151	2.00E-12	75.8
38	0	100.0	1	194877	194914	2.00E-12	75.8
38	0	100.0	1	184781	184818	2.00E-12	75.8
38	1	97.4	2	77332	77369	2.00E-10	67.9
38	1	97.4	1	53309	53346	4.00E-10	67.9
38	1	97.4	1	197269	197306	4.00E-10	67.9
38	1	97.4	1	67274	67311	4.00E-10	67.9
38	2	94.7	2	120360	120397	5.00E-08	60.0
38	2	94.7	1	61240	61277	9.00E-08	60.0
38	2	94.7	1	57326	57363	9.00E-08	60.0
38	2	94.7	1	57326	57363	9.00E-08	60.0
38	2	94.7	1	214876	214913	9.00E-08	60.0
38	2	94.7	1	169405	169442	9.00E-08	60.0
38	2	94.7	1	184783	184820	9.00E-08	60.0
38	2	94.7	1	33645	33682	9.00E-08	60.0
37	0	100.0	1	57328	57364	6.00E-12	73.8
37	0	100.0	1	58818	58854	6.00E-12	73.8
37	1	97.3	1	20244	20280	1.00E-09	65.9
37	1	97.3	1	225358	225394	1.00E-09	65.9
36	0	100.0	2	81410	81445	1.00E-11	71.9

36	0	100.0	2	69210	69245	1.00E-11	71.9
36	0	100.0	1	99382	99417	2.00E-11	71.9
36	0	100.0	1	55896	55931	2.00E-11	71.9
36	0	100.0	1	231528	231563	2.00E-11	71.9
36	0	100.0	1	186736	186771	2.00E-11	71.9
36	0	100.0	1	187432	187467	2.00E-11	71.9
36	0	100.0	1	52295	52330	2.00E-11	71.9
36	1	97.2	2	109165	109200	3.00E-09	63.9
36	1	97.2	1	96694	96729	6.00E-09	63.9
36	1	97.2	1	239669	239704	6.00E-09	63.9
36	1	97.2	1	194871	194906	6.00E-09	63.9
35	0	100.0	2	81411	81445	5.00E-11	69.9
35	0	100.0	2	89113	89147	5.00E-11	69.9
35	0	100.0	2	69042	69076	5.00E-11	69.9
35	0	100.0	2	43875	43909	5.00E-11	69.9
35	0	100.0	2	89113	89147	5.00E-11	69.9
35	0	100.0	2	93279	93313	5.00E-11	69.9
35	0	100.0	1	4896	4930	1.00E-10	69.9
35	0	100.0	1	74513	74547	1.00E-10	69.9
35	0	100.0	1	105012	105046	1.00E-10	69.9
35	0	100.0	1	156119	156153	1.00E-10	69.9
35	0	100.0	1	42447	42481	1.00E-10	69.9
35	0	100.0	1	187433	187467	1.00E-10	69.9
35	0	100.0	1	229296	229330	1.00E-10	69.9
35	0	100.0	1	229296	229330	1.00E-10	69.9
35	1	97.1	2	56912	56946	1.00E-08	61.9
35	1	97.1	1	229297	229331	2.00E-08	61.9
34	0	100.0	4	3127	3160	2.00E-11	67.9
34	0	100.0	1	156811	156844	4.00E-10	67.9
34	1	97.1	2	43876	43909	5.00E-08	60.0
34	1	97.1	2	56912	56945	5.00E-08	60.0
34	1	97.1	1	169437	169470	9.00E-08	60.0
34	1	97.1	1	222775	222808	9.00E-08	60.0
33	0	100.0	3	7201	7233	8.00E-11	65.9
33	0	100.0	2	131641	131673	8.00E-10	65.9
33	0	100.0	1	208851	208883	1.00E-09	65.9
33	0	100.0	1	194877	194909	1.00E-09	65.9
32	0	100.0	2	35923	35954	3.00E-09	63.9
32	0	100.0	1	45845	45876	6.00E-09	63.9
31	0	100.0	2	55906	55936	1.00E-08	61.9
31	0	100.0	2	105138	105168	1.00E-08	61.9
31	0	100.0	1	92974	93004	2.00E-08	61.9
31	0	100.0	1	208851	208881	2.00E-08	61.9
30	0	100.0	3	3337	3366	5.00E-09	60.0

30	0	100.0	2	63483	63512	5.00E-08	60.0
30	0	100.0	1	179713	179742	9.00E-08	60.0
30	0	100.0	1	113074	113103	9.00E-08	60.0

[1]Identity calculations do not include sites that contain indels.

M. herrei

Length (bp)	Mismatches	Identity (%)[1]	Scaffold	Positions		e-value	Bitscore
991	156	84.3	3	27563	28553	0	728.0
991	156	84.3	9	296048	297038	0	728.0
599	24	92.2	6	56944	57537	0	821.0
599	24	92.2	9	148111	148691	0	821.0
547	89	82.6	3	26802	27342	1.00E-90	333.0
547	89	82.6	9	297043	297589	9.00E-90	333.0
473	85	82.0	5	7208	7680	6.00E-70	264.0
473	85	82.0	3	18616	19088	7.00E-70	264.0
455	8	97.1	5	23260	23709	0	799.0
455	8	97.1	9	156381	156835	0	799.0
447	22	93.7	3	36457	36903	0	666.0
447	22	93.7	9	70200	70640	0	666.0
415	18	92.1	9	147414	147823	2.00E-158	561.0
415	18	92.1	9	182021	182425	2.00E-158	561.0
414	19	95.2	6	56496	56909	0	654.0
414	19	95.2	9	148696	149108	0	654.0
400	10	96.3	2	1572	1971	0	674.0
400	10	96.3	4	1	395	0	674.0
364	21	94.2	9	146755	147118	1.00E-156	555.0
364	21	94.2	9	182737	183100	1.00E-156	555.0
346	48	86.1	3	28706	29051	2.00E-82	305.0
346	48	86.1	9	295332	295677	2.00E-81	305.0
316	7	96.5	2	2844	3155	5.00E-153	537.0
316	7	96.5	4	1971	2286	2.00E-152	537.0
279	11	95.7	9	147145	147422	5.00E-125	450.0
279	11	95.7	9	182447	182725	5.00E-125	450.0
263	18	90.1	1	24164	24423	2.00E-84	311.0
263	18	90.1	8	1	258	2.00E-84	311.0
261	3	98.9	2	2353	2613	6.00E-140	494.0
261	3	98.9	4	1256	1516	2.00E-139	494.0
248	45	81.9	4	14923	15170	2.00E-31	135.0
248	45	81.9	9	183622	183869	4.00E-30	135.0
246	41	83.3	3	26471	26716	2.00E-39	163.0
246	41	83.3	9	297675	297920	2.00E-38	163.0
221	45	79.6	3	18275	18495	6.00E-15	81.8
221	45	79.6	9	88031	88251	5.00E-14	81.8
173	7	94.2	3	36812	36984	1.00E-68	260.0

173	7	94.2	9	302576	302745	1.00E-67	260.0
170	11	90.0	9	302591	302754	5.00E-51	204.0
170	11	90.0	9	331612	331781	5.00E-51	204.0
165	3	98.2	2	3702	3866	1.00E-82	303.0
165	3	98.2	4	2793	2957	4.00E-82	303.0
164	6	94.5	8	15511	15674	5.00E-66	250.0
164	6	94.5	9	23966	24126	1.00E-64	250.0
161	13	91.3	3	21303	21462	9.00E-51	200.0
161	13	91.3	9	349418	349578	8.00E-50	200.0
158	11	93.0	5	6105	6262	1.00E-58	226.0
158	11	93.0	3	36942	37099	2.00E-58	226.0
156	4	94.2	2	1494	1649	6.00E-63	238.0
156	4	94.2	4	407	557	2.00E-62	238.0
145	1	98.6	9	64524	64667	7.00E-69	264.0
145	1	98.6	9	71705	71849	7.00E-69	264.0
137	1	99.3	9	140737	140873	7.00E-69	264.0
137	1	99.3	9	145982	146118	7.00E-69	264.0
136	3	97.8	9	145693	145828	2.00E-63	246.0
136	3	97.8	9	187726	187861	2.00E-63	246.0
134	25	81.3	3	18759	18892	9.00E-11	67.9
134	25	81.3	9	87644	87777	8.00E-10	67.9
129	0	100.0	5	23969	24097	1.00E-67	256.0
129	0	100.0	9	156206	156334	2.00E-66	256.0
129	23	82.2	5	7351	7479	1.00E-12	73.8
129	23	82.2	9	87649	87777	1.00E-11	73.8
121	21	82.6	2	1039	1159	2.00E-13	73.8
121	21	82.6	4	2621	2741	8.00E-13	73.8
119	17	84.9	4	8813	8931	2.00E-16	85.7
119	17	84.9	3	39106	39223	4.00E-16	85.7
112	7	92.9	3	37130	37241	8.00E-36	151.0
112	7	92.9	9	186460	186570	7.00E-35	151.0
112	9	87.5	1	13605	13716	4.00E-24	111.0
112	9	87.5	7	29098	29204	7.00E-24	111.0
112	19	83.0	5	6969	7080	4.00E-12	71.9
112	19	83.0	3	18377	18488	6.00E-12	71.9
107	12	88.8	8	519	625	5.00E-26	117.0
107	12	88.8	1	24687	24793	7.00E-26	117.0
102	2	94.1	9	30093	30190	2.00E-35	153.0
102	2	94.1	9	70682	70783	2.00E-35	153.0
101	0	100.0	1	21923	22023	6.00E-51	200.0
101	0	100.0	9	302925	303025	8.00E-50	200.0
99	1	99.0	2	4713	4811	5.00E-48	188.0
99	1	99.0	9	24922	25020	3.00E-46	188.0
96	0	100.0	3	4050	4145	9.00E-48	190.0

96	0	100.0	9	44640	44735	8.00E-47	190.0
94	16	83.0	4	14428	14521	1.00E-08	60.0
94	16	83.0	9	184447	184540	2.00E-07	60.0
93	2	96.8	3	14452	14543	2.00E-36	153.0
93	2	96.8	9	170943	171035	2.00E-35	153.0
92	1	98.9	9	70200	70291	5.00E-42	174.0
92	1	98.9	9	302576	302667	5.00E-42	174.0
83	1	98.8	5	1	83	1.00E-37	157.0
83	1	98.8	9	64780	64862	1.00E-36	157.0
79	1	98.7	9	71694	71772	3.00E-34	149.0
79	1	98.7	9	302197	302275	3.00E-34	149.0
79	3	96.2	8	10517	10595	9.00E-31	133.0
79	3	96.2	9	29854	29932	2.00E-29	133.0
79	5	93.7	3	7358	7436	1.00E-25	117.0
79	5	93.7	9	137674	137752	1.00E-24	117.0
78	0	100.0	2	5690	5767	7.00E-38	155.0
78	0	100.0	8	19269	19346	2.00E-37	155.0
78	0	100.0	1	23602	23679	3.00E-37	155.0
78	0	100.0	1	22668	22745	3.00E-37	155.0
78	0	100.0	9	222480	222557	4.00E-36	155.0
78	0	100.0	9	302803	302880	4.00E-36	155.0
78	1	98.7	8	6857	6934	6.00E-35	147.0
78	1	98.7	9	219298	219375	1.00E-33	147.0
78	2	97.4	5	25469	25546	2.00E-32	139.0
78	2	97.4	9	192418	192495	3.00E-31	139.0
78	7	91.0	9	41218	41295	2.00E-19	99.6
78	7	91.0	9	54264	54341	2.00E-19	99.6
77	1	92.2	2	1673	1749	6.00E-23	105.0
77	1	92.2	6	13544	13615	6.00E-22	105.0
76	1	92.1	1	23924	23999	1.00E-21	103.0
76	1	92.1	9	30004	30074	1.00E-20	103.0
75	1	98.7	3	8427	8501	7.00E-33	141.0
75	1	98.7	9	319178	319252	7.00E-32	141.0
74	0	100.0	4	6717	6790	6.00E-35	147.0
74	0	100.0	3	1	74	1.00E-34	147.0
74	3	96.0	4	16594	16667	9.00E-28	123.0
74	3	96.0	9	135898	135971	2.00E-26	123.0
74	4	94.6	1	22114	22187	3.00E-25	115.0
74	4	94.6	9	275306	275379	4.00E-24	115.0
73	1	98.6	4	12245	12317	6.00E-32	137.0
73	1	98.6	9	156843	156915	1.00E-30	137.0
73	2	97.3	4	8660	8732	1.00E-29	129.0
73	2	97.3	6	60665	60737	4.00E-29	129.0
72	5	93.1	4	102	173	8.00E-22	103.0

72	5	93.1	6	13544	13615	2.00E-21	103.0
71	5	93.0	1	22073	22143	4.00E-21	101.0
71	5	93.0	3	37026	37096	6.00E-21	101.0
70	0	98.6	1	22022	22091	1.00E-27	123.0
70	0	98.6	9	302786	302854	2.00E-26	123.0
69	0	100.0	7	1	69	1.00E-31	137.0
69	0	100.0	7	26851	26919	1.00E-31	137.0
69	0	100.0	9	43292	43360	1.00E-30	137.0
69	0	100.0	9	226963	227031	1.00E-30	137.0
68	0	100.0	1	24075	24142	3.00E-31	135.0
68	0	100.0	9	29816	29883	4.00E-30	135.0
68	2	97.1	3	36827	36894	3.00E-26	119.0
68	2	97.1	9	70209	70276	2.00E-25	119.0
68	2	97.1	9	331612	331679	2.00E-25	119.0
68	2	97.1	9	331612	331679	2.00E-25	119.0
68	1	97.1	9	64601	64667	6.00E-23	111.0
68	1	97.1	9	302197	302264	6.00E-23	111.0
67	1	91.0	5	6152	6218	3.00E-16	85.7
67	1	91.0	3	36989	37055	4.00E-16	85.7
67	6	91.0	9	186970	187036	3.00E-15	85.7
67	6	91.0	9	242977	243043	3.00E-15	85.7
67	1	91.0	9	70746	70807	3.00E-15	85.7
67	1	91.0	9	70746	70807	3.00E-15	85.7
66	0	100.0	8	3536	3601	3.00E-30	131.0
66	0	100.0	5	30914	30979	6.00E-30	131.0
66	0	100.0	7	892	957	8.00E-30	131.0
66	0	100.0	9	332612	332677	6.00E-29	131.0
65	2	96.9	2	3637	3701	2.00E-25	113.0
65	2	96.9	4	2556	2620	9.00E-25	113.0
65	5	90.8	3	7355	7418	1.00E-12	73.8
65	5	90.8	6	44507	44571	2.00E-12	73.8
64	2	96.9	8	12604	12667	3.00E-24	111.0
64	2	96.9	7	12604	12667	7.00E-24	111.0
64	7	89.1	1	2273	2336	4.00E-12	71.9
64	7	89.1	9	254185	254248	5.00E-11	71.9
64	3	87.5	1	23931	23994	9.00E-10	63.9
64	3	87.5	9	223591	223649	1.00E-08	63.9
63	6	90.5	4	7577	7639	5.00E-14	77.8
63	6	90.5	9	124799	124861	8.00E-13	77.8
62	0	100.0	9	101701	101762	2.00E-26	123.0
62	0	100.0	9	302682	302743	2.00E-26	123.0
62	1	96.8	2	4817	4877	4.00E-21	99.6
62	3	95.2	7	12600	12661	3.00E-20	99.6
62	1	96.8	9	25014	25075	2.00E-19	99.6

62	3	95.2	9	345364	345425	2.00E-19	99.6
61	6	90.2	4	78	138	8.00E-13	73.8
61	6	90.2	9	230933	230993	1.00E-11	73.8
60	1	98.3	9	245349	245408	6.00E-23	111.0
60	1	98.3	9	346459	346518	6.00E-23	111.0
59	1	98.3	8	15351	15409	1.00E-23	109.0
59	1	98.3	9	106236	106294	2.00E-22	109.0
59	4	93.2	9	30009	30067	3.00E-15	85.7
59	4	93.2	9	223591	223649	3.00E-15	85.7
58	1	98.3	8	12605	12662	5.00E-23	107.0
58	1	98.3	3	36927	36984	1.00E-22	107.0
58	1	98.3	9	331715	331772	9.00E-22	107.0
58	1	98.3	9	345363	345420	9.00E-22	107.0
58	2	87.9	8	17954	18011	1.00E-08	60.0
58	2	87.9	9	185241	185293	2.00E-07	60.0
56	0	100.0	9	51401	51456	6.00E-23	111.0
56	0	100.0	9	331736	331791	6.00E-23	111.0
55	0	100.0	9	101708	101762	2.00E-22	109.0
55	0	100.0	9	331716	331770	2.00E-22	109.0
55	1	98.2	3	36928	36982	6.00E-21	101.0
55	1	98.2	9	101708	101762	6.00E-20	101.0
55	5	90.9	2	1649	1703	3.00E-12	69.9
55	5	90.9	9	230933	230987	2.00E-10	69.9
54	5	90.7	6	44483	44536	1.00E-10	67.9
54	5	90.7	9	137705	137758	8.00E-10	67.9
53	0	100.0	5	3114	3166	3.00E-22	105.0
53	0	100.0	6	3370	3422	6.00E-22	105.0
53	1	98.1	1	1042	1094	6.00E-20	97.6
53	1	98.1	3	20280	20332	1.00E-19	97.6
53	1	98.1	6	23402	23454	2.00E-19	97.6
53	1	98.1	9	284595	284647	9.00E-19	97.6
52	0	100.0	4	3851	3902	8.00E-22	103.0
52	0	100.0	9	145534	145585	1.00E-20	103.0
52	1	98.1	9	70124	70175	3.00E-18	95.6
52	1	98.1	9	70124	70175	3.00E-18	95.6
52	1	98.1	9	302695	302746	3.00E-18	95.6
52	1	98.1	9	331722	331773	3.00E-18	95.6
52	4	92.3	3	37048	37099	6.00E-12	71.9
52	4	92.3	9	35004	35055	5.00E-11	71.9
51	0	100.0	3	36934	36984	6.00E-21	101.0
51	0	100.0	9	70125	70175	6.00E-20	101.0
51	1	98.0	6	47443	47493	2.00E-18	93.7
51	1	98.0	9	37612	37662	1.00E-17	93.7
51	5	90.2	4	9013	9063	3.00E-09	61.9

51	5	90.2	3	38937	38987	5.00E-09	61.9
50	1	98.0	2	4644	4693	9.00E-19	91.7
50	1	98.0	9	24885	24934	5.00E-17	91.7
49	0	100.0	1	22073	22121	6.00E-20	97.6
49	0	100.0	9	181830	181878	9.00E-19	97.6
49	0	100.0	9	263498	263546	9.00E-19	97.6
49	0	100.0	9	35007	35055	9.00E-19	97.6
49	1	98.0	9	70127	70175	2.00E-16	89.7
49	1	98.0	9	101714	101762	2.00E-16	89.7
49	2	95.9	6	11640	11688	9.00E-15	81.8
49	2	95.9	9	185159	185207	5.00E-14	81.8
48	0	100.0	1	4214	4261	2.00E-19	95.6
48	0	100.0	8	15346	15393	2.00E-19	95.6
48	0	100.0	3	2106	2153	4.00E-19	95.6
48	0	100.0	9	128728	128775	3.00E-18	95.6
48	0	100.0	9	10056	10103	3.00E-18	95.6
48	0	100.0	9	70758	70805	3.00E-18	95.6
47	0	100.0	9	100805	100851	1.00E-17	93.7
47	0	100.0	9	238676	238722	1.00E-17	93.7
47	4	91.5	7	21722	21768	6.00E-09	61.9
47	4	91.5	9	319127	319173	5.00E-08	61.9
46	0	100.0	6	50508	50553	9.00E-18	91.7
46	0	100.0	6	15714	15759	9.00E-18	91.7
46	0	100.0	9	51411	51456	5.00E-17	91.7
46	0	100.0	9	302709	302754	5.00E-17	91.7
46	0	100.0	9	311729	311774	5.00E-17	91.7
46	0	100.0	9	279958	280003	5.00E-17	91.7
46	3	93.5	1	2305	2350	6.00E-11	67.9
46	3	93.5	3	38643	38688	9.00E-11	67.9
46	4	91.3	8	15409	15454	1.00E-08	60.0
46	4	91.3	7	32705	32750	2.00E-08	60.0
46	4	91.3	9	35017	35062	2.00E-07	60.0
46	4	91.3	9	223517	223562	2.00E-07	60.0
45	0	100.0	9	63340	63384	2.00E-16	89.7
45	0	100.0	9	142363	142407	2.00E-16	89.7
45	1	97.8	6	27601	27645	9.00E-15	81.8
45	1	97.8	9	20158	20202	5.00E-14	81.8
45	1	97.8	9	124283	124327	5.00E-14	81.8
45	1	97.8	9	302388	302432	5.00E-14	81.8
45	2	95.6	4	14229	14273	8.00E-13	73.8
45	2	95.6	9	184696	184740	1.00E-11	73.8
44	3	93.2	4	7553	7596	7.00E-10	63.9
44	3	93.2	9	221399	221442	1.00E-08	63.9
44	3	93.2	9	317959	318002	1.00E-08	63.9

44	3	93.2	9	141598	141641	1.00E-08	63.9
43	0	100.0	9	10061	10103	3.00E-15	85.7
43	0	100.0	9	106252	106294	3.00E-15	85.7
43	2	95.4	5	6105	6147	2.00E-11	69.9
43	2	95.4	5	6105	6147	2.00E-11	69.9
43	2	95.4	9	331730	331772	2.00E-10	69.9
43	2	95.4	9	302703	302745	2.00E-10	69.9
43	3	93.0	5	6105	6147	4.00E-09	61.9
43	3	93.0	9	70125	70167	5.00E-08	61.9
42	0	100.0	3	36988	37029	1.00E-15	83.8
42	0	100.0	5	6151	6192	1.00E-15	83.8
42	0	100.0	9	76029	76070	1.00E-14	83.8
42	0	100.0	9	82000	82041	1.00E-14	83.8
42	0	100.0	9	51361	51402	1.00E-14	83.8
42	0	100.0	9	51361	51402	1.00E-14	83.8
42	1	97.6	2	5468	5509	5.00E-14	75.8
42	1	97.6	9	101778	101819	3.00E-12	75.8
42	1	97.6	9	223552	223593	3.00E-12	75.8
42	1	97.6	9	186331	186372	3.00E-12	75.8
42	2	95.2	9	100693	100734	8.00E-10	67.9
42	2	95.2	9	182415	182456	8.00E-10	67.9
42	3	92.9	4	12767	12808	1.00E-08	60.0
42	3	92.9	9	186267	186308	2.00E-07	60.0
41	0	100.0	1	17	57	4.00E-15	81.8
41	0	100.0	3	13674	13714	6.00E-15	81.8
41	0	100.0	6	49840	49880	9.00E-15	81.8
41	0	100.0	9	237230	237270	5.00E-14	81.8
41	0	100.0	9	209788	209828	5.00E-14	81.8
41	0	100.0	9	216962	217002	5.00E-14	81.8
41	1	97.6	9	230420	230460	1.00E-11	73.8
41	1	97.6	9	315887	315927	1.00E-11	73.8
41	2	95.1	8	5038	5078	2.00E-10	65.9
41	2	95.1	5	6105	6145	3.00E-10	65.9
41	2	95.1	6	1018	1058	5.00E-10	65.9
41	2	95.1	9	101722	101762	3.00E-09	65.9
40	0	100.0	5	6220	6259	2.00E-14	79.8
40	0	100.0	9	70092	70131	2.00E-13	79.8
40	0	100.0	9	331815	331854	2.00E-13	79.8
40	0	100.0	9	222441	222480	2.00E-13	79.8
40	1	97.5	9	34951	34990	5.00E-11	71.9
40	1	97.5	9	213263	213302	5.00E-11	71.9
39	0	100.0	9	221404	221442	8.00E-13	77.8
39	0	100.0	9	315883	315921	8.00E-13	77.8
39	0	100.0	9	98190	98228	8.00E-13	77.8

