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Brief Communication

Dramatic difference in rate of chromosome number evolution among sundew (*Drosera* L., Droseraceae) lineages

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Abstract

Chromosome number change is a driver of speciation in eukaryotic organisms. Carnivorous sundews in the plant genus *Drosera* L. exhibit single chromosome number variation both among and within species, especially in the Australian *Drosera* subg. *Ergaleium* D.C., potentially linked to atypical centromeres that span much of the length of the chromosomes. We critically reviewed the literature on chromosome counts in *Drosera*, verified the taxonomy and quality of the original counts, and reconstructed dated phylogenies. We used the BiChrom model to test whether rates of single chromosome number increase and decrease, and chromosome number doubling differed between *D.* subg. *Ergaleium* and the other subgenera and between self-compatible and self-incompatible lineages. The best model for chromosome evolution among subgenera had equal rates of chromosome number doubling but higher rates of single chromosome number change in *D.* subg. *Ergaleium* than in the other subgenera. Contrary to expectation, self-incompatible lineages had a significantly higher rate of single chromosome loss than self-compatible lineages. We found no evidence for an association between differences in single chromosome number changes and diploidization after polyploidy or centromere type. This study presents an exemplar for critically examining published cytological data and rigorously testing factors that may impact the rates of chromosome number evolution.

Keywords: BiChrom model, chromosome number change, diploidization, RevBayes, holocentric chromosomes, carnivorous plants

Chromosome evolution events, such as duplication, inversion, fusion, and fission, are universal across the eukaryotic tree of life but appear to be more common in some lineages than others (reviewed in Coghlan et al., 2005). These chromosomal changes have long been considered driving forces of speciation and lineage diversification (Coyne & Orr, 2004; Grant, 1981; Stebbins, 1971). Therefore, identifying lineages with unusually high or low rates of chromosome number change and the intrinsic and environmental factors influencing these rates is critical to our understanding of evolutionary processes in general.

Recent developments in macroevolutionary modeling approaches have explored the association of chromosome evolution with trait evolution and lineage diversification (Baniaga et al., 2019; Freyman & Höhna, 2018; Mayrose et al., 2011; Román-Palacios et al., 2020; Zenil-Ferguson et al., 2019; Zhan et al., 2021). However, most of this work has focused on the role of chromosome doubling. Putative factors influencing the occurrence of single chromosome change include post-polyploidy rediploidization (Mandáková & Lysak, 2018) and centromere type (Luceño & Guerra, 1996; Mayrose & Lysak, 2020; Ruckman et al., 2020). Factors influencing the establishment of a new karyotype have only been explored in relation to polyploidy but likely impact single chromosome evolution as well (Husband et al., 2013; Van Drunen & Husband, 2019; Weiss-Schneeweiss et al., 2013). For example, outcrossing results in deleterious heterozygote karyotypes and can hinder the proliferation of the new karyotype (Husband et al., 2013; Van Drunen & Husband, 2019). However, the relative importance of selfing in the establishment of single chromosome changes remains largely unknown.

Despite the importance of quantifying chromosome number change, obtaining a data set of chromosome numbers with a matching phylogeny is challenging. A well-resolved phylogeny with a comprehensive species-level sampling is not always available. More importantly, chromosome counts require fresh root tips or flower buds, and counts are often limited for lineages with broad geographic distributions. In addition to incomplete sampling, the quality of chromosome count data sets can be eroded by chromosome counting errors

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(Windham & Yatskievych, 2003), reporting errors in chromosome number databases (Rivero et al., 2019), and taxonomic issues from species misidentification and taxonomic changes resulting in a mismatch between the count data versus the molecular phylogeny.

The carnivorous plants known as sundews (genus *Drosera* L.; family Droseraceae; order Caryophyllales) are exceptionally well-studied cytologically, with chromosome counts available for about half of the ca. 260 species. *Drosera* species occur in a wide variety of habitats from boreal peatlands to tropical savannahs and subtropical sandplain heathlands and rock outcrops (Fleischmann et al., 2018). Hotspots of species diversity include Australia (ca. 170 species), Africa (ca. 40 species), and South America (ca. 40 species; Fleischmann et al., 2018). *Drosera* consists of four well-supported subgenera (Fleischmann et al., 2018): two subgenera *D.* subg. *Regiae* Seine & Barthlott and *Arcturia* (Planch.) Schlauer that include only one and two species each, respectively, and two subgenera *D.* subg. *Drosera* L. and *Ergaleium* D.C. comprising ca. 110 and ca. 150 species, respectively. Cytological studies on *Drosera* have been undertaken for over 120 years (Huie, 1897; Rosenberg, 1903), resulting in a rich literature record comprising more than 600 individual chromosome counts for ca. 140 species (e.g., Chen, 1998; Kress, 1970; Rivadavia, 2005; Rothfels & Heimburger, 1968; Sheikh & Kondo, 1995).

