



Genome size changes by duplication, insertion and divergence in *Caenorhabditis* worms

Joshua D. Millwood,¹ John M. Sutton,^{1,2} Paula E. Adams,^{1,3} Jason Pienaar^{4,5,7} and Janna L. Fierst^{6,7*}

¹Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, ²Current address: Absci, Vancouver, WA 98663, ³Current address: Department of Biological Sciences, Auburn University, Auburn, AL 36830, ⁴Institute of the Environment, ⁵Center for Tropical Botany, ⁶Biomolecular Sciences Institute and ⁷Department of Biological Sciences, Florida International University, Miami, FL 33199

*Corresponding author. jfierst@fiu.edu

FOR PUBLISHER ONLY Received on Date Month Year; revised on Date Month Year; accepted on Date Month Year

Abstract

Genome size has been measurable since the 1940s but we still do not understand the basis of genome size variation. *Caenorhabditis* nematodes show strong conservation of chromosome number but vary in genome size between closely related species. Androdioecy, where populations are composed of males and self-fertile hermaphrodites, has evolved from outcrossing, female-male dioecy, three times in this group. Androdioecious genomes are 10–30% smaller than dioecious species but large phylogenetic distances and rapid protein evolution have made it difficult to pinpoint the basis of these changes. Here, we analyze the genome sequences of *Caenorhabditis* and test three hypotheses explaining genome evolution: 1) genomes evolve through deletions and ‘genome shrinkage’ in androdioecious species; 2) genome size is determined by transposable element (TE) expansion and DNA loss through large deletions (the ‘accordion model’); and 3) TE dynamics differ in androdioecious and dioecious species. We find no evidence for these hypotheses in *Caenorhabditis*. Across both short and long evolutionary distances *Caenorhabditis* genomes evolve through small structural variant (SV) mutations including frequent duplications and insertions, predominantly in genic regions. *Caenorhabditis* have rapid rates of gene family expansion and contraction and we identify 71 protein families with significant, parallel decreases across self-fertile *Caenorhabditis*. These include genes involved in the sensory system, regulatory proteins and membrane-associated immune responses, reflecting the shifting selection pressures that result from self-fertility. Our results suggest that the rules governing genome evolution differ between organisms based on ecology, life style and reproductive system.

Key words: structural variants, mutation, reproductive transitions, molecular divergence

Introduction

Genetic variation is the fuel for adaptation (Fisher, 1930). Molecular evolutionary studies have focused on genomic variation at single nucleotide

polymorphisms (SNPs) but gene deletions, duplications and other SV mutations may play important roles in adaptation and species divergence. Long-read DNA sequencing technologies have recently expanded our capability to accurately characterize SVs and we can

now address the evolutionary significance of SVs in genomic evolution.

Nematodes have high rates of genomic polymorphisms and are a compelling system for studying the evolution of genetic and genomic variation. Outcrossing dioecious *Caenorhabditis* are characterized by molecular ‘hyper diversity’ and little linkage disequilibrium (Dey et al., 2013; Cutter et al., 2006) while the self-fertile androdioecious *C. elegans* has remarkably low levels of genetic variation (Cutter et al., 2009) and little global population structure (Andersen et al., 2012). Despite this there is substantial genomic divergence between *C. elegans* strains. The genome of the ‘Hawaiian’ CB4856 strain contains an extra 4Mb of genomic sequence when compared with the laboratory standard ‘Bristol’ N2 (Kim et al., 2019; Thompson et al., 2015). Sequencing and analysis across hundreds of wild-collected *C. elegans* strains indicates that 2-10% difference in genome size is common (Cook et al., 2017) with copy number (Maydan et al., 2010) and gene presence-absence variation (Lee et al., 2022) contributing to these differences.

Across the *Caenorhabditis* group self-fertile species have genomes 10-30% smaller than related outcrossing species (Bird et al., 2005; Haag et al., 2007). Similar patterns of smaller self-fertile genomes and larger outcrossing genomes have been reported in plants including *Arabidopsis* (Hu et al., 2011) and *Capsella* (Slotte et al., 2013) with equivocal support for different mechanisms of genome expansion and reduction. In *Caenorhabditis* genome size differences reflect outcrossing species higher gene number and protein-coding genome content (Stevens et al., 2019; Teterina et al., 2020; Thomas et al., 2012; Fierst et al., 2015; Noble et al., 2021), but it is unclear if this divergence occurred through ‘shrinkage’ across self-fertile species (Yin et al., 2018) or growth across outcrossing species (Kanzaki et al., 2018). There is also a substantial phylogenetic component to genome size in *Caenorhabditis*, further complicating analyses (Stevens et al., 2019).

Mutation accumulation studies focusing on the self-fertile *C. elegans* show SV mutations are common. Gene deletion and duplication are frequent (Lipinski et al., 2011; Farslow et al., 2015; Katju and Bergthorsson, 2013) with duplications occurring at an estimated 2.9×10^{-5} /gene per generation and deletions at 5×10^{-6} /gene per generation (Konrad et al., 2018). In comparison, single nucleotide base substitutions occur at 10^{-9} to 10^{-8} per generation (Denver et al., 2004).

The evolutionary significance of these SV patterns has been difficult to address in *C. elegans* because the most closely related known species, *C. inopinata*, diverged roughly 12 million years ago (Kanzaki et al., 2018). Outcrossing species have resisted the inbreeding necessary to create homozygous genome sequences (Dolgin et al., 2007) and residual allelism has prevented rigorous genomic analyses (Barriere et al., 2009). Self-fertility has evolved at least three times in the *Caenorhabditis* group but the known self-fertile species diverged more than 30 million years ago (Cutter, 2008). This span of divergence means that genomic comparisons between these species can not increase our understanding of SVs across evolutionary scales.

Here, we study SVs across a range of evolutionary divergence times. We start very small and compare the genomes of three strains of the outcrossing nematode *C. remanei*. We include *C. latens*, a dioecious species originally classified as a strain of *C. remanei* and later defined as a separate species (Dey et al., 2012). The *Caenorhabditis Elegans* group represents over 100 million years of evolutionary divergence and we broaden our comparisons to the outcrossing species *C. nigoni*, *C. inopinata* and *C. sinica* and the self-fertile *C. elegans*, *C. briggsae* and *C. tropicalis*.

Assembled genome sequences are available for more than 25 *Caenorhabditis* species but many of these remain fractured and incomplete (Stevens et al., 2019). The genic regions of these draft sequences are sufficient for phylogenetic reconstruction and other targeted analyses but not sufficient for fine-scale studies of genomic features. Transposable elements (TEs) retain high sequence similarity and these nearly-similar sequences are often collapsed or missing from draft assembled sequences (Ekblom and Wolf, 2014). Models of genome evolution often invoke TE-related changes and accurate quantification and characterization is necessary to test theoretical predictions and infer relationships between pattern and process. For example, the ‘accordion model’ of genome evolution proposes that genome size changes primarily through TE expansion and large segmental deletions (Kapusta et al., 2017). Population genetic theory predicts that Class I TEs involving an extrachromosomal RNA intermediate (retroelements) may be differentially affected by the evolution of self-fertility when compared with Class II TEs (DNA elements) with a ‘cut-and-paste’ mechanism (Boutin et al., 2012; Dolgin and Charlesworth, 2006). Here, we

analyze chromosome-scale genome sequences that permit reliable characterization of protein-coding gene content, TE content and TE type. We focus on the *Caenorhabditis Elegans* group and test three hypotheses of genome evolution: 1) ‘genome shrinkage’ in self-fertile species (Yin *et al.*, 2018) ; 2) the ‘accordion model’ (Kapusta *et al.*, 2017); and 3) differing Class I/Class II TE dynamics in androdioecious and dioecious species (Boutin *et al.*, 2012; Dolgin and Charlesworth, 2006).

We find *Caenorhabditis* genome evolution is characterized by numerous small-scale rearrangements. These occur frequently, even between closely related strains within species and the majority of rearrangements are within genes. TE-associated rearrangements are less frequent but have a larger mean and median size. Ancestral genome reconstructions show that duplicated sequence is the most common mode of genomic change with the larger outcrossing genomes due to an excess of duplications in comparison with the smaller self-fertile genomes. We find no evidence for the ‘accordion model’ of genome evolution in these worms, and no evidence for differential evolution of Class I and Class II TEs in outcrossing and self-fertile *Caenorhabditis*. Gene families show high birth and death rates across *Caenorhabditis* and there are no gene families changing in parallel

across outcrossing *Caenorhabditis*. We find 71 gene families have decreased across self-fertile *Caenorhabditis* while no gene families have increased in parallel across these species. Significant, parallel reductions have occurred in self-fertile species sensory systems, regulatory proteins and membrane-associated immune responses. These protein changes reflect the shifts in selection that occur with self-fertility including a reduced need to find a mate or deal with pathogens. Overall, our results point to variable, dynamic rules of genome evolution across phylogenetic groups.

Results

Variation in protein-coding gene number between closely related *Caenorhabditis*

Similar to previous work (Thomas *et al.*, 2012; Yin *et al.*, 2018; Fierst *et al.*, 2015) we found that genome size varied with reproductive mode (Fig. 1). The genomes of outcrossing *Caenorhabditis* were 120.37-130.48Mb in size while self-fertile *Caenorhabditis* genomes were 80.98-105.42 Mb. The number of protein-coding genes in these species also varied with reproductive mode with outcrossing genomes containing 21,443-34,696 genes while self-fertile genomes contained 19,997-21,210 genes. The fragmented *C. sinica* sequence (Supplementary

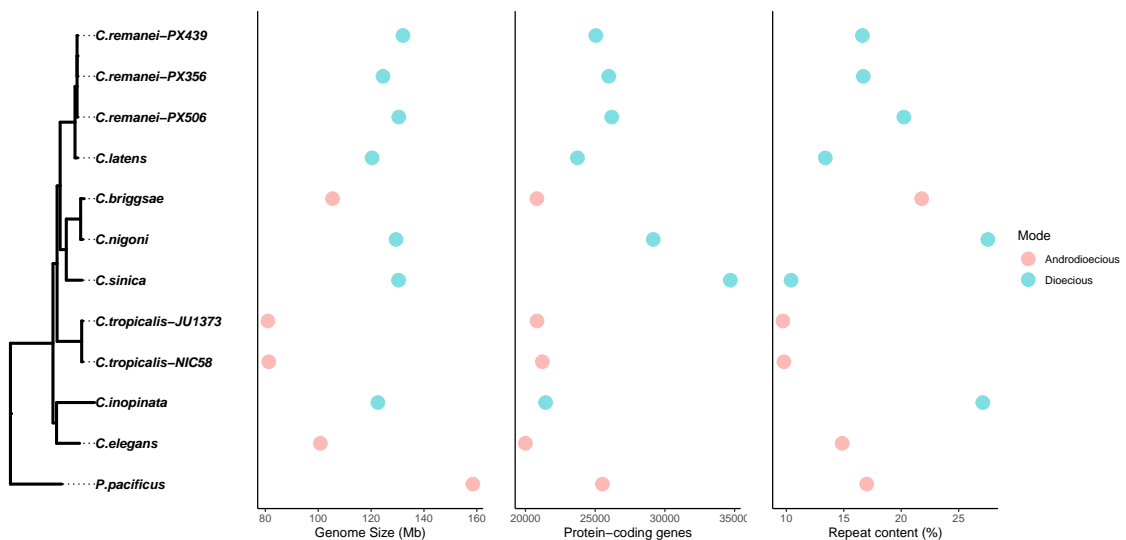


Fig. 1: Genome size and number of protein-coding genes varied with reproductive mode while repeat content did not.

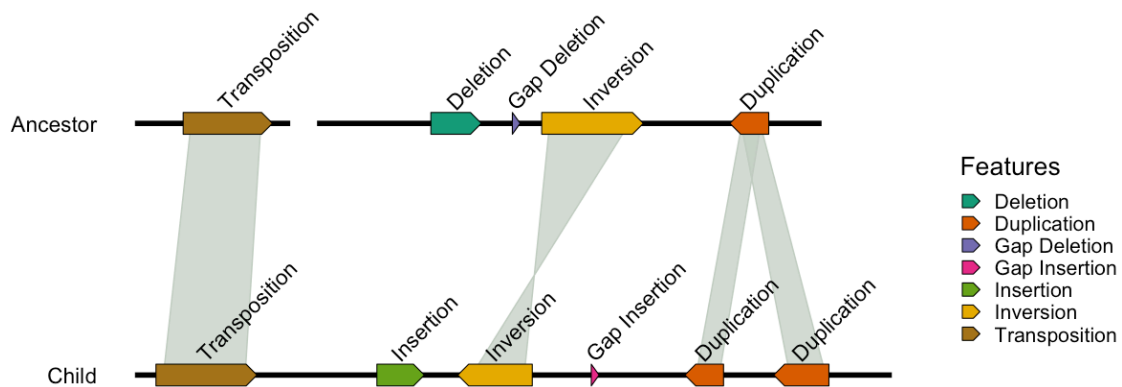


Fig. 2: Genomic changes from ancestor to child can happen by transposition, small (gap) and large deletions, small (gap) and large insertions, inversions and duplications.

Table 1) may have an artificially inflated estimated gene number of 34,696. Excluding *C. sinica* the mean number of genes in outcrossing genomes was 25,258 as compared to 20,714 in self-fertile genomes.

Caenorhabditis genome evolution is characterized by extensive gene-associated insertions and duplications

Genomic changes from ancestor to child can occur in multiple ways (Fig. 2). The most common nucleotide changes across the *Caenorhabditis Elegans* group were insertions and duplications (Fig. 3). Deletions represented a small fraction of observed changes between both closely and more distantly related species pairs. Rapid nucleotide divergence could account for some of these differences as highly divergent nucleotide regions could escape classification as substitutions and result in an overrepresentation of 'inserted' sequences.