39	0	100.0	9	204106	204144	8.00E-13	77.8
39	0	100.0	9	26632	26670	8.00E-13	77.8
39	0	100.0	9	28784	28822	8.00E-13	77.8
39	1	97.4	8	15422	15460	1.00E-11	69.9
39	1	97.4	9	223535	223573	2.00E-10	69.9
39	2	94.9	1	6363	6401	3.00E-09	61.9
39	2	94.9	6	47474	47512	8.00E-09	61.9
39	2	94.9	6	1728	1766	8.00E-09	61.9
39	2	94.9	9	188758	188796	5.00E-08	61.9
39	2	94.9	9	186549	186587	5.00E-08	61.9
39	2	94.9	9	211641	211679	5.00E-08	61.9
38	0	100.0	5	6181	6218	3.00E-13	75.8
38	0	100.0	3	13274	13311	4.00E-13	75.8
38	0	100.0	3	37018	37055	4.00E-13	75.8
38	0	100.0	6	27571	27608	6.00E-13	75.8
38	0	100.0	6	56904	56941	6.00E-13	75.8
38	0	100.0	6	232	269	6.00E-13	75.8
38	0	100.0	9	229180	229217	3.00E-12	75.8
38	0	100.0	9	246196	246233	3.00E-12	75.8
38	0	100.0	9	51419	51456	3.00E-12	75.8
38	0	100.0	9	70124	70161	3.00E-12	75.8
38	0	100.0	9	105893	105930	3.00E-12	75.8
38	0	100.0	9	30093	30130	3.00E-12	75.8
38	0	100.0	9	30093	30130	3.00E-12	75.8
38	0	100.0	9	108333	108370	3.00E-12	75.8
38	1	97.4	6	9892	9929	1.00E-10	67.9
38	1	97.4	6	50517	50554	1.00E-10	67.9
38	1	97.4	6	9888	9925	1.00E-10	67.9
38	1	97.4	6	9888	9925	1.00E-10	67.9
38	1	97.4	7	21719	21756	1.00E-10	67.9
38	1	97.4	9	135716	135753	8.00E-10	67.9
38	1	97.4	9	211932	211969	8.00E-10	67.9
38	1	97.4	9	257494	257531	8.00E-10	67.9
38	1	97.4	9	28912	28949	8.00E-10	67.9
38	1	97.4	9	300794	300831	8.00E-10	67.9
38	1	97.4	9	196603	196640	8.00E-10	67.9
38	1	97.4	9	315883	315920	8.00E-10	67.9
38	1	97.4	9	221404	221441	8.00E-10	67.9
38	1	97.4	9	237074	237111	8.00E-10	67.9
38	2	94.7	1	25097	25134	1.00E-08	60.0
38	2	94.7	8	16445	16482	1.00E-08	60.0
38	2	94.7	8	17353	17390	1.00E-08	60.0
38	2	94.7	7	8391	8428	2.00E-08	60.0
38	2	94.7	6	59744	59781	3.00E-08	60.0

38	2	94.7	9	189966	190003	2.00E-07	60.0
38	2	94.7	9	216571	216608	2.00E-07	60.0
38	2	94.7	9	292669	292706	2.00E-07	60.0
38	2	94.7	9	1537	1574	2.00E-07	60.0
38	2	94.7	9	353577	353614	2.00E-07	60.0
37	0	100.0	1	9965	10001	9.00E-13	73.8
37	0	100.0	3	4949	4985	1.00E-12	73.8
37	0	100.0	3	36948	36984	1.00E-12	73.8
37	0	100.0	6	47475	47511	2.00E-12	73.8
37	0	100.0	9	138512	138548	1.00E-11	73.8
37	0	100.0	9	51420	51456	1.00E-11	73.8
37	0	100.0	9	119080	119116	1.00E-11	73.8
37	0	100.0	9	211933	211969	1.00E-11	73.8
37	1	97.3	1	13729	13765	2.00E-10	65.9
37	1	97.3	1	9967	10003	2.00E-10	65.9
37	1	97.3	3	13273	13309	3.00E-10	65.9
37	1	97.3	3	13273	13309	3.00E-10	65.9
37	1	97.3	5	17830	17866	3.00E-10	65.9
37	1	97.3	7	16787	16823	4.00E-10	65.9
37	1	97.3	7	29224	29260	4.00E-10	65.9
37	1	97.3	6	9892	9928	5.00E-10	65.9
37	1	97.3	6	27572	27608	5.00E-10	65.9
37	1	97.3	6	56905	56941	5.00E-10	65.9
37	1	97.3	6	9890	9926	5.00E-10	65.9
37	1	97.3	9	230014	230050	3.00E-09	65.9
37	1	97.3	9	257499	257535	3.00E-09	65.9
37	1	97.3	9	311738	311774	3.00E-09	65.9
37	1	97.3	9	336829	336865	3.00E-09	65.9
37	1	97.3	9	311739	311775	3.00E-09	65.9
36	0	100.0	1	9968	10003	4.00E-12	71.9
36	0	100.0	1	9968	10003	4.00E-12	71.9
36	0	100.0	7	16788	16823	6.00E-12	71.9
36	0	100.0	6	46558	46593	9.00E-12	71.9
36	0	100.0	6	50515	50550	9.00E-12	71.9
36	0	100.0	6	50516	50551	9.00E-12	71.9
36	0	100.0	6	937	972	9.00E-12	71.9
36	0	100.0	6	9893	9928	9.00E-12	71.9
36	0	100.0	9	118065	118100	5.00E-11	71.9
36	0	100.0	9	211935	211970	5.00E-11	71.9
36	0	100.0	9	230418	230453	5.00E-11	71.9
36	0	100.0	9	140071	140106	5.00E-11	71.9
36	0	100.0	9	311736	311771	5.00E-11	71.9
36	0	100.0	9	311737	311772	5.00E-11	71.9
36	0	100.0	9	292668	292703	5.00E-11	71.9

36	0	100.0	9	316054	316089	5.00E-11	71.9
36	0	100.0	9	211935	211970	5.00E-11	71.9
36	0	100.0	9	230418	230453	5.00E-11	71.9
36	0	100.0	9	241354	241389	5.00E-11	71.9
36	0	100.0	9	209458	209493	5.00E-11	71.9
36	0	100.0	9	221406	221441	5.00E-11	71.9
36	0	100.0	9	315885	315920	5.00E-11	71.9
36	1	97.2	1	9967	10002	9.00E-10	63.9
36	1	97.2	6	56905	56940	2.00E-09	63.9
36	1	97.2	6	27572	27607	2.00E-09	63.9
36	1	97.2	6	50518	50553	2.00E-09	63.9
36	1	97.2	7	16788	16823	2.00E-09	63.9
36	1	97.2	9	230421	230456	1.00E-08	63.9
36	1	97.2	9	167050	167085	1.00E-08	63.9
36	1	97.2	9	70740	70775	1.00E-08	63.9
36	1	97.2	9	30101	30136	1.00E-08	63.9
36	1	97.2	9	317969	318004	1.00E-08	63.9
36	1	97.2	9	299764	299799	1.00E-08	63.9
36	1	97.2	9	275350	275385	1.00E-08	63.9
36	1	97.2	9	275350	275385	1.00E-08	63.9
36	1	97.2	9	108335	108370	1.00E-08	63.9
36	1	97.2	9	108335	108370	1.00E-08	63.9
36	1	97.2	9	340771	340806	1.00E-08	63.9
35	0	100.0	1	9967	10001	1.00E-11	69.9
35	0	100.0	1	9967	10001	1.00E-11	69.9
35	0	100.0	8	6816	6850	1.00E-11	69.9
35	0	100.0	6	50517	50551	3.00E-11	69.9
35	0	100.0	9	18220	18254	2.00E-10	69.9
35	0	100.0	9	211936	211970	2.00E-10	69.9
35	0	100.0	9	184714	184748	2.00E-10	69.9
35	0	100.0	9	230419	230453	2.00E-10	69.9
35	0	100.0	9	225713	225747	2.00E-10	69.9
35	0	100.0	9	51422	51456	2.00E-10	69.9
35	0	100.0	9	101728	101762	2.00E-10	69.9
35	0	100.0	9	288263	288297	2.00E-10	69.9
35	0	100.0	9	219269	219303	2.00E-10	69.9
35	0	100.0	9	311738	311772	2.00E-10	69.9
35	1	97.1	1	22109	22143	3.00E-09	61.9
35	1	97.1	8	10015	10049	3.00E-09	61.9
35	1	97.1	5	6189	6223	4.00E-09	61.9
35	1	97.1	3	16223	16257	5.00E-09	61.9
35	1	97.1	6	50517	50551	8.00E-09	61.9
35	1	97.1	6	9892	9926	8.00E-09	61.9
35	1	97.1	9	311738	311772	5.00E-08	61.9

35	1	97.1	9	211935	211969	5.00E-08	61.9
35	1	97.1	9	315886	315920	5.00E-08	61.9
35	1	97.1	9	221407	221441	5.00E-08	61.9
35	1	97.1	9	315888	315922	5.00E-08	61.9
35	1	97.1	9	317969	318003	5.00E-08	61.9
35	1	97.1	9	340771	340805	5.00E-08	61.9
35	1	97.1	9	340772	340806	5.00E-08	61.9
35	1	97.1	9	340771	340805	5.00E-08	61.9
35	1	97.1	9	340772	340806	5.00E-08	61.9
35	1	97.1	9	340772	340806	5.00E-08	61.9
35	1	97.1	9	211935	211969	5.00E-08	61.9
35	1	97.1	9	38843	38877	5.00E-08	61.9
35	1	97.1	9	189674	189708	5.00E-08	61.9
34	0	100.0	1	9968	10001	6.00E-11	67.9
34	0	100.0	3	13680	13713	9.00E-11	67.9
34	0	100.0	6	50517	50550	1.00E-10	67.9
34	0	100.0	6	50517	50550	1.00E-10	67.9
34	0	100.0	9	311738	311771	8.00E-10	67.9
34	0	100.0	9	211936	211969	8.00E-10	67.9
34	0	100.0	9	315887	315920	8.00E-10	67.9
34	0	100.0	9	221408	221441	8.00E-10	67.9
34	0	100.0	9	311738	311771	8.00E-10	67.9
34	0	100.0	9	211936	211969	8.00E-10	67.9
34	0	100.0	9	221408	221441	8.00E-10	67.9
34	0	100.0	9	315887	315920	8.00E-10	67.9
34	0	100.0	9	209789	209822	8.00E-10	67.9
34	0	100.0	9	211645	211678	8.00E-10	67.9
34	0	100.0	9	18255	18288	8.00E-10	67.9
34	0	100.0	9	158561	158594	8.00E-10	67.9
34	0	100.0	9	315887	315920	8.00E-10	67.9
34	0	100.0	9	221408	221441	8.00E-10	67.9
34	0	100.0	9	211645	211678	8.00E-10	67.9
34	0	100.0	9	230420	230453	8.00E-10	67.9
34	1	97.1	1	2307	2340	1.00E-08	60.0
34	1	97.1	1	2307	2340	1.00E-08	60.0
34	1	97.1	1	9898	9931	1.00E-08	60.0
34	1	97.1	1	9967	10000	1.00E-08	60.0
34	1	97.1	7	16791	16824	2.00E-08	60.0
34	1	97.1	7	16790	16823	2.00E-08	60.0
34	1	97.1	7	16790	16823	2.00E-08	60.0
34	1	97.1	6	9892	9925	3.00E-08	60.0
34	1	97.1	9	230420	230453	2.00E-07	60.0
34	1	97.1	9	340772	340805	2.00E-07	60.0
34	1	97.1	9	230420	230453	2.00E-07	60.0

34	1	97.1	9	4128	4161	2.00E-07	60.0
34	1	97.1	9	70742	70775	2.00E-07	60.0
34	1	97.1	9	30101	30134	2.00E-07	60.0
34	1	97.1	9	344905	344938	2.00E-07	60.0
34	1	97.1	9	211935	211968	2.00E-07	60.0
33	0	100.0	1	9968	10000	2.00E-10	65.9
33	0	100.0	1	9969	10001	2.00E-10	65.9
33	0	100.0	7	16791	16823	4.00E-10	65.9
33	0	100.0	6	50518	50550	5.00E-10	65.9
33	0	100.0	6	9893	9925	5.00E-10	65.9
33	0	100.0	6	50517	50549	5.00E-10	65.9
33	0	100.0	6	9892	9924	5.00E-10	65.9
33	0	100.0	9	315888	315920	3.00E-09	65.9
33	0	100.0	9	311739	311771	3.00E-09	65.9
33	0	100.0	9	230421	230453	3.00E-09	65.9
33	0	100.0	9	221409	221441	3.00E-09	65.9
33	0	100.0	9	211936	211968	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	221408	221440	3.00E-09	65.9
33	0	100.0	9	230420	230452	3.00E-09	65.9
33	0	100.0	9	311738	311770	3.00E-09	65.9
33	0	100.0	9	315887	315919	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	317969	318001	3.00E-09	65.9
33	0	100.0	9	230018	230050	3.00E-09	65.9
33	0	100.0	9	257499	257531	3.00E-09	65.9
33	0	100.0	9	344906	344938	3.00E-09	65.9
33	0	100.0	9	317969	318001	3.00E-09	65.9
32	0	100.0	1	6156	6187	9.00E-10	63.9
32	0	100.0	5	3857	3888	1.00E-09	63.9
32	0	100.0	6	39088	39119	2.00E-09	63.9
32	0	100.0	7	16792	16823	2.00E-09	63.9
32	0	100.0	9	257499	257530	1.00E-08	63.9
32	0	100.0	9	230019	230050	1.00E-08	63.9
32	0	100.0	9	344906	344937	1.00E-08	63.9

32	0	100.0	9	317969	318000	1.00E-08	63.9
32	0	100.0	9	17559	17590	1.00E-08	63.9
32	0	100.0	9	163170	163201	1.00E-08	63.9
32	0	100.0	9	186855	186886	1.00E-08	63.9
32	0	100.0	9	230019	230050	1.00E-08	63.9
31	0	100.0	6	2399	2429	8.00E-09	61.9
31	0	100.0	9	229017	229047	5.00E-08	61.9
31	0	100.0	9	280027	280057	5.00E-08	61.9
31	0	100.0	9	82619	82649	5.00E-08	61.9
31	0	100.0	9	215646	215676	5.00E-08	61.9
31	0	100.0	9	242752	242782	5.00E-08	61.9
30	0	100.0	1	19736	19765	1.00E-08	60.0
30	0	100.0	4	5208	5237	1.00E-08	60.0
30	0	100.0	8	5116	5145	1.00E-08	60.0
30	0	100.0	5	6118	6147	2.00E-08	60.0
30	0	100.0	6	6581	6610	3.00E-08	60.0
30	0	100.0	6	3553	3582	3.00E-08	60.0
30	0	100.0	6	50025	50054	3.00E-08	60.0
30	0	100.0	9	106701	106730	2.00E-07	60.0
30	0	100.0	9	124810	124839	2.00E-07	60.0
30	0	100.0	9	157006	157035	2.00E-07	60.0
30	0	100.0	9	119058	119087	2.00E-07	60.0
30	0	100.0	9	51420	51449	2.00E-07	60.0

[1]Identity calculations do not include sites that contain indels.

Table S6. Read-pair support for the predominant and alternative configuration of single-copy sequences flanking two-copy repeats in the *M. ciliata* and *M. herrei* mitochondrial genomes.[1]

M. ciliata

Repeat pair		Reads supporting each configuration[3]				AC	Scaffolds and positions	
Length (bp)	Identity[2] (%)	AB	CD	BD	CA	support[4] (%)	AB	CD
1309	83.4%	0	0	0	0	N/A	1.116980-118288	1.143575-142267
346	86.1%	230	205	0	0	0.0%	1.118668-119013	1.141894-141549
291	81.4%	254	213	0	0	0.0%	1.118313-118603	1.142249-141959
246	83.3%	234	291	0	0	0.0%	1.116649-116894	1.143906-143661
143	93.0%	437	321	0	0	0.0%	1.40797-40939	2.115777-115635
127	94.5%	281	242	0	0	0.0%	1.219187-219313	3.11248-11374

M. herrei

Repeat pair		Reads supporting each configuration[3]				AC	Scaffolds and positions	
Length (bp)	Identity[2] (%)	AB	CD	BD	CA	support[4] (%)	AB	CD
991	84.3%	0	0	0	0	N/A	3.27563-28553	9.297038-296048
599	92.2%	0	93	0	0	0.0%	6.56944-57537	9.148691-148111
473	82.0%	124	118	0	0	0.0%	3.18616-19088	5.7208-7680
346	86.1%	165	149	0	0	0.0%	3.28706-29051	9.295677-295332
164	94.5%	450	470	1	0	0.1%	8.15511-15674	9.23966-24126
161	91.3%	506	285	0	0	0.0%	3.21303-21462	9.349578-349418
158	93.0%	452	493	2	1	0.3%	3.36942-37099	5.6105-6262
145	98.6%	399	410	2	0	0.3%	9.64524-64667	9.71849-71705
134	81.3%	558	595	0	0	0.0%	3.18759-18892	9.87777-87644
129	82.2%	542	599	0	0	0.0%	5.7351-7479	9.87777-87649
112	92.9%	502	534	0	0	0.0%	3.37130-37241	9.186460-186570
112	87.5%	310	630	1	0	0.1%	1.13605-13716	7.29098-29204
102	94.1%	503	564	0	0	0.0%	9.30093-30190	9.70783-70682
101	100.0%	548	568	3	3	0.5%	1.21923-22023	9.303025-302925

[1]This table does not include repeats ≤ 100 bp in size. Nor does it include repeats that contain plastid-derived DNA or are within 800 bp of scaffold or contig ends, as these conditions preclude accurate measurement of frequencies of all four repeat configurations.
[2]Identity calculations are exclusive of gaps.
[3]Number of reads supporting the predominant configurations AB and CD (these are the configurations present in the genome assembly, with AB designating the pair of single-copy sequences flanking one repeat copy, and CD the pair flanking the other repeat) and the alternative, minority configurations BD and CA.
[4]Support for alternative configurations (AC) is calculated as the percentage of all reads that support configurations BD and CA.

CHAPTER 2

Horizontal transfer via gene conversion with existing foreign sequences

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Abstract

Background

Horizontal gene transfer (HGT) is relatively common between mitochondrial DNAs (mtDNAs) of angiosperms, with the transfer of multiple genes observed in several cases. Gene conversion often occurs between foreign and native sequences in angiosperm mitochondria, overwriting sequence patterns in native regions and occasionally introducing completely new sequences.

Results

We examined seven recently sequenced mtDNAs from *Monsonia* (Geraniaceae) for evidence of HGT from Solanales donors. Phylogenetic analysis revealed the presence of six foreign genes in mtDNA of *Monsonia emarginata* that were acquired from at least two Solanales donors. We found many intergenic regions in the seven *Monsonia* mtDNAs that, on the basis of their exclusivity to *Monsonia* and the Solanales and exceptionally low divergence between the two groups, were probably horizontally

transferred from the Solanales to a common ancestor of these *Monsonia* species. *M. emarginata* contains 17 species-specific foreign sequences (including the six genes), all of which abut on one or both ends the foreign intergenic sequences acquired in a *Monsonia* common ancestor. Divergence patterns and identical endpoints of these shared intergenic regions indicate that the recent transfers in *M. emarginata* involved gene conversion between foreign sequences acquired early in *Monsonia* evolution and those acquired recently.

Conclusions

Monsonia mtDNAs contain many foreign sequences from members of the Solanales. At least one horizontal transfer event and perhaps many more occurred in the mtDNA of the common ancestor of the sampled *Monsonia* species between 9 and 23 million years ago. At least four transfer events occurred within *Monsonia* evolution, with most of this Solanales DNA acquired within the last 3.7 million years by the *M. emarginata* lineage via at least two transfer events from different donors. These *M. emarginata*-specific transfers occurred through the conversion and extension of existing foreign sequences put in place by the earliest transfer(s) from Solanales donors. These recently acquired sequences exemplify a new mode of HGT in angiosperm mtDNA.

Introduction

Horizontal gene transfer (HGT) is the transmission of genetic material between individuals not related by parent-offspring relationships. Although HGT is widely known to be very important in the evolution of prokaryotes (Ochman et al. 2000; Beiko et al. 2005; Zhaxybayeva and Doolittle 2011; Soucy et al. 2015), it is now broadly understood

to be more widespread and important in eukaryote evolution than previously thought (Keeling and Palmer 2008; Leger et al. 2018; Husnik and McCutcheon 2018). Angiosperm mitochondrial DNAs (mtDNAs) are especially prone to HGT (Bergthorsson et al. 2003; Richardson and Palmer 2007, Mower et al. 2012; Sanchez-Puerta 2014). In several cases, multiple genes have been horizontally transferred from the mtDNA of one angiosperm to another, distantly related one (Mower et al. 2010; Rice et al. 2013; Xi et al. 2013; Park et al. 2015; Sanchez-Puerta et al. 2017; Cusimano and Renner 2019). Mitochondrion-to-mitochondrion HGT is thought to generally occur through fusion of foreign and native mitochondria followed by recombination between their mtDNAs (Rice et al. 2013; Sanchez-Puerta et al. 2019).