Previous cytological studies in *Drosera* have found strikingly elevated levels of single chromosome number variation in *D.* subg. *Ergaleium* (almost every haploid number from $1n$ = 3 to 23, with numbers up to $1n$ = 45; tuberous, pygmy, and wooly sundews of Australia; Supplementary Table S1; Hoshi & Kondo, 1998; Rivadavia et al., 2003; Sheikh & Kondo 1995; Shirakawa et al., 2011). In contrast, the other three subgenera exhibit primarily polyploid chromosome number series (n = 10, 14, 15, 20, 30, 40; Hoshi & Kondo, 1998; Rivadavia et al., 2003). The increased single chromosome number variation has been attributed to the presence of holocentric chromosomes in *Drosera* (Sheikh et al., 1995). Holocentric chromosomes have a single centromere groove (holocentromere) that extends across much of the length of the chromosome rather than the localized centromere in the typical monocentric chromosome (Wanner et al., 2015). Holocentric chromosomes can segregate properly even in individuals heterozygous for a chromosome break (Jankowska et al., 2015; Luceño & Guerra, 1996; Ruckman et al., 2020) and therefore have been associated with increased chromosome fission producing a higher number of smaller chromosomes (Cuacos et al., 2015; Ruckman et al., 2020). In *Drosera*, centromere type has not been directly inferred using the gold standards of centromere protein (like centromeric histone 3) staining or α-tubulin localization along the chromosome. Multiple indirect experimental approaches have been used to investigate the presence of holocentromeres in mitotic tissue of *Drosera*. Depending on the experimental approach, all the species investigated in each study either showed evidence for being monocentric (three species; Demidov et al., 2014) or holocentric (eight species; Furuta & Kondo, 1999; Kolodin et al., 2018; Sheikh et al., 1995; Shirakawa et al., 2011; Zedek et al., 2016). This suggests that the experimental approaches may be inconclusive, and/or the elevated levels of chromosome number variation in *Drosera* may not correspond to the presence of holocentromeres. Aside from centromere type, the contrasting levels of chromosome number variation could also result from different ages of the lineages, uneven taxon sampling, counting errors, and taxonomic issues (e.g., the misidentification of *D. spatulata* as *D. aliciae* due to morphological similarity; see Kress 1970; of *D. montana* and closely allied taxa due to taxonomic revisions; see Rivadavia, 2005). A critical evaluation of chromosome count data across all original records is required to lay the foundations for subsequent analyses. Furthermore, the rate of chromosome number change has yet to be tested taking the phylogenetic history into consideration. This phylogenetic modeling framework would also allow the investigation of associations between rates of chromosome number evolution and traits such as centromere type, life history, and mating system.

In this study, we quantified the rate of chromosome doubling and single chromosome gain and loss on dated phylogenies of *Drosera*. We tested whether the rates of chromosome evolution differ significantly between *D.* subg. *Ergaleium* and the other three subgenera and between lineages of different mating systems. To do so, we critically evaluated previously published chromosome counts, verified voucher specimens to identify possible taxonomic updates or misidentifications, and used the BiChrom (binary state linked to chromosome number change) models (Zenil-Ferguson et al., 2017) and Bayes factors to compare models of subgeneric differences in rates of chromosome evolution in a genus-wide phylogenetic context. We also analyzed the rate of chromosome change in self-compatible versus self-incompatible species using BiChrom. An ancestral state reconstruction based on the resulting best-fit model was compared with genome size and centromere type to explore potential factors associated with different chromosome evolution rates between *Drosera* subgenera.

Methods

Literature review and evaluation of chromosome counts

Lists of original references for *Drosera* chromosome counts were obtained from the Chromosome Counts Database (Rice et al., 2015), Index of Plant Chromosome Numbers (Goldblatt & Johnson, 1979), citations referenced by publications on karyotypes in *Drosera* (Dawson, 2000; Kondo, 1969; Rivadavia et al., 2003; Veleba et al., 2017), and searches on Google Scholar and the library databases of the University of Minnesota, Curtin University, and University of Western Australia. Voucher specimen information, chromosome count methodology, and provenance data were recorded for every chromosome count either from the original publication or from subsequent literature in the case of 14 counts (six publications) where the original data could not be obtained.

We excluded chromosome counts from subsequent analyses where the count was uncertain (12 counts), where counts were made from first-generation hybrids (31 counts; we kept allopolyploid species), or where taxonomic issues existed (72 counts). Count uncertainty included chromosome number uncertainty expressed by the original publication (8 counts), a count based on a single cell, and a different chromosome number cited by the voucher versus the corresponding publication (2 counts). Taxonomic issues included (a) counts that lack both species identification and voucher specimen; (b) species with taxonomy updates after the karyotype publication (especially in the case of species complexes) that lack sufficient provenance, character description, or any voucher specimen

with which to assign the taxon to the updated species name; (c) counts made from cultivated material of a species often misidentified in cultivation; or (d) a mismatch between the voucher specimen and the name associated with the count. See Supplementary Material S1 for details on evaluating published chromosome count data and Supplementary Table S1 for how extraneous situations were filtered.

After filtering, if multiple chromosome numbers were reported for a species, all chromosome numbers with more than one count were used for subsequent modeling analyses. In cases where all chromosome numbers for a species had only one count, all counts for that species were used.

Phylogenetic reconstruction for comparative analyses

In order to estimate chloroplast and nuclear chronograms for modeling chromosome number evolution, *rbc*L and ITS sequences for *Drosera* species and outgroup taxa from noncore Caryophyllales were retrieved from the GenBank (Supplementary Table S2).