For closely related species like the *C. remanei*/*C. latens* species group nucleotide changes can be tracked at a higher resolution. For example, for *C. remanei* PX506 we find that 1.33Mb of sequence was deleted relative to the ancestor (1% of the *C. remanei* PX506 genome size), 2% of nucleotides changed through substitution, 5% of the nucleotides were inserted and 14% of the nucleotides were duplicated (Supplementary Data 2). In comparison, 92% of the nucleotides were aligned/matched to the reconstructed ancestor sequence. Nucleotide changes can be encased in larger genomic rearrangements and multiply represented in the dataset and the total nucleotide changes sums to a representation greater

than the current genome size (*i.e.*, greater than 100%). Despite this, we can compare the relative contribution of each type of genomic change to genome size in the dataset and reject deletions as the predominant mode of genome evolution in this group ($df = 21, t = 4.75, p = 5.47 \times 10^{-5}$).

We associated the mutations identified by Progressive Cactus with genomic features in each dataset. We found that most genomic changes in the *Caenorhabditis Elegans* group occurred within genic sequences or unannotated regions (Fig. 3; $df = 10, t = 2.46, p = 0.017$). Changes within and close to TEs were less frequent but of larger mean and median size in all genomes. Inversions and transpositions occurred at a frequency of 1-2 orders of magnitude less than insertions with mean and median sizes 1-2 orders of magnitude greater than insertion-associated changes. The landscape of insertions, inversions and transpositions by chromosome is shown for the self-fertile *C. briggsae* in Fig. 4 and its outcrossing relative *C. nigoni* in Fig. 5. Both species showed similar patterns with mutations more frequent at the edges of chromosomes and slightly less frequent in chromosome centers.

For the majority of the *Caenorhabditis* species mutations were distributed across all chromosomes equally with the exception of the *C. remanei*/*C. latens* species complex. For example, in *C. remanei* PX506 both gene-associated and TE-associated mutations were less frequent on the X chromosome (Fig. 6).

Insertions, inversions and transpositions were calculated in the species genome coordinates while

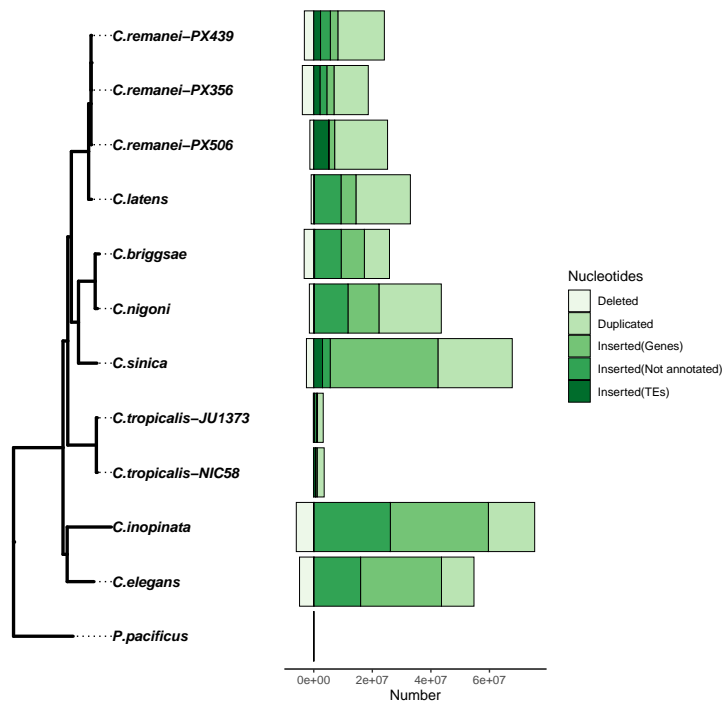


Fig. 3: *Caenorhabditis* genome evolution is characterized by duplications and insertions with infrequent deletions. For most species insertions map to gene regions with the exception of *C. remanei* PX506.

deletions and duplications were calculated in the ancestral genome coordinates. This limited our ability to infer overlap with genomic features as the ancestral genomes are not reconstructed at a sufficiently high resolution to annotate protein-coding genes and TEs. Duplications were more frequent than deletions and had larger mean and median sizes. Deletion and duplication patterns were similar for both self-fertile and outcrossing *Caenorhabditis*. For example, the mean and median sizes of deletions and duplications were similar for both the self-fertile *C. briggsae* and the outcrossing *C. nigoni* (Fig. 7).

Transposable element content is not predicted by reproductive mode

The ‘accordion model’ of genome evolution (Kapusta *et al.*, 2017) proposes that genomes grow and shrink by a balance of TE-associated expansions and segmental deletions. We find no evidence for this model of genome evolution in the *Caenorhabditis* *Elegans* group. Despite consistent differences in

genome size between outcrossing and self-fertile species, there are no consistent differences in repeat content. Genome size variation within self-fertile species and outcrossing species also does not reflect differences in repeat content. For example, the nematode *C. inopinata* has one of the smallest outcrossing genomes at 122Mb but one of the highest repeat contents at 27.1%. Outcrossing genomes varied between 10.41 and 27.54% repetitive content and self-fertile genomes varied between 9.7 and 21.78% repetitive content (Fig. 1).

It has also been proposed that Class I and Class II TEs might vary predictably with reproductive mode (Boutin *et al.*, 2012; Dolgin and Charlesworth, 2006). We find that in the *Caenorhabditis* *Elegans* group Class II TEs varied similarly across reproductive modes with ranges of 6.96-19.63% in outcrossing genomes and 6.13-16.38% in self-fertile genomes. However, outcrossing genomes had higher proportions of Class I TEs (0.4-4.85%) when compared with self-fertile species (0.68-1%).

To statistically evaluate this Class I TE difference and the ‘accordion model’ of genome

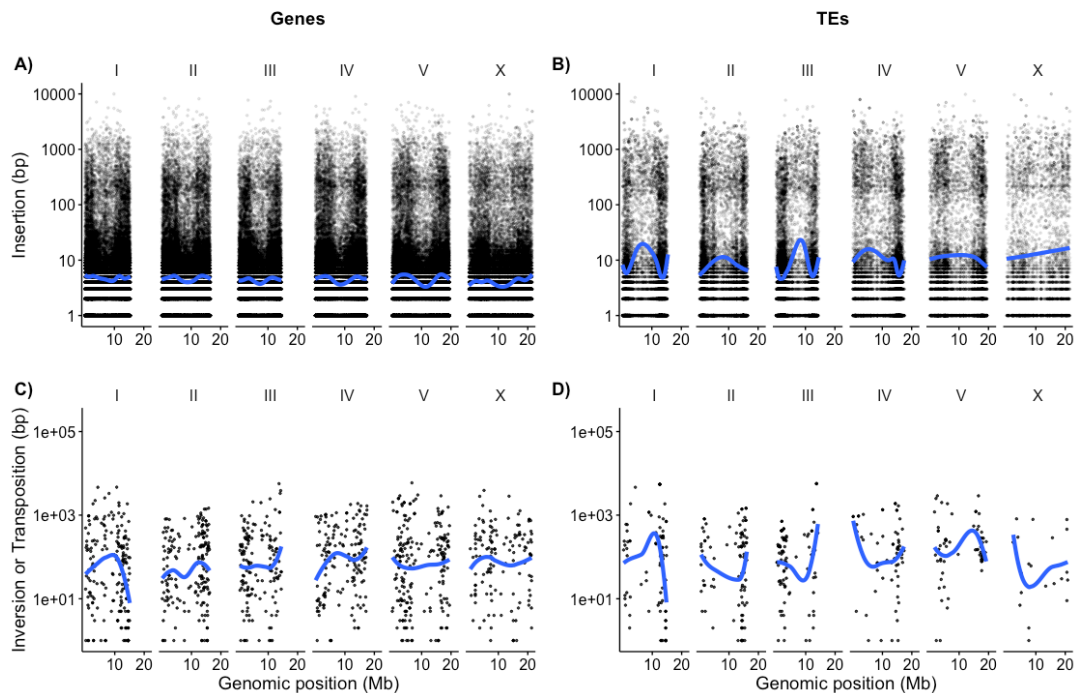


Fig. 4: The most frequent type of genomic changes between *C. briggsae* and its ancestor were small gene-associated insertions.

size differences via TE expansion we performed a phylogenetic comparative analysis. We mapped the discrete ‘selfer’ or ‘outcrosser’ states onto the phylogeny and distinguished “equal rates”, “symmetrical” and “all rates different” transition matrix models using the small sample size corrected Akaike Information Criteria (AICc). We found the “symmetrical” and “equal” rate transition matrix models both outperformed the “all rates different” model, but were indistinguishable from each other by AICc (Table 1). In all but one case, either the Brownian Motion or the Ornstein-Uhlenbeck global optima model performed best, and although not always distinguishable from each other, always outperformed the Ornstein-Uhlenbeck separate optima model by more than two AICc units (the criteria for significantly different suggested by Burnham and Anderson, 2002).

The Ornstein-Uhlenbeck model with separate optima for self-fertile and outcrossing species performed best only for the DNA transposon Mutator and by 1.79 AICc units better than the next best single optima model. The estimates of primary

optima for self-fertile species (2.97% +/- 0.58) and outcrossing species (6.19% +/- 0.44) were greater than 2 standard errors different from each other and given the low level of phylogenetic inertia estimated ($t_{1/2} = 0.11\%$ of the total tree height), were similar to the currently observed mean values within self-fertile and outcrossing species. Mutator elements in self-fertile genomes ranged from 5,234 in *C. briggsae* to 12,076 in *C. tropicalis* JU1373. In comparison, Mutator elements in outcrossing genomes ranged from 24,803 in *C. nigoni* to 47,268 in *C. inopinata*. However, we note that this DNA transposon makes up just 6% and 3% of the respective genomes and cannot explain the much larger difference in overall genome sizes (12-66%) between these two groups.

Gene family turnover is high across *Caenorhabditis*

We used orthofinder (Emms and Kelly, 2015) to associate protein-coding genes to 24,574 different orthogroups or gene families. We eliminated orthogroups that were not present in at least half

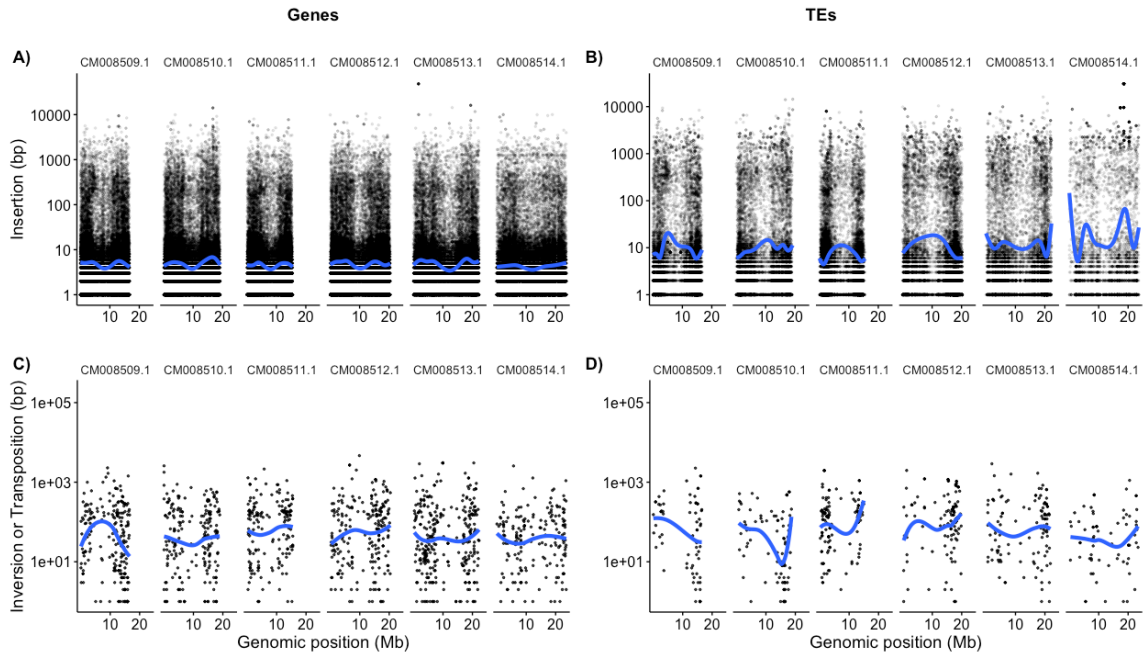


Fig. 5: The genome of *C. nigoni*, the most closely related outcrossing species to *C. briggsae*, also diverged from their common ancestor primarily through small gene-associated insertions.

of our *Caenorhabditis* genomes and retained 14,590 gene families. We estimated gene birth and death with the CAFE5 software (Mendes *et al.*, 2020; Han *et al.*, 2013; Hahn *et al.*, 2005) for these gene families. We tested models with 1-9 different birth/death rates (λ s) for gene family change and found that a model with 2 birth/death rates (λ s) had the highest likelihood. The estimated birth/death rate across the *Caenorhabditis* group ($\lambda = 0.4293$) was 1-2.5 orders of magnitude higher than that reported in *Drosophila* (Hahn *et al.*, 2007) or *Saccharomyces* (Han *et al.*, 2013). Rapid gene family expansion and contraction was not limited to one clade or individual species (Fig. 8).