Although HGT is known to introduce new sequences outright, the overwriting of existing sequences through gene conversion by more-or-less distantly related foreign DNA is also a well-documented outcome of HGT (Mau et al. 2006; Klasson et al. 2009; Didelot and Maiden 2010). Indeed, many cases have been identified in which portions of angiosperm mitochondrial genes have been replaced through conversion by foreign homologs (Bergthorsson et al. 2003; Barkman et al. 2007; Hao and Palmer 2009; Hao and Palmer 2010; Mower et al. 2010; Hepburn et al. 2012; Wang et al. 2012; Sloan, Müller et al. 2012; Park et al. 2015; Sanchez-Puerta et al. 2017).

Horizontal transfer may yield both outcomes, adding new sequences and converting tracts flanking them. A plethora of such cases involves the only intron present in angiosperm *cox1* genes (Adams et al. 1998; Cho et al. 1998; Sanchez-Puerta et al.

2008). This homing group I intron has been acquired hundreds if not thousands of times across angiosperms after its introduction to the group from fungi. The insertion of this intron, mediated by its encoded endonuclease, is accompanied mechanistically by the coconversion of the exonic region immediately flanking the 3' end of the intron.

We examined seven recently sequenced mtDNAs (Cole et al. 2018) from *Monsonia* (Geraniaceae) for evidence of HGT from Solanales mitochondrial donors. We focused on Solanales because of initial evidence that all foreign genes in *Monsonia* mtDNAs were acquired from this order. We identified a new mode of HGT in the mtDNA of *Monsonia emarginata* (dysentery herb, Geraniaceae), one in which existing foreign sequence is converted and extended by homologs acquired more recently from related donors. This process has resulted in the transfer of many intergenic regions and six regions containing genes.

Methods

Phylogenetic analysis of mitochondrial genes

Individual alignments of the 27 mitochondrial protein genes in *Monsonia emarginata* with those from a diversity of 42-57 other angiosperms were constructed with MUSCLE 3.8.29 (Edgar 2004) using default parameters. The gymnosperm *Cycas* was included in the alignments as an outgroup. Variably present introns in *cox1* and *cox2* were excluded from the alignment. Phylogenetic analysis of the alignments was performed in PhyML 3.1.2 (Guidon and Gascuel 2003). Substitution models were selected on the basis of the Bayesian information criterion as implemented in jModeltest 2.1.6 (Darriba

et al. 2012). Bootstrap supports were calculated for the resulting trees using 1000 pseudoreplicates. For those six *M. emarginata* genes with two copies, this most likely unconstrained topology was compared to the most likely topology constrained under the hypothesis of vertical transmission and recent gene duplication (i.e., with the two copies placed as sisters and within the Geraniaceae) using the AU test (Shimodaira 2002) as implemented in CONSEL 0.20 (Shimodaira and Hasegawa 2001).

Identification of other horizontally transferred DNA

Early in this study we discovered six *M. emarginata* genes that were all acquired from Solanales donors and found that the sequences flanking these genes are present exclusively in *Monsonia* and Solanales. These findings led us to search for all potentially foreign DNA in *Monsonia* of Solanales origin by BLASTing entire *Monsonia* mtDNAs against five sequenced Solanales mtDNAs using ungapped BLASTn with a raw score cutoff of 30. The same BLAST search was conducted between the *Monsonia* mtDNAs and 166 diverse other angiosperm mtDNAs in order to distinguish between *Monsonia*/Solanales shared sequences that are present in a diversity of angiosperms and those present exclusively in *Monsonia* and Solanales. The latter set of sequences were considered to be good candidates for horizontal transfer between Solanales and *Monsonia*.

To further explore this hypothesis of horizontal transfer, these *Monsonia* and Solanales sequences were aligned with MUSCLE 3.8.29 (Edgar 2004) using default parameters, and divergence was calculated in HyPhy version 2.2 (Pond et al. 2005). These

divergences were compared to those for all other *Monsonia*/Solanales shared sequences, which are presumably vertically transmitted as they are present widely across angiosperms and most are genes, and to synonymous-site divergences between *Monsonia* and Solanales calculated with the method of Gojobori and Nei (1986) using a concatenated alignment of the 27 protein genes found in *Monsonia* mtDNAs.

Prediction of RNA editing sites

RNA editing sites were predicted using PREP-mt (Mower 2009) with a cutoff value of 0.5.

Results

Phylogenetic evidence of horizontal gene transfer in Monsonia emarginata

Inspection of the seven *Monsonia* mtDNAs sequenced by Cole et al. (2018) identified a total of six genes (*atp4, atp8, atp9, cox1, cox2,* and *nad4L*), all in *M. emarginata*, for which there are two substantially different copies (table 1; figs. 1 and S1). Phylogenetic analysis placed one member of each *M. emarginata* gene pair as expected for a native gene, i.e., within the rosid family Geraniaceae and in all but one case as sister to *M. speciosa*, the only other species of *Monsonia* included in these analyses. In contrast, the second member of each gene pair was always embedded within the asterid order Solanales. Five of these six foreign gene sequences have highly supported placements in the Solanales (≥ 99% bootstrap) (table 1; figs. 1 and S1). These results are corroborated by the AU test (table 1). The hypothesis of the most likely tree topology, which indicates the horizontal transfer of one gene copy, was significantly preferred ($p <$

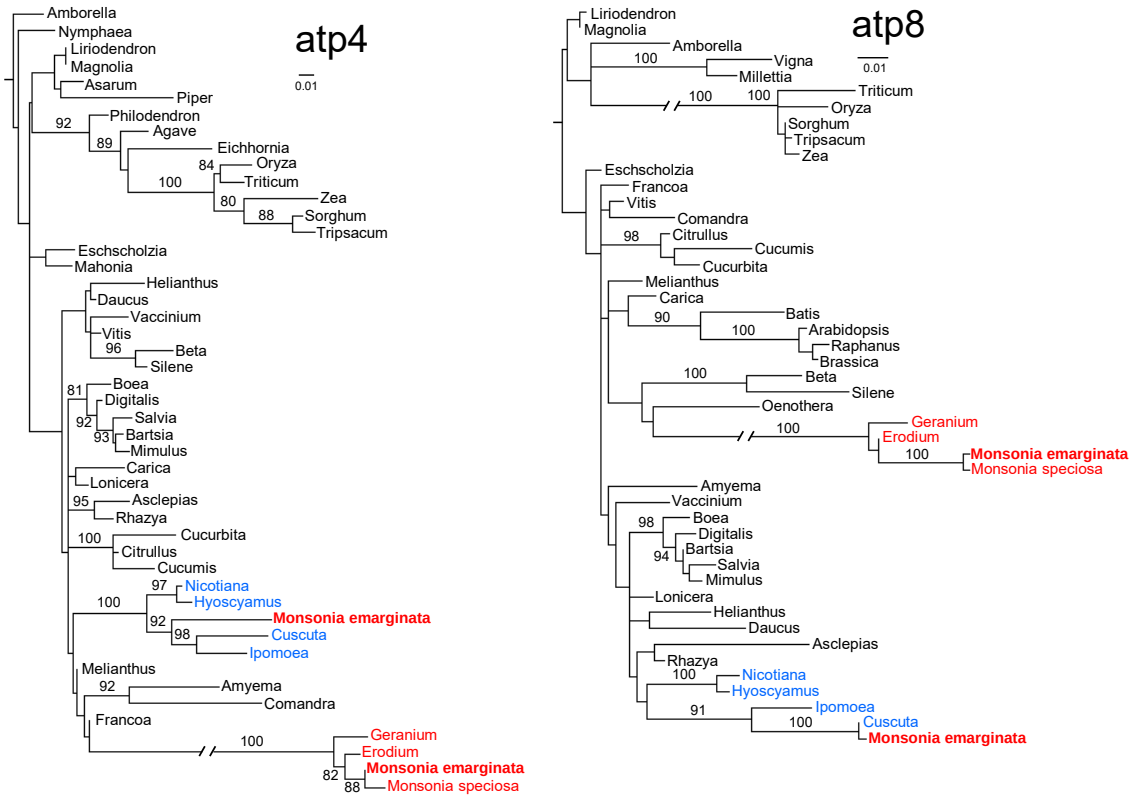

Figure 1. Phylograms of *atp4* and *atp8* showing horizontally transferred genes. Taxa in the Geraniaceae are in red, and those in the Solanales are in blue. Branches with a broken line are displayed at one-half length. Bootstrap values ≥ 80% are displayed. Scale bars are in units of substitutions per site. The outgroup (*Cycas*) is not shown.

0.01 in all cases and < 0.001 in four; table 1) over the most likely topology in which the two *M. emarginata* copies are sister to one another and placed within the Geraniaceae, as indicative of vertical transfer and gene duplication. Taken together, the bootstrap values and AU results constitute strong evidence for the horizontal transfer of these six gene copies in *M. emarginata*.

Three of the foreign genes (*atp8*, *cox1*, and *cox2*) are sister to the only included species of *Cuscuta* with moderate to strong support (table 1; figs. 1 and S1). This raises the possibility that two or all three of these genes were acquired via the same transfer event, with the donor being a species of *Cuscuta* or a close relative in the Convolvulaceae. The foreign *atp4* gene was placed as sister to a *Cuscuta+Ipomoea* clade with strong support and was thus probably acquired from a different member of the Convolvulaceae (Refulio-Rodriguez and Olmstead, 2014). The foreign *atp9* and *nad4L* genes were placed in unresolved positions within the Solanales. The six foreign genes in *M. emarginata* were therefore acquired through as few as two and as many as six transfer events, all from Solanales donors.

Consistent with their phylogenetic placement, the foreign genes in *M. emarginata* have Solanales-like complements of sites predicted to undergo C-to-U RNA editing (table S2). The *M. emarginata* native copies, which have very few sites of predicted RNA editing (10 sites across the six genes with three genes having no sites), share none of

Table 1. Features of horizontally transferred gene duplicates.

Gene	Length (bp)	Foreign integrity[1]	AU test p-value[2]	Phylogenetic placement (BS)[3]			
				Within Sol	Sister to Con	Within Con	Sister to Cuscuta
atp4	472	Ψ (Δ80)	< 0.001	100%	92%	-	-
atp8	484	Ψ(N)	< 0.001	-	-	91%	100%
atp9	123	Ψ (Δ95)	0.008	31%	-	-	-
cox1	849	Ψ (Δ732)	< 0.001	-	-	100%	80%
cox2	774	I	< 0.001	-	-	91%	100%
nad4L	303	I	0.005	99%	-	-	-

[1]"I" indicates an intact copy whereas "Ψ" indicates a pseudogene. For pseudogenes, "Δ" indicates truncation followed by the base pairs lost whereas "N" indicates a nonsense mutation resulting in a premature stop codon.
[2]The p-value is that of the approximately unbiased test comparing the mostly likely tree constrained to vertical transfer (gene copies in *M. emarginata* resolving as sisters) and the most likely tree which shows horizontal transfer.
[3]Bootstrap support values for the relevant phylogenetic placements. "Sol" stands for Solanales, and "Con" for Convolvulaceae.

the 45 editing sites present in the foreign copies. All five examined Solanales genomes possess at least 39 of these 45 editing sites, with *Cuscuta* and *Ipomoea* containing all 45 sites.

Four of six foreign genes (*atp4*, *atp8*, *atp9*, and *cox1*) are either truncated or possess an internal stop codon, indicating that they are probably nonfunctional (table 1). The two intact foreign genes (*cox2* and *nad4L*) may either still be functional or be undiagnosed pseudogenes, owing for example to lack of transcription or to RNA editing incompatibilities between Solanales and *Monsonia*. It would not be surprising to find

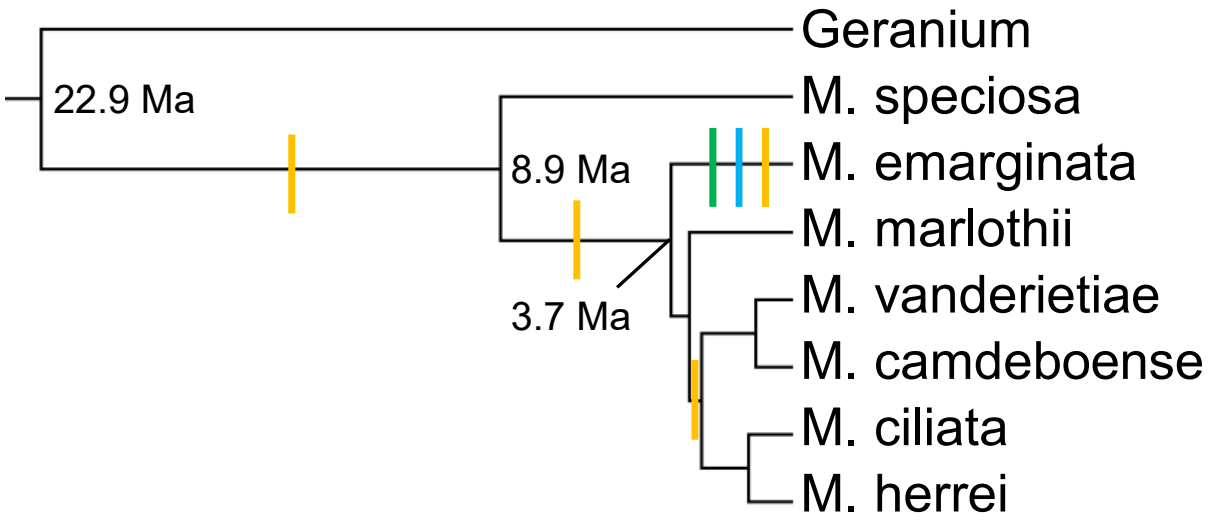

Figure 2. Summary of horizontal gene transfer in *Monsonia*. The tree is scaled to absolute time (topology and divergence times are from Cole et al. 2018). The blue bar indicates transfer from a *Cuscuta*-like donor, the green bar indicates transfer from an uncertain Convolvulaceae donor, and the orange bars indicate transfer from uncertain Solanales donors. The 40 sequences acquired in a *Monsonia* common ancestor result from as few as a single transfer and as many as 40 transfers, while the 17 bridge or extension sequences acquired in the *M. emarginata* lineage result from at least two transfers (figs. 1 and S1; table 1) and as many as 17. The other two bars represent transfer of a single sequence.

Table 2. Foreign regions in *Monsonia*[1] mtDNAs: Length and identity to Solanales.

Region[2]	Length (bp)[3]								Identity (%)[3,4]							
	Msp	Mem[5,6]		Mma	Mva	Mca	Mci	Mhe	Msp	Mem[5]		Mma	Mva	Mca	Mci	Mhe
atp4f1	337	337	**795**	337	83	337	337	337	99.7	100.0	**99.6**	100.0	100.0	100.0	100.0	99.7
atp8f1	302	302	**1102**	302	301	301	301	301	99.0	100.0	**99.3**	99.0	98.7	98.7	99.0	99.0
atp8f2	361	361		361	361	361	361	361	98.9	100.0		98.9	98.9	98.9	98.9	98.9
atp9f1	400	400	**507**	400	146	146	400	400	99.5	100.0	**99.6**	99.8	99.8	99.8	99.5	99.8
cox1f1	326	326	**1671**	326	326	326	326	326	99.1	99.7	**99.6**	98.8	98.8	98.8	98.2	98.8
cox2f1	233	233	**1281**	233	233	233	165	233	97.9	99.1	**99.4**	97.9	97.4	97.9	99.4	97.9
cox2f2	307	307		307	307	192	307	307	99.0	99.7		99.0	99.0	99.5	99.0	99.0
nad4Lf1	267	267	**843**	267	267	-	267	267	99.7	100.0	**99.5**	99.7	99.7	-	99.7	99.7
nad4Lf2	306	306		306	306	306	101	306	99.3	100.0		99.3	99.0	99.3	98.0	99.3
i1f1	151	151	**688**	151	-	151	151	-	99.3	99.3	**99.4**	99.3	-	99.3	99.3	-
i1f2	177	177		177	177	177	177	-	98.9	98.9		98.9	98.9	98.9	98.9	-
i2f1	211	309	**1699**	211	211	211	211	211	99.1	100.0	**99.4**	99.1	99.1	99.1	99.1	99.1
i2f2	358	358		145	145	145	-	-	98.6	98.9		99.3	99.3	99.3	-	-
i3f1	411	411	**1064**	411	411	411	411	411	98.1	98.5	**99.3**	98.1	98.1	98.1	98.1	98.1
i3f2	378	378		378	378	378	378	378	98.7	99.7		98.7	98.7	98.7	98.7	98.4
i4f1	373	373	**663**	373	373	202	373	373	98.4	98.9	**99.5**	98.4	98.4	99.0	98.4	98.4
i4f2	189	189		189	189	189	189	189	97.9	98.4		97.9	97.9	97.9	97.9	97.9
i5f1	351	351	**1519**	351	-	-	-	351	98.3	99.4	**99.3**	98.3	-	-	-	98.3
i5f2	380	380		380	-	-	-	380	98.7	98.9		98.7	-	-	-	98.7
i6f1	122	279	**956**	122	122	122	-	122	100.0	100.0	**99.5**	100.0	99.2	100.0	-	100.0
i6f2	181	181		181	181	181	181	181	98.3	99.4		98.3	98.3	98.3	98.3	98.3
i7f1	456	676	**1591**	676	676	309	-	676	99.3	100.0	**99.4**	98.8	98.8	99.0	-	98.8
i7f2	211	211		211	211	211	211	211	99.5	100.0		99.5	99.5	99.5	99.5	99.5
i8f1	417	417	**822**	300	-	-	-	-	98.8	99.5	**99.3**	98.3	-	-	-	-
i9f1	395	520	**830**	-	520	520	-	-	98.7	99.8	**99.6**	-	98.8	98.8	-	-
i9f2	-	-		-	230	230	-	-	-	-		-	99.1	99.1	-	-
i10f1	158	158	**280**	-	-	-	158	158	99.4	99.4	**98.6**	-	-	-	99.4	99.4
i10f2	-	-		-	-	-	140	140	-	-		-	-	-	99.3	99.3
i11f1	666	666	**917**	666	-	-	-	-	98.6	99.5	**99.2**	98.6	-	-	-	-
i11f2	-	-		295	-	-	-	-	-	-		99.3	-	-	-	-
i12	147	147	n.a.	147	147	147	147	147	99.3	100.0	n.a.	99.3	99.3	99.3	99.3	99.3
i13	125	125	n.a.	125	125	125	125	125	100.0	100.0	n.a.	100.0	100.0	100.0	100.0	100.0
i14	303	303	n.a.	-	303	303	303	303	99.7	99.7	n.a.	-	99.7	99.7	99.7	99.7
i15	201	201	n.a.	-	201	201	201	201	99.5	100.0	n.a.	-	99.5	99.5	99.5	99.5
i16	261	-	n.a.	261	261	261	261	261	100.0	-	n.a.	100.0	100.0	100.0	100.0	100.0
i17	115	-	n.a.	115	115	115	115	115	100.0	-	n.a.	100.0	100.0	100.0	100.0	100.0
i18	151	-	n.a.	151	151	151	151	-	99.3	-	n.a.	99.3	99.3	99.3	99.3	99.3
i19	427	-	n.a.	-	427	427	427	427	99.5	-	n.a.	-	99.1	99.5	99.5	99.5
i20	200	200	n.a.	200	-	-	-	200	99.5	99.5	n.a.	99.5	-	-	-	99.5
i21	127	-	n.a.	127	127	-	-	127	100.0	-	n.a.	100.0	100.0	-	-	99.2
i22	-	125	n.a.	122	122	-	-	122	-	100.0	n.a.	100.0	100.0	-	-	100.0
i23	-	-	n.a.	-	179	179	179	179	-	-	n.a.	-	98.9	98.9	98.9	98.9

[1]*Monsonia* species are denoted by the first letter of their genus names and first two letters of their species names.
[2]"**f**" refers to foundation regions.
[3]"**-**" indicates absence of that region.
[4]Identity calculations do not include gap regions. In those cases where identities were different when compared to different Solanales sequences, the number pertains to the comparison for which the region is most completely covered (with *Capsicum* except for *i9f2*, *i10f2*, *i11f2*, and *i23*, which are comparisons with *Ipomoea*, and *I22*, which is with *Cuscuta*).

[5]The 10 bridge cases in *M. emarginata* are bolded, while the seven extension cases are bolded and underlined. The lengths in these 17 cases encompass the entire foreign region, i.e., the foundation(s) and the accompanying bridge or extension region.
[6]Lengths for the entire region of *i9-i11* in *M. emarginata* are shorter than in table 4 because entries here are based on the region that is recovered for *M. emarginata,* i.e. a single foundation and partial bridge.
[7]These are the only two of the 27 *M. emarginata* foundations that are not the same size as in at least one and usually most other *Monsonia* species; importantly, however, these size differences are due entirely to indels, and thus the outer boundary of these foundations is the same in *M. emarginata* as in the other species.

intact pseudogenes given how recently, within the past 3.7 million years, these genes were acquired (fig. 2).

The 21 single-copy mitochondrial protein genes in *M. emarginata* were all placed as sister to *M. speciosa,* with these two species grouped with other Geraniaceae. Therefore, these genes were likely not subject to HGT. This result combined with the findings for the six gene pairs provides no evidence for gene replacement via HGT in *M. emarginata* mtDNA.

Horizontal transfer of intergenic mtDNA

We identified 42 intergenic regions (table 2) present in one or more (usually most) *Monsonia* mtDNAs that were very likely horizontally transferred from the Solanales. The inference of HGT *per se* rests on three lines of evidence. First, all 42 sequences are present only in mtDNAs of *Monsonia* and Solanales among the many diverse angiosperm mtDNAs examined. The alternative hypothesis, of vertical-transmission, would invoke at minimum 12 independent losses of each sequence in the context of the current, scanty sampling of angiosperms (fig. S2) and undoubtedly many more losses with complete sampling. Second, all 42 of these sequences show much higher levels of sequence identity between the two groups than expected for sequences related by purely vertical descent. For example, these sequences from *M. speciosa* are, in

Table 3. Divergence[1] between Solanales and *Monsonia*[2] mtDNA sequences.