For *rbc*L, five sequences were removed due to ambiguous nucleotide sites. The taxonomy for sequences with herbarium vouchers at M and SPF (herbarium acronyms following Index Herbariorum) was updated as noted in Supplementary Table S2. For species with multiple *rbc*L sequences, the longest sequence was kept.

Sequences were aligned with default settings using the MAFFT (Katoh & Standley, 2013) plug-in for Geneious version 11.1.5 (Kearse et al., 2012). The ends of sequences that were only present in two outgroup species were trimmed. Priors for molecular dating in BEAST version 2.6.4 (Bouckaert et al., 2014) followed previous molecular dating analysis across the Caryophyllales (Yao et al., 2019) using a lognormal relaxed molecular clock and the birth–death model of speciation. For each fossil constraint, the prior was set to a lognormal distribution with a mean of 1.0, an *SD* of 0.5, and an offset based on the age of the fossil. As in Yao et al. (2019), fossil *Aldrovanda intermedia* and *A. ovata* (family Droseraceae) were used to set the prior for the most recent common ancestor (MRCA) of *Dionaea* and *Aldrovanda* with an offset of 41.2 Ma, and *Polygonocarpum johnsonii* was used to constrain the MRCA of the Polygonoideae (family Polygonaceae) included with an offset of 66.0 Ma. The MRCA of noncore Caryophyllales was constrained to 115 Ma with a normal distribution and a standard deviation of 4.0 Ma, representing the 95% confidence interval in the posterior distribution of the dating analysis of Yao et al. (2019). The Markov Chain Monte Carlo (MCMC) was run for 100,000,000 generations, sampling every 1,000 generations. The BEAST input file and data are available at https://doi.org/10.5281/zenodo.6081366. The resulting summary statistics were visualized in Tracer version 1.7.1 (Rambaut et al., 2018).

Similar to *rbc*L, for species with multiple ITS sequences, the longest sequence was kept. Alignment and BEAST settings followed those above except that the *Polygonocarpum johnsonii* fossil was not used due to different taxon sampling for ITS, and the root constraint was placed at the divergence of the carnivorous Caryophyllales from other noncore Caryophyllales represented by *Psylliostachys suworowii* (family Plumbaginaceae).

For both *rbc*L and ITS, the obtained phylogenetic trees were summarized in TreeAnnotator version 2.6.2 (Drummond & Rambaut, 2007) with a 10% burn-in, and the maximum clade credibility tree was visualized in FigTree version 1.4.4 (Rambaut, 2018). The chronograms (ape R package; Paradis & Schliep, 2019) and chromosome count matrices were trimmed to species shared by both the gene and the chromosome data sets for subsequent analyses.

Modeling chromosome number evolution

We used the binary trait linked to chromosome number change model (BiChrom; Zenil-Ferguson et al., 2017) and implemented it in RevBayes software version 1.1.0 (Höhna et al., 2016) to estimate the differences in three rates of chromosome number evolution for each binary state (Figure 1): γ (a single chromosome gain, by duplication or fission), δ (a single chromosome loss, by rearrangement, fusion, or loss), and ρ (a polyploidy event). The binary state is defined as whether a taxon belongs to *D.* subg. *Ergaleium* (state E) or not, in which case it belongs to *D.* subg. *Drosera*, *Arcturia*, or *Regiae* (state D). By defining our binary state in this fashion, we estimate a transition rate *q*, which is a nuisance parameter but allows us to correctly compare rates of chromosome change between the two groups using the phylogenetic structure of our estimated trees. Species were assigned state E or state D sensu Fleischmann et al. (2018).

We first defined a *Q*-matrix describing the dynamics of chromosome number change between two chromosome numbers within a given state (E or D) or a change between the E and D states given a fixed chromosome number (Supplementary Figure S1; Mayrose et al., 2010; Zenil-Ferguson et al., 2017). The *Q*-matrix allows us to define a continuous-time Markov chain for the discrete trait of chromosome number. However, this *Q*-matrix can be numerically difficult to use because of its large dimensions and many rates being equal to zero (e.g., the instantaneous transition rate between $1n = 10$ to $1n = 17$ is zero since the change is not a doubling, or a single increase or decrease in chromosome number). Therefore, limiting the maximum number of chromosomes, hence smaller matrix dimensions, is necessary for the convergence of estimates (Zenil-Ferguson et al., 2018). Since our data set had $2n$ chromosome numbers ranging from 8 to 80, we set the haploid ($1n$) chromosome number as a state for the *Q*-matrix to range from 1 to 40 and a 40+ state for taxa with more than $1n = 40$ to make it computationally feasible (Supplementary Figure S1; Zenil-Ferguson et al., 2017, 2018). We removed records of B-chromosomes, as these small satellite chromosomes do not segregate normally during cell division, and *Drosera lanata* ($2n = 19$) to avoid non-integer haploid chromosome numbers. The resulting matrix had 82 rows and 82 columns reflecting 1–40 and more than 40 chromosome numbers for both states E and D (Supplementary Figure S1). Since we expect the chromosome evolution rate in *Drosera* outside of *D.* subg. *Ergaleium* to be more similar to the rate in most angiosperms, we considered state D the ancestral state and state E the derived state and only allowed transitions from state D to state E. The probabilities of the root being 1 to more than 40 chromosomes in either state D or E were set equal.