As an illustration of this we studied the evolution of F-box FBA2 (fbx-a) genes in the *C. elegans*-*C. nigoni* selfing-outcrossing species pair. F-box genes are involved in protein-protein interactions (Kipreos and Pagano, 2000) and implicated in the sexual system in *Caenorhabditis*. For example, recruitment of an F-box gene to a pathway regulating *C. elegans* hermaphrodite development is a crucial piece of evidence for the 3 independent origins of self-fertility in the *Caenorhabditis* *Elegans* group (Guo *et al.*,

2009). F-box genes occur in *Caenorhabditis* genomes in high numbers with at least 377 in *C. elegans* (Wang *et al.*, 2021). Of this, 222 are annotated as F-box FBA2 (fbx-a) genes characterized by an F-box domain and an FBA2 domain. The proteins are unevenly spread across the chromosomes with 6 on chromosome IV in *C. elegans* and 92 on chromosome V.

We identified a 2.1kb region of ancestral sequence (Fig. 9) that aligns to multiple regions within a 500kb sections of the *C. elegans* chromosome III, labeled duplicated sequence by ProgressiveCactus (Armstrong *et al.*, 2020). The same region of ancestral sequence was not identified in the *C. inopinata* genome and instead aligned poorly to small, disjunct regions across the chromosome. Biological function is not known for F-box FBA genes but they appear to have arisen in high numbers in *Caenorhabditis* through tandem duplications (Wang *et al.*, 2021). F-box and F-box FBA genes are abundant in outcrossing *Caenorhabditis* as well, with 1,358 annotated F-box proteins and 412 annotated fbx-a proteins in the outcrossing *C. remanei* PX506. Rapid gene birth and death via small structural

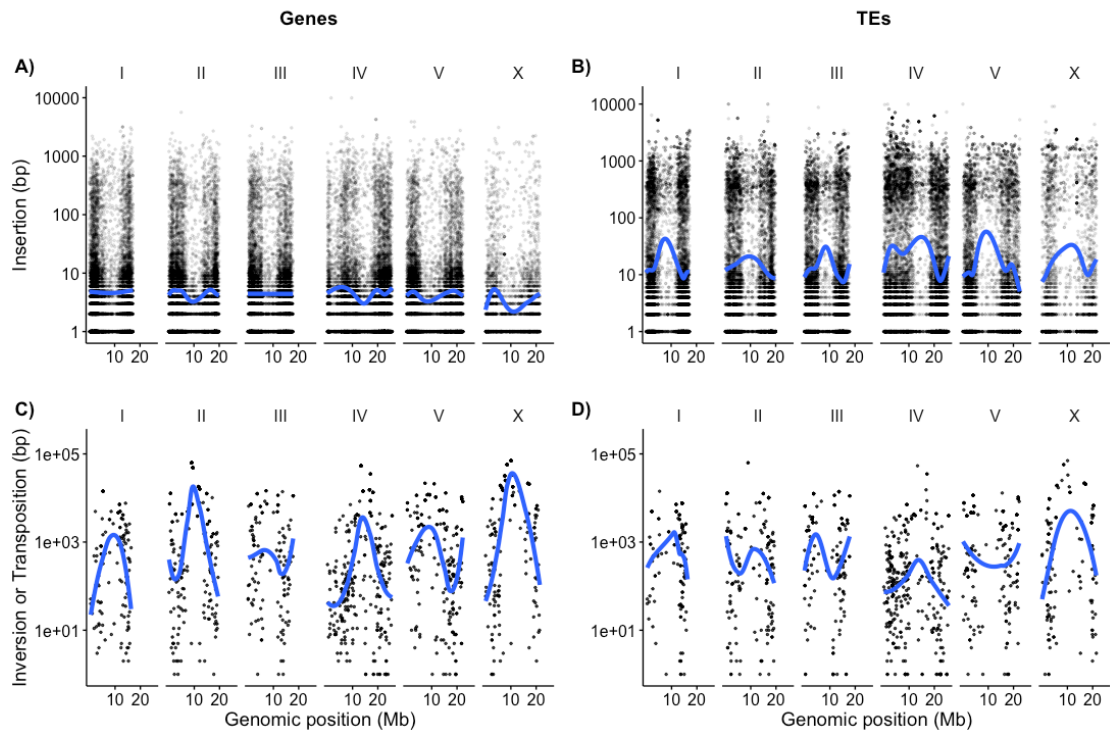


Fig. 6: The outcrossing *C. remanei* PX506 diverged from its ancestor through small gene-associated insertions.

mutations were common in both outcrossing and self-fertile in the *Caenorhabditis Elegans* group.

Few gene families show parallel changes across self-fertile species

We found that of the 14,590 gene families 595 were expanding or contracting significantly at $p < 0.01$. Of these, 71 were decreasing in self-fertile species relative to outcrossing species. We identified an annotated protein domain or *C. elegans* orthologous protein for genes in 30 of these families. Six of these families involved serpentine receptors, chemoreceptors known to be important for *Caenorhabditis* ability to navigate its environment. Three of the families encode F-box associated genes and 10 of the families encode regulatory proteins including transcription factors, DNA polymerase-associated domains, RNA export domains, histone-lysine N-methyltransferases and kinases. We also identified 8 families encoding membrane associated immune and protein responses including peptidases,

C-type lectins, ankyrin repeats and actin and chitin associated proteins.

Caenorhabditis genomes contain large numbers of each of these protein families and changes in gene number could simply reflect overall changes at the genomic level. We found that F-box associated proteins and kinases were not changing significantly while serpentine receptors ($p = 0.0142$), transcription factors (0.0001) and peptidases were ($p = 0.0026$). Thus, gene family expansions and contractions likely reflect a mix of true selection and drift mediated by high molecular turnover in these species. We did not identify gene families consistently increasing in parallel in selfing species or increasing or decreasing in outcrossing species.

Discussion

We have presented a fine-scaled analysis of genome evolution across the *Caenorhabditis Elegans* group. Our results contribute to a body of work establishing protein-coding changes as the basis of genome

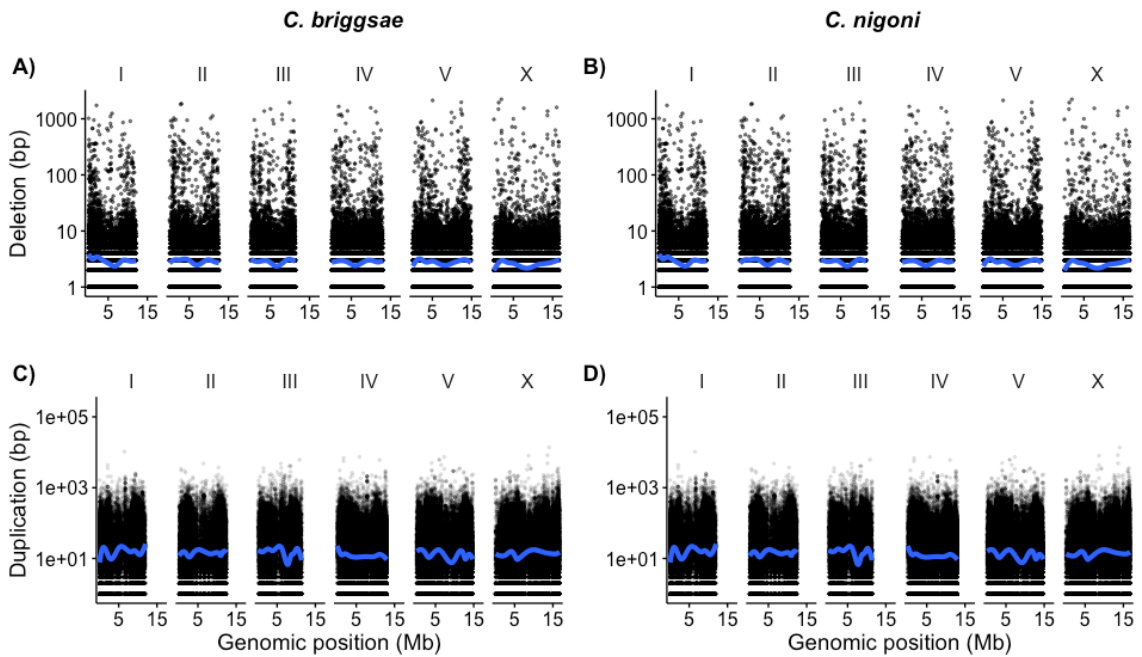


Fig. 7: The genomes *C. nigoni*, the most closely related outcrossing species to *C. briggsae*, experienced similar mean and median sizes of Deletions and Duplications

size differences in these worms (Stevens *et al.*, 2019; Teterina *et al.*, 2020; Thomas *et al.*, 2012; Yin *et al.*, 2018; Fierst *et al.*, 2015; Kanzaki *et al.*, 2018; Noble *et al.*, 2021). We tested hypotheses proposing different sources of genome size variation between outcrossing and self-fertile species including ‘genome shrinkage’ (Yin *et al.*, 2018), the ‘accordion’ model of TE expansion and segmental deletion (Kapusta *et al.*, 2017) and differences in Class I and Class II TE dynamics (Boutin *et al.*, 2012; Dolgin and Charlesworth, 2006). We found no evidence for these hypotheses as the basis of genome size differences in these species. Instead, our results show genome evolution in *Caenorhabditis* was characterized by high rates of structural variant mutations, particularly insertions and duplications, coupled with rapid nucleotide divergence. Differential genome size in these worms evolved through an excess of duplications and insertions in outcrossing species relative to their self-fertile relatives.

Caenorhabditis genomes: Duplications, insertions and high rates of gene family evolution

The dominant hypothesis for genome evolution after the advent of self-fertility has been deletion of genes (Fierst *et al.*, 2015; Thomas *et al.*, 2012; Yin *et al.*, 2018; Rodelsperger *et al.*, 2018) and loss of DNA (Shimizu and Tsuchimatsu, 2015; Roessler *et al.*, 2019; Hu *et al.*, 2011). We found no evidence for this in *Caenorhabditis*. Our results suggest instead that *Caenorhabditis* genomes evolved size differences through differential expansion via numerous duplications and insertions coupled with rapid nucleotide divergence. Similar patterns of genome size varying with selfing and outcrossing have been observed in plants including *Arabidopsis* (Hu *et al.*, 2011) and *Capsella* (Slotte *et al.*, 2013). These comparisons have attempted to test hypotheses regarding mechanisms but reported equivocal results due to rapid evolution, low sample sizes and strong phylogenetic signals.

For example, a large-scale study describing the genomes of 10 newly discovered *Caenorhabditis* reported a substantial phylogenetic influence on genome size with the *Drosophilae* Super-group (the

Table 1. Specific TE elements and the AICc scores for a Brownian Motion (BM), single optimum Ornstein-Uhlenbeck model (OU1) and separate self-fertile and outcrossing optima Ornstein-Uhlenbeck model (OU2). Underlined AICc values indicated either the single best model or in some cases the best model and next best model that could not be distinguished by more than 2 AICc units. Eighteen of the 21 TEs were best modeled by a BM process and four of these could not be distinguished from a single optimum OU process. Five TEs were best modeled as a single optima OU process, two of which could not be distinguished from a BM model. Only one of the 21 TEs (the DNA transposon Mutator) was best modeled by a separate optimum OU model. The phylogenetic half-life and stationary variance for Mutator were 0.0011 and 1.35 respectively with greater than 60% of the variance explained by the two optima model ($r^2=0.64$).

Element	AICc (BM)	AICc (OU1)	AICc (OU2)
Repeats(Total)	<u>73.83</u>	<u>74.86</u>	77.94
GC %	<u>24.33</u>	28.26	27.59
Retroelements	<u>38.06</u>	40.64	43.84
Penelope	<u>-51.02</u>	-47.09	-43.06
LINEs	<u>1.49</u>	<u>1.59</u>	6.61
L2 CR1 Rex	<u>-12.83</u>	<u>-14.25</u>	-10.29
R2 R4 NeSL	-37.05	<u>-42.28</u>	-37.49
RTE Bov B	<u>-17.74</u>	-13.81	-9.21
LTR elements	<u>34.49</u>	37.32	39.91
BEL Pao	<u>-19.89</u>	<u>-19.86</u>	-15.10
Gypsy	<u>20.76</u>	24.48	27.45
DNA transposons	<u>57.25</u>	61.17	64.09
hobo Activator	<u>-1.73</u>	2.20	7.03
TC1 IS630 Pogo	<u>42.08</u>	46.01	50.93
PiggyBac	<u>-5.95</u>	-2.04	0.37
Mutator	51.41	<u>51.00</u>	<u>49.21</u>
Other (Mirage, P elements, Transib)	62.05	<u>58.63</u>	62.87
Helitrons/Rolling Circles	<u>15.93</u>	19.86	25.10
Unclassified Repeats	72.05	<u>42.61</u>	45.13
Total interspersed repeats	<u>72.78</u>	<u>73.52</u>	75.91
Satellites	<u>12.51</u>	16.44	21.66
Simple repeats	<u>-25.85</u>	-21.92	-23.05

clade at the base of the *Elegans* group) having genome sizes 65-91Mb (Stevens *et al.*, 2019) and protein-coding gene counts dipping to just 17,134. Notably, all of these species are outcrossing. The lower bound of these estimates is roughly half that observed for both genome size and gene count in outcrossing *Caenorhabditis* in the *Elegans* group and these two clades comprise the upper and lower bounds for estimates of genome size and gene count across the genus. These results suggest that reproductive transitions and the associated influences on genome evolution in *Caenorhabditis* occur against a background of dynamic genome evolution characterized by frequent SV mutations and genome size changes.

We found that *Caenorhabditis* had high rates of gene family turnover including both birth and death rates, and resulting expansions and contractions across the phylogeny. These rates were 1-2.5 orders

of magnitude higher than those previously reported for other metazoans (Hahn *et al.*, 2005, 2007; Han *et al.*, 2013; Schrader *et al.*, 2021). Although the SV mutations are randomly generated, they appear to be contributing to rapid rates of gene duplication and in some instances deletion. These high rates have been experimentally observed in *C. elegans* (Konrad *et al.*, 2018; Lipinski *et al.*, 2011), to the point that duplications and deletions arise in parallel in replicate populations (Farslow *et al.*, 2015).