Comparison	Native sequence				Foreign sequence			
	Syn. sites		All		Foundation		Other[3]	
	div.	bp	div.	bp	div.[4]	bp	div.	bp
Capsicum/Ms	0.112	9,030	0.057	57,444	0.011	8,424	0.003	2,057
Capscium/Me	0.112	9,030	0.055	51,003	0.005	9,024	0.002	1,101
Hyoscyamus/Ms	0.117	9,030	0.050	48,391	0.012	7,901	0.003	2,057
Hyoscyamus/Me	0.117	9,030	0.048	51,616	0.006	8,501	0.002	1,101
Nicotiana/Ms	0.126	9,030	0.050	54,203	0.008	7,756	0.002	1,754
Nicotiana/Me	0.126	9,030	0.047	48,877	0.004	8,356	0.001	798
Cuscuta/Ms	0.131	9,030	0.053	44,744	0.015	2,225	0.000	451
Cuscuta/Me	0.131	9,030	0.048	49,930	0.011	2,225	0.000	326
Ipomoea/Ms	0.112	9,030	0.054	55,283	0.005	6,879	0.003	1,607
Ipomoea/Me	0.112	9,030	0.047	57,617	0.003	6,574	0.002	651

[1]Divergence (div.) is in substitutions per site, with gapped regions excluded.
[2]*M. speciosa* is denoted as Ms and *M. emarginata* as Me.
[3]These correspond to sequences i12-i23 in Table S1 and thus are neither foundation nor bridge or extension sequences, the last two of which are not included in this table.
[4]Foundation-sequence divergence is consistently lower in comparisons with *M. emarginata* than with *M. speciosa* owing to gene conversion of *M. emarginata* sequences by a recent Solanales donor (see text, figure 3, and table 4).

aggregate, six and 12 times more similar to those from *Capsicum* (Solanales) than are putatively vertically transmitted regions and synonymous sites, respectively (Table 3). In absolute terms, the sequences from across *Monsonia* are 97.9 to 100% identical (median = 99.3%, mean = 99.2%) to their counterparts in Solanales (table 2). Third, nine intergenic regions abut the six genes for which an HGT origin in *M. emarginata* is well-supported by phylogenetic inference.

The inference that transfer was from Solanales to *Monsonia* and not the reverse follows from the preceding point for nine intergenic sequences and from the greater antiquity of these sequences in Solanales for 41 of the 42 sequences. In 34 cases these sequences are found in members of both the Solanaceae and Convolvulaceae (table S1), indicating an origin prior to the 56-67 Ma common ancestry of the two families (Naumann et al. 2013; Tank et al. 2015; Sun et al. 2018). This is much earlier than their appearance 9-23 Ma in a *Monsonia* common ancestor. For those seven sequences

restricted to multi-taxa subclades of either Solanaceae or Convolvulaceae, respectively (table S1), the older estimated appearances of these clades (24-30 and 33-35 Ma, respectively; Naumann et al. 2013; Särkinen et al. 2013; Sun et al. 2018) are also indicative of Solanales-to-*Monsonia* transfer. In addition, two of these seven sequences appear to have been acquired more recently, within *Monsonia* evolution (see below). For only one of the 42 sequences (*i9F2*, present in only a single examined Solanales mtDNA) is the direction of transfer unclear.

Between 23 and 37 (median = 33, mean = 30) of the 42 foreign intergenic sequences are present in any given *Monsonia* mtDNA, where they total 7.1-18.3 kb (median = 8.8 kb, mean = 10.0 kb) and 1.5-3.2% (median = 1.7%, mean = 1.9%) of the genome (Table 2). These totals may underestimate the amount of Solanales-derived DNA given our strict criterion for inclusion (we only considered those intergenic sequences exclusive to *Monsonia* and the Solanales). Also, the shortest possible region that could satisfy our search cutoff would be a 30-bp region with 100% identity to Solanales, so very short or divergent regions that were horizontally transferred from the Solanales may have been missed.

Our search has certain limitations that introduce the possibility that a few of these regions are not exclusive to *Monsonia* and the Solanales. First, the sequenced angiosperm mtDNAs examined in this study represent only 11% (44/416) of angiosperm families and are highly biased toward several economically important families. Second, this approach may fail to detect sequences from those highly divergent angiosperm

mtDNAs that have been subject to high substitution rates (Cho et al. 2004; Mower et al. 2007; Parkinson et al. 2005; Sloan, Alverson et al. 2012). Third, sequences from non-angiosperms obviously would not be detected by this method; however, searches (for these intergenic regions in the NCBI nucleotide collection (nr/nt) database using the search parameters given in Methods yielded no positive results.

These 42 foreign regions are absent from *Geranium* and *Erodium*, close relatives of *Monsonia* and all but two were probably acquired in a common ancestor of all seven sampled *Monsonia* species, between 9 and 23 million years ago (Ma) (fig. 2, table 2). Two foreign intergenic region were probably acquired more recently, 2.8-3.1 Ma in the case of *i23* and 3.7-8.9 Ma for *i22* (fig. 2; table 2).

Bridge and extension patterns of foreign sequences

Seventeen of the 23 foreign regions in *M. emarginata* are substantially longer (498-1689 bp, mean = 1076 bp, median = 1064 bp) than in all other *Monsonia* species (122-666 bp, mean = 312 bp, median = 326 bp), with these shorter regions homologous (usually across their lengths) to one end or the other of the longer regions in *M. emarginata* (tables 2 and 4). In 10 of these 17 cases, one or more (usually most) of the six other *Monsonia* genomes possess short regions corresponding to both ends of the longer region in *M. emarginata*, while in three cases a somewhat similar situation exists (see second paragraph below). We refer to these 13 cases as having a “bridge” pattern. Those four cases in which the other *Monsonia* genomes possess DNA corresponding to only one end of a longer region in *M. emarginata* are referred to as having an “extension” pattern. The shorter regions are referred to as “foundations”, the regions

between foundations in *M. emarginata* bridge patterns are referred to as “bridges,” and the regions adjacent to the single foundation in extension patterns are referred to as “extensions.” Six of the 23 foreign sequences in *M. emarginata* do not exhibit either the bridge or extension pattern; these are short sequences (125-303 bp) of the same length (or in one case, 3 bp longer) as foreign homologs present in other *Monsonia* species (table 2). Whereas these six *M. emarginat*a sequences were probably acquired

Table 4. Features of foreign regions in *M. emarginata* mtDNA that exhibit a bridge or extension pattern.

Region	Pattern	Differences (bp)[1]		Lengths (bp)			
		F1[2]	F2[3]	F1	F2	B/E[4]	Region
atp4	extension	0	n.a.	337	n.a.	458	795
atp8	bridge	3	4	302	361	439	1102
atp9	extension	1	n.a.	400	n.a.	107	507
cox1	extension	3	n.a.	326	n.a.	1345	1671
cox2	bridge	3	2	233	307	741	1281
nad4L	bridge	1	2	267	306	270	843
i1	bridge	0	0	151	177	360	688
i2	bridge	4	1	309	358	1032	1699
i3	bridge	2	4	411	378	275	1064
i4	bridge	2	1	373	189	101	663
i5	bridge	4	1	351	380	788	1519
i6	bridge	2	2	279	181	496	956
i7	bridge	8	1	676	211	704	1591
i8	extension	3	n.a.	417	n.a.	405	822
i9	bridge[5]	4	n.a.	520	230	395	1145
i10	bridge[5]	0	n.a.	158	140	200	498
i11	bridge[5]	6	n.a.	666	295	401	1362

[1]Nucleotides shared between *M. emarginata* and Solanales to the exclusion of all other *Monsonia* species.
[2]First foundation.
[3]Second foundation.
[4]Bridge or extension.
[5]These three cases are inferred to be bridge HGTs but possess only one foundation sequence in *M. emarginata*. This inference is based on the presence of a candidate second foundation sequence in other *Monsonia* species, this foundation being located in Solanales only a short distance (≤ 401 bp) from the first foundation. The absence of this second foundation in *M. emarginata* precludes calculation of shared *M.emarginata*/ Solanales nucleotides, hence, "n.a." in the F2 column for i9-i11. In these three cases, F2 length is calculated as the length of the candidate foundation sequence in other *Monsonia* species, bridge length is calculated as the aforementioned distance between foundations in Solanales, and region length is calculated as the sum of bridge length and both foundation lengths. This results in longer lengths for the entire region than those given in the *M. emarginata* entries in table 2 because the latter are based on the region that is recovered for *M. emarginata,* i.e. a single foundation and a partial bridge.

by HGT in a *Monsonia* common ancestor, the 17 bridge and extension sequences in *M. emarginata* were probably acquired on the terminal branch leading to this species (fig. 2).

Three of the six foreign genes present only in *M. emarginata* are located in bridge-pattern regions, while the other three are in extension-pattern regions (table 4; fig. 3). The nine foundations in these cases all have small overlaps with the genes (13 to 31 bp in size). Foundation regions flanking foreign genes are not homologous with the sequences flanking native homologs in *M. emarginata* but rather are homologous to the sequences flanking these genes in all examined Solanales.

Seven sets of foreign intergenic sequences (*i1*-*i7* in tables 2 and 4) clearly exhibit the bridge pattern, while three others (*i9*-*i11*) show some evidence for the bridge pattern. In these three cases, the putative bridge region is truncated in *M. emarginata* relative to Solanales such that it has only one foundation, as does *M. speciosa*. Importantly, though, certain other *Monsonia* species have a candidate second foundation, with this sequence being located in Solanales only a short distance (≤ 401 bp) from the foundation present in *M. emarginata*. One intergenic region (*i8*) exhibits the extension pattern. Twelve intergenic regions (i12-i23) show neither bridge nor extension patterns (table 2).

Evidence for horizontal transfer via gene conversion with existing foreign sequence

The presence of 17 bridge and extension regions in *M. emarginata* only and of their 27 linked foundations in most other *Monsonia* species (Table 2) – above all, in all 27 cases for *M. speciosa*, the sister to the rest of *Monsonia* – indicates that the ancestral state for *Monsonia* was the absence of bridge and extension sequences but presence of foundations. For three reasons, we hypothesize that *M. emarginata* acquired its bridge and extension regions through gene conversion of these ancestrally acquired foundation regions by homologous sequences from a more recent set of Solanales donors. First, twenty-three of the 27 foundation sequences that flank bridges or extensions in *M. emarginata* differ from all homologous *Monsonia* foundations at between one and eight sites (64 total) at which *M. emarginata* is instead identical to homologs from the Solanales (fig. 3; table 4). Consequently, these *M. emarginata* foundation sequences are less divergent from Solanales homologs than are foundations from other *Monsonia* species (tables 2 and 3). Second, the outer boundaries of all 27 foundation sequences in *M. emarginata* are precisely the same as in most other *Monsonia* species, including *M. speciosa* in all but two cases (Table 2). Third, there is no evidence of any extra, ancestral-like foundation elements in the *M. emarginata* genome. Each of these three findings fits perfectly with the gene-conversion hypothesis; together they provide compelling evidence that conversion between newer and older foundation sequences was fundamental to the integration of most if not all bridge and extension sequences in the *M. emarginata* mitochondrial genome (fig. 4).

The best alternative explanation we can provide for these findings fits them very poorly. This postulates that entire Solanales regions (i.e., ones containing “extensions” or “bridges” together with their one or two “foundations”) were integrated in the *M. emarginata* genome as is, i.e., physically apart from the ancestral *Monsonia* foundations, and without any gene conversion. This hypothesis fits with the first line of evidence for the conversion hypothesis, as under it the 64 *M. emarginata*/Solanales shared sites would simply reflect the recency of the *M. emarginata* transfers. Crucially,

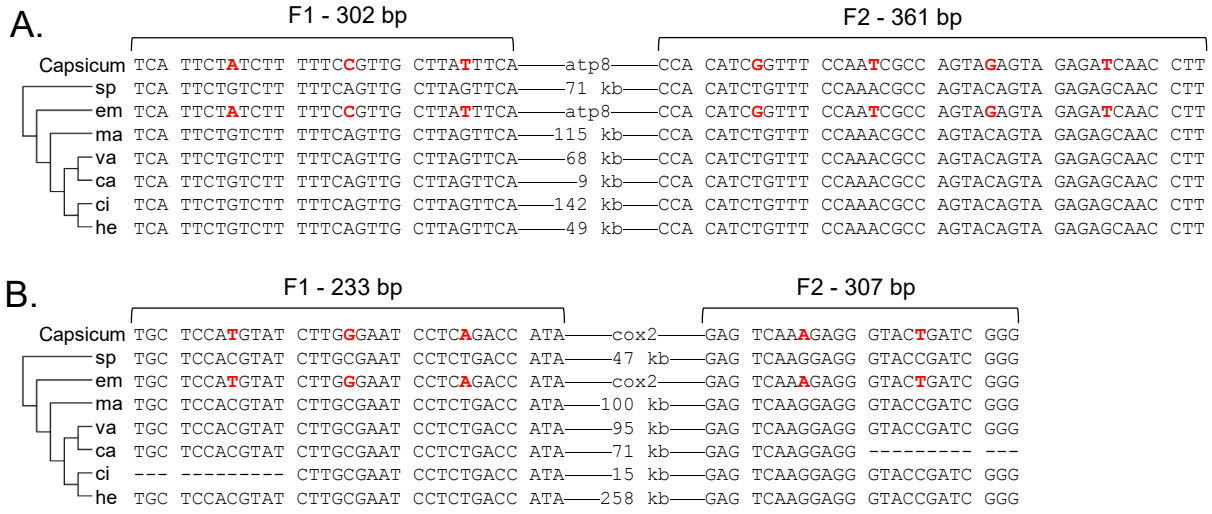

Figure 3. Evidence for HGT in *M. emarginata* via conversion of existing Solanales-derived sequences by sequences from a subsequent Solanales transfer. Displayed are alignments of portions of foundation sequences (F1 and F2) for the (A) *atp8* and (B) *cox2* bridge-pattern cases in *M. emarginata*; portions not displayed are indicated by spaces, while numbers above the sequences indicate the entire length of F1 and F2 in *M. emarginata*. Single nucleotide differences shared by *Capsicum annum* (a member of the Solanales; *C. annum* has the most intact bridge regions of sampled Solanales) and *M. emarginata* are in red. The distances between foundation sequences in those *Monsonia* species that do not possess the indicated bridge sequence are given in kb. The trees are from Cole et al. 2018, with *Monsonia* species abbreviated by the first two letters of their species epithets.

though, it is very unlikely that a “foundation” element acquired in even just one of the 17 bridge and extension cases would, by sheer coincidence (i.e., in the absence of conversion), have precisely the same outer boundary as its homolog acquired in a *Monsonia* common ancestor; it is unthinkable that such a coincidence would occur multiple times. Furthermore, the alternative predicts that the *M. emarginata* genome would contain two copies of a number of foundation-like sequences, one from the ancestral *Monsonia* transfers and one from the *M. emarginata* transfers, but no such foundation duplications were found. In conclusion, it is very likely that all 17 bridges and extensions, together with their associated foundations, arose through the conversion model described in the preceding paragraph and illustrated in fig. 4.

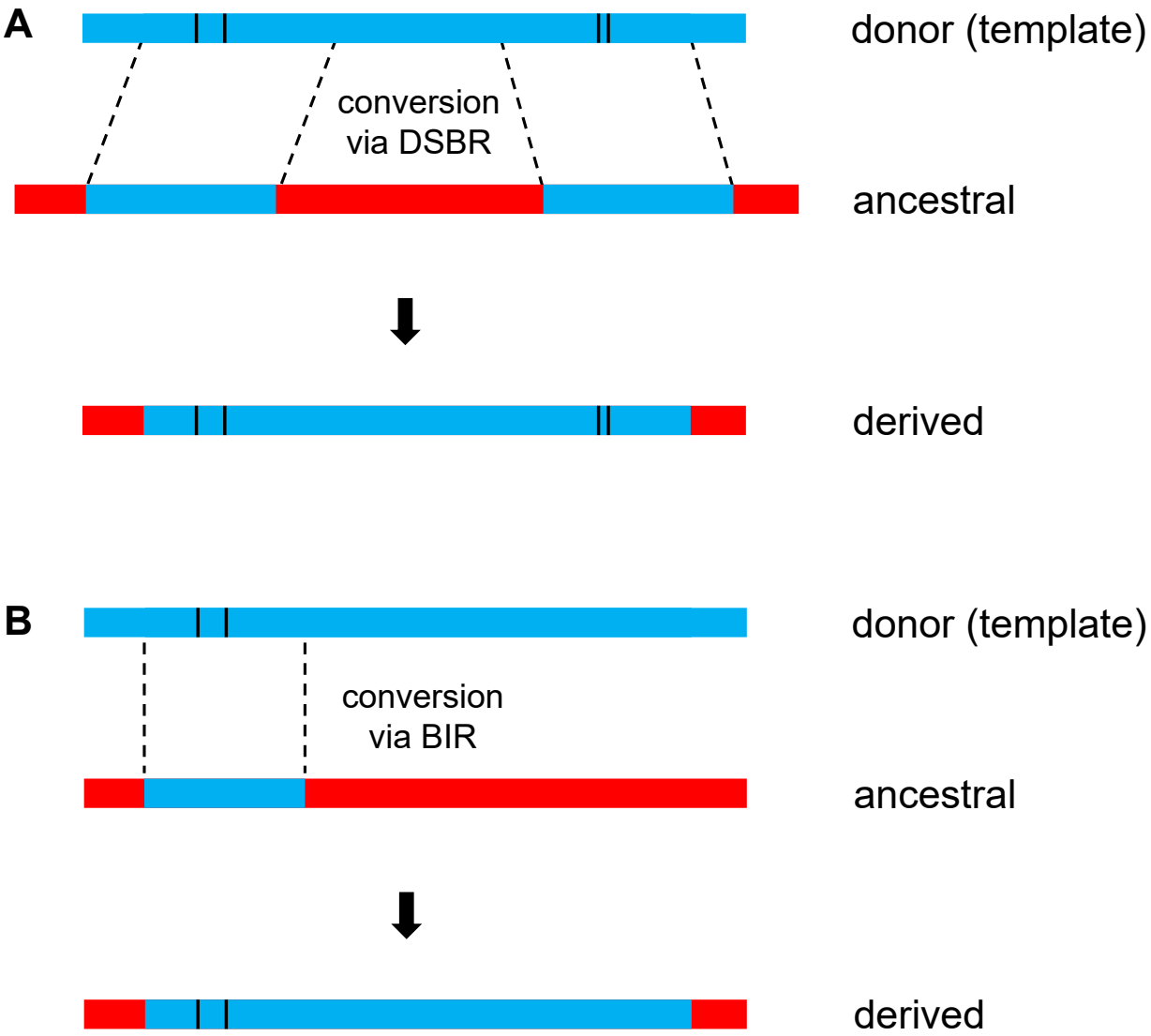

Figure 4. Schematic diagrams of bridge (A) and extension (B) HGT. Red bars represent native sequence, and blue bars represent foreign sequence. Black lines within the blue bars mark nucleotide differences shared by donor and derived sequences relative to the ancestral sequence (see fig. 3 and table 4). Dashed lines connect foundation sequences, which are stretches of shared homology used during conversion.

Discussion

Monsonia mtDNAs contain many foreign sequences from members of the Solanales. These sequences were acquired through at least five transfer events and perhaps many more (fig. 2). The earliest set of transfers, which introduced some 40 regions of Solanales mtDNA, occurred in a common ancestor of the examined *Monsonia* species between 8.9 and 22.9 Ma. At least two transfers, which introduced 17 more regions, occurred within the last 3.7 million years on the terminal branch leading to *M. emarginata*, while two additional transfers occurred at other points within *Monsonia* evolution (fig. 2). The horizontal transfer of intergenic sequences introduced in a *Monsonia* common ancestor is evidenced by the restriction of these sequences to *Monsonia* and the Solanales and the very low levels of divergence between these sequences from the two groups (table 3). The foreign origin of those genes introduced by the recent HGT events is supported by phylogenetic analyses (figs. 1 and S1; table 1).

Many nucleotides in foundation regions are shared by *M. emarginata* and Solanales to the exclusion of all other *Monsonia* species (fig. 3; table 4), yet the endpoints of foundation sequences in *M. emarginata* are identical to those of other *Monsonia* species. These data strongly support a model in which Solanales sequences acquired in the ancestral *Monsonia* mtDNA facilitated – via gene conversion – the subsequent acquisition of those introduced in the *M. emarginata* lineage. These recently acquired sequences exemplify new modes of HGT that we term bridge and extension HGT.

In the next section, we discuss these new HGT modes in the context of underlying pathways and mechanisms, especially with regard to the quandary presented by the drastically different physical dispositions of foundation pairs in *M. emarginata* mtDNA vs. other *Monsonia* mtDNAs. We then place our findings in broad perspective with regard to what is currently known about HGT in plant mitochondria and their implications for what we might learn through future investigations into mitochondrial HGT in plants.

Mechanisms of bridge and extension HGT

Our results indicate that most if not all transfers involved DNA intermediates rather than RNA intermediates. The presence of numerous unedited sites of predicted C-to-U editing (three to 14 per gene and 45 total across the six genes; table S2) in foreign gene copies is strong evidence that transfer did not involve an RNA intermediate as the majority of editing sites are fully edited in studies of mitochondrial transcript pools in angiosperms (Giege and Brennicke 1999; Sloan et al. 2010; Grewe et al. 2014). Furthermore, the transfer of many intergenic sequences and a *cox1* intron sequence is also indicative of a DNA-mediated mechanism (but see Wu et al. 2015; Lima and Smith 2017).

Two recombination-based DNA repair pathways, both of which are common in plant mitochondria (Davila et al. 2011; Christensen 2014; Garcia et al. 2019), were probably involved in the bridge- and extension-type HGTs described here (fig. 4). Bridge-type HGT most likely involves two foundations that serve as homology substrates for the two Holliday junctions that are involved in the dHj double-strand break repair (DSBR)

pathway, resulting in an introduced bridge and two converted foundations (fig. 4). Extension-type HGT probably involves the break-induced replication pathway (BIR) which predominates when a double-strand break has only one end (Llorente et al. 2008; Garcia et al. 2019). This pathway has been shown to utilize lengths of homology in plant mitochondria (Davila et al. 2011) that are similar to the lengths observed for foundation sequences. An alternative, more complicated, and thus less likely pathway leading to the extension pattern is DSBR-mediated bridge HGT followed by loss of one foundation and some adjacent bridge sequence. One would also have to invoke parallel losses of this foundation in *M. speciosa* and in the common ancestor of the five other examined *Monsonia* species.

In those 10 of the 13 inferred cases of bridge HGT in which *M. emarginata* contains both foundations, the bridge length, i.e., the distance separating a given pair of foundations, is short (101-1032 bp; mean = 521 bp; median = 468 bp; Tables 4 and S3), with most of these distances equal in length to the distances between homologous foundation pairs in Solanales (Table S4), and with these bridge regions sharing the same endpoints in both groups. In contrast, in all 50 bridge cases in which a bridgeless *Monsonia* genome contains both foundations, these sequences are much farther apart, – on average at least 247 times farther apart than in *M. emarginata* (median = 101 times; range = 15-2,656 times; Table S3). In 38 cases, a given pair of foundations are on the same scaffold, 7-268 kb apart (mean = 71 kb; median = 65 kb; Table S3). In the other 12 cases, the two foundations are on different scaffolds, allowing a minimum estimate of separation size (22-166 kb; mean = 78 kb; median = 70 kb) corresponding

to the sum of the shortest distance between each foundation and the ends of the scaffold in which it resides. In short, foundation pairs in bridgeless *Monsonia* genomes are effectively randomized in position relative to one another, whereas they are close together in *M. emarginata*. We consider three scenarios to account for this striking discrepancy in foundation-pair separations.