Three nested models were used for testing the difference of chromosome number evolution between *D.* subg. *Ergaleium* (state E) and the rest of the genus (state D). The full model (H2) allowed rates (ρ = chromosome doubling, δ = chromosome loss, γ = chromosome gain) to vary independently in states D and E. The fixed-polyploid model (denoted as H1: $\rho_D = \rho_E$) constrained the rate of chromosome doubling to be

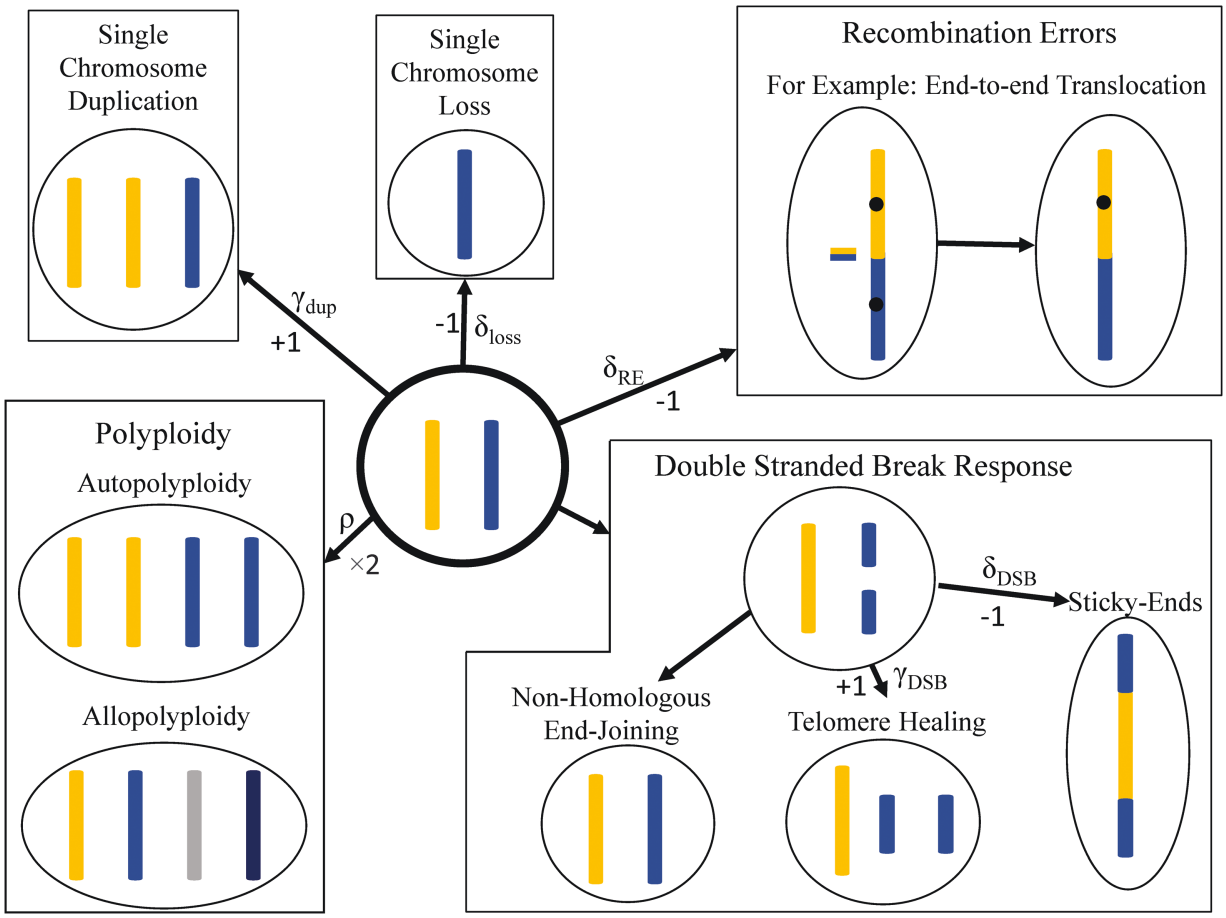

Figure 1. Processes that give rise to changes in chromosome number. Each cell is depicted in haploid form. The original cell (center) starts with two haploid chromosomes. Arrows indicate changes in chromosomes and, where possible, are labeled with the type of change (+1, −1, ×2) and the symbol used in BiChrom models (γ, δ, and ρ, respectively; Mayrose & Lysak, 2020). Since +1 and −1 can occur via multiple mechanisms, a subscript is used to distinguish the cause of change. Therefore, $\gamma_{DSB} + \gamma_{dup} = \gamma$, and $\delta_{DSB} + \delta_{RE} + \delta_{loss} = \delta$. The centromere is shown as a black spot in the "Recombination Error" box to emphasize the steps required to handle an additional centromere. An increase in one chromosome can be due to telomere healing after a chromosome break or a single chromosome duplication; a single chromosome decrease can be due to a recombination error (Mayrose & Lysak, 2020), two chromosomes fusing after a breakage, or the loss of a single chromosome. Single chromosome loss is unlikely except after polyploidy (Luceno & Guerrra, 1996). A doubling of all chromosomes can be due to an autopolyploidy or allopolyploidy. Holocentromeres are expected to alleviate issues caused by acentric fragments after double stranded breaks and tangling of bicentric chromosomes after fusion (Cuacos et al., 2015).

the same between states D and E. Finally, the null model (H0) constrained all three rates to be equal for states D and E (H0: $\rho_D = \rho_E$, $\gamma_D = \gamma_E$, $\delta_D = \delta_E$). Rate prior distributions for all chromosome transition rates were defined using an exponential distribution with a mean equal to 3 changes per million years (Myr). The prior distribution had a large variance allowing for a wide range of initial potential values for transition rates.