Given this rich mutational spectrum, it can be challenging to infer which gene families evolved as a consequence of reproductive mode. We found no gene families expanding or contracting consistently in outcrossing species and no gene families were consistently expanding in self-fertile species. Of the 595 gene families that were significantly expanding or contracting across the *Caenorhabditis Elegans* phylogeny, 71 (12%) were contracting in parallel

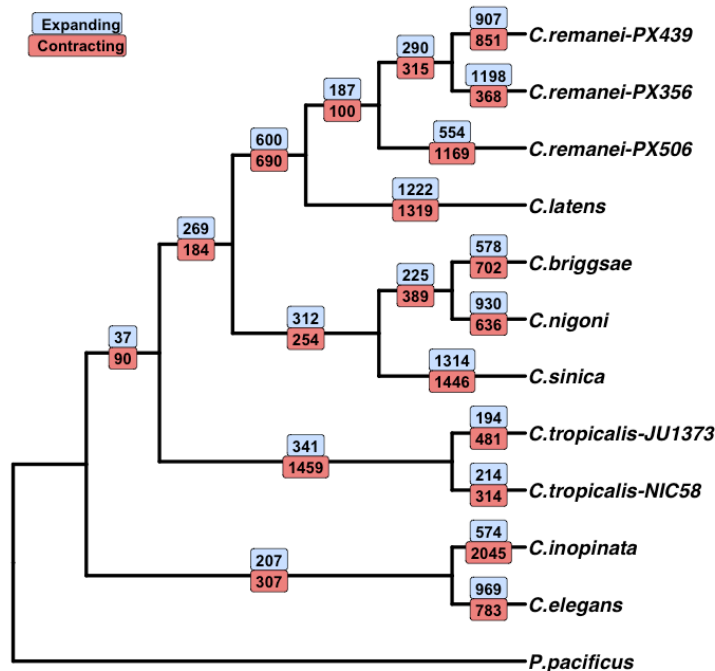


Fig. 8: The number of gene family expansions (in blue) and contractions (in red) on each branch of the *Caenorhabditis Elegans* phylogeny.

across self-fertile genomes. A smaller protein-coding content in self-fertile species has been repeatedly observed (Fierst *et al.*, 2015; Yin *et al.*, 2018; Shimizu and Tsuchimatsu, 2015; Roessler *et al.*, 2019; Hu *et al.*, 2011) and two transcriptome-based studies have reported smaller proteomes and parallel gene family loss in self-fertile *Caenorhabditis* (Thomas *et al.*, 2012) and *Pristionchus* (Rodelsperger *et al.*, 2018) nematodes. The average gene is 3kb in *Caenorhabditis* and our results show that these reductions contribute little to overall genome size differences. Several gene families were changing in proportion to their overall genome composition and likely reflect genetic drift at the level of SVs.

However, single genes, proteins and protein families can be extremely significant for organismal evolution and our results are not mutually exclusive with important gene losses mediated by the evolution of self-fertility. An important example of this is the male secreted short or *mss* protein family that is required for sperm competition in outcrossing species and has been largely lost in self-fertile *Caenorhabditis* (Yin *et al.*, 2018; Yin and Haag,

2019). *Caenorhabditis* have an extremely short mutational path from dioecious outcrossing to self-fertility (Baldi *et al.*, 2009), but the transition to androdioecy puts the population into an entirely different selection regime. An outcrossing organism must be able to navigate its environment to find the opposite sex, successfully mate, and frequently deal with many more pathogens and parasites (Andersson, 1994). In multiply-mating species these behavioral and environmental demands exert strong selective pressures on males (and. J Wade, 2003) and, accordingly, male-biased and male-associated genes are preferentially missing in both *Caenorhabditis* (Thomas *et al.*, 2012; Yin *et al.*, 2018) and *Pristionchus* (Rodelsperger *et al.*, 2018). Our results suggest that similar patterns are occurring in proteins responsible for sensory recognition, regulatory systems and membrane associated immune and protein responses. These genomic changes reflect the comprehensive environmental selection pressures that mating systems impose. Thus, while the overall size dynamics were not explained by differential loss

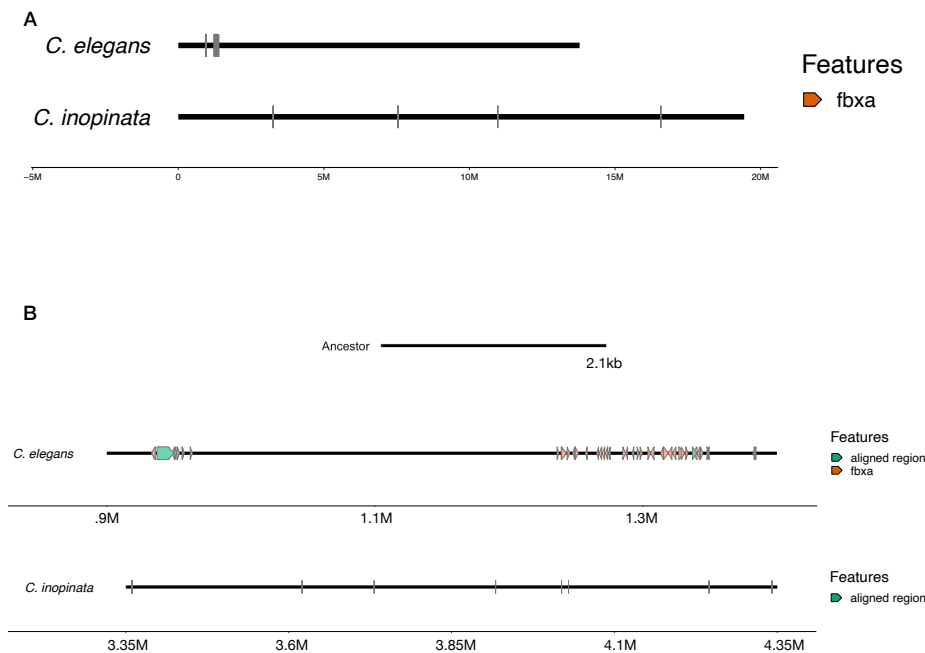


Fig. 9: A) A 500kb region of *C. elegans* chromosome III contains 46 annotated fbx-a genes. In comparison there are 4 fbx-a genes spread across 15Mb of chromosome III in *C. inopinata*. B) A 2.1kb region of ancestral sequence aligns to this region multiple times in *C. elegans* with high sequence similarity but aligns weakly to small, disjunct regions in *C. inopinata*.

of genes or DNA there were still important functional patterns of loss and change influenced by reproductive mode in *Caenorhabditis*.

Genome size is not predicted by TE content

We found the observed differences in *Caenorhabditis* genome size were not due to TEs. There was no evidence for differences in overall TE content, differential expansion and deletion (the accordion model) (Kapusta *et al.*, 2017) or dynamics of Class I/Class II TEs (Boutin *et al.*, 2012; Dolgin and Charlesworth, 2006). Our results add to studies showing little change in TE abundance after the evolution of self-fertility (Hu *et al.*, 2011; Slotte *et al.*, 2013) and little difference in the evolutionary dynamics of Class I and Class II TEs (Nowell *et al.*, 2021). However, this absence of evidence is not conclusive evidence against these hypotheses. Models addressing reproductive systems and their influence on TE dynamics make specific predictions based

on the relative age of the sexual system and TE invasion into the genome (i.e., recent vs ancient) and are sensitive to variation in parameters like quantitative rates of outcrossing and transposition. These subtle variations may act in populations but not be discernible in broad-scale comparative analyses across multiple types of TEs and long evolutionary divergence times. Further fine-scale studies that are better able to match experimental data with theoretical parameters may result in greater insights into the interactions of reproductive systems and TE evolution.

The one exception to this general lack of differentiation in TEs was the DNA transposon Mutator. Mutator-like elements (MLEs) draw their name from their mutagenic abilities as the TEs are highly active near genes and frequently acquire host gene fragments (Dupeyron *et al.*, 2019). Our phylogenetic comparative analysis found the outcrossing primary optimum was roughly twice that of the self-fertile primary optimum for Mutator

TEs, which results in two interesting biological questions. First, why is the Mutator content higher in outcrossing species? And second, are Mutator dynamics responsible for the high rates of rearrangements we observed in *Caenorhabditis* genomes?

Mutator transposons were first discovered in maize where it was found that lines with the TE had mutation rates 30x higher than those without (Robertson, 1978). Since then Mutator transposons and Mutator-Like Elements (MULEs) have been extensively studied in maize and used as forward and reverse mutagenesis systems (Lisch, 2015). In addition to high rates of transposition activity Pack-Mutator-like transposable elements (Pack-MULEs) modify genes through biased acquisition of GC-rich sequences and preferential insertion near the 5' end of transcripts (Jiang *et al.*, 2011). Mutator-based mutagenesis overwhelmingly affects genes because of these unique sequence-level mechanisms. Multiple generations of self-fertilization can silence Mutator transposons in maize (Robertson, 1986) through DNA methylation (Chandler and Walbot, 1986; Martienssen and Baron, 1994; Slotkin, 2005) that heritably modifies histones (Guo *et al.*, 2021).

These results in maize suggest that Mutator transposons may be differentially affected by outcrossing and self-fertilization, and the contribution to mutational dynamics in maize may also occur in nematodes. However, mutator transposons have only recently been discovered in metazoans and there is little known about how their dynamics may be similar to or differ from those observed in plants (Liu and Wessler, 2017). For example, despite the interesting parallel with self-fertilization DNA methylation is absent in *Caenorhabditis* (Simpson *et al.*, 1986; Wenzel *et al.*, 2011) and any mechanism of Mutator control in worms would have to occur through a separate mechanism. Mutator transposons in nematodes, particularly *Caenorhabditis*, may be an exciting avenue for future studies of mutational dynamics.

Variation in genome size and protein-coding gene number within conspecific lineages and between interfertile species

Previous studies of genome structure in *Caenorhabditis* have compared between distantly related self-fertile species (Stein *et al.*, 2003; Hillier *et al.*, 2007), outcrossing species and distantly related self-fertile species (Thomas *et al.*, 2012; Fierst *et al.*, 2015)

or closely related outcrossing-selfing pairs (Kanzaki *et al.*, 2018; Yin *et al.*, 2018). We sought to evaluate the significance of these comparisons by comparing genome structure and sequence between the conspecifics *C. remanei* PX356, PX439 and PX506 and the interfertile *C. latens*. Similar comparisons have been performed for globally distributed strains of *C. elegans* (Cook *et al.*, 2017; Kim *et al.*, 2019; Lee *et al.*, 2019) and identified a somewhat paradoxical pattern of low nucleotide diversity coupled with high intraspecific tolerance for structural variations to the level of presence-absence variation in entire genes (Lee *et al.*, 2022). We find that this pattern is echoed in outcrossing *Caenorhabditis* with variation in genome size, protein-coding gene number and gene family size across strains within the *C. remanei* species.

This genomic structure where a complement of genes is shared among all members of a group and substantial gene presence-absence variation occurs between genomes has been recognized in prokaryotes and termed the “core genome” and “pangenome” (Tettelin *et al.*, 2005). The pangenome concept has been applied to a broad range of organisms including fungi, plants and animals (Golicz *et al.*, 2020). However, the scale of variation within and between core and pangenomes varies dramatically across these groups. For example in microbes the core genome is frequently <10% of the full pangenome reference (van Tonder *et al.*, 2014) while in fungi (McCarthy and Fitzpatrick, 2019) and plants (Gao *et al.*, 2019) the core genome comprises 80-90% of the pangenome reference. In humans the pangenome fraction is smaller still, comprising an estimated 0.5-1.2% of the reference sequence (Miga and Wang, 2021).

Within *Caenorhabditis* species a core-pangenome structure is difficult to define because many presence-absence variant genes are members of large, diverse protein families (Lee *et al.*, 2022). Identifying one-to-one orthologous relationships given an array of highly divergent paralogs within each genome is difficult in *Caenorhabditis* and other groups characterized by rapid molecular changes (Zallot *et al.*, 2016). A more useful division for *Caenorhabditis* protein families may be those that are sufficient in small number and those that proliferate within individual genomes. For example, the small worms are extremely dependent on external environment and have highly developed repertoires of chemoreceptors with some 10-20% of the total gene complement comprised

of chemoreceptors (Robertson and Thomas, 2005; Thomas and Robertson, 2008).

Holocentric chromosomes and sexually antagonistic selection

In addition to the mutagenic potential of Mutator TEs, there is a cellular mechanism that may contribute to the generation of SV mutations in nematodes. Holocentric chromosomes with diffuse centromeres have been noted in plant and animal species including *C. elegans* (Maddox *et al.*, 2004). Holocentrism can stabilize double stranded DNA breaks in meiosis and facilitated gene conversion in the silkworm *Bombyx mori* (Mon *et al.*, 2011). High rates of SVs and rearrangements have been noted in other holocentric species including aphids (Mathers *et al.*, 2021) and lepidopterans (Hill *et al.*, 2019).

One of the most compelling questions to emerge from our analyses is why outcrossing species would experience selection for increased genome size and protein-coding content. Theory suggests that sexual conflict, when two sexes have opposing optima (Rowe *et al.*, 1994), can select for the retention and divergence of duplicated genes (Connallon and Clark, 2011). This has been observed in *D. melanogaster* with the tandem duplicate genes *Apollo* and *Artemis* supporting a scenario of duplication and divergence resolving sexually antagonistic selection (VanKuren and Long, 2018). Going from theoretical predictions and single gene studies to broad-scale quantification of the contribution of sexually antagonistic selection to genome size variation across taxonomic groups is challenging. It will require high-quality genome sequences, robust characterizations of protein-coding genes and a developed understanding of the dynamics of sexual and natural selection.