One scenario is that when *M. emarginata* acquired its bridges, its foundation pairs were comparably far apart as in bridgeless *Monsonia* genomes. DSBR-mediated gene conversion by newly acquired Solanales template DNA then led to the replacement of the large regions separating these foundations by the short regions that separate foundations in extant Solanales. It is well established that DSBR operates this way, e.g., spreading indels that differentiate meiotic homologs or yeast nuclear chromosomes and plasmids introduced to effect transformation (Eberhardt and Hohmann 1995; Andersen and Sekelsky 2010). A major obstacle for transfer under this scenario is that the average estimated distance between putatively essential transcription units in *M. emarginata* is 13 kb, as compared to the average distance, of ≥ 73 kb, between bridge foundation pairs in bridgeless *Monsonia* genomes. Therefore most conversions would be lethal. A second potential obstacle is that the ≥ 73-kb bridgeless mean separation is extremely large compared to the size of the bridge-associated foundations in *M. emarginata* (mean = 310 bp); this could diminish the likelihood of successful conversion.

A second, related scenario follows from the complicated *in vivo* condition of the mitochondrial genome in angiosperms, and of mitochondria themselves. Angiosperm

mtDNA usually assembles *in silico* as a single circular chromosome, the predominant arrangement of the genome containing its entire sequence content (Palmer and Shields 1984; Mower et al. 2012; Sloan et al. 2013). However, *in vivo* it appears to exist largely as a complicated mixture of linear and branched molecules, with active recombination between a multitude of repeats of a range of sizes (as in *Monsonia*; Cole et al., 2018) leading to a potentially huge number of different sequence arrangements of highly variable abundance (Oldenburg and Bendich 1996; Backert and Börner 2000; Manchekar et al. 2006; Sloan et al. 2013). Furthermore, there are many copies of the mitochondrial genome in each plant cell, with ongoing cyles of mitochondrial fission and fusion leading to near-complete mixing of the population of mitochondria and their genomes (Arimura et al. 2004; Sheahan et al. 2005; Arimura 2018). Consequently, at a given point in time, a plant cell may harbor an mtDNA subpopulation in which one or more pairs of foundations are relatively close together. This should increase both the likelihood of their gene-conversional recombination with the closely-spaced foundation pairs acquired from Solanales donors and the likelihood of fixation of this event (because no essential gene would be lost). The problem with this scenario is that it's unlikely to operate on more than one foundation pair for any one HGT event; if it occurred in every bridge sequence this would mean 10-13 separate transfers on the *M. emarginata* lineage.

A third, unrelated scenario involves two successive BIR-mediated conversion events. The first is conversion of a single *M. emarginata* foundation by newly acquired Solanales DNA, with this event extending through part of the nearby Solanales-derived

foundation. This partial foundation is then “completed” through conversion by the cognate, existing *M. emarginata* foundation. A major problem with this scenario is that it predicts that the “completed” foundation would not show any evidence of conversion by the newly acquired foreign DNA. Only one of the 10 clear-cut cases of bridge HGT meets this prediction (Table 4). Also, if this scenario were common, one would expect to find extra copies of bridge-associated foundations in *M. emarginata*, and as emphasized earlier we do not find any such copies.

The introduction of new sequence (not just the overwriting of sequence) via conversion of existing sequence is reminiscent of prior cases of serial HGT. Serial HGT has been rarely substantiated but postulated to have been the cause of aberrant phylogenetic analyses (Ragan et al. 2006; Nosenko and Bhattacharya 2007). Indeed, the bridge and extension HGT that we describe could be considered a type of serial HGT that is contingent upon the occurrence of an older HGT event and which operates through a homologous recombination-based repair pathway (DSBR or BIR) with this existing foreign sequence. It does, however, differ from prior substantiated cases (Rice et al. 2013; Taylor et al. 2015), or possible cases where the concept has been invoked (Nosenko and Bhattacharya 2007), in that there is direct sequence-based evidence for many introductions of new sequence via serial HGT at sites of prior transfer.

HGT, BIR, and mitochondrial genome expansion

HGT that adds new sequences (as opposed to simply replacing existing ones by gene conversion) intrinsically drives some level of genome expansion. In certain

mitochondrial cases this expansion is considerable (Rice et al. 2013; Sanchez-Puerta et al. 2016, 2019), and thus HGT can account for a significant portion of the extensive genome-size variation in angiosperm mitochondria (Ward et al. 1981; Sloan, Alverson et al. 2012; Skippington et al. 2015). To the extent that prior HGT lays the foundation for bridge and extension HGT, these transfers will be autocatalytic in fueling further genome expansion. The import of nuclear DNA into mtDNA and the proliferation of mitochondrial repeats are also proposed to be major forces driving mitochondrial genome expansion in angiosperms (Alverson et al. 2011; Goremykin et al. 2012; Christensen 2013). To a varying extent, all three of these genome-expansion processes may involve BIR, fundamentally in the case of repeat proliferation (Christensen 2013; Garcia et al. 2019) and to the extent that extension transfer occurs in the case of HGT and import of nuclear DNA.

Comparison with other cases of HGT in angiosperm mitochondria

Phylogenetic analyses show that six genes in *M. emarginata* are of foreign origin, with all of them acquired from Solanales donors (figs. 1 and S1; table 1). Although there are many published cases of the transfer of a single mitochondrial gene or intron in angiosperms (e.g., Bergthorsson et al. 2003; Davis and Wurdock 2004; Nickrent et al. 2004; Schonenberger et al. 2005; Cho et al. 1998; Sanchez-Puerta et al. 2008), this is only the seventh case of multigene mitochondrion-to-mitochondrion HGT (Mower et al. 2010; Rice et al. 2013; Xi et al. 2013; Park et al. 2015; Sanchez-Puerta et al. 2017; Cusimano and Renner 2019). One of these cases is in *Geranium*, making *Monsonia* the second genus in Geraniaceae for which multigene mitochondrion-to-mitochondrion HGT

has been observed (Park et al. 2015).

There are several reasons, in addition to robust phylogenetic placement, why *Cuscuta*, a genus of parasitic plants commonly known as dodders, or a close relative, is a reasonable candidate as a donor for at least one of the HGT events in *M. emarginata*. First, there is strong phylogenetic evidence for a *Cuscuta* donor in two other cases of multi-gene mitochondrion-to-mitochondrion HGT in angiosperms, including the *Geranium* case mentioned above (Mower et al., 2010; Park et al. 2015). Along these lines, there is also evidence for a *Cuscuta* donor in HGT between nuclear genomes (Vogel et al. 2018), perhaps facilitated by the trans-species exchange of RNAs (Kim et al. 2014; Johnson and Axtell 2019). Third, *Cuscuta* has been shown to form intimate parasitic relationships with other members of the Geraniaceae (De Bock and Fer 1992) and to produce cellulose-degrading enzymes that can breach host cell walls (Nagar et al. 1984; Kaiser et al. 2015), providing a potential opportunity for HGT via mitochondrial fusion (Rice et al. 2013; Arimura 2018) or some other mechanism. Fourth, the co-occurrence of *Cuscuta* and *M. emarginata* in southern Africa (Venter 1983; Welman 2003) provides ample opportunity for these plants to come in contact. Finally, among the sampled *Monsonia* species, *M. emarginata* occupies a unique high altitude niche (García-Aloy et al. 2017), and *Cuscuta* has been shown to have greater population densities at higher altitudes (Pennings and Callaway 1996). This may, in part, explain why *M. emarginata* has undergone more HGT, especially apparently from *Cuscuta,* than the other examined *Monsonia* species.

There is much evidence for parasitism playing a role in mitochondrion-to-mitochondrion HGT in angiosperms. For example, in several taxa, i.e., *Amborella* (Rice et al. 2013), *Plantago* (Mower et al. 2010), *Geranium* (Park et al. 2015), *Rafflesia* (Xi et al. 2013), *Lophophytum (*Sanchez-Puerta et al. 2017; Sanchez-Puerta et al. 2019*), Pilostyles* (Nickrent et al. 2004; Barkman et al. 2007), and *Cynomorium* (Cusimano and Renner 2019), there is phylogenetic evidence of a parasitic relationship (some recovered relationships involving known parasitic taxa such as *Cuscuta*, the Rafflesiaceae, or the Santalales) bringing host and parasite into close contact in a manner that may have allowed HGT to occur. The plausibility of several of these cases is also supported by known examples of parasitism of these plants or their close relatives and geographic co-occurrence of the recipient plant and its putative parasite (Barkman et al. 2007; Mower et al. 2010; Xi et al. 2013; Park et al. 2015; Sanchez-Puerta et al. 2017), much like in *Monsonia*.

There are both similarities and differences between bridge HGT in *M. emarginata* and transfer of the *cox1* group I homing intron described earlier. These two processes have similar outcomes, resulting in both added sequence (bridges and the *cox1* intron) and conversion of existing flanking sequence (in foundations and in one or possibly both adjacent exons). These similar outcomes may reflect a similar mechanism; these foreign, insertion-containing regions may have been used as templates for the conversion of native flanking sequence through the DSBR pathway (Sanchez-Puerta et al. 2011). Although both scenarios involve the introduction of new foreign sequence, the inserted bridge regions in *M. emarginata* have, unlike the *cox1* intron, probably replaced

some existing sequence. Also, whereas *cox1* intron transfer is mediated by an endonuclease encoded by the intron, bridge transfer must be mediated by normal processes of mtDNA recombination and repair. The mobility that this homing endonuclease confers has enabled the rapid and pervasive spread of the *cox1* intron across angiosperms; such spread is hard to imagine for bridge HGT.

How much bridge and extension HGT in angiosperm mtDNA?

The discovery of bridge and extension HGT in *M. emarginata* mtDNA naturally raises the question of how frequently these processes occur in angiosperm mtDNA overall. Even in *M. emarginata* this question is germane because we searched only for foreign DNA from mitochondrial genomes of Solanales. Uncovering the full extent of bridge and extension HGT in any one mtDNA will require phylogenetically comprehensive sequencing of green plant mtDNAs (the primary donor pool for HGT in angiosperm mtDNA; Rice et al. 2013), as well as even denser sequencing of mtDNA from relatives of that mtDNA. For example, if the only sequenced *Monsonia* mtDNA available had been that of *M. emarginata*, we would have had no reason to suspect that most of its Solanales-derived DNA were the product of bridge and extension HGT. Systematic searches for these types of HGT should therefore commence with the relatively few genera and families of angiosperms for which many mtDNAs have been sequenced. These searches should also be conducted, following sequencing of close relatives, for HGT-laden angiosperm mtDNAs (Mower et al. 2010; Rice et al. 2013; Xi et al. 2013; Park et al. 2015; Sanchez-Puerta et al. 2016, 2019; Cusimano and Renner 2019). Pursuing these searches is important because recurrent transfer or conversion of the

same locus either during or after HGT may have a dramatic effect on phylogenetic inference or the identification of HGT donors (Keeling and Palmer 2001; Ragan et al. 2006; Nosenko and Bhattacharya 2007; Hao and Palmer 2011). More generally, they are important in order to gain a full and unskewed understanding of HGT with respect to mechanisms and processes, frequency, and the extent of HGT-associated gene conversion.

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Research contributions

Jeff Mower found initial evidence suggesting potential genic HGT in *M. emarginata*. Logan Cole performed all other analyses and prepared all tables and figures.

References

Adams K, Clements MJ, Vaughn JC. 1998. The *Peperomia* mitochondrial *cox1* group 1 intron: timing of horizontal transfer and subsequent evolution of the intron. *J Mol Evol.* 46(6): 689–696.

Alverson AJ, Zhuo S, Rice DW, Sloan DB, Palmer JD. 2011. The mitochondrial genome of the legume *Vigna radiata* and the analysis of recombination across short repeats. *PLoS One.* 6(1): e16404.

Anderson SL, Sekelsky J. 2010. Meiotic versus mitotic recombination: two different routes for double-strand break repair: the different functions of meiotic versus mitotic DSB repair are reflected in different pathway usage and different outcomes. *Bioessays*. 32(12): 1058–1066.

Arimura S, Yamamoto J, Aida GP, Nakazono, M, Tsutsumi, N. 2004. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proc Natl Acad Sci USA.* 101(20): 7805–7808.

Arimura S. 2018. Fission and fusion of plant mitochondria, and genome maintenance. *Plant Physiol.* 176(1): 152–161.

Backert S, Börner, T. 2000. Phage T4-like intermediates of DNA replication and recombination in the mitochondria of the higher plant *Chenopodium album* (L.). *Curr*

Genet. 37(5): 304–314.

Barkman TJ, McNeal JR, Lim S, Coat G, Croom HB, Young ND, dePamphilis CW. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evol Biol.* 7(1): 248.

Beiko RG, Harlow TJ, Ragan, MA. 2005. Highways of gene sharing in prokaryotes. *Proc Natl Acad Sci USA.* 102(40): 14332–14337.

Bergthorsson U, Adams KL, Thomason B, Palmer JD. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* 424(6945): 197–-201.

Cheng N, Lo Y-S, Ansari MI, Ho, K-C, Jeng S-T, Lin N-S, Dai, H. 2017. Correlation between mtDNA complexity and mtDNA replication mode in developing cotyledon mitochondria during mung bean seed germination. *New Phytol.* 213(2): 751–763.

Cho Y, Mower JP, Qiu YL, Palmer, JD. 2004. Mitochondrial substitution rates are extraordinarily high and elevated within a genus of flowering plants. *Proc Natl Acad Sci USA.* 101(51): 17741–17746.

Cho Y, Qui Y-L, Kuhlman P, Palmer JD. 1998. Explosive invasion of plant mitochondria by a group I intron. *Proc Natl Acad Sci USA.* 95(24): 14244–-14249.

Christen AC. 2013. Plant mitochondrial genome evolution can be explained by DNA repair mechanisms. *Genome Biol Evol.* 5(6): 1079–1086.

Christensen AC. 2014. Genes and junk in plant mitochondria—repair mechanism and selection. *Genome Biol Evol.* 6(6): 1448–1453.

Cole LW, Guo W, Mower JP, Palmer JD. 2018. High and variable rates of repeat-mediated mitochondrial genome rearrangement in a genus of plants. *Mol Biol Evol.* 35(11): 2773–2785.

Cusimano N, Renner, SS. 2019. Sequential horizontal gene transfers from different hosts in a widespread Eurasian parasitic plant, *Cynomorium coccineum*. *Am J Bot.* 106(5): 679–689.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModeltest 2: more models, new heuristics and parallel computing. *Nat Methods.* 9(8): 772.

Davila JL, Arrieta-Montiel MP, Wamboldt Y, Cao J, Hagmann J, Shedge V, Xu YZ, Weigel D, Mackenzie SA. 2011. Double-strand break repair processes drive evolution of the mitochondrial genome in Arabidopsis. *BMC Biol* 9(1): 64.

Davis CC, Wurdock KJ. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from the malpighiales. *Science* 305(5684): 676–678.

De Bock F, Fer A. 1992. Effects of abscisic acid on the transfer of sucrose from host, *Pelargonium zonale* (L.) Aiton, to a phanerogamic parasite, *Cuscuta reflexa* Roxb. *Aust J Plant Physiol.* 19(6): 679–691.

Didelot X, Maiden MCJ. 2010. Impact of recombination on bacterial evolution. *Trends Microbiol.* 18(7): 315–322.

Eberhardt I, Hohmann S. 1995. Strategy for deletion of complete open reading frames in Saccharomyces cerevisiae. *Current Genetics* 27(4): 306–308

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32(5): 1792–1797.

García-Aloy S, Sanmartín I, Kadereit G, Vitales D, Millanes AM, Roquet C, Vargas P, Alarcón M, Aldasoro JJ. 2017. Opposite trends in the genus *Monsonia* (Geraniaceae): specialization in the African deserts and range expansions throughout eastern Africa. *Sci Rep.* 7(1): 9872.

Garcia, L.E., Zubko, M.K., Zubko, E.I., and Sanchez-Puerta, M.V. 2019. Elucidating genomic patterns and recombination events in plant cybrid mitochondria*. Plant Molecular Biology* 100(4–5): 433–450.

Giege P, Brennike A. 1999. RNA editing in *Arabidopsis* mitochondria effects 441 C to U changes in ORFs. *Proc Natl Acad Sci USA* 96(26): 15324–15329.

Goremykin VV, Lockhart PJ, Viola R, Velasco R. 2012. The mitochondrial genome of *Malus domestica* and the import-driven hypothesis of mitochondrial genome expansion in seed plants. *Plant J.* 71(4): 615–626.

Grewe F, Edger PP, Keren I, Sultan L, Pires JC, Ostersetzer-Biran O, Mower JP. 2014. Comparative analysis of 11 Brassicales mitochondrial genomes and the mitochondrial transcriptome of *Brassica oleracea*. *Mitochondrion.* 19B(1): 135–143.

Hao W, Palmer JD. 2009. Fine-scale mergers of chloroplast and mitochondrial genes create functional, transcompartmentally chimeric mitochondrial genes. *Proc Natl Acad Sci USA.* 106(39): 16728–16733.

Hao W, Palmer JD. 2011. Confounding phylogenetic influence of duplicative horizontal transfer and differential gene conversion. *Mob Genet Elements.* 1(4): 256–261.

Hao W, Richardson AO, Zheng Y, Palmer, JD. 2010. Gorgeous mosaic of mitochondrial genes created by horizontal transfer and gene conversion. *Proc Natl Acad Sci USA.* 107(50): 21576–21581.

Hepburn NJ, Schmidt DW, and Mower JP. 2013. Loss of two introns from the *Magnolia*

tripetala mitochondrial *cox2* gene implicates horizontal gene transfer and gene conversion as a novel mechanism of intron loss. *Mol Biol Evol.* 29(10): 3111–3120.

Husnik F, McCutcheon JP. 2018. Functional horizontal gene transfer from bacteria to eukaryotes. *Nat Rev Microbiol.* 16(2): 67–79.

Johnson NR, Axtell MJ. 2019. Small RNA warfare: exploring origins and function of trans-species microRNAs from the parasitic plant *Cuscuta*. *Curr Opin Plant Biol.* 50(1): 76–81.

Kaiser B, Vogg G, Furst UB, Albert M. 2015. Parasitic plants of the genus *Cuscuta* and their interaction with susceptible and resistant host plants. *Front Plant Sci.* 6(1): 45.

Keeling PJ, Palmer JD. 2001. Lateral transfer at the gene and subgenic levels in the evolution of the eukaryotic enolase. *Proc National Acad Sci USA.* 98(19): 10745–10750.

Keeling PJ, Palmer JD. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat Rev Genet.* 9(8): 605–618.

Kim G, LeBlanc ML, Wafula EK, dePamphilis CW, Westwood JH. 2014. Genome-scale exchange of mRNA between a parasitic plant and its hosts. *Science* 345(6198): 808–811.

Klasson L, Westberg J, Sapountzis P, Naslund K, Lutnaes Y, Darby AC, Veneti Z, Chen L, Braig HR, Garrett R, Bourtzis K, Andersson SGE. 2009. The mosaic structure of the *Wolbachia* wRi strain infecting *Drosophila simulans. Proc Natl Acad Sci USA.* 106(14): 5725–5730.

Lee H-O, Choi J-W, Bae JH, Oh J-H, Lee S-C, Kim C-K. 2018. Assembly of the mitochondrial genome in the Campanulaceae family using Illumina low-coverage. *Genes (Basel).* 9(8): 383.

Leger MM, Eme L, Stairs CW, Roger AJ. 2018. Demystifying eukaryote lateral gene transfer. *BioEssays.* 40(5): 170242.

Lima MS, Smith DR. 2017. Pervasive transcription of mitochondrial, plastid, and nucleomorph genomes across diverse plastid-bearing species. *Genome Biol Evol.* 9(10): 2650–2657.

Llorente B, Smith CE, Symington LS. 2008. Break-induced replication: what is it and what is it for? *Cell Cycle.* 7(7): 859–864.

Manchekar M, Scissum-Gunn K, Song D, Khazi F, McLean SL, Nielsen BL. 2006. DNA recombination activity in soybean mitochondria. *J Mol Biol.* 356(2): 288–299.

Mau B, Glasner JD, Darling AE, Perna NT. 2006. Genome-wide detection and analysis

of recombination among sequenced strains of *Escherichia coli*. *Genome Biol.* 7(5): R44.

Mishra P, Chan DC. 2016. Metabolic regulation of mitochondrial dynamics. *J Cell Biol.* 212(4): 379–387.

Mower JP, Jain K, Hepburn NJ. 2012. The role of horizontal transfer in shaping the plant mitochondrial genome. In: Maréchal-Drouard L, editor. *Advances in Botanical Research* Vol. 63. Amsterdam, Netherlands: Elsevier. p. 41–69.

Mower JP, Stefanovic, S, Hao W, Gummow JS, Jain K, Ahmed D, Palmer JD. 2010. Horizontal acquisition of multiple mitochondrial genes from a parasitic plant followed by gene conversion with host mitochondrial genes. *BMC Biol.* 8(1):150.

Mower JP, Stefanovic S, Young GJ, Palmer JD. 2004. Plant genetics: gene transfer from parasitic to host plants. *Nature* 432(7014): 165–166.

Mower JP, Touzet P, Gummow JS, Delph LF, Palmer JD. 2007. Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. *BMC Biol.* 7(1): 135.

Nagar R, Singh M, Sanwal GG. 1984. Cell wall degrading enzymes in *Cuscuta reflexa* and its hosts. *J Exp. Bot.* 35(8): 1104–1112.

Naumann J, Salomo K, Der JP, Wafula EK, Bolin JF, Maass E, Frenzke L, Samain M-S, Neinhus C, de Pamphilis CW, Wanke S. 2013. Single copy nuclear genes place haustorial Hydnoraceae within Piperales and reveal a Cretaceous origin of multiple parasite lineages. *PLoS One.* 8(11): e79204.

Nei M, Gojobori T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol Biol Evol.* 3(5): 418–426.

Nickrent DL, Blarer A, Qiu YL, Vidal-Russell R, Anderson FE. 2004. Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. *BMC Evol Biol.* 4(1): 40.

Nosenko T, Bhattacharya D. 2007 Horizontal gene transfer in chromalveolates. *BMC Evol Biol.* 7(1): 173.

Ochman H, Lawrence JG, Groisman EA. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405(6784): 299–304.

Oldenburg DJ, Bendich AJ. Size and structure of replicating mitochondrial DNA in cultured tobacco cell. *Plant Cell* 8(1): 447–461.

Palmer JD, Shields CR. 1984. Tripartite structure of *Brassica campestris* mitochondrial genome. *Nature* 307(5950): 437–440.