We ran our custom MCMC scripts in RevBayes (Höhna et al., 2016) for 1,000,000 generations. Using Tracer (Rambaut et al., 2018), we ensured convergence had been reached and verified that effective sample sizes for all the parameters were above 200. Concurrently, for the best model, we reconstructed ancestral states using marginal posterior probabilities for each of the internal nodes as part of the inference following Freyman & Höhna (2018) and Zenil-Ferguson et al. (2019). The RevBayes input data and scripts are available at https://doi.org/10.5281/zenodo.6081366.

The three models were compared by estimating their marginal log-likelihoods to calculate the test statistic κ representing the Bayes factors, done in RevBayes as well (Höhna et al., 2016). The marginal likelihood, which is the probability of a model integrated over all the parameter space, allows us to assess model fit in a Bayesian framework similar to the Akaike information criterion statistic in a likelihood framework (Xie et al., 2011). To compare models, we subtracted the marginal log-likelihood of a given pair of models, which is κ = log marginal likelihood of Model 1 – log marginal likelihood of Model 2. We consider κ > 6 as evidence for strong support for Model 1, κ > 1 as moderate support for Model 1, a value of κ between −1 and 1 as no evidence in favor of either model, and κ < −1 as support for Model 2 (Kass & Raftery, 1995).

All the MCMC outputs were analyzed using Tracer with the first 10% discarded as burn-in. The ancestral state reconstruction results for the best-supported model were visualized with the RevGadgets R package (Tribble et al., 2021).

Branch length and topology uncertainty

To evaluate the effect of phylogenetic uncertainty on the estimated rates, the best BiChrom model (H1) was fitted to the last ten *rbc*L trees sampled in BEAST and on the ITS chronogram. Before running, *D. indica* and *D. collinsiae* were removed from the ITS phylogeny due to their placement outside the corresponding phylogenetically defined sections

(Fleischmann et al., 2018). The MCMC outputs of both analyses were analyzed using Tracer with a burn-in of 10% discarded.

Genome size and mating system

Drosera genome sizes were obtained from Veleba et al. (2017) or newly generated in this study for 17 species at the Flow Cytometry Core Lab at the Benaroya Research Institute (Seattle, WA, USA). For each genome size, four flow cytometry measurements were taken against a known size standard. Source, voucher, and size standards used for generating new flow cytometry data are listed in Supplementary Table S3.1. We used the average genome size for each species for subsequent analyses. Self-compatibility data for 98 species of *Drosera* were obtained from publications (Supplementary Table S3.2). Recent observations (Fleischmann, in ed) suggest all *D. auriculata* populations studied are self-compatible, contrary to a doubtful previously published report by Chen et al. (1997).

We repeated the full BiChrom model for over 200,000 generations with self-compatibility as the binary state. In this analysis, the transition rate between self-compatibility and self-incompatibility (q) was permitted in either direction, and the rate prior distribution for this transition was defined using an exponential distribution with a mean of 1 change per 10 Myr. For species with populations that varied in compatibility, the tip state included both states in every combination with the chromosome number for that species.

Results

Chromosome counts for 127 *Drosera* species show distinctive patterns of variation between *D.* subgenus *Ergaleium* and other subgenera

An initial data set of 676 chromosome counts in *Drosera* from 150 species or hybrids was compiled (Supplementary Table S1). After removing hybrids and low-quality counts, 510 counts from 127 species were used for downstream analyses. These counts included 48% of all named species in *Drosera*. Across the geographic distribution of *Drosera*, the filtered counts included 32% of named species from Africa, 45% from South America, 51% from Australia, 60% from Asia, and all species from North America and Europe.

Among the four subgenera, *Drosera* subg. *Arcturia*, *D.* subg. *Drosera*, *D.* subg. *Ergaleium*, and *D.* subg. *Regiae* each had 50%, 43%, 51%, and 100% of the named species represented. Almost every even chromosome number from $2n$ = 6–46 was reported from *D.* subg. *Ergaleium*, and scattered chromosome number variation was observed within 21 species (Figure 2A). In contrast, *D.* subg. *Drosera* has chromosome numbers from $2n$ = 16–80, with variation primarily in polyploid series both within and among species ($2n$ = 20, 30, 40, 60, 80; Figure 2A). Despite more counts have been reported in *D.* subg. *Drosera*, only seven species have within-species chromosome number variation reported. Chromosome number for *D. arcturi* (*D.* subg. *Arcturia*) was $2n$ = 20 and for *D. regia* (*D.* subg. *Regiae*) was $2n$ = 34.