Until recently, the technology used to assemble and characterize genome sequences has had an outsize influence on the inference of genome size, protein-coding genes and TEs and our ability to test theoretical predictions regarding genome evolution. For example, a prominent hypothesis linking large effective population sizes with small genome sizes (Lynch and Conery, 2003) generated predictions and explanatory theory for a pattern *opposite* to that observed in worms, plants and other self-fertile/outcrossing species. As the field of evolutionary biology shifts to newer long read DNA technologies we will be increasingly able to quantify patterns of genome evolution as they apply to phylogenetic groups, reproductive modes

and functional systems like chromosome pairing and meiosis. The ambitious Darwin Tree of Life programme at the Wellcome Sanger Institute is aiming to sequence the 70,000 eukaryotic species in the UK and Ireland with long-read technology and permit high-quality, chromosome-scale assemblies (Blaxter *et al.*, 2022). These resources will be critical for definitively rejecting and critically evaluating hypotheses regarding genome evolution across eukaryotic life.

Conclusions

The evolution of genome size is a fundamental question in biology (Gregory, 2005b). Comparative studies across eukaryotes have found that genome size variation across large phylogenetic and physical scales is often explained by repeat content (Elliott and Gregory, 2015; Gregory, 2005a; Kapusta *et al.*, 2017). Here, we asked if these dynamics explain genome size variation at smaller physical and phylogenetic scales in species with defined reproductive transitions and correlated genome size differences. We found that smaller scale genome size variation is determined by separate factors. Both outcrossing and self-fertile *Caenorhabditis* experienced numerous gene-associated SV mutations with genomes evolving through duplications, insertions and rapid divergence. TEs and repeats do not explain genome size variation among strains within species, between species or between reproductive modes. Within this landscape of frequent rearrangements we found significant, parallel reductions in gene families across self-fertile species. These encode proteins important for the sensory system, regulatory molecules and membrane-associated immune and protein responses, likely reflecting the global shift in selection pressures created by the transition to self-fertility.

Materials and methods

Assembling chromosome-scale sequences for the outcrossing *C. remanei* PX356 and PX439 and *C. latens*

We used Oxford Nanopore Technologies to generate DNA libraries for 3 strains of outcrossing *Caenorhabditis*; *C. remanei* PX356, PX439 and *C. latens* PX534 (Sutton *et al.*, 2021). The two species are closely related (Felix *et al.*, 2014) and partially interfertile (Dey *et al.*, 2014). We assembled genome sequences in <100 contiguous sequences for each

strain and used the contiguous *C. remanei* PX506 assembled sequence (Teterina *et al.*, 2020) to scaffold the genome sequences into chromosome-scale pseudomolecules (Supplementary Table 1). Each of the assembled sequences we studied was contained in 6-155 contiguous sequences with the exception of *C. sinica* which is contained in 15,261 sequences. We included *C. sinica* in analyses despite this fragmentation as a representative outcrossing species closer to the root of the *Caenorhabditis Elegans* group. We also included the distantly related *Pristionchus pacificus* to orient and root our phylogenetic analyses.

Nematode laboratory culture

The *C. remanei* PX356 and PX439 and *C. latens* PX534 strains were graciously provided by the lab of Patrick C. Phillips. Nematodes were cultured on 100mm Nematode Growth Media (NGM) plates seeded with *E. coli* OP50 (Stiernagle, 2006). For sequencing we collected worms from 5-10 100mm plates by washing with M9 media into 15mL conical tubes. Tubes were placed on a tabletop rocker for 1 hour and then centrifuged to pellet nematodes. To minimize *E. coli* contamination we removed the M9, added fresh M9, mixed the tubes and pelleted the worms by centrifugation, repeating this process 5 times.

DNA extraction and sequencing

Our protocol for DNA extraction was described in Sutton *et al.* (2021). Briefly, we froze pelleted worms in liquid nitrogen to rupture cuticles and combined 1.2mL lysis buffer (100mM EDTA, 50mM Tris, 1% SDS) with 20 μ L Proteinase K (100mg/mL) before incubating at 56C for 30 minutes with shaking. We used phenol chloroform for extraction following Sambrook (2001) and the Short Read Eliminator Kit from Circulomics Inc. (Baltimore, MD) to select high molecular weight DNA.

We used the Oxford Nanopore Technologies (Oxford, UK) SQK-LSK109 ligation sequencing kit for DNA library preparation. Approximately 500-900ng of DNA was sequenced for 48 hours on R9.4.1 RevD flowcells via a gridION X5. We used Guppy v.4.0.11 for basecalling in the ‘high-accuracy’ mode.

Genome assembly strategy

We used the Canu v2.0 software package to correct Nanopore libraries (Koren *et al.*, 2017). Briefly, Canu’s correction module creates an all-vs-all overlap

dataset, uses this to correct individual reads, selects the longest available reads and creates a dataset of user-specified coverage. For the data presented here we used 40x coverage based on an estimated genome size of 130Mb. We assembled the Canu-corrected reads with Flye v.2.8.2 (Kolmogorov *et al.*, 2019) and polished the assembled sequences with Pilon v1.23 (Walker *et al.*, 2014). We designed this correction, assembly and polishing protocol after extensive simulations and testing (described in Sutton *et al.*, 2021). For polishing we used paired-end Illumina libraries previously generated by the laboratory of Patrick C. Phillips and obtained from the NCBI SRA in December 2020 (accessions are listed at the end of this article). We eliminated microbial and other contaminants after polishing with the SDR software (Fierst and Murdock, 2017). Briefly, SDR uses ensemble-based machine learning to train a model of sequence identity (i.e., target or contaminant) based on measured predictor variables. Here, the predictors were sequence GC content, read depth of Nanopore libraries aligned to the assembled sequences and *k*-mer frequency distributions with *k*=19.

Residual allelism has been a problem with previous *Caenorhabditis* genome sequences (Barriere *et al.*, 2009). We used the purge_haplotigs software version 1.1.1 (Roach *et al.*, 2018) to identify possible heterozygous regions of the assembled sequences. Briefly, we aligned the ONT libraries to the assembled sequences and produced a read-depth histogram to identify regions of abnormal sequencing depth. The method works under the assumption that alleles will result in ‘split coverage’ with read depth approximately 0.5 that of the homozygous contigs. The read-depth histogram did not have noticeable regions of abnormal coverage and the software identified less than 400,000bp of possible haplotigs in the genome sequences of *C. remanei* PX356, *C. remanei* PX439 and *C. latens* PX534. This represented <0.33% of any of the assembled genome sequences and we chose to retain these sequences in the assembled genome because we could not reliably identify them as ‘haplotigs.’

Creating pseudo-molecules

Previous studies have shown remarkable conservation of large-scale synteny between *Caenorhabditis* (Yin *et al.*, 2018; Fierst *et al.*, 2015; Stein *et al.*, 2003; Teterina *et al.*, 2020). We assumed this large-scale synteny is conserved within the interfertile

C. remanei/*C. latens* species complex and used the chromosome-scale *C. remanei* strain PX506 assembled genome sequence (Teterina *et al.*, 2020) to construct pseudo-molecules for *C. remanei* PX356 and PX439 and *C. latens* PX534 with the RagTag software version 2.1.0 (Alonge *et al.*, 2021). Briefly, RagTag performs homology-based scaffolding by aligning query sequences to a reference assembled sequence with the minimap2 software (Li, 2018).

Gene annotation

We annotated protein-coding genes in the assembled sequences of *C. remanei* PX356 and PX439 and *C. latens* PX534 with the BRAKER2 v2.1.6 software (Bruna *et al.*, 2021). We used RepeatModeler v2.0.2 (Smit *et al.*, 2015) for *de novo* repeat identification and the queryRepeatDatabase.pl script inside RepeatMasker/util to extract Rhabditida repeats (Bao *et al.*, 2015). We combined these files to create a library of known and *de novo* repeats and used these with RepeatMasker v4.1.2-p1 (Smit *et al.*, 2015) to softmask repeats in the assembled sequence. We aligned RNA-Seq libraries extracted from mixed stage nematode populations to the softmasked sequences with STAR aligner v2.7.9a (Dobin *et al.*, 2013). We used this in BRAKER2 with the protein sequences from *C. remanei* PX506 as homology evidence. Only the RNA-Seq libraries were used for training gene predictors. We obtained the assembled genome sequences, protein-coding gene annotations and coding sequence files for the remaining *Caenorhabditis* species from WormBase Parasite (Howe *et al.*, 2017) in December 2020 (version WBPS15) with the exception of *C. tropicalis*, which was obtained from the NCBI in February 2021. We performed all of the following analyses (functional annotation, transposable element annotation, whole genome alignment) on the *C. remanei* and *C. latens* assembled genome sequences and protein-coding gene annotations produced by our group and the *Caenorhabditis* assembled genome sequences and protein-coding gene annotations obtained from WormBase Parasite and the NCBI. We used the AGAT suite to calculate gene statistics (Dainat, v080).

Functional annotation

We used the Interproscan software v5.19 (Finn *et al.*, 2017; Zdobnov and Apweiler, 2001) to annotate protein domains and motifs, gene ontologies (Consortium, 2000) and pathway information (Kanehisa

et al., 2016; Kanehisa and Goto, 2000). Briefly, Interproscan searches multiple databases for protein information including PRINTS (Attwood and Beck, 1994; Attwood *et al.*, 1994), Pfam (Punta *et al.*, 2012), ProDom (Bru *et al.*, 2005) and PROSITE (Hulo *et al.*, 2005).

Whole genome alignment

We used ProgressiveCactus version 2.0.4 (Armstrong *et al.*, 2020) to align the *Caenorhabditis* genome sequences and measure the spectrum of mutational events. ProgressiveCactus permits reference-free alignment and uses phylogenetic information to estimate parental (ancestral) genome sequences. We used the *Caenorhabditis* phylogeny estimated by Stevens *et al.* (2019) as the basis for the alignment and added the strains *C. remanei* PX356, PX439, PX506 and *C. tropicalis* JU1373 and NIC58 as polytomies. There is some genetic differentiation between strains within each species but the scale of protein divergence at loci conserved across Phylum Nematoda is minimal within strains when compared with the entire phylum (Bird *et al.*, 2005). Additionally, the inbreeding process necessary to create homozygous strains can lead to unusual fixations and genetic sampling effects that do not accurately represent the species (Adams *et al.*, 2022; Roessler *et al.*, 2019). We used *Pristionchus pacificus* as an outgroup to root the alignments. *P. pacificus* is a distant relative of the *Caenorhabditis* group (Dieterich *et al.*, 2008) but is the closest relative to the group with a high-quality assembled genome sequence suitable for use as a reference.

The ProgressiveCactus alignment algorithm reconstructs an ancestral sequence for branch points along the phylogeny and mutational events are measured between ancestor and child genomes. ProgressiveCactus defines deletions, insertions, gap deletions and gap insertions by size, where gap deletions and insertions are <5bp. Transpositions involve transfer of sequence from one chromosomal region to another while inversions are regions that have reversed orientation. Duplications are sequences that occurred singly in the ancestor and in multiple copies in the child genome.

The output of a ProgressiveCactus (Armstrong *et al.*, 2020) alignment is a binary hierarchical alignment (HAL) file (Hickey *et al.*, 2013). We used the halSummarizeMutations function to calculate substitutions, transitions, transversions, insertions, deletions, duplications and transpositions. The

halSummarizeMutations function calculates these quantities for each genome relative to the ancestral genome and for estimated ancestral genomes relative to each estimated parent node. We used the halBranchMutations function to create a BED-formatted file with the genomic locations of each of the mutations and the bedtools intersect function to associate these mutations with annotated repeat elements and protein-coding genes (Quinlan and Hall, 2010). We used the hal2fasta function to print the estimated ancestral genome sequences, annotated repeat elements in these with the EDTA software (Ou *et al.*, 2019) and associated mutations in ancestral genomes with annotated repeats using bedtools intersect. We could not reconstruct protein-coding genes in estimated ancestral sequences because the accuracy of protein-coding gene annotation is dependent on nucleotide-level features like start codons and accurate intron-exon boundaries that are not prioritized in Progressive Cactus alignment. However, this approach did allow us to analyze insertions and deletions in repeat elements and specific TE families.

TE annotation

We used the Extensive *de novo* TE Annotator software version 2.0 (EDTA; Ou *et al.*, 2019) to identify repeats in *Caenorhabditis* genome sequences. EDTA uses a number of different open-source tools to identify TE candidates in genome sequences and combines these with annotated repeats using known coding sequences (CDS) to eliminate false positive TEs. We used the Rhabditida repeats extracted from RepeatMasker (Bao *et al.*, 2015) and the CDS obtained from BRAKER2 (Bruma *et al.*, 2021) annotation, WormBase Parasite (Howe *et al.*, 2017) and the NCBI Long terminal repeat retrotransposons (LTRs) were identified with LTR Harvest (Ellinghaus *et al.*, 2008), LTR Finder (Xu and Wang, 2007), LTR retriever (Ou and Jiang, 2018, 2019) and Generic Repeat Finder (Shi and Liang, 2019). Generic Repeat Finder (Shi and Liang, 2019) was also used to identify Terminal Direct Repeats (TDRs), Miniature Inverted repeat Transposable Elements (MITEs) and Terminal Inverted Repeats (TIRs). TIRs were also identified with TIR-Learner (Su *et al.*, 2019). Helitron transposons were identified with HelitronScanner (Xiong *et al.*, 2014) and TEsorter used to classify identified TEs (Zhang *et al.*, 2019). EDTA (Ou *et al.*, 2019) calculates the number, base pairs and percentage of the genome

covered by different TEs but a large proportion of the TIR (DNA) elements were not assigned to families found in the Sequence Ontology database and classified as generic 'repeat region.' To accurately characterize these we used the RepeatMasker (Bao *et al.*, 2015) annotation table with the EDTA-identified TEs to calculate the number, base pairs and percentage of the genome covered by different classes and types of TEs.