Park S, Grewe F, Zhu A, Ruhlman TA, Sabir J, Mower JP, Jansen RK. 2015. Dynamic evolution of *Geranium* mitochondrial genomes through multiple horizontal and intracellular gene transfers. *New Phytol.* 208(2): 570–583.

Parkinson CL, Mower JP, Qiu YL, Shirk YL, Song K, Young ND, dePamphilis CW. 2005. Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. *BMC Evol Biol.* 5(1): 73.

Pennings SC, Callaway RM. 1996. Impact of a parasitic plant on the structure and dynamics of salt marsh vegetation. *Ecology* 77(5): 1410–1419.

Pond SLK, Frost, SDW, Muse SV. 2005. HyPhy: hypothesis testing using phylogenies. *Bioinformatics* 21(5): 676–679.

Ragan MA, Harlow TJ, Beiko RG. 2006. Do different surrogate methods detect lateral genetic transfer events of different relative ages? *Trends Microbiol.* 14(1): 4–8.

Refulio-Rodriguez NF, Olmstead RG. 2014. Phylogeny of Lamiidae. *Am J Bot.* 101(2): 287-299.

Rice DW, Alverson AJ, Richardson AO, Young GJ, Sanchez-Puerta MV, Munzinger J, Barry K, Boore JL, Zhang Y, dePamphilis CW, Knox EB, Palmer JD. 2013. Horizontal

transfer of entire genomes via mitochondrial fusion in the angiosperm *Amborella.* *Science* 342(6165): 1468–1473.

Richardson AO, Palmer JD. 2007. Horizontal gene transfer in plants. *J Exp Bot.* 58(1): 1–9.

Sanchez-Puerta MV. 2014. Involvement of plastid, mitochondrial and nuclear genomes in plant-to-plant horizontal gene transfer. *Acta Soc Bot Pol.* 83(4): 317–323.

Sanchez-Puerta MV, Abbona CC, Zhuo S, Tepe EJ, Bohs L, Olmstead RG, Palmer JD. 2011. Multiple recent horizontal transfers of the *cox1* intron in Solanaceae and extended co-conversion of flanking exons. *BMC Evol Biol* 11(1): 277.

Sanchez-Puerta MV, Cho Y, Mower JP, Alverson AJ, Palmer JD. 2008. Frequent, phylogenetically local horizontal transfer of the *cox1* group I intron in flowering plant mitochondria. *Mol Biol Evol*. 25(8): 1762–1777.

Sanchez-Puerta MV, Edera A, Gandini CL, Williams AV, Howell KA, Nevill PG, Small I. 2019. Genome-scale transfer of mitochondrial DNA from legume hosts to the holoparasitic *Lophophytum mirabile* (Balanophoraceae). *Mol. Phylogenetics Evol.* 132(1): 243–250.

Sanchez-Puerta MV, Garcia LE, Wohlfeiler J, Ceriotti LF. 2017. Unparalleled

replacement of native mitochondrial genes by foreign homologs in a holoparasitic plant. *New Phytol.* 214(1): 376–387.

Sanchez-Puerta MV, Zubko MK, Palmer JD. 2015. Homologous recombination of a single form of most genes shape the highly chimeric mitochondrial genome of a cybrid plant. *New Phytol.* 206(1): 381–396.

Särkinen T, Bohs L, Olmstead RG, Knapp S. 2013. A phylogenetic framework for the evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree. *BMC Evol Biol* 13(1): 214.

Schonenberger J, Anderberg AA, Sytsma KJ. 2005. Molecular phylogenetics and patterns of floral evolution in the Ericales. *Int J Plant Sci.* 166(2): 265–268.

Sheahan MB, McCurdy DW, Rose RJ. 2005. Mitochondria as a connected population: ensuring continuity of the mitochondrial genome during plant cell dedifferentiation through massive mitochondrial fusion. *Plant J.* 44(5): 744–755.

Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst Biol.* 51(3): 492–508.

Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17(12): 1246–1247.

Skippington E, Barkman TJ, Rice DW, Palmer JD. 2015. Miniaturized mitogenome of the parasitic plant *Viscum scurruloideum* is extremely divergent and dynamic and has lost all nad genes. *Proc Natl Acad Sci USA.* 112(27): E3515–E3524.

Sloan DB. 2013. One ring to rule them all? Genome sequencing provides new insights into the 'master circle' model of plant mitochondrial DNA structure. *New Phytol* 200(4): 978–985.

Sloan DB, MacQueen AH, Alverson AJ, Palmer JD, Taylor DR. 2010. Extensive loss of RNA editing sites in rapidly evolving *Silene* mitochondrial genomes: selection vs retroprocessing as the driving force. *Genetics* 185(4):1369–1380.

Sloan DB. 2013. One ring to rule them all? Genome sequencing provides new insights into the 'master circle' model of plant mitochondrial DNA structure. *New Phytol* 200(4): 978–985.

Sloan DB, Alverson AJ, Chuckalovcak JP, Wu M, McCauley DE, Palmer JD, Taylor DR. 2012. Rapid evolution of enormous, multichromosomal genomes in flowering plant mitochondria with exceptionally high mutation rates. *PLoS Biol.* 10(1): e1991241.

Sloan DB, Müller K. McCauley DE, Taylor DR, Štorchová H. 2012. Intraspecific variation in mitochondrial genome sequence, structure, and gene content in *Silene vulgaris*, an

angiosperm with pervasive cytoplasmic male sterility. *New Phytol.*196(4): 1228–1239.

Soucy SM, Huang J, Gogarten JP. 2015. Horizontal gene transfer: building the web of life. *Nat Rev Genet.* 16(8): 472–482.

Stevens PF. (2001 onwards). Angiosperm Phylogeny Website. Version 14. Updated April 4, 2018. http://www.mobot.org/MOBOT/research/APweb/

Sun G, Xu Y, Liu H, Sun T, Zhang J, Hettenhausen C, Shen G, Qi J, Qin Y, Li J, Wang L, Chang W, Guo Z, Baldwin IT, Wu J. 2018. Large-scale gene losses underlie the genome evolution of parasitic plant *Cuscuta australis*. *Nat Commun*. 9(1): 2683.

Tank DC, Eastman JM, Pennell MW, Soltis, PS, Soltis DE, Hinchliff CE, Brown JW, Sessa EB, Harmon LJ. 2015. Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. *New Phytol.* 207: 454–467.

Taylor ZN, Rice DW, Palmer JD. 2015. The complete moss mitochondrial genome in the angiosperm Amborella is a chimera derived from two moss whole-genome transfers. *PLoS One.* 10(11): e0137532.

Venter HJT. 1983. Phytogeography and interspecies relationships in *Monsonia* (Geraniaceae). *Bothalia* 14(3–4): 865–869.

Vogel A, Schwacke R, Denton AK, Usadel B, Hollmann J, Fischer K, Bolger A, Schmidt, MHW, Boldger ME, Gundlach H, Mayer KFX, Weiss-Schneeweiss H, Temsch EM, Krause K. 2018. Footprints of parasitism in the genome of the parasitic plant *Cuscuta campestris*. *Nat Commun.* 9(1): 2515.

Wang D, Rousseau-Gueutin M, Timmis JN. 2012. Plastid sequences contribute to some plant mitochondrial genes. *Mol Biol Evol.* 29(7): 1707–1711.

Ward B, Anderson R, Bendich A. 1981. The size of the mitochondrial genomes is large and variable in a family of plants (Cucurbitaceae). *Cell* 25(3): 793–803.

Welman WG. 2003. *Cuscuta.* In: Germishuizen G, Meyer NL, editors. *Plants of southern Africa: an annotated checklist.* Strelitzia Vol. 15. Pretoria, South Africa: South African National Biodiversity Institute (SANBI Publishing). p. 373–374.

Wu Z, Stone JD, Storchova H, Sloan DB. 2015. High transcript abundance, RNA editing, and small RNAs in intergenic regions within the massive mitochondrial genome of the angiosperm *Silene noctiflora*. *BMC Genomics* 16(1): 938.

Zhaxybayeva, O. and Doolittle, W.F. 2011. Lateral gene transfer. *Curr Biol.* 21(7): R242–R246.

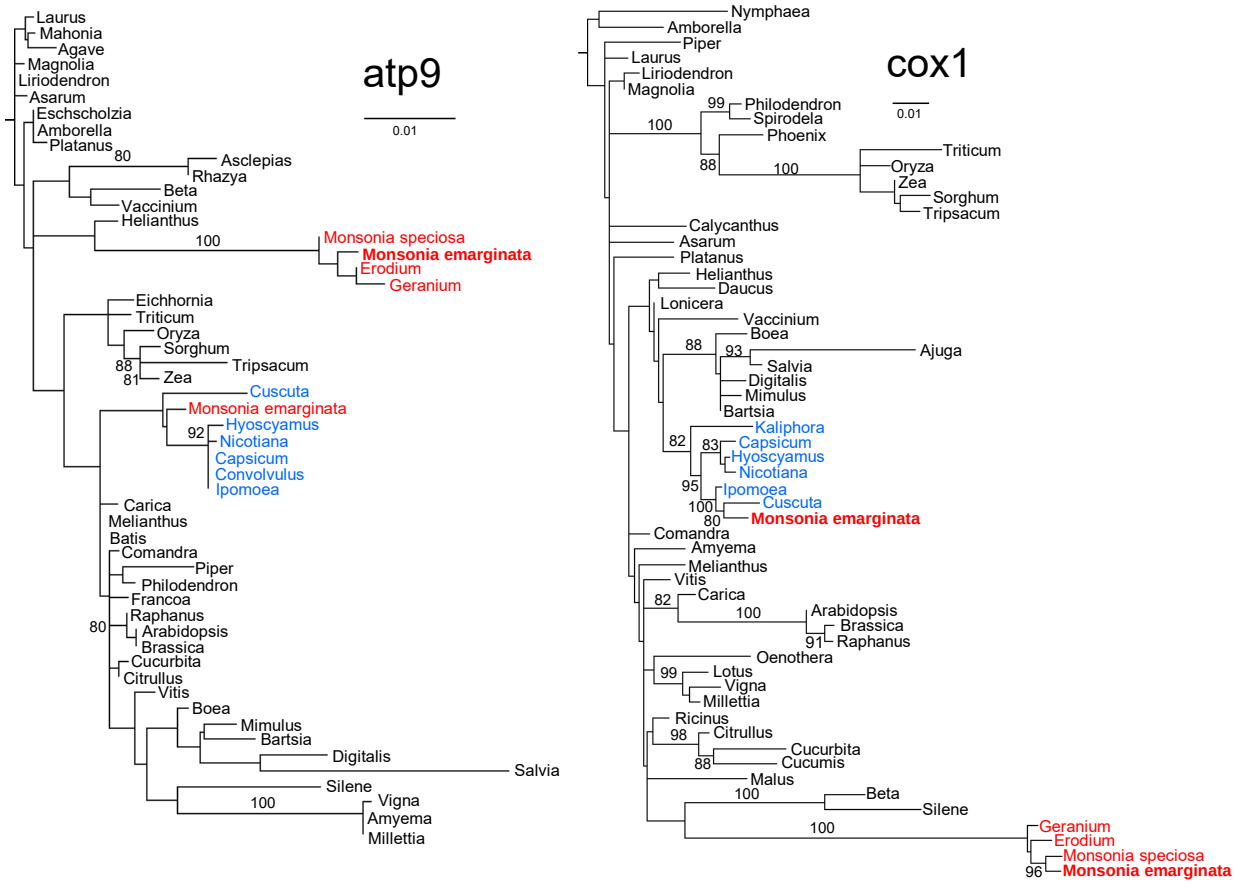
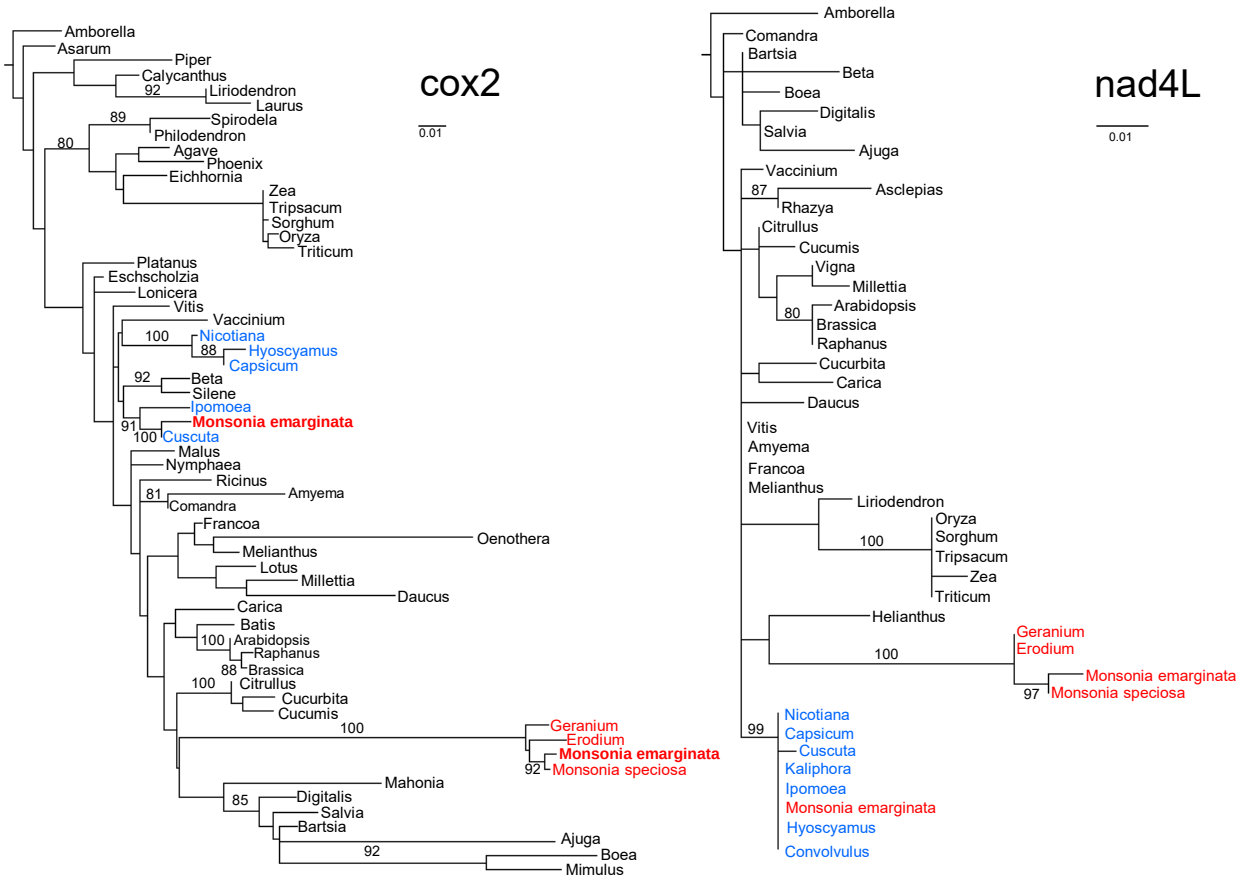


Figure S1. Phylograms of *atp9*, *cox1*, *cox2*, and *nad4L* showing horizontally transferred genes. Taxa in the Geraniaceae are in red, and those in the Solanales are in blue. Bootstrap values ≥ 80% are displayed. Scale bars are in units of substitutions per site. The outgroup (*Cycas*) is not shown.



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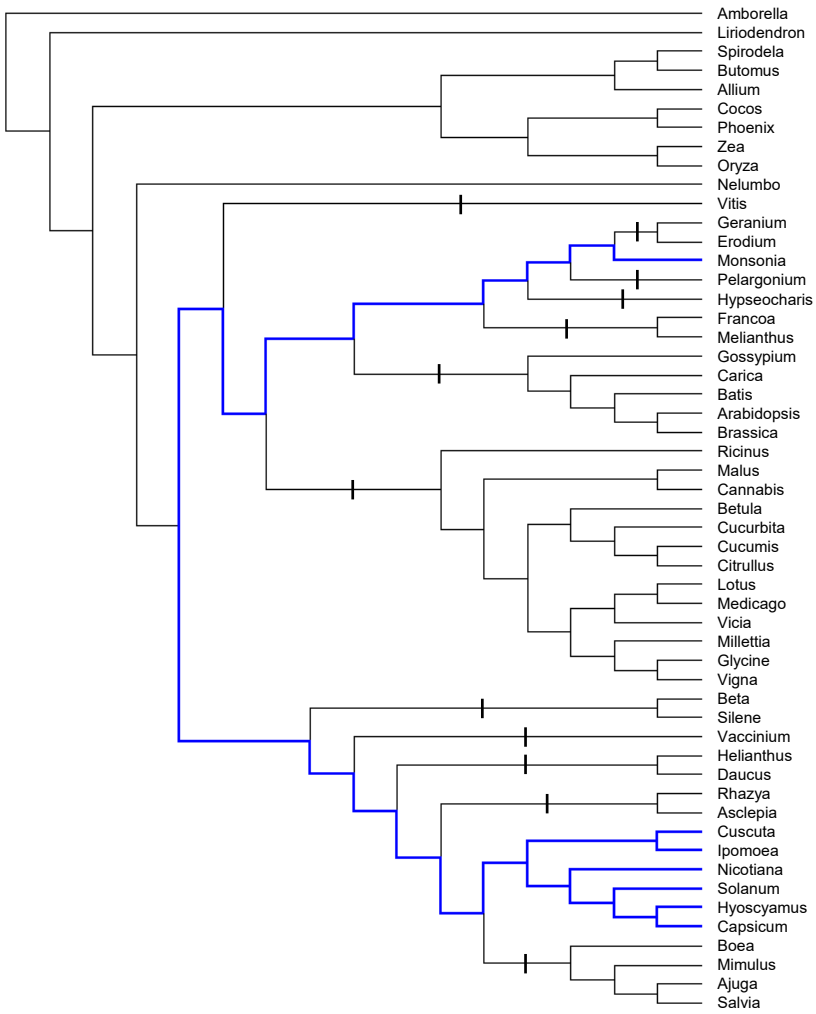

Figure S2. Scenario involving vertical transmission and differential loss rather than horizontal transfer for the phylogenetic distribution of nongenic putatively transferred mtDNA regions present in *Monsonia* mtDNAs. The cladogram shows organismal relationships for a subset of the 178 examined mtDNAs (topology from Stevens et al. 2001 onwards). The blue path indicates the line of vertical descent connecting Solanales and *Monsonia*, with the vertical bars marking the minimum set of losses under the vertical transmission/differential loss scenario. This scenario postulates that nongenic sequences currently present only in in Solanales and *Monsonia* were actually present in the common ancestor of the two groups and subsequently lost in all lineages except the Solanales and *Monsonia*. Note that for certain regions, additional losses would have occurred within the Solanales and *Monsonia* (tables 3 and S1).

Table S1. Lengths of regions donated to *Monsonia* in Solanales mtDNAs.

Region[1]	Length (bp)[2,3]				
	Capsicum	Hyoscyamus	Nicotiana	Cuscuta	Ipomoea
atp4f1	337	337	337	45	45 292
atp8f1	302	302	302	85 117	85 217
atp8f2	361	61 143	361	72 110	72 110
atp9f1	400	400	81 126	68 90 35	311 89
cox1f1	326	326	326	52	326
cox2f1	233	233	72 121	32 71	32
cox2f2	307	307	307	40	307
nad4Lf1	267	267	267	71	267
nad4Lf2	306	306	306	88	306
i1f1	151	151	151	71 56	151
i1f2	177	177	177	-	177
i2f1	309	309	309	-	309
i2f2	358	358	358	89 124	89 171
i3f1	411	411	411	-	411
i3f2	378	378	378	121 160	378
i4f1	373	373	373	-	201
i4f2	189	189	120	-	91 98
i5f1	351	-	-	-	351
i5f2	380	380	380	-	70
i6f1	279	279	279	279	279
i6f2	181	166	166	-	-
i7f1	676	676	676	80	-
i7f2	211	211	211	72	211
i8f1	417	417	417	-	-
i9f1	520	520	305 215	-	520
i9f2	-	-	-	-	240
i10f1	158	158	158	80 62	80 62
i10f2	-	-	-	103 40	150
i11f1	666	666	666	55	398 68
i11f2	-	-	-	201 94	295
i12	147	147	147	-	-
i13	125	125	125	42	125
i14	303	303	-	-	303
i15	201	201	201	-	83
i16	261	261	261	97 112	171 90
i17	115	115	115	115	115
i18	151	151	151	-	151
i19	427	427	427	-	132 213
i20	200	81 119	200	-	97
i21	127	127	127	85	127
i22	-	-	-	125	43
i23	-	-	-	94 45	179

[1]"f" refer to foundation regions.

[2]"-" indicates absence of that region.

[3]Split cells indicate regions that appear to have been split, with the lengths being those of their constituent parts. For split foundation regions, the leftmost number is for that part that abuts the bridge.

Table S2. Number of predicted C-to-U RNA-editing sites for foreign gene copies in *M. emarginata* and native homologs in Solanales.

Gene	Editing sites[1]						
	M. emarginata		Solanaceae			Convolvulaceae	
	Native	Foreign	Capsicum	Hyoscyamus	Nicotiana	Cuscuta	Ipomoea
atp4[2]	7 (9)	9	9	9	9	9	9
atp8	0 (3)	3	4	4	4	3	3
atp9[2]	0 (3)	3	3	3	3	3	3
cox1[2]	0 (5)	5	5(4)	6(4)	4(3)	5	5
cox2	2(11)	11	9(2)	10(2)	9(2)	11	11
nad4L	1(14)	14	14	14	14	14	14
Shared[3]	0	45	39	39	40	45	45

[1]Numbers in parenthesis are those editing sites that are absent in that copy but present in the foreign copies of *M. emarginata*. The foreign copies in *M. emarginata* share no common edit sites with the native copies. All edit sites in the foreign copies of *M. emarginata* are shared by *Ipomoea* and *Cuscuta.*
[2]Parts of the genes that are truncated in the foreign *M. emarginata* copies are not considered.
[3]Total number of editing sites shared with the foreign copy of *M. emarginata.*

Table S3. Separation distances across *Monsonia* between pairs of foundation regions for bridge HGTs in *M. emarginata*[1].