Chronogram reconstruction

The trimmed *rbc*L matrix included 1,440 bases with 478 variable sites across the 17 outgroup and 79 ingroup species. The trimmed ITS matrix included 1,133 bases with 783 variable sites across 7 outgroup and 50 ingroup species. After the burn-in, the ESS was greater than 200 for all statistics in both ITS and *rbc*L analyses. The *rbc*L tree placed *D. regia* in a clade with *Aldrovanda* and *Dionaea* with strong to moderate support (Supplementary Figure S2). The ITS tree placed *D. regia* sister to the rest of *Drosera*, consistent with the cladogram from Fleischmann et al. (2018; Supplementary Figure S2). BEAST analyses estimated the crown age of *Drosera* (including *D. regia*) at around 69.9 Mya based on *rbc*L and 80.1 Mya based on ITS with overlapping confidence intervals (Supplementary Figure S2).

Drosera subgenus *Ergaleium* differs from other subgenera in single-chromosome evolution rates

The chromosome counts and *rbc*L data overlapped for 59 species: 25 from *D.* subg. *Ergaleium*, 32 from *D.* subg. *Drosera*, and 1 species each from *D.* subg. *Arcturia* and *D.* subg. *Regiae*.

In the full model (H2), the mean posterior rate of gaining (γ_E = 0.16 per 1 million years) or losing (δ_E = 0.17) one chromosome in *D.* subg. *Ergaleium* was 7.3-fold and 370-fold higher than other subgenera (γ_D = 0.022; δ_D = 0.00046; Figure 3; Supplementary Table S4). However, the rate of chromosome gain for *D.* subg. *Drosera*, *Arcturia*, and *Regiae* fell within the first quartile of the rate of chromosome gain for *D.* subg. *Ergaleium* and only the 95% credible interval for the rates of single chromosome loss was distinct (95% HPD δ_E = 0.036–0.36; 95% HPD $\delta_D = 3.6 \times 10^{-7}$ to 1.0×10^{-3}; Figure 3; Supplementary Table S4). The rates of polyploidy largely overlapped (Figure 3).

Compared with rates estimated in the full model, the null model (H0) estimated an intermediate rate for losing one chromosome, while the estimated rate of polyploidy doubled and the rate for gaining a chromosome decreased (Figure 3). Comparing Bayes factors for the full model and null model on the *rbc*L results strongly favored the full model (κ = 15.0), supporting that chromosome evolution rates were different between *D.* subg. *Ergaleium* and the other subgenera.

Given the largely overlapping polyploidy rates for state D versus state E, we tested an additional model H1, which linked the polyploidy rates for states D and E, but estimated rates for chromosome loss and gain for the two states separately. We found a moderate preference for H1 over the full model (H2; κ = 5.9; Figure 3; Supplementary Table S4).

The best-fit model (H1) with separate chromosome loss (δ) and gain (γ) rates for state D versus state E but equal ploidy, showed both higher chromosome loss and chromosome gain rates in *D.* subg. *Ergaleium* and 95% credible intervals similar to the full model (Figure 3; Supplementary Table S4). The mean δ_E was 358-fold higher than δ_D, and the 95% HPD did not overlap (Figure 3; Supplementary Table S4). With overlapping 95% HPDs, the mean γ_E was over 6.0-fold higher than γ_D (Figure 3; Supplementary Table S4).

Under the H1 model, the ancestral state reconstruction estimated the most probable value of the MRCA of *Drosera* to be a haploid chromosome number of eight. The base of *D.* subg. *Ergaleium* also had a most probable haploid chromosome number of eight. The difference in single chromosome change between subgenera is supported across the reconstruction by the stability of chromosome number in *D.* subg. *Drosera* and repeated changes in *D.* subg. *Ergaleium*. Based on the reconstruction, polyploidization events occurred four times in *D.* subg. *Ergaleium*, three times in *D.* subg. *Drosera*, and once in *D.* subg. *Regiae* (Figure 4).