Phylogenetic comparative analyses

Hansen (1997) introduced the use of an Ornstein-Uhlenbeck model to test hypotheses of trait adaptation to different niches mapped on a phylogeny. The Ornstein-Uhlenbeck process includes parameters that capture deterministic movement of species trait values toward optimal states that can vary as a function of environmental or ecological variables. Hansen (1997) termed these states "primary optima" defined as the average expected trait values (the local optima) for many species adapting to a given primary niche. The idea is that "secondary" selective factors average out across species, leaving the common effect of the primary niche on trait values. Hypotheses about adaptation can be tested by estimating the primary optima for different states of the environmental or ecological variables and asking if they differ as predicted by the hypotheses (see Hansen (2014) for a detailed argument).

Mathematically, a simple Ornstein-Uhlenbeck process is described by the stochastic differential equation:

$$dy = -\alpha(y - \theta)dt + \sigma dW, \quad (1)$$

where dy is the change in a given species' mean trait value, y , over a short time interval dt , θ is the primary optimum, α determines the rate of adaptation toward the primary optimum, dW represents independent normally distributed stochastic changes with mean zero and unit variance over a unit of time, and σ is the standard deviation of these changes. The σ parameter is more readily interpretable when expressed as a stationary variance of the process, $v = \sigma^2/2\alpha$, which is the variance among species within a niche after a long period of independent evolution. Using SLOUCH (Hansen *et al.*, 2008; Kopperud *et al.*, 2018), one of many R packages that implement these methods, we investigated whether nematodes sharing a given niche (here, dioecious or androdioecious

reproduction) tend to be more similar compared to taxa sharing a different niche in terms of their TE compositions whilst simultaneously estimating and controlling for the levels of adaptation and phylogenetic inertia in the clade (see Hansen, 2014, Mahler and Ingram, 2014 and O'Meara and Beaulieu, 2014 for general reviews). We analyzed differences in total repeat content and number for 21 different classes of repeats including retroelements like SINES, LINES and LTR elements, DNA transposons like PiggyBac and Mutator, rolling circle/Helitrons, unclassified elements and simple repeats (Table 1).

We mapped 'selfer' and 'outcrosser' as discrete states onto the phylogeny using maximum likelihood employed by the ace function in the Ape R package (Paradis and Schliep, 2018). The ancestral states along each branch were discretized by choosing the state with the highest probability on each branch for the best model. We used SLOUCH (Hansen et al., 2008; Kopperud et al., 2018) to fit a Brownian motion model and two Ornstein-Uhlenbeck models, one with a single global optimum and one with separate primary optima for selfers and outcrossers, for each of the twenty-two TEs studied here. The small sample size corrected Akaike Information Criteria (AICc) was used to determine which of the three models best captured TE evolution.

Orthology assignment

We used OrthoFinder version 2.5.4 (Emms and Kelly, 2015) to identify orthologous and paralogous genes in our *Caenorhabditis* genomes. We selected the longest isoform for each gene with the OrthoFinder.primary_transcript.py. Briefly, OrthoFinder aligns proteomes with DIAMOND (Buchfink et al., 2015, 2021) and uses a Markov Cluster Algorithm to assign proteins to orthogroups.

Gene birth and death analyses

We used the orthogroups assigned by OrthoFinder (Emms and Kelly, 2015) to calculate gene family expansions and contractions with the CAFE5 software (Mendes et al., 2020). CAFE5 assumes that each gene family has at least one representative at the base of the tree and we eliminated the distantly related *P. pacificus* from the analysis. *C. sinica* has the most fragmented assembled sequence of the species we studied here but its annotated protein-coding gene complement is large enough to fit this requirement and we included it throughout this manuscript to orient our analyses

and reduce bias that might be introduced by overly distant species like *P. pacificus*, assembled sequences with retained allelism like the outcrossing *C. brenneri* (Barriere et al., 2009) or possible reduced complements of protein-coding genes like the self-fertile *C. elegans* (Stevens et al., 2019). We used phytools (Revell, 2012) to create a dichotomous, ultrametric phylogenetic tree from the Stevens et al. (2019) estimated phylogeny.

We estimated an error model with the CAFE5 '-e' option and used this error model in further analyses. CAFE5 uses maximum-likelihood estimation to fit gene birth-death rate parameters or λ values based on a user specifying the number of discrete λ values. Gene families changing significantly are those experiencing rapid expansions or contractions across segments of the phylogenetic tree. We fit models with 2-8 λ values and found that the model with $\lambda=2$ had the highest likelihood given the dataset.

To focus on gene families that were expanding or contracting in parallel in selfing and outcrossing lineages we extracted orthogroups that were identified as significant ($p < 0.01$) in the CAFE5 analyses and implemented a set of Boolean rules. If an orthogroup was stable or decreasing in all selfing lineages and stable or increasing in all outcrossing lineages we defined it as 'Decreasing in Selfers.' Similarly, if an orthogroup was stable or decreasing in all outcrossing lineages and stable or increasing in all selfing lineages we defined it as 'Decreasing in Outcrossers.' If an orthogroup was stable or increasing in all selfing lineages and stable or decreasing in all outcrossing lineages we defined it as 'Increasing in Selfers.' Similarly, an orthogroup that was stable or decreasing in all outcrossing lineages and stable or increasing in all selfing lineages was 'Decreasing in Outcrossers.' We searched the functional annotations for information on any member of these orthogroups and searched *C. elegans* for orthologous genes as these may also provide functional information.

Accessions

C. remanei PX356 Bioproject PRJNA248909
C. remanei PX439 Bioproject PRJNA248911
C. remanei PX506 Bioproject PRJNA577507
C. latens PX534 Bioproject PRJNA248912
C. briggsae AF16 Bioproject PRJNA20855
C. nigoni JU1422 Bioproject PRJNA384657
C. elegans N2 Bioproject PRJNA158, PRJNA13758
C. tropicalis NIC58, JU1373 Bioproject PRJNA662844

C. inopinata NKZ35 Bioproject PRJDB5687

C. sinica ZZY0401 Bioproject PRJNA194557

Bioinformatic scripts, software and workflows associated with this project are located at <https://github.com/jannafierst/Worm-nomics>.

Supplementary Material

Supplementary materials are available at XXXX.

Acknowledgments

The authors would like to thank Rohit Kapila, Victoria (Tori) Eggers, Justin Rosario, Ana Perez-Sanchez, Jessica Gonzalez and Adam Trautwig for helpful comments and feedback. PEA and JMS were supported by National Alumni Association Fellowship through the University of Alabama Alumni Association. JP was supported by the US National Science Foundation award DEB 2225683 and Florida International University start-up funds. JLF was supported by the US National Science Foundation awards EF 1921585 and DEB 1941854 and Florida International University start-up funds.

References

- Adams, P. E., Crist, A. B., Young, E. M., Willis, J. H., Phillips, P. C., and Fierst, J. L. 2022. Slow recovery from inbreeding depression generated by the complex architecture of segregating deleterious mutations. *Molecular Biology and Evolution*, 39(1): msab330.
- Alonge, M., Lebeigle, L., Kirsche, M., Aganezov, S., Wang, X., Lippman, Z. B., Schatz, M. C., and Soyk, S. 2021. Automated assembly scaffolding elevates a new tomato system for high-throughput genome editing. *BioRxiv*, doi: <https://doi.org/10.1101/2021.11.18.469135>.
- and J. Wade, S. M. S. 2003. *Mating systems and strategies*. Princeton University Press Princeton New Jersey.
- Andersen, E. C., Gerke, J. P., Shapiro, J. A., Crissman, J. R., Ghosh, R., Bloom, J. S., Felix, M.-A., and Kruglyak, L. 2012. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nature Genetics*, 44(3): 285–290.
- Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, New Jersey.
- Armstrong, J., Hickey, G., Diekhans, M., Fiddes, I. T., Novak, A. M., Deran, A., Fang, Q., Xie, D., Feng, S., Stiller, J., Genereux, D., Johnson, J., Marinescu, V. D., Alfoldi, J., Harris, R. S., Lindblad-Toh, K., Haussler, D., Karlsson, E., Jarvis, E. D., Zhang, G., and Paten, B. 2020. Progressive Cactus is a multiple-genome aligner for the thousand-genome era. *Nature*, 587: 246–251.
- Attwood, T. K. and Beck, M. E. 1994. PRINTS— a protein motif fingerprint database. *Protein Engineering*, 7: 841–848.
- Attwood, T. K., Beck, M. E., JBleasby, A., and Parry-Smith, D. J. 1994. PRINTS— a database of protein motif fingerprints. *Nucleic Acids Research*, 22: 3590–3596.
- Baldi, C., Cho, S., and Ellis, R. E. 2009. Mutations in two independent pathways are sufficient to create hermaphroditic nematodes. *Science*, 326(5955): 1002–1005.
- Bao, W., Kojima, K. K., and Kohany, O. 2015. Repbase update, a database of repetitive elements in eukaryotic genomes. *Mobile DNA*, 6: 4–9.
- Barriere, A., Yang, S.-P., Pekarek, E., Thomas, C. G., Haag, E. S., and Ruvinsky, I. 2009. Detecting heterozygosity in shotgun genome assemblies: Lessons from obligately outcrossing nematodes. *Genome Research*, 19: 470–480.
- Bird, D. M., Blaxter, M. L., McCarter, J. P., Mitreva, M., Sternberg, P. W., and Thomas, W. K. 2005. A white paper on nematode comparative genomics. *Journal of Nematology*, 37(4): 408–416.
- Blaxter, M., Archibald, J. M., Childers, A. K., Coddington, J. A., Crandall, K. A., Di Palma, F., Durbin, R., Edwards, S. V., Graves, J. A. M., Hackett, K. J., Hall, N., Jarvis, E. D., Johnson, R. N., Karlsson, E. K., Kress, W. J., Kuraku, S., Lawnczak, M. K. N., Lindblad-Toh, K., Lopez, J. V., Moran, N. A., Robinson, G. E., Ryder, O. A., Shapiro, B., Soltis, P. S., Warnow, T., Zhang, G., and Lewin, H. A. 2022. Why sequence all eukaryotes? *Proceedings of the National Academy of Sciences*, 119(4).
- Boutin, T. S., Le Rouzic, A., and Capy, P. 2012. How does selfing affect the dynamics of selfish transposable elements? *Mobile DNA*, 3: 5.
- Bru, C., Courcelle, E., Carrere, S., Beausse, Y., Dalmar, S., and Kahn, D. 2005. The ProDom database of protein domain families: more emphasis on 3D. *Nucleic Acids Research*, 33(s1)(D212–D215).
- Bruna, T., Hoff, K. J., Lomsadze, A., Stanke, M., and Borodovsky, M. 2021. BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database. *NAR Genomics and Bioinformatics*, 3(1): lqaa108.
- Buchfink, B., Xie, C., and Huson, D. H. 2015. Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, 12: 59–60.
- Buchfink, B., Reuter, K., and Drost, H. G. 2021. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nature Methods*, 18: 366–368.
- Burnham, K. P. and Anderson, D. R. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, Second Edition*. Springer, Berlin, Heidelberg.