Region	Separation distance (bp)[2,3]						
	Msp	Mem	Mma	Mva	Mca	Mci	Mhe
atp8	71,118	439	114,926	68,019	9,298	142,434	49,040
cox2	47,102	741	100,328	94,829	71,112	14,697	257,773
nad4L	21,904	270	6,849	>69,541	-	>139,402	121,419
i1	133,310	360	84,331	-	17,212	31,249	-
i2	>33,465	1,032	33,329	120,011	98,853	-	-
i3	>41,216	275	13,551	11,995	62,871	28,912	>111,635
i4	12,522	101	>105,713	268,264	92,543	71,334	>41,500
i5	100,258	788	48,505	-	-	-	12,115
i6	88,972	496	21,274	>166,403	>101,229	-	>21,667
i7	>61,124	704	>41,505	27,118	55,890	-	71,060
Region	Ratio of bridgeless *Monsonia* to *M. emarginata* separations[2,3]						
	Msp	Mem	Mma	Mva	Mca	Mci	Mhe
atp8	162	n.a.	261	154	21	324	111
cox2	63	n.a.	135	127	95	19	347
nad4L	81	n.a.	25	>257	-	>516	449
i1	370	n.a.	234	-	47	86	-
i2	>32	n.a.	32	116	95	-	-
i3	>149	n.a.	49	43	228	105	>405
i4	123	n.a.	>1046	2,656	916	706	>410
i5	127	n.a.	61	-	-	-	15
i6	179	n.a.	42	>335	>204	-	>43
i7	>86	n.a.	>58	38	79	-	100

[1]*Monsonia* species are denoted by the first letter of their genus names and first two letters of their species names.
[2]In cases in which the two foundations are on different scaffolds, a minimum estimate of separation size corresponding to the sum of the shortest distance between each foundation and the ends of the scaffold in which it resides was calculated. These cases are denoted as greater than (>) this minimum.
[3]"-" indicates one or both foundation regions are absent, precluding calculation of a separation distance.

Table S4. Distances in *M. emarginata* and Solanales between pairs of foundation regions implicated in bridge HGTs.

Region	Separation distance (bp)[1]					
	M. emarg.	Capsicum	Hyoscyamus	Nicotiana	Cuscuta	Ipomoea
atp8	439	**439**	438	561	441	441
cox2	741	1,648	2,278	2,110	1,915	2,093
nad4L	270	**270**	**270**	**270**	**270**	**270**
i1	360	**360**	**360**	**360**	-	**360**
i2	1,032	1,008	1,008	1,008	-	**1,032**
i3	275	**275**	**275**	**275**	-	**275**
i4	101	**101**	**101**	**101**	-	**101**
i5	788	**788**	-	-	-	**788**
i6	496	**496**	**496**	**496**	-	-
i7	704	**704**	**704**	640	**704**	-

[1]Distances in Solanales that are equal to those in *M. emarginata* are bolded. "-" indicates one or both foundation regions are absent, precluding calculation of separation distance.

CHAPTER 3

The evolution of per-cell organelle number

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Abstract

Organelles with their own distinct genomes, such as plastids and mitochondria, are found in most eukaryotic cells. As these organelles and their host cells have evolved, the partitioning of metabolic processes and the encoding of interacting gene products have created an obligate codependence. This relationship has played a role in shaping the number of organelles in cells through evolution. Factors such as stochastic evolutionary forces acting on genes involved in organelle biogenesis, organelle–nuclear gene interactions, and physical limitations may, to varying degrees, dictate the selective constraint that per-cell organelle number is under. In particular, coordination between nuclear and organellar gene expression may be important in maintaining gene product stoichiometry, which may have a significant role in constraining the evolution of this trait.

Introduction

With a few exceptions (Cavalier-Smith 1987; Karnkowska et al. 2016), mitochondria or plastids are found in all eukaryotic cells. As these organelles and their host cells have evolved together, the partitioning of metabolic processes and the encoding of interacting gene products have created an obligate codependence. This relationship has obviously

played an important role in shaping the genomes of these different compartments (Howe et al. 2002; Adams and Palmer 2003), but what has not been widely considered is how this intimate relationship has constrained the number of organelles in a cell through evolution.

Typically, a multicellular eukaryote has hundreds or thousands of these organelles in each of their cells. These numbers not only vary among species but also among tissue types (Veltri et al. 1990; Li et al. 2001; Moller 2004) and temporally during the cell cycle, across development, and in response to stress (Holloszy 1967; Posakony et al. 1977; Boffey and Leech 1982; Visser et al. 1995; Moller 2004; Kowald and Kirkwood 2011). These organelles serve necessary metabolic functions and create gene products that are necessary not only for their own function but for that of the entire cell. This, along with the transfer of genes from the organelle to nuclear genomes over evolutionary time (Blanchard and Schmidt 1995; Howe et al. 2002; Adams and Palmer 2003), has created a necessary coordination between these entities. These factors, to some extent, have constrained the number of organelles in a cell through selection. Other factors, such as stochastic evolutionary forces, which vary depending on the population genetic environment of the genes involved in organelle biogenesis and organelle–nuclear interactions, or physical limitations may obscure the selective constraint that this feature is under.

Estimating organelle number across the eukaryotes

Organelles can be counted directly using molecular staining (Williamson and Fennell 1979; Chazotte 2011) and microscopy, but this is very labor intensive. Alternatively, organelles can be quantified with some uncertainty through flow cytometry (Mattiasson 2004), which has the drawback of underestimation that results from the isolation process, or through the use of biochemical probes (Robin and Wong 1988). Recently, some have been using micro-dissection to estimate organelle number by extrapolating counts from cross sections (Inuda and Wildermuth 2004; Kubinova et al. 2014). Some would argue that the methods that involve direct counting are the only ones appropriate to properly estimate the number of organelles in a cell, but they are impractical when it comes to some cases, such as animal cells, that have thousands of mitochondria.

These techniques are useful for low error quantification of organelle number within a cell but, with the advent of sequencing technology, one can use organelle genome copy number to estimate organelle number (Moraes 2001). This, of course, comes with the consideration that there tends to be between 1 and 10 copies of mtDNA per mitochondrion in animals (Satoh and Kuroiwa 1991; Weisner et al. 1992; Bereiter-Hahn and Voth 1996; Iborra et al. 2004; Legros et al. 2004; Brown et al. 2011; Kukat et al. 2011), between 50 and 200 copies of mtDNA in yeast (Williamson and Fennell 1979; Azpiroz and Butow 1993; Solieri 2010), and between 0 and 2 copies of mtDNA in plants (Suyama and Bonner 1966; Bendich and Gauriloff 1984; Preuten et al. 2010), though this estimate may be influenced by the presence of substoichiometric mtDNA molecules (Palmer and Shields 1984; Preuten et al. 2010; Mower et al. 2012), and up to ~1000

copies of cpDNA per mature chloroplast (Boffey and Leech 1982). These per-organelle genome copy numbers can also vary temporally; they can either change over the life span of the organelle as is the case with most chloroplasts (Lamppa et al. 1980; Scott and Possingham 1980, 1983; Baumgartner et al. 1989; Kuroiwa 1991), the lifespan of the organism as is the case with some mitochondria (Hartmann et al. 2011) and chloroplasts (Zoschke et al. 2007), or change erratically and unpredictably due to the unequal redistribution of nucleoid material between organelles during fusion and fission, which has been shown to be the case in some mitochondria (Arimura, Aida et al. 2004).

A major caveat with these methods of quantification is that they tend to estimate organelle number at a particular time point or over a very small time range, while it is known that the number of organelles is a very labile trait that can vary a great deal over time (Stoecker and Silver 1990; Arimura, Aida et al. 2004).

Like many unicellular eukaryotes, which can have as few as a single mitochondrion or a few dozen mitochondria per-cell (Gray et al. 2004) and can also have on the order of 105 mitochondria (Okie et al. 2016), multicellular eukaryotes tend to have a wide range of per-cell mitochondria numbers with estimates in mammalian somatic cells ranging from ~80 to ~2000 (Robin and Wong 1988; Bogenhagen 2011; Kukat et al. 2011) and from ~200 to ~600 in plant mesophyll cells (Logan 2007). Yeast have been shown to vary in terms of per-cell mitochondria number based on substrate despite relatively consistent per-cell mtDNA copy number—for example, yeast grown on a glucose substrate were shown to contain 2–3 mitochondria that are branched in structure while

those grown on an ethanol substrate contain 20–30 tubular-shaped mitochondria (Visser et al. 1995). Occasionally, there can be tens of thousands (Satoh and Kuroiwa 1991) or even millions of mitochondria in an animal oocytes, as is the case in the oocytes of *Xenopus laevis* which have been estimated to have on the order of 10^7 mitochondria (Marinos 1985). Mitochondria are very dynamic entities that are subject to frequent fusion and fission (Arimura, Aida et al. 2004; Kowald and Kirkwood 2011) so this can change over time. Both mitochondria (Gurdon et al. 2016) and chloroplasts (Thyssen et al. 2012) have been shown to move between cells in plants, even among those of different species, which may have some effect on per-cell counts of organelles. Some unicellular algae will tend to have a single chloroplast (Itoh et al. 1996) whereas some cells from plants, such as mesophyll cells, have been seen to have an average of ~50 chloroplasts at their earliest stages of development (Boffey and Leech 1982) and up to hundreds of chloroplasts (Moller 2004) with an average of 155 at their latest stages of development (Boffey and Leech 1982). Similarly, mitochondria number has been shown to fall 25–50% during leaf senescence in *Vitis vinifera* (Ruberti et al. 2014). These numbers suggest that those early eukaryotes had one or a few of these organelles, as in some modern unicellular eukaryotes, and then underwent an increase followed by a diversification in organelle number, resulting in the variation in organelle number we see among multicellular eukaryotes.

These numbers not only vary significantly across organisms, but among tissue types. For example, there are more chloroplasts in leaf tissue than in other tissue (Li et al. 2001). Moist, leafy tissue in plants has been shown to have more mitochondria than

woody and stem tissue (Moller 2004). This is the result of leafy, green tissue being the important focal tissue of photosynthesis. Mesophyll and stomata cells have also been shown to lose mitochondria at different rates during leaf senescence (Ruberti et al. 2014). Studies of the mouse have shown that tissue of the liver, kidney, heart, and brain have different numbers of mitochondria per-cell (Veltri et al. 1990). Organelle number also varies in in terms of lability across tissue types. For example, muscle cells are known to vary greatly in mitochondrial content with organismal physical activity (Holloszy 1967). The differences in mitochondria number among tissue types may be the result of varying energetic constraints (Holloszy 1967; Veltri et al. 1990). It has also been shown that there can be large reductions in chloroplast number in stressful high and low light conditions in a variety of green plants (Higa and Wada 2016).

The extent to which per-cell mitochondria number varies within tissue is largely unknown. Many methods used to count mitochondria within multicellular eukaryotes involve large samples of tissue with flow cytometry or other methods that represent an aggregate of many cells within a tissue (Mattiasson 2004). *In vivo* estimates would require microscopy of many cells within the same tissue of the same organism. Variability has been assessed within a HeLa cell line, which was shown to contain between 383 and 882 mitochondria per-cell (Posakony et al. 1977), though this is mostly the result of differences in cell cycle stages. Furthermore, this should not be interpreted as a reflection of natural or *in vivo* variation of within-tissue organelle number. Within-tissue variation in chloroplast number, however, has been shown to be rather extensive with between ~2 and ~140 fold variation in chloroplast number in

palisade tissue from different green plants (Higa and Wada 2016). To what extent and how within-tissue per-cell organelle number variation is maintained will require more data and this will serve as a prerequisite to analyses with methods from quantitative genetics, developmental biology, and physiology.

Organelle biogenesis

At the molecular level, per-cell organelle number is underpinned by series of transcriptional pathways. These processes are distinct but involve related components in chloroplasts and mitochondria that mostly control the pattern of division of organelles and the replication and transcription of their genomes. An understanding of what genes are involved in organelle biogenesis is necessary to fully understand the evolution of organelle number in cells.

The so-called “master regulators” of mitochondrial biogenesis are members of the PGC (peroxisome proliferator-activated receptor-γ) family of co-transcriptional regulatory factors (Irrcher et al. 2003; Ventura-Clapier et al. 2008). In particular, PGC-1α is known to activate different transcription factors that interact with Tfam (mitochondrial transcription factor A), which is involved in the replication and transcription of mtDNA and the transcription of nuclear-encoded mitochondrial components (Virbasius and Scarpulla 1994). Though the connection between PGC-1α and the physical division of mitochondria is not fully understood, it has been shown to increase respiration in cells (Wu et al. 1999). The division of mitochondria is known to involve the dynamin-like proteins (Arimura, Yamamot et al. 2004) and Fis-type proteins (Lu et al. 2011). These

pathways are mostly understood from the perspective of human and mouse mitochondria, so these processes may be quite different in the mitochondria of other eukaryotes.

Plastid biogenesis is much less well-understood than mitochondrial biogenesis because many factors implicated in this process are often only known from the phenotyping of Arabidopsis mutants (López-Juez 2007). Those factors whose functions are well-understood seem to mostly be involved in the formation of plastid division rings including dynamin-like proteins (Gao et al. 2003), like those involved in mitochondrial biogenesis. As such, the process of plastid division is mostly understood to be a mechanical one with daughter plastid components mostly being the result of random, unequal segregation of the contents of the parent cell (López-Juez 2007), though some components of the plastid, such as thykaloids, seem to have an equitable redistribution between daughter plastids as regulated through the ARTEMIS and PEND proteins (Fulgosi et al. 2002; Terasawa and Sato 2005).

The population genetic environment of genes involved in organelle number

The genes involved in determining organelle number mostly fall within one of two categories: (1) those that are in the nuclear genome, including those that control the division of organelles and the over two-hundred genes that encode products that interact with organelle-encoded proteins (van Wijk 2004; Giegé et al. 2005; van Wijk and Baginsky 2011) and (2) those that encode proteins in the organelle genomes themselves, especially those that interact with nuclear proteins, whose potential

importance will be explained later in the essay. The three compartments in which these genes reside present different population genetic environments that presumably play a large role in dictating the relative power of selection in the evolution of organelle number.

Population mutation rate (often represented by π or θ), which is a reflection of the population genetic environment as influenced by mutation and drift, is $2N_g u$ where u is the mutation rate per nucleotide per generation and N_g is the effective number of genes at a locus (Lynch 2006). For diploid nuclear genomes, this term is equal to $4N_e u$ where N_e is the effective population size. This is important to consider as genes that dictate organelle number, like those that regulate mitochondrial biogenesis such as the PGC family (Ventura-Clapier et al. 2008) and those that encode the dynamin-like proteins and Fis-type proteins that are involved in plastid and mitochondrial division (Gao et al. 2003; Arimura, Yamamot et al. 2004; Lu et al. 2011), are encoded in the nucleus.

The argument has been made that, due to the haploidy and maternal transmission of organelles, population mutation rate is simply equal to $N_e u$ in organelle genomes (Palumbi et al. 2001). Alternatively, it has been argued that this idea assumes an incorrect parity of selection and recombination between nuclear and organelle genomes and equivalency between males and females with respect to progeny (Lynch et al. 2006). Furthermore, organelle mutation rate is thought to vary much between different taxa. For example, when compared to nuclear genomes, animal mitochondrial mutation

rates appear to be much higher (Brown et al. 1979) and plant mitochondrial mutation rates appear to be much lower (Palmer and Herbon 1988).

Regardless of any of these considerations, the effective population size is generally thought to be smaller in organelle genomes (Palumbi et al. 2001; Lynch et al. 2006) and, as such, the strength of drift is thought to be greater than that of the nucleus, but the efficacy of selection could be just as great (Cooper et al. 2015) and vary greatly between different taxa with different mutation (Brown et al. 1979; Palmer and Herbon 1988) and recombination regimes (Rokas et al. 2003; McCauley 2013). As a result of these differences, for example, if organelle-encoded genes are important in the determination of per-cell organelle number, the efficacy of selection on organelle number may be more so in animals than in plants because of the greater mutation rate (Brown et al. 1979; Palmer and Herbon 1988) and lower incidence of recombination (McCauley 2013) in animal mitochondrial genomes.

Metabolism as a potential selective constraint

The metabolic needs of a cell and the capacity for organelles to fulfill these needs may act as selective constraints on the number of organelles in a cell. Mitochondria, for example, perform a few different metabolic processes, such as the production of ATP through primarily aerobic respiration, regulation of cellular metabolic processes (McBride et al. 2006), and steroid synthesis (Rossier 2006). Chloroplasts perform photosynthesis, producing NADPH and ATP through light reactions and glucose

through the Calvin cycle, and are also involved in fatty acid (Rawsthorne 2002) and amino acid synthesis (Burgess 1989).

Metabolic needs have been thought to drive the relationship between the total amount of mitochondria and body mass across organisms according to a power law (Kleiber 1947). Per-cell mitochondria content is also seen to change with cell size across the cell cycle (Posakony et al. 1977). Single-celled eukaryotes do not appear to follow Kleiber's power law for mitochondria or chloroplasts, but do appear to follow linear and sublinear scaling, respectively, for organelle number with cell size (Okie et al. 2016). This study also shows that organelle size does not appear to scale strongly with cell size in single-celled eukaryotes, suggesting that per-cell organelle number is more important than organelle size as a means of modulating energetic requirements at the scale of the cell. Rafelski et al. (2012), however, did note a sublinear, positive correlation between cell volume and total organelle volume in yeast. Given the relationship between cell size and per-cell mitochondrial content, perhaps there is an optimal per-cell mitochondria number given cell size and the nature of mitochondrial biogenesis.

It may soon be possible to obtain an estimate of whole-cell energetic requirements per unit time and use this to determine the optimal per-cell mitochondrial content to further understand the role of selection on this trait through quantitative genetics. Though it is not yet possible to completely understand the energetic needs per unit time in a eukaryotic cell, there has been some progress in developing theoretical models that can

estimate the metabolic and energetic needs of a single cell (Suthers et al. 2009; Karr et al. 2012; Lynch and Marinov 2016).

Coordination between the nucleus and organelles: transcription and translation

Since the original endosymbiosis events, there has been a substantial integration of the function of organelles and their hosts, some of which is due to the transfer of genes from organelle to nuclear genomic compartments over evolutionary time (Blanchard and Schmidt 1995). As a result, most protein products encoded in the organelles form complexes with those that are encoded in the nucleus. There have also evolved to be nuclear-encoded transcription factors (Leigh-Brown et al. 2010) and proteins involved in post-transcriptional modification such as RNA editing (Schmitz-Linneweber and Small 2008) that regulate the expression of mitochondrial genes. As such, some level of stoichiometric balance has to be maintained between nuclear- and organelle-encoded factors during the processes of transcription and translation to ensure the proper allocation of resources and to prevent waste in terms of energy and macromolecules. Maintenance of proper stoichiometric ratios for gene products is necessary, at least to some degree, in maintaining some semblance of physiological homeostasis at both the cellular and organismal levels.

Many mitochondrial encoded proteins (Giegé et al. 2005) and almost all plastid encoded proteins (van Wijk 2004; van Wijk and Baginsky 2011) form multi-subunit complexes with nuclear encoded products, of which there are about two-hundred. Among these complexes are ribosomes and ATP synthases of both organelles. Rubisco, PEP, and

the major photosynthetic complexes of the plastid and all four major complexes of the electron transport chain in the mitochondrion are also among those formed by complexing with nuclear subunits. These are formed from specific stoichiometric ratios of subunits. The number of organelles could play a significant role in influencing the number of these products produced in a cell and, as such, the nuclear-organelle subunit stoichiometry could act as a significant constraint on the number of organelles in the cell.

Though levels of transcription do differ between genes, mitochondrial transcription in animals is mostly performed at a constitutive level (Bendich 1988) because it occurs over the entire circular mitochondrial genome, one strand at a time (Lee and Clayton 1998). This constitutive transcription and the tenuous connection between metabolic activity and gene expression in mitochondrial genomes (Bendich 1988) suggest that, in order to maintain the appropriate numbers of transcripts, other means at other levels must be utilized by the cell. Another potential means of controlling gene product stoichiometry may be by adjusting organelle DNA copy number per organelle, but it has been shown that, since this quantity varies so little (Boffey and Leech 1982; Weisner et al. 1992), per-cell organelle number rather than per-organelle DNA copy number is the major driver of per-cell organelle DNA copy number (Robin and Wong 1988; Zoschke et al. 2007). Adjusting the number of organelles could act as a broad-scale blunt means of regulation for these genes, as an alternative to regulating expression on a gene-by-gene or genome-by-genome basis. As such, per-cell organelle number could play an

important role in maintaining the stoichiometry between nuclear- and organelle-encoded subunits.

Given a particular cell, there could be an optimal number of organelles to maintain the stoichiometry between nuclear and organelle gene products. Counting gene products can be performed at the level of transcription by looking at the number of particular mRNAs in a cell (Itzkovitz et al. 2012) or at the level of translation by counting the number of particular proteins in a cell (Huang et al. 2004). By doing this, we could see how closely these quantities track the expected stoichiometry in some cases. Since we can estimate the number of organelles in a cell by the methods stated earlier, it may be possible to survey many cells of the same type for the density of organelles and see if there is an optimum that gives a ratio of gene products closest to the expected stoichiometric ratio.

If we can make the assumption that it is most fit for cells to produce the least amount of excess gene product for those proteins that form obligatory complexes, looking at many cells may be able to tell us which have the closest to the optimum per-cell organelle number. A comparison of the typical per-cell organelle density to our best estimate of the optimum per-cell organelle density may tell about the role of evolution in shaping organelle number. For example, if most cells have a far-from-optimal organelle number per-cell, it may merit further investigation into the possibility of there being a drift barrier (Lynch 2011) or some other, previously not understood, physical constraint that is preventing most cells from reaching this stoichiometric optimum.

Conclusion

Metabolism and the production of organelle encoded gene products are both important to consider in the context of organelle and cell evolution. Cells have energetic requirements and need to maintain some semblance of particular gene product quantities in order to function properly. Work in the fields of biophysics and physiology will allow us to determine how these processes are impacting fitness and constraining per-cell organelle number. To what extent these processes constrain per-cell organelle number through selection will be more clear as the field of cell-scale proteomics becomes more empirically and computationally tractable. As these techniques become increasingly practical, we will be able to apply the analytical approaches of population and quantitative genetics to better understand this issue from an evolutionary perspective.

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References

Adams KL., Palmer JD. 2003. Evolution of mitochondrial gene content: gene loss and transfer to the nucleus. *Mol. Phylogenet. Evol.* 29(3): 380–395.

Arimura S, Aida GP, Fujimoto M, Nakazono M, Tsutsumi B. 2004. *Arabidopsis* dynamin-like protein 2a (ADL2a), like ADL2b, is involved in plant mitochondrial division. *Plant Cell Physiol.* 45(2): 236–242.

Arimura S, Yamamot J, Aida GP, Nakazono M, Tsutsumi N. 2004. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proc. Natl. Acad. Sci. U.S.A.* 101(20): 7805–7808.

Azpiroz R, Butow RA. 1993. Patterns of mitochondrial sorting in yeast zygotes. *Mol. Biol. Cell.* 4(1): 21–36.

Baumgartner BJ, Rapp JC, Mullet JE. 1989. Plastid transcription activity and DNA copy number increase early in barley chloroplast development. *Plant Physiol.* 89(3): 1011–1018.