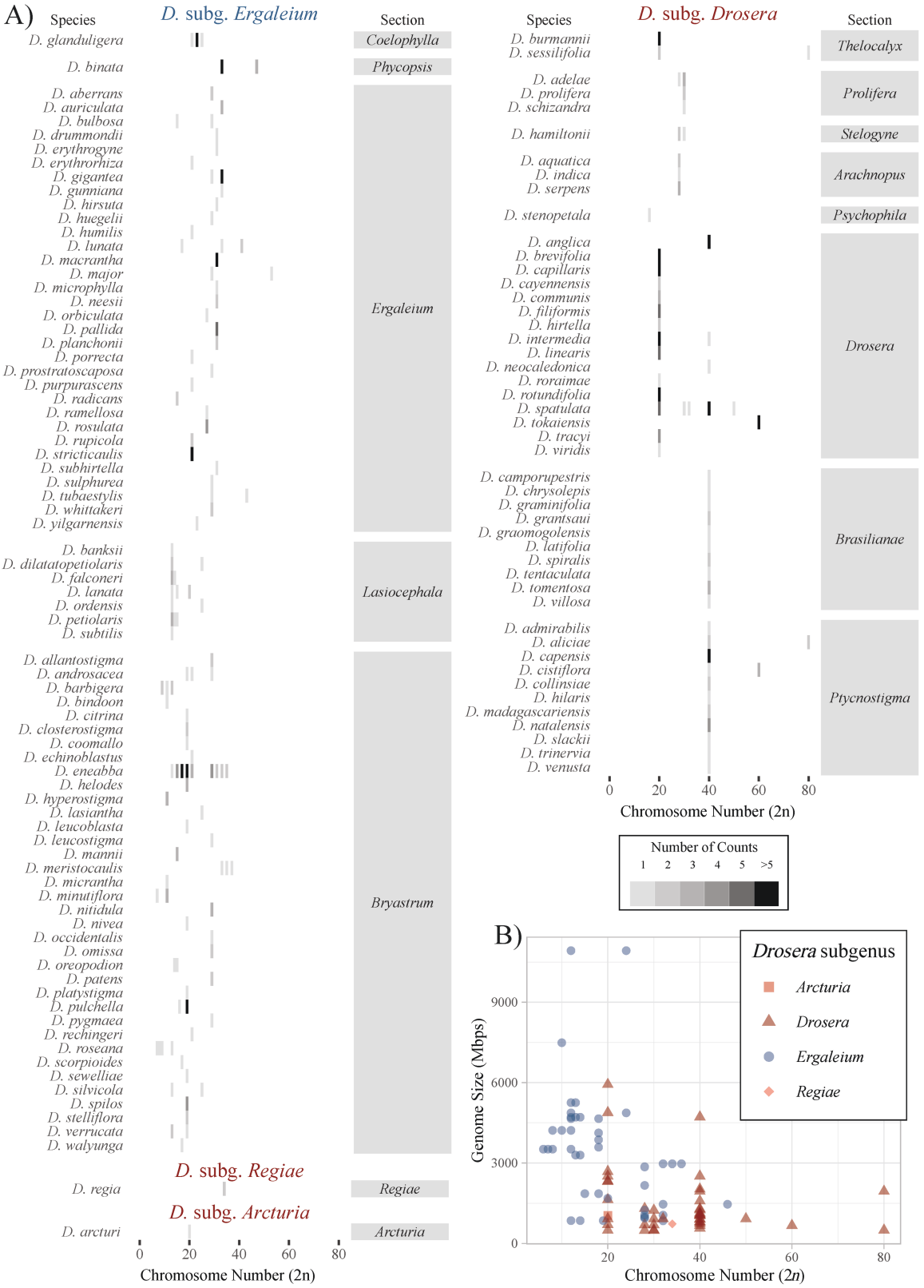

Figure 2. Chromosome and genome size variation in *Drosera*, showing the full data set. (A) *Drosera* subg. *Ergaleium* (left) exhibited marked single chromosome number variation both among and within species. In contrast, both among- and within-species chromosome number variation in *D.* subg. *Drosera* (right) fell primarily into polyploidy series. The shade of the bar indicates the number of samples for each chromosome number, emphasizing that the lower level of variation in *D.* subg. *Drosera* is not due to a lack of counts. (B) *Drosera* species with larger chromosome numbers tend to have smaller genome sizes.