- Chandler, V. L. and Walbot, V. 1986. DNA modification of a maize transposable element correlates with loss of activity. *Proceedings of the National Academy of Sciences of the United States of America*, 83: 1767–1771.
- Connallon, T. and Clark, A. G. 2011. The resolution of sexual antagonism by gene duplication. *Genetics*, 187: 919–937.
- Consortium, T. G. O. 2000. Gene ontology: Tool for the unification of biology. *Nature Genetics*, 25(1): 25–29.
- Cook, D. E., Zdraljevic, S., Roberts, J. P., and Andersen, E. C. 2017. CeNDR, the *Caenorhabditis elegans* natural diversity resource. *Nucleic Acids Research*, 45: D650–D657.
- Cutter, A. D. 2008. Divergence times in *Caenorhabditis* and *Drosophila* inferred from direct estimates of the neutral mutation rate. *Molecular Biology and Evolution*, 25(4): 778–786.
- Cutter, A. D., Baird, S. E., and Charlesworth, D. 2006. High nucleotide polymorphism and rapid decay of linkage disequilibrium in wild populations of *Caenorhabditis remanei*. *Genetics*, 174: 901–913.
- Cutter, A. D., Dey, A., and Murray, R. L. 2009. Evolution of the *Caenorhabditis elegans* genome. *Molecular Biology and Evolution*, 26(6): 1199–1234.
- Dainat, J. v0.8.0. AGAT: another gff analysis toolkit to handle annotations in any GTF/GFF format. *Zenodo*, <https://www.doi.org/10.5281/zenodo.3552717>.
- Denver, D. R., Morris, K., Lynch, M., and Thomas, W. K. 2004. High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature*, 430(7000): 679–682.
- Dey, A., Jeon, Y., Wang, G., and Cutter, A. D. 2012. Global population genetic structure of *Caenorhabditis remanei* reveals incipient speciation. *Genetics*, 191: 1257–1269.
- Dey, A., Chan, C. K. W., Thomas, C. G., and Cutter, A. D. 2013. Molecular hyperdiversity defines populations of the nematode *Caenorhabditis brenneri*. *Proceedings of the National Academy of Sciences of the United States of America*, 110(27): 11056–11060.
- Dey, A., Jin, Q., Chen, Y., and Cutter, A. D. 2014. Gonad morphogenesis defects drive hybrid male sterility in asymmetric hybrid breakdown of *Caenorhabditis* nematodes. *Evolution and Development*, 16(6): 362–372.
- Dieterich, C., Clifton, S. W., Schuster, L. N., Chinwalla, A., Delehaunty, K., Dinkelacker, I., Fulton, L., Fulton, R., Godfrey, J., Minx, P., Mitreva, M., Roessler, W., Tian, H., Witte, H., Yang, S.-P., Wolsen, R. K., and Sommer, R. J. 2008. The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nature Genetics*, 40(10): 1193–1198.
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T. R. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1): 15–21.
- Dolgin, E. S. and Charlesworth, B. 2006. The fate of transposable elements in asexual populations. *Genetics*, 174(2): 817–27.
- Dolgin, E. S., Charlesworth, B., Baird, S. E., and Cutter, A. D. 2007. Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution*, 61(6): 1339–1352.
- Dupeyron, M., Singh, K. S., Bass, C., and Hayward, A. 2019. Evolution of *Mutator* transposable elements across eukaryotic diversity. *Mobile DNA*, 10: 12.
- Eklom, R. and Wolf, J. B. W. 2014. A field guide to whole-genome sequencing, assembly and annotation. *Evolutionary Applications*, 7: 1026–1042.
- Ellinghaus, D., Kurtz, S., and Willhoeft, U. 2008. LTRharvest, an efficient and flexible software for *de novo* detection of LTR retrotransposons. *BMC Bioinformatics*, 9: 18.
- Elliott, T. A. and Gregory, T. R. 2015. What’s in a genome? the C-value enigma and the evolution of eukaryotic genome content. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370: 20140331.
- Emms, D. M. and Kelly, S. 2015. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biology*, 16: 157.
- Farslow, J. C., Lipinski, K. J., Packard, L. B., Edgley, M. L., Taylor, J., Flibotte, S., Moerman, D. G., Katju, V., and Bergthorsson, U. 2015. Rapid increase in frequency of gene copy-number variants during experimental evolution in *Caenorhabditis elegans*. *BMC Genomics*, 16(1): 1–18.
- Felix, M.-A., Braendle, C., and Cutter, A. D. 2014. A Streamlined System for Species Diagnosis in *Caenorhabditis* (Nematoda: Rhabditidae) with Name Designations for 15 Distinct Biological Species. *Plos One*, 9(4): e94723.
- Fierst, J. L. and Murdock, D. A. 2017. Decontaminating eukaryotic genome assemblies with machine learning. *BMC Bioinformatics*, 18(1): 533.
- Fierst, J. L., Willis, J. H., Thomas, C. G., Wang, W., Reynolds, R. M., Ahearne, T. E., Cutter, A. D., and Phillips, P. C. 2015. Reproductive mode and the evolution of genome size and structure in *Caenorhabditis* nematodes. *PLoS Genetics*, 11(6): e1005323.
- Finn, R. D., Attwood, T. K., Babbitt, P. C., Bateman, A., Bork, P., Bridge, A. J., Chang, H. Y., Dosztanyi, Z., El-Gebali, S., Fraser, M., Gough, J., Haft, D., Holliday, G. L., Huang, H., Huang, X., Letunic, I., Lopez, R., Lu, S., Marchler-Bauer, A., Mi, H., Mistry, J., Natale, D. A., Necci, M., Nuka, G., Orengo, C. A., Park, Y., Pesseat, S., Piovesan, D., Potter, S. C., Rawlings, N. D., Redaschi, N., Richardson, L., Rivoire, C., Sangrador-Vegas, A., Sigrist, C., Sillitoe, I., Smithers, B., Squizzato, S.,

- Sutton, G., Thanki, N., Thomas, P. D., Tosatto, S. C., Wu, C. H., Xenarios, I., Yeh, L. S., Young, S. Y., and Mitchell, A. L. 2017. Interpro in 2017-beyond protein family and domain annotations. *Nucleic Acids Res*, 45(D1): D190–d199.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Dover Publications, New York.
- Gao, L., Gonda, I., Sun, H., Ma, Q., Bao, K., Tieman, D. M., Burzynski-Chang, E. A., Fish, T. L., Stromberg, K. A., Sacks, G. L., THannhauser, T. W., Foolad, M. R., Diez, M. J., Blanca, J., Canizares, J., Xu, Y., van der Knaap, E., Huang, S., Klee, H. J., Giovannoni, J. J., and Fei, Z. 2019. The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. *Nature Genetics*, 51: 1044–1051.
- Golicz, A. A., Bayer, P. E., Bhalla, P. L., Batley, J., and Edwards, D. 2020. Pangenomics comes of age: from bacteria to plant and animal applications. *Trends In Genetics*, 36(2): 132–145.
- Gregory, T. R. 2005a. The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Annals of Botany*, 95: 133–146.
- Gregory, T. R. 2005b. Genome size evolution in animals. In T. R. Gregory, editor, *The Evolution of the Genome*. Elsevier, San Diego.
- Guo, W., Wang, D., and Lisch, D. 2021. RNA-directed DNA methylation prevents rapid and heritable reversal of transposon silencing under heat stress in *Zea mays*. *PLoS Genetics*, 17(6): e1009326.
- Guo, Y., Lang, S., and Ellis, R. E. 2009. Independent recruitment of F box genes to regulate hermaphrodite development during nematode evolution. *Current Biology*, 19: 1853–1860.
- Haag, E. S., Chamberlin, H., Coghlan, A., Fitch, D. H., Peters, A. D., and Schulenburg, H. 2007. *Caenorhabditis* evolution: If they all look alike, you aren't looking hard enough. *Trends in Genetics*, 23(3): 101–104.
- Hahn, M. W., De Bie, T., Stajich, J. E., Nguyen, C., and Cristianini, N. 2005. Estimating the tempo and mode of gene family evolution from comparative genomic data. *Genome Research*, 15: 1153–1160.
- Hahn, M. W., Han, M. V., and Han, S. 2007. Gene family evolution across 12 *Drosophila* genomes. *PLoS Genetics*, 3(11): e197.
- Han, M. V., Thomas, G. W. C., Lugo-Martinez, J., and Hahn, M. W. 2013. Estimating gene gain and loss rates in the presence of error in genome assembly and annotation using CAFE 3. *Molecular Biology and Evolution*, 30: 1987–1997.
- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution*, 51(5): 1341–1351.
- Hansen, T. F. 2014. *Modern phylogenetic comparative methods and their application in evolutionary biology*, chapter Use and misuse of comparative methods in the study of adaptation. Springer, Berlin, Heidelberg.
- Hansen, T. F., Pienaar, J., and Orzack, S. H. 2008. A comparative method for studying adaptation to a randomly evolving environment. *Evolution*, 62(8): 1965–1977.
- Hickey, G., Paten, B., Earl, D., Zerbino, D., and Haussler, D. 2013. HAL: a hierarchical format for storing and analyzing multiple genome alignments. *Bioinformatics*, 29(10): 1341–1342.
- Hill, J., Rastas, P., Horne, E. A., Neethiraj, R., Clark, N., Morehouse, N., de la Paz Celorio-Mancera, M., Carnicer Cols, J., Dirksen, H., Meslin, C., Keehn, N., Pruischer, P., Sikkink, K., Vives, M., Vogel, H., Wiklund, C., Woronik, A., Boggs, C. L., Nylin, S., and Wheat, C. W. 2019. Unprecedented reorganization of holocentric chromosomes provides insights into the enigma of lepidopteran chromosome evolution. *Science Advances*, 5(6): eaau3648.
- Hillier, L. W., Miller, R. D., Baird, S. E., Chinwalla, A., Fulton, L. A., Koboldt, D. C., and Waterston, R. H. 2007. Comparison of *C. elegans* and *C. briggsae* genome sequences reveals extensive conservation of chromosome organization and synteny. *PLoS Biology*, 5(7): e167.
- Howe, K. L., Bolt, B. J., Shafie, M., Kersey, P., and Berriman, M. 2017. WormBase ParaSite: a comprehensive resource for helminth genomics. *Molecular and Biochemical Parasitology*, 215: 2–10.
- Hu, T. T., Pattyn, P., Bakker, E. G., Cao, J., Cheng, J.-F., Clark, R. M., Fahlgren, N., Fawcett, J. A., Grimwood, J., Gundlach, H., Haberer, G., Hollister, J. D., Ossowski, S., Ottillar, R. P., Salamov, A. A., Schneeberger, K., Spannagl, M., Wang, X., Yang, L., Nasrallah, M. E., Bergelson, J., Carrington, J. C., Gaut, B. S., Schmutz, J., Mayer, K. F. X., Van de Peer, Y., Grigoriev, I. V., Nordborg, M., Weigel, D., and Guo, Y.-L. 2011. The *Arabidopsis lyrata* genome sequence and the basis of rapid genome size change. *Nature Genetics*, 43(5): 476–481.
- Hulo, N., Bairoch, A., Bulliard, V., Cerutti, L., De Castro, E., Legendijk-Genevaux, P. S., and nd C J A Sigrist, M. P. 2005. The PROSITE database. *Nucleic Acids Research*, 34(s1): D227–D230.
- Jiang, N., Ferguson, A. A., Slotkin, R. K., and Lisch, D. 2011. Pack-mutator-like transposable elements (pack-MULEs) induce directional modification of genes through biased insertion and DNA acquisition. *Proceedings of the National Academy of Sciences of the United States of America*, 108(4): 1537–1542.
- Kanehisa, M. and Goto, S. 2000. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, 28(1): 27–30.
- Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. 2016. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Research*, 44(D1): D457–D462.
- Kanzaki, N., Tsai, I. J., Tanaka, R., Hunt, V. L., Liu, D., Tsuyama, K., Maeda, Y., Namai, S., Kumagai, R., Tracey, A., Holroyd, N., Doyle, S. R., Woodruff, G. C., Murase, K., Kitazume, H., Chai, C., Akagi,

- A., Panda, O., Ke, H., Schroeder, F. C., Wang, J., Berriman, M., Sternberg, P. W., Sugimoto, A., and Kikuchi, T. 2018. Biology and genome of a newly discovered sibling species of *Caenorhabditis elegans*. *Nature Communications*, 9: 3216.
- Kapusta, A., Suh, A., and Feschotte, C. 2017. Dynamics of genome size evolution in birds and mammals. *Proceedings of the National Academy of Sciences of the United States of America*, 114(8): E1460–E1469.
- Katju, V. and Bergthorsson, U. 2013. Copy-number changes in evolution: rates, fitness effects and adaptive significance. *Frontiers in Genetics*, 4: 273.
- Kim, C., Kim, J., Kim, S., Cook, D. E., Evans, K. S., Andersen, E. C., and Lee, J. 2019. Long-read sequencing reveals intra-species tolerance of substantial structural variations and new subtelomere formation in *C. elegans*. *Genome Research*, 29(1023-1035).
- Kipreos, E. T. and Pagano, M. 2000. The F-box protein family. *Genome Biology*, 1(5): REVIEWS3002.
- Kolmogorov, M., Yuan, J., Lin, Y., and Pevzner, P. A. 2019. Assembly of long, error-prone reads using repeat graphs. *Nature Biotechnology*, 37: 540–546.
- Konrad, A., Flibotte, S., Taylor, J., Waterston, R. H., Moerman, D. G., Bergthorsson, U., and Katju, V. 2018. Mutational and transcriptional landscape of spontaneous gene duplications and deletions in *Caenorhabditis elegans*. *Proceedings Of The National Academy Of Sciences*, 115(28): 7386–7391.
- Kopperud, B. T., Pienaar, J., Voje, K. L., Orzack, S. H., Hansen, T. F., and Grabowski, M. 2018. *SLOUCH v2.0: Stochastic linear Ornstein-Uhlenbeck models for comparative hypotheses*. <https://cran.r-project.org/web/packages/slouch/index.html>.
- Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., and Phillippy, A. M. 2017. Canu: Scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Research*, 27(5): 722–736.
- Lee, B. Y., Kim, J., and Lee, J. 2022. Intraspecific de novo gene birth revealed by presence-absence variant genes in *Caenorhabditis elegans*. *NAR Genomics and Bioinformatics*, 4(2): lqac031.
- Lee, D., Zdravljic, S., Cook, D. E., Frezal, L., Hsu, J., Sterken, M. G., Riksen, J. A. G., Wang, J., Kammenga, J. E., Braendle, C., Felix, M., Schroeder, F. C., and Andersen, E. C. 2019. Selection and gene flow shape niche-associated copy-number variation of pheromone receptor genes. *BioRxiv*.
- Li, H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, 34(18): 3094–3100.
- Lipinski, K. J., Farslow, J. C., Fitzpatrick, K. A., Lynch, M., Katju, V., and Bergthorsson, U. 2011. High spontaneous rate of gene duplication in *Caenorhabditis elegans*. *Current Biology*, 21(4): 306–310.
- Lisch, D. 2015. Mutator and MULE transposons. *Microbiology Spectrum*, 3(2): MDNA3–0032.
- Liu, K. and Wessler, S. R. 2017. Functional characterization of the active mutator-like transposable element, mutal from the mosquito *Aedes aegypti*. *Mobile DNA*, 8: 1–12.
- Lynch, M. and Conery, J. S. 2003. The origins of genome complexity. *Science*, 302: 1401–1404.
- Maddox, P. S., Oegema, K., Desai, A., and Cheeseman, I. M. 2004. Holo than though: chromosome segregation and kinetochore function in *C. elegans*. *Chromosome Research*, 12: 641–653.
- Mahler, D. L. and Ingram, T. 2014. *Modern phylogenetic comparative methods and their application in evolutionary biology*, chapter Phylogenetic comparative methods for studying clade-wide convergence, pages 425–450. Springer, Berlin, Heidelberg.
- Martienssen, R. and Baron, A. 1994. Coordinate suppression of mutations caused by robertson's mutator transposons in maize. *Genetics*, 136: 1157–1170.
- Mathers, T. C., Wouters, R. H. M., Mugford, S. T., Swarbreck, D., van Oosterhout, C., and Hogenhout, S. A. 2021. Chromosome-scale genome assemblies of aphids reveal extensively rearranged autosomes and long-term conservation of the X chromosome. *Molecular Biology and Evolution*, 38(3): 856–875.
- Maydan, J. S., Lorch, A., LEDgley, M., Flibotte, S., and Moerman, D. G. 2010. Copy number variation in the genomes of twelve natural isolates of *Caenorhabditis elegans*. *BMC Genomics*, 11: 62.
- McCarthy, C. G. and Fitzpatrick, D. A. 2019. Pan-genome analyses of model fungal species. *Microbial Genomics*, 5(2): mgen.0.000243.
- Mendes, F. K., Vanderpool, D., Fulton, B., and Hahn, M. W. 2020. CAFE 5 models variation in evolutionary rates among gene families. *Bioinformatics*, page btaa1022.
- Miga, K. H. and Wang, T. 2021. The need for a human pangenome reference. *Annual Review of Ecology Evolution and Systematics*, 22: 81–102.
- Mon, H., Lee, J., Kawaguchi, Y., and Kusakabe, T. 2011. Double-strand breaks repair by gene conversion in silkworm holocentric chromosomes. *Molecular Genetics and Genomics*, 286(3-4): 215–24.
- Noble, L. M., Yuen, J., Stevens, L., Moya, N., Persaud, R., Moscatelli, M., Jackson, J. L., Zhang, G., Chitrakar, R., Baugh, L. R., Braendle, C., Andersen, E. C., Seidel, H. S., and Rockman, M. V. 2021. Selfing is the safest sex for *Caenorhabditis tropicalis*. *eLife*, 10: e62587.
- Nowell, R. M., Wilson, C. G., Almeida, P., Schiffer, P. H., Fontaneto, D., Becks, L., Rodriguez, F., Arkhipova, I. R., and Barraclough, T. G. 2021. Evolutionary dynamics of transposable elements in bdelloid rotifers. *eLife*, 10: e63194.