Bendich A. 1988. Why do chloroplasts and mitochondria contain so many copies of their genome? *Bioessays* 6(6): 279–282.

Bendich AJ, Gauriloff LP. 1984. Morphometric analysis of cucurbit mitochondria: the relationship between chondriome volume and DNA content. *Protoplasma* 119(1-2): 1–7.

Bereiter-Hahn J, Voth M. 1996. Distribution and dynamics of mitochondrial nucleoids in animal cell in culture. *Exp. Biol.* 1:4.

Blanchard JL, Schmidt GW. 1995. Pervasive migration of organellar DNA to the nucleus in plants. *J. Mol. Evol.* 41(4): 397–406.

Boffey SA, Leech RM. 1982. Chloroplast DNA levels and the control of chloroplast division in light-grown wheat leaves. *Plant Physiol.* 69(6): 1387–1391.

Bogenhagen DF. 2011. Mitochondrial DNA nucleoid structure. *Biochim. Biophys. Acta* 1819(9-10): 914–920.

Brown TA, Tkachuk AN, Shtengel G, Kopek BG, Bogenhagen DF, Hess H, Clayton DA. 2011. Super-resolution fluorescence imaging of mammalian mitochondrial nucleiods. *Mol. Cell. Biol.* 31(24): 4994–5010.

Brown WM, George M, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.* 76(4): 1967–1971.

Burgess J. 1989. *An Introduction to Plant Cell Development.* Cambridge: Cambridge University Press.

Cavalier-Smith T. 1987. Eukaryotes with no mitochondria. Nature 326(6111): 332–333.

Chazotte B. 2011. Labeling mitochondria with MitoTracker dyes. *Cold Spring Harb. Protoc.* 8: 990–992.

Cooper BS, Burrus CR, Ji C, Hahn MW, Montooth KL. 2015. Similar efficacies of selection shape mitochondrial and nuclear genes in both *Drosophila melanogaster* and *Homo sapiens*. *G3* 5(10): 2165–2176.

Fulgosi H, Gerdes L, Westphal S, Glockmann C, Soll J. 2002. Cell and chloroplast division requires ARTEMIS. *Proc. Natl. Acad. Sci. U.S.A.* 100(17): 4328–4333.

Gao H, Kadirjan-Kalbach D, Froehlich JE, Osteryoung KW. 2003. ARC5, a cytosolic dynamin-like protein from plants, is part of the chloroplast division machinery. *Proc. Natl. Acad. Sci. U.S.A.* 100(7): 4328–4333.

Giegé P, Sweetlove LJ, Cognat V, Leaver CJ. 2005. Coordination of nuclear and mitochondrial genome expression during mitochondrial biogenesis in Arabidopsis. *Plant Cell* 17(1): 1497–1512.

Gray MW, Lang BF, Burger G. 2004. Mitochondria of protists. *Annu. Rev. Genet.* 38(1): 477–524.

Gurdon C, Svab Z, Feng Y, Kumar D, Maliga P. 2016. Cell-to-cell movement of mitochondria in plants. *Proc. Natl. Acad. Sci. U.S.A.* 113(12): 3395–3400.

Hartmann N, Reichwald K, Wittig I, Dröse S, Schmeisser S, Lück C, Hahn C, Graf M, Gausmann U, Terzibasi E, Cellerino A, Ristow M, Brandt U, Platzer M, Englert C. 2011. Mitochondrial DNA copy number and function decrease with age in the short-lived fish *Nothobranchius furzeri*. *Aging Cell* 10(1): 824–831.

Higa T, Wada M. 2016. Chloroplast avoidance movement is not functional in plants grown under strong sunlight. *Plant Cell Environ.* 39(4): 871–882.

Holloszy JO. 1967. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem.* 242(9): 2278–2282.

Howe CJ, Barbrook AC, Koumandou VL, Nisbet RER, Symington HA, Wightman TF. 2002. Evolution of the chloroplast genome. *Philos. Trans. R. Soc. B.* 358(1429): 99–107.

Huang LR, Cox EC, Austin RH, Sturm JC. 2004. Continuous particle separation through deterministic lateral displacement. *Science* 304(5673): 987–990.

Iborra FJ, Kimura H, Cook PR. 2004. The functional organization of mitochondrial genomes in human cells. *BMC Biol.* 2:9.

Inuda N, Wildermuth MC. 2004. Novel tissue preparation method and cell-specific marker for laser microdissection of Arabidopsis mature leaf. *Planta* 221(1): 9–16.

Irrcher I, Adhihetty PJ, Sheehan T, Joseph AM, Hood DA. 2003. PPARγ co-activator-1α expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. *Am. J. Physiol. Cell Physiol.* 284(6): C1669–1677.

Itoh R, Takahashi H, Toda K, Kuroiwa H, Kuroiwa T. 1996. Aphidicolin uncouples the chloroplast division cycle from the mitotic cycle in the unicellular red alga *Cyanidioschyzon merolae*. *Eur. J. Cell Biol.* 71(3): 303–310.

Itzkovitz S, Lyubimova A, Blat IC, Maynard M, van Es J, Lees J, Clevers H, van Oudenaarden A. 2012. Single-molecule transcript counting of stem-cell markers in the mouse intestine. *Nature Cell Biol.* 14(1): 106–114.

Karnkowska A, Vacek V, Zubáčová Z, Treitli SC, Petrželková R, Eme L, Novák L, Zárský V, Barlow LD, Herman EK, Soukal P, Hroudová M, Doležal P, Stairs CW, Roger

AJ, Eliás M, Dacks JB, Vlcek C, Hampl V. 2016. A eukaryote without a mitochondrial organelle. *Curr. Biol.* 26(1): 1274–1284.

Karr JR, Sanghvi JC, Macklin DN, Gutschow MV, Jacobs JM, Bolival B, Assad-Garcia N, Glass JI, Covert MW. 2012. A whole-cell computational model predicts phenotype from genotype. *Cell* 150(2): 389–401.

Kleiber M. 1947. Body size and metabolic rate. *Physiol. Rev.* 27(4) 511–541.

Kowald A, Kirkwood TBL. 2011. Evolution of the mitochondrial fusion-fission cycle and its role in aging. *Proc. Natl. Acad. Sci. U.S.A.* 108(25): 10237–10242.

Kubínová Z, Janáček J, Lhotáková Z, Kubínová L, Albrechtová J. 2014. Unbiased estimation of chloroplast number in mesophyll cells: advantage of a genuine three-dimensional approach. *J. Exp. Bot.* 65(2): 609–620.

Kukat C, Wurm CA, Spåhr H, Falkenberg M, Larsson NG, Jakobs S. 2011. Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. *Proc. Natl. Acad. Sci. U.S.A.* 108(33): 13534–13539.

Kuroiwa T. 1991. The replication, differentiation, and inheritance of plastids with emphasis on the concept of organelle nuclei. *Int. Rev. Cytol.* 128(1): 1–62.

Lamppa GK, Elliot LV, Bendich AJ. 1980. Changes in chloroplast number during development of pea (*Pisum sativum*). *Plant Physiol.* 64(1): 126–130.

Lee DY, Clayton DA. 1998. Initiation of mitochondrial DNA replication by transcription and r-loop processing. *J. Biol. Chem.* 273(46): 30614–30621.

Legros F, Malka F, Franchon P, Lombès A, Rojo M. 2004. Organization and dynamics of human mitochondrial DNA. J. Cell Sci. 117(Pt. 13): 2653–2662.

Leigh-Brown S, Enriquez JA, Odom DT. 2010. Nuclear transcription factors in mammalian mitochondria. *Genome Biol.* 11: 215.

Li H, Bai D, Hu Y, Kuang T, Lin J. 2001. Differences between the number and structure of chloroplasts in leaves and in non-leaf organs of wheat. *Belg. J. Bot.* 134(2): 121–126.

Logan DC. 2007. *Annual Plant Reviews: Plant Mitochondria.* Oxford: Blackwell Publishing Ltd.

López-Juez E. 2007. Plastid biogenesis, between light and shadows. *J. Exp. Bot.* 58(1): 11–26.

Lu Y, Rolland SG, Conradt B. 2011. A molecular switch that governs mitochondrial fusion and fission mediated by the BCL2-like protein CED-9 of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 108(41): E813–E822.

Lynch M. 2006. The origins of eukaryotic gene structure. *Mol. Biol. Evol.* 23(2): 450–468.

Lynch M. 2011. The lower bound to the evolution of mutation rates. *Genome Biol. Evol*. 3: 1107–1118.

Lynch M, Koskella B, Schaack S. 2006. Mutation pressure and the evolution of organelle genomic architecture. *Science* 311(5768): 1727–1730.

Lynch M, Marinov G. 2016. The bioenergetic costs of a gene. *Proc. Natl. Acad. Sci. U.S.A.* 112(51): 11690–11695.

Marinos E. 1985. The number of mitochondria in *Xenopus laevis* ovulated oocytes. *Cell Differ.* 16(2): 139–143.

Mattiasson G. 2004. Flow cytometric analysis of isolated liver mitochondria to detect changes relevant to cell death. *Cytometry A* 60(2): 145–154.

McBride HM, Neuspiel M, Wasiak S. 2006. Mitochondria: more than just a powerhouse. *Curr. Biol.* 16(14): R551–560.

McCauley DE. 2013. Paternal leakage, heteroplasmy, and the evolution of plant mitochondrial genomes. *New Phytol.* 200(4): 966–977.

Moller SG. 2004. *Plastids.* Oxford: Blackwell Publishing Ltd.

Moraes CT. 2001. What regulates mitochondrial DNA copy number in animal cells? *Trends Genet.* 17(4): 199–205.

Mower JP, Case AL, Floro ER, Willis JH. 2012. Evidence against equimolarity of large repeat arrangements and a predominant master circle structure of the mitochondrial genome from a monkeyflower (*Mimulus guttatus*) lineage with cryptic CMS. *Genome Biol. Evol*. 4(5): 670–686.

Okie JG, Smith VH, Martin-Cereceda M. 2016. Major evolutionary transitions of life, metabolic scaling and the number and size of mitochondria and chloroplasts. *Proc. R. Soc. B.* 283(1831): 20160611.

Palmer JD., Herbon LA. 1988. Plant mitochondrial DNA evolved rapidly in structure, but slowly in sequence*. J. Mol. Evol.* 28(1-2): 87–97.

Palmer JD, Shields CR. 1984. Tripartite structure of the *Brassica campestris* mitochondrial genome. *Nature* 307(5950): 437–440.

Palumbi SR, Cirpriano F, Hare MP. 2001. Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. *Evolution* 55(5): 859–868.

Posakony JW, England JM, Attardi G. 1977. Mitochondrial growth and division during the cell cycle in HeLa cells. *J. Cell Biol.* 74(2): 468–491.

Preuten T, Cincu E, Fuchs J, Zosche R, Liere K, Börner T. 2010. Fewer genes than organelles: extremely low and variable gene copy numbers in mitochondria of somatic plant cells. *Plant J.* 64(6): 948–959.

Rafelski SM, Viana MP, Zhang Y, Chan YHM, Thorn KS, Yam P, Fung JC, Li H, Costa Lda F, Marshall WF. 2012. Mitochondrial network size scaling in budding yeast. *Science* 338(6108): 822–824.

Rawsthorne S. 2002. Carbon flux and fatty acid synthesis in plants. *Prog. Lipid Res.* 41(2): 182–196.

Robin ED, Wong R. 1988. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J. Cell. Physiol.* 136(3): 507–513.

Rokas A, Ladoukakis E, Zouros E. 2003. Animal mitochondrial DNA revisited. *Trends Ecol. Evol.* 18(8): 411–417.

Rossier MF. 2006. T channels and steroid biosynthesis: in search of a link with mitochondria. *Cell Calcium* 40(2): 155–164.

Ruberti C, Barizza E, Bodner M, La Rocca N, De Michelle R, Carimi F, Schiavo FL, Zottini M. 2014. Mitochondria change dynamics and morphology during grapevine leaf senescence. *PLoS One* 9:E102012.

Satoh M, Kuroiwa T. 1991. Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. *Exp. Cell Res.* 196(1): 137–140.

Schmitz-Linneweber C, Small I. 2008. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci.* 13(12): 663–670.

Scott NS, Possingham JV. 1980. Plastid replication and development in the life cycle of higher plants. *Annu. Rev. Plant Physiol.* 31(1): 113–129.

Scott NS, Possingham JV. 1983. Changes in chloroplast DNA levels during growth of spinach leaves. *J. Exp. Bot.* 34(12): 1756–1767.

Solieri L. 2010. Mitochondrial inheritance in budding yeasts: towards an integrated understanding. *Trends Microbiol.* 18(11): 521–530.

Stoecker DK, Silver MW. 1990. Replacement and aging of chloroplasts in *Strombidium capitatum* (Ciliophora: Oligotrichida). *Mar. Biol.* 107(3): 491–502.

Suthers PF., Dasika MS, Kumar VS, Denisov G, Glass JI, Marana CD. 2009. A genome-scale metabolic reconstruction of *Mycoplasma genitalium* iPS189. *PLoS Comput. Biol.* 5:e1000285.

Suyama Y, Bonner WD. 1966. DNA from plant mitochondria. *Plant Physiol.* 41(3): 383–388.

Terasawa K, Sato N. 2005. Visualization of plastid nucleoids *in situ* using the PEND-GFP fusion protein. *Plant Cell Physiol.* 46(4): 649–660.

Thyssen G, Svab Z, Maliga P. 2012. Cell-to-cell movement of plastids in plants. *Proc. Natl. Acad. Sci. U.S.A.* 109(7): 2439–2443.

van Wijk KJ. 2004. Plastid proteomics. *Plant Physiol. Biochem.* 42(12): 963–977.

van Wijk KJ, Baginsky S. 2011. Plastid proteomics in higher plants: current state and future goals. *Plant Physiol.* 155(F): 1578–1588.

Veltri KL, Espiritu M, Singh G. 1990. Distinct genomic copy number in mitochondria of different mammalian organs. *J. Cell. Physiol.* 143(1): 160–164.

Ventura-Clapier R, Garnier A, Veksler V. 2008. Transcriptional control of mitochondrial biogenesis: the central role of PGC-1α. *Cardiovasc. Res.* 79(2): 208–217.

Virbasius JV, Scarpulla RC. 1994. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 91(4): 1309–1313.

Visser W, van Spronsen EA, Nanninga N, Pronk JT, Gijs Kunen J, van Dijken JP. 1995. Effects of growth conditions on mitochondrial morphology in *Saccharomyces cerevisiae*. *Antonie Leeuwenhoek* 67(3): 243–253.

Weisner RJ, Ruegg JC, Morano I. 1992. Counting target molecules by exponential polymerase chain reaction: copy number of mitochondrial DNA in rat tissues. *Biochem. Biophys. Res. Comm.* 183(2): 553–559.

Williamson DH, Fennell DJ. 1979. Visualization of yeast mitochondrial DNA with the fluorescent stain “DAPI”. *Methods Enzymol.* 56(1): 728–733.

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98(1): 115–124.

Zoschke R, Liere K, Börner T. 2007. From seedling to mature plant: arabidopsis plastidal genome copy number, RNA accumulation and transcription are differentially regulated during leaf development. *Plant J.* 50(4): 710–722.

Logan W. Cole
Bloomington, Indiana

Research interests: Genome evolution; molecular evolution; phylogenetics

EDUCATION

Indiana University - Bloomington, Indiana — September 2019
PhD in Evolution, Ecology, and Behavior with a minor in Bioinformatics
Thesis: The radical structural evolution of genomes in flowering plant mitochondria
Advisor: Jeffrey D. Palmer

University at Buffalo - Buffalo, New York — May 2012
BS in Biological Sciences - Graduated *magna cum laude* and with high honors
Honors Advisor: Charlotte Lindqvist

AWARDS, SCHOLARSHIPS, & HONORS

SMBE Conference Registration Award	2017
SSE Outreach Grant with W. Shoemaker and D. Schwab ($1,000)	2017
Charles B. Heiser Fellowship in Plant Evolution ($2,000)	2016–2017
SMBE Young Investigator's Travel Award ($2,000)	2016
SSB Ernst Mayr Award Finalist	2015
Ralph Cleland Travel Award ($500)	2015
SSE Student Travel Stipend ($500)	2015
Louise Constable Hoover Fellowship ($1,000)	2015
Bayard Floyd Plant & Fungal Biology Summer Fellowship ($12,519)	2013–2017
Departmental Honors - UB Department of Biological Sciences	2012
UB Foundation Teaching Stipend ($400)	2011
UB CURCA Undergraduate Research Award ($495)	2011
PennAg Industries Association Scholarship ($500)	2010
New York State Academic Competitiveness Grant ($1,300)	2009
University at Buffalo Provost Scholarship ($10,000)	2008
New York State Attorney General Andrew Cuomo's Triple "C" Award	2008

RESEARCH APPOINTMENTS

Indiana University - Department of Biology — 2012–2019
Graduate Research - Advisor: Jeffrey D. Palmer

University at Buffalo - Department of Biological Sciences — 2010–2012
Undergraduate Research - Faculty Supervisor: Charlotte Lindqvist

TEACHING APPOINTMENTS

Indiana University - Department of Biology
Associate Instructor for undergraduate courses

BIOL-S 318 - Honors Evolution - upper division honors course	2017
BIOL-L 318 - Evolution - upper division course	2016
BIOL-L 111 - Evolution and Diversity - introductory course	2012, 2016

Indiana University - Foundations in Science and Mathematics (IU FSM)
Instructor for summer high school courses
Our Evolving World - supplementary course on evolution 2017
Introductory Biology - preparatory course for high school biology 2015, 2016

University at Buffalo - Department of Biological Sciences
Teaching assistant for undergraduate courses
Basic Training in the Biological Sciences Workshop - preparatory course 2011
BIO 200 - Evolutionary Biology - introductory course 2009–2011

PROFESSIONAL SERVICE
Curriculum development - IU FSM 2015–2018
Co-founder and co-chair - IU Evolution Discussion Group (EDG) 2015–2018
Member - Activities committee for IU Biology GRW 2017
Member - Panel for IU EEB Orientation entitled “Grad School 101” 2016
Member - Talks committee at the Midwest Ecology and Evolution meeting 2015
Chair - Session on plant organelle genome evolution at Evolution meeting 2014

Manuscript review: *Genome Biology and Evolution* and *Molecular Biology and Evolution*

PUBLICATIONS
Cole LW, Mower JP, Palmer JD. Horizontal transfer via gene conversion with existing foreign sequences. *In prep.*

Cole LW, Palmer JD. Unexpectedly high and variable tempo of plastid DNA integration in mitochondrial genomes of angiosperms. *In prep.*

Schwab DB, Cole LW, Desai KM, Hemann J, Hummels KR, Maltese AV. 2018. A summer STEM outreach program run by graduate students: successes, challenges, and recommendations for implementation. *J Res STEM Educ.* 4(2): 117–129.

Cole LW, Guo W, Mower JP, Palmer JD. 2018. High and variable rates of repeat-mediated mitochondrial genome rearrangement in a genus of plants. *Mol Biol Evol.* 35(11): 2773–2785.

Cole LW. 2016. The evolution of per-cell organelle number. *Front Cell Dev Biol.* 4:85.

Roy T, Cole LW, Chang TH, Lindqvist C. 2015. Untangling reticulate evolutionary relationships among New World and Hawaiian mints (Stachydeae, Lamiaceae). *Mol Phylogenet Evol.* 89: 46–62.

PRESENTATIONS
Symposium talks
Cole LW. 2019. The radical structural evolution of genomes in flowering plant mitochondria. **Selected for Great Lakes Evolutionary Genetics Symposium at GLAM-EvoGen.** Amherst, NY.

Cole LW, Mower JP, Palmer JD. 2016. Unexpectedly high and variable tempo of plastid DNA integration in mitochondrial genomes of *Monsonia*. **Selected for "Mitochondria in the Age of Genomics" symposium at SMBE 2016.** Gold Coast, Australia.

Cole LW, Mower JP, Palmer JD. 2015. A complex history of plastid-derived insertions and horizontal gene transfer in *Monsonia*. **Selected for Ernst Mayr Symposium at Evolution 2015.** Guarujá, Brazil.

Cole LW, Chang TH, Lindqvist C. 2012. Phylogenetic analyses of low-copy nuclear loci show reticulate evolutionary patterns among Hawaiian endemic mints and New World *Stachys* relatives. University at Buffalo Department of Biological Sciences Honors Symposium. Amherst, NY.

Contributed conference talks

Cole LW, Mower JP, Palmer JD. 2015. A complex history of plastid-derived insertions and horizontal gene transfer in *Monsonia*. SMBE Satellite Meeting on Mutation, Repair, and Evolution. Bloomington, IN.

Cole LW, Mower JP, Palmer JD. 2015. Horizontal gene transfer in the mitochondrial genome of *Monsonia emarginata*. Midwest Ecology and Evolution Conference 2015. Bloomington, IN.

Cole LW, Mower JP, Palmer JD. 2014. Horizontal gene transfer in the mitochondrial genome of *Monsonia emarginata*. Evolution 2014. Raleigh, NC.

Cole LW, Chang TH, Lindqvist C. 2012. Phylogenetic analyses of low-copy nuclear loci show reticulate evolutionary patterns among Hawaiian endemic labiates and New World *Stachys* relatives. Botany 2012. Columbus, OH.

Intradepartmental talks and workshops at Indiana University in Bloomington, IN

Cole LW. 2017. Basic phylogenetics as a tool for applications in other fields. Ecolunch.

Cole LW. 2017. The evolution of mitochondrial genomes in flowering plants. EEB Brownbag.

Cole LW. 2015. A complicated history of rearrangement, plastid-derived insertions, and horizontal gene transfer in the mitochondrial genomes of *Monsonia*. EEB Brownbag.

Cole LW. 2015. Reticulate evolutionary patterns between angiosperm genomes. EDG.

Cole LW. 2014. Phylogenetics as a tool for applications in other fields. Ecolunch.

Poster presentations

Cole LW, Mower JP, Palmer JD. 2017. Extensive and variable repeat-mediated mitochondrial genome rearrangement in a genus of plants. SMBE 2017. Austin, TX.

Cole LW, Mower JP, Palmer JD. 2013. Horizontal gene transfer in the mitochondrial genome of *Monsonia emarginata*. SMBE 2013. Chicago, IL.

Cole LW, Chang TH, Lindqvist C. 2011. An American Origin for the Hawaiian Native Mints. UB CURCA Celebration of Academic Excellence. Amherst, NY.

PROFESSIONAL AFFILIATIONS

Society for Molecular Biology and Evolution (SMBE)
Society for the Study of Evolution (SSE)
Society of Systematic Biologists (SSB)
Indiana Academy of Science
National Center for Science Education