- O'Meara, B. C. and Beaulieu, J. 2014. *Modern phylogenetic comparative methods and their application in evolutionary biology*, chapter Modelling stabilizing selection: the attraction of Orstein-Uhlenbeck models, pages 381–393. Springer, Berlin, Heidelberg.
- Ou, S. and Jiang, N. 2018. LTR retriever: A highly accurate and sensitive program for identification of long terminal repeat retrotransposons. *Plant Physiology*, 180(4): 1803–1815.
- Ou, S. and Jiang, N. 2019. LTR FINDER parallel: parallelization of LTR FINDER enabling rapid identification of long terminal repeat retrotransposons. *Mobile DNA*, 10(1): 48.
- Ou, S., Su, W., Liao, Y., CHougule, K., Agda, J. R. A., Hellinga, A. J., Santiago, C., Lugo, B., Elliott, T. A., Ware, D., Peterson, T., Jiang, N., Hirsch, C. N., and Hufford, M. B. 2019. Benchmarking transposable element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome Biology*, 20(275).
- Paradis, E. and Schliep, K. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, 35(3): 526–528.
- Punta, M., Coghill, P. C., Eberhardt, R. Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., Heger, A., Holm, L., Sonnhammer, E. L. L., Eddy, S. R., Bateman, A., and Finn, R. D. 2012. The Pfam protein families database. *Nucleic Acids Research*, 40(D1): D290–D301.
- Quinlan, A. R. and Hall, I. M. 2010. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics*, 26(6): 841–842.
- Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3(2): 217–223.
- Roach, M. J., Schmidt, S. A., and Borneman, A. R. 2018. Purge halotigs: allelic contig reassignment for third-gen diploid genome assemblies. *BMC Bioinformatics*, 19: 460.
- Robertson, D. S. 1978. Characterization of a mutator system in maize. *Mutational Research*, 51: 21–8.
- Robertson, D. S. 1986. Genetic studies on the loss of mu mutator activity in maize. *Genetics*, 113: 765–773.
- Robertson, H. M. and Thomas, J. S. 2005. The putative chemoreceptor families of *C. elegans*. In *WormBook*. The *C. elegans* Research Community, <http://www.wormbook.org>.
- Rodelsperger, C., Roseler, W., Prabh, N., Yoshida, K., Weiler, C., Herrmann, M., and Sommer, R. J. 2018. Phylotranscriptomics of *Pristionchus* nematodes reveals parallel gene loss in six hermaphroditic lineages. *Current Biology*, 28(19): 3123–3127.
- Roessler, K., Muyle, A., Diez, C. M., Gaut, G. R. J., Bousios, A., Stitzer, M. C., Seymour, D. K., Doebley, J. F., Liu, Q., and Gaut, B. S. 2019. The genome-wide dynamics of purging during selfing in maize. *Nature Plants*, 5(9): 980–990.
- Rowe, L., Arnqvist, G., Krups, J., and Sih, A. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology and Evolution*, 9: 289–293.
- Sambrook, J. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, third edition.
- Schrader, L., Pan, H., Bollazzi, M., Schiott, M., Larabee, F. J., Bi, X., Deng, Y., Zhang, G., Boomsma, J. J., and Rabeling, C. 2021. Relaxed selection underlies genome erosion in socially parasitic ant species. *Nature Communications*, 12: 2918.
- Shi, J. and Liang, C. 2019. Generic repeat finder: A high-sensitivity tool for genome-wide de novo repeat detection. *Plant Physiology*, 180(4): 1803–1815.
- Shimizu, K. K. and Tsuchimatsu, T. 2015. Evolution of selfing: recurrent patterns in molecular adaptation. *Annual Review of Ecology Evolution and Systematics*, 46: 593–622.
- Simpson, V. J., Johnson, T. E., and Hammen, R. F. 1986. *Caenorhabditis elegans* DNA does not contain 5-methylcytosine at any time during development or aging. *Nucleic Acids Research*, 14: 6711–6719.
- Slotkin, R. K. 2005. *The heritable epigenetic silence of mutator transposons by Mu killer*. Ph.D. thesis, University of California at Berkeley, Berkeley, California.
- Slotte, T., Hazzouri, K. M., Ågren, J. A., Koenig, D., Maumus, F., Guo, Y.-L., Steige, K., Platts, A. E., Escobar, J. S., Newman, L. K., Wang, W., Mandáková, T., Vello, E., Smith, L. M., Henz, S. R., Steffen, J., Takuno, S., Brandvain, Y., Coop, G., Andolfatto, P., Hu, T. T., Blanchette, M., Clark, R. M., Quesneville, H., Nordborg, M., Gaut, B. S., Lysak, M. A., Jenkins, J., Grimwood, J., Chapman, J., Prochnik, S., Shu, S., Rokhsar, D., Schmutz, J., Weigel, D., and Wright, S. I. 2013. The *Capsella rubella* genome and the genomic consequences of rapid mating system evolution. *Nature Genetics*, 45(7): 831–835.
- Smit, A. F. A., Hubley, R., and Green, P. 2013–2015. Repeatmasker open-4.0.
- Stein, L. D., Bao, Z., Blasiar, D., Blumenthal, T., Brent, M. R., Chen, N., Chinwalla, A., Clarke, L., Clee, C., Coghlan, A., Coulson, A., D'Eustachio, P., Fitch, D. H. A., Fulton, L. A., Fulton, R. E., Griffiths-Jones, S., Harris, T. W., Hillier, L. W., Kamath, R., Kuwabara, P. E., Mardis, E. R., Marra, M. A., Miner, T. L., Minx, P., Mullikin, J. C., Plumb, R. W., Rogers, J., Schein, J. E., Sohrmann, M., Spieth, J., Stajich, J. E., Wei, C., Willey, D., Wilson, R. K., Durbin, R., and Waterston, R. H. 2003. The genome sequence of *Caenorhabditis briggsae*: A platform for comparative genomics. *PLoS Biology*, 1(2): e5.
- Stevens, L., Felix, M., Beltran, T., Braendle, C., Caurcel, C., Fausett, S., Fitch, D., Frezal, L., Gosse, C., Kaur, T., Kiontke, K., Newton, M. D., Noble,

- L. M., Richaud, A., Rockman, M. V., Sudhaus, W., and Blaxter, M. 2019. Comparative genomics of 10 new *Caenorhabditis* species. *Evolution Letters*, 3(2): 217–236.
- Stiernagle, T. 2006. *Maintenance of C. elegans*.
- Su, W., Gu, X., and Peterson, T. 2019. TIR-Learner, a new ensemble method for TIR transposable element annotation, provides evidence for abundant new transposable elements in the maize genome. *Molecular Plant*, 12: 447–60.
- Sutton, J. M., Millwood, J. D., McCormack, A. C., and Fierst, J. L. 2021. Optimizing experimental design for genome sequencing and assembly with oxford nanopore technologies. *GigaByte*, doi:10.46471/gigabyte.27.
- Teterina, A., Willis, J., and Phillips, P. 2020. Chromosome-level assembly of the *caenorhabditis remanei* genome reveals conserved patterns of nematode genome organization. *Genetics*, 214(4): 769–780.
- Tettelin, H., Massignani, V., Cieslewicz, M. J., Donati, C., Medini, D., Ward, N. L., Angiuoli, S. V., Crabtree, J., Jones, A. L., Durkin, A. S., DeBoy, R. T., Davidsen, T. M., Mora, M., Scarselli, M., Margarit y Ros, I., Peterson, J. D., Hauser, C. R., Sundaram, J. P., Nelson, W. C., Madupu, R., Brinkac, L. M., Dodson, R. J., Rosovitz, M. J., Sullivan, S. A., Daugherty, S. C., Haft, D. H., Selengut, J., Gwinn, M. L., Zhou, L., Zafar, N., Khouri, H., Radune, D., Dimitrov, G., Watskins, K., O'Connor, K. J. B., Smith, S., Utterback, T. R., White, O., Rubens, C. E., Grandi, G., Madoff, L. C., Kasper, D. L., Telford, J. L., Wessels, M. R., Rappuoli, R., and Fraser, C. M. 2005. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial "pan-genome". *Proceedings of the National Academy of Sciences of the United States of America*, 102(39): 13950–13955.
- Thomas, C. G., Li, R., Smith, H. E., Woodruff, G. C., Oliver, B., and Haag, E. S. 2012. Simplification and desexualization of gene expression in self-fertile nematodes. *Current Biology*, 22(22): 2167–2172.
- Thomas, J. H. and Robertson, H. M. 2008. The *Caenorhabditis* chemoreceptor gene families. *BMC Biology*, 6(1): 42.
- Thompson, O. A., Snoek, L. B., Nijveen, H., Sterken, M. G., Volkers, R. J. M., Brenchley, R., van't Hof, A., Bevers, R. P. J., Cossins, A. R., Yanai, I., Hajnal, A., Schmid, T., Perkins, J. D., Spencer, D., Kruglyak, L., Andersen, E. C., Moerman, D. G., Hillier, L. W., Kammenga, J. E., and Waterston, R. H. 2015. Remarkably divergent regions punctuate the genome assembly of the *Caenorhabditis elegans* hawaiian strain cb4856. *Genetics*, 200(3): 975–989.
- van Tonder, A. J., Mistry, S., Bray, J. E., Hill, D. M. C., Cody, A. J., Farmer, C. L., Klugman, K. P., von Gottberg, A., Bentley, S. D., Parkhill, J., Jolley, K. A., Maiden, M. C. J., and Brueggemann, A. B. 2014. Defining the estimated core genome of bacterial populations using a bayesian decision model. *PLoS Computational Biology*, 10(8): e1003788.
- VanKuren, N. W. and Long, M. 2018. Gene duplicates resolving sexual conflict rapidly evolved essential gametogenesis function. *Nature Ecology and Evolution*, 2: 705–712.
- Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., and Earl, A. M. 2014. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE*, 9(11): e112963.
- Wang, A., Chen, W., and Tao, S. 2021. Genome-wide characterization, evolution, structure, and expression analysis of the f-box genes in *Caenorhabditis*. *BMC Genomics*, 22: 889.
- Wenzel, D., Palladino, F., and Jedrusik-Bode, M. 2011. Epigenetics in *C. elegans*: facts and challenges. *Genetics*, 49: 647–661.
- Xiong, W., He, L., Lai, J., Dooner, H. K., and Du, C. 2014. HelitronScanner uncovers a large overlooked cache of helitron transposons in many plant genomes. *Proceedings Of The National Academy Of Sciences*, 111: 10263–8.
- Xu, Z. and Wang, H. 2007. LTR Finder: an efficient tool for the prediction of full-length LTR retrotransposons. *Nucleic Acids Research*, 35: W265–8.
- Yin, D. and Haag, E. S. 2019. Evolution of sex ratio through gene loss. *Proceedings Of The National Academy Of Sciences*, 116(26): 12919–12924.
- Yin, D., Schwarz, E. M., Thomas, C. G., Felde, R. L., Korf, I. F., Cutter, A. D., Schartner, C. M., Ralston, E. J., Meyer, B. J., and Haag, E. S. 2018. Rapid genome shrinkage in a self-fertile nematode reveals sperm competition proteins. *Science*, 359(6371): 55–61.
- Zallot, R., Harrison, K. J., Kolaczowski, B., and de Crecy-Lagard, V. 2016. Functional annotations of paralogs: a blessing and a curse. *Life (Basel)*, 6(3): 39.
- Zdobnov, E. M. and Apweiler, R. 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics*, 17: 847–848.
- Zhang, R., Wang, Z., Ou, S., and Li, G. 2019. TESorter: lineage-level classification of transposable elements using conserved protein domains. *BioRxiv*, 800177.