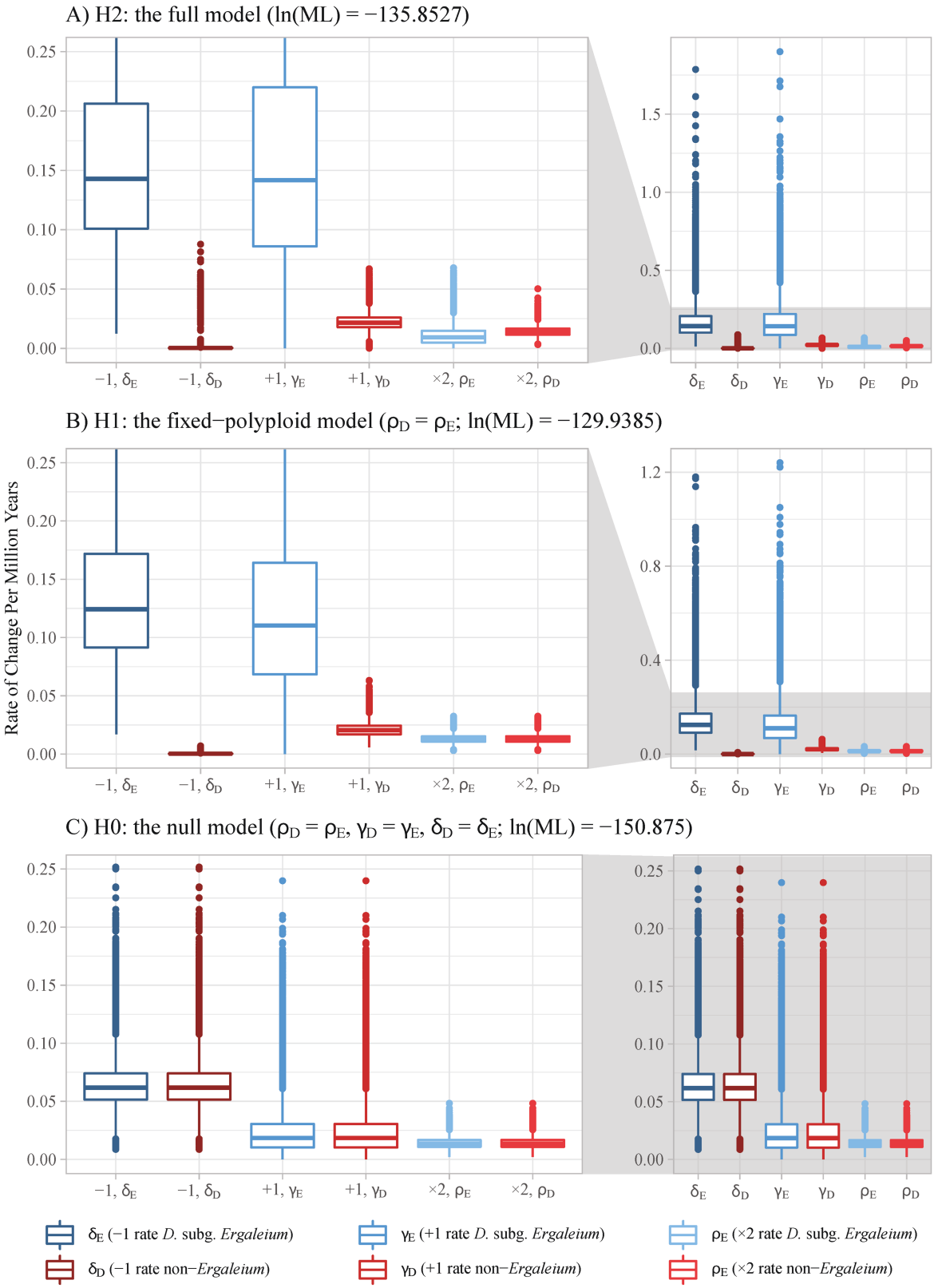

Figure 3. The posterior distribution of chromosome evolution rates for three BiChrom models. These models are (A) H2, where all rates (δ = chromosome number decrease by one, γ = chromosome number increase by one, and ρ = chromosome number doubling) were estimated independently for *Drosera* subg. *Ergaleium* (state E) versus the other three *Drosera* subgenera (state D); (B) H1, where all rates were independent across *Drosera* except ρ ($\rho_D = \rho_E$); and (C) H0, where all rates were equal across *Drosera* ($\rho_D = \rho_E$, $\gamma_D = \gamma_E$, $\delta_D = \delta_E$). δ_D and δ_E were significantly distinct in H2 and H1. All remaining rates were not significantly different between states E and D.

Results from rbc*L* were robust when considering phylogenetic uncertainty and when using the ITS data set

The results of the H1 model (fixed-polyploidy) on the 10 *rbc*L trees from the BEAST MCMC sampling all found higher single chromosome gain and loss in *D*. subg. *Ergaleium* than the other subgenera despite differences in branch lengths and topology (Supplementary Figure S3). The ITS BiChrom results had higher levels of uncertainty, likely due to only 47 species overlapping between the chromosome count and ITS data after filtering. Nonetheless, the ITS analysis once again supported higher rates of single chromosome gain and loss in *D*. subg. *Ergaleium* than the other subgenera. The rates of gaining (γ_E = 0.11) or losing (δ_E = 0.11) one chromosome in *D*. subg. *Ergaleium* were both eightfold higher than those of the other subgenera (γ_D = 0.014; δ_D = 0.013; Supplementary Figure S3).

Self-compatible species have a lower rate of single chromosome number decrease than self-incompatible species

In *D*. subg. *Ergaleium*, 48 of the 60 (80%) species with known mating systems are self-incompatible in at least some populations (Figure 4; Supplementary Table S3.2). In contrast, only three distantly related species of the 38 species (8%) in the remaining three subgenera are self-incompatible (Figure 4; Supplementary Table S3.2). Our BiChrom modeling found a significantly higher rate of single chromosome number decrease in self-incompatible species (δ_{SI} = 0.65 per million years) than in self-compatible species (δ_{SC} = 0.0048) of *Drosera*, but no significant difference in single chromosome number increase or polyploidy between these two groups (Supplementary Figure S4; Supplementary Table S4).

Genome size decreases as chromosome number increases across *Drosera*

Our newly generated genome size estimates ranged from 630 to 5,249 Mbps (Supplementary Table S3.1). Many were similar to previous publications, but a few appear to be polyploids such as *D. spatulata*. Across *Drosera*, genome size remained the same or decreased as chromosome number increased (Figure 2B). By visually comparing the genome sizes of polyploid taxa and those of the closely related diploid taxa, the polyploid taxa generally have similar or smaller genome sizes except in the more recent polyploid event of *D. anglica* (Figure 4).

Discussion

Rates of single chromosome number change significantly differ among *Drosera* subgenera and between mating systems

In this study, we carefully reviewed primary cytological literature and voucher information to correct for counting and taxonomic issues. We then modeled chromosome evolution taking both time and phylogenetic history into consideration. We found that the rate of polyploidy in *Drosera* (0.014 per Myr) did not significantly differ between subgenera or mating systems and was very similar to the polyploidy rate previously reported for perennial angiosperms (0.015 per Myr; Van Drunen & Husband, 2019) and within angiosperm families (median 0.025 per Myr; Zhan et al., 2021). The single chromosome gain (0.021) and loss rate (0.00040) for *Drosera* subgenera other than *D*. subg. *Ergaleium* were higher and lower, respectively, than the average within family rates (0.0061 and 0.016, respectively) across angiosperms (Zhan et al., 2021). In contrast, the rate of single chromosome number shifts in *D*. subg. *Ergaleium* was 6-fold (gain) and 350-fold (loss) higher than in the remainder of the genus, and higher single chromosome evolution rates would likely be detected in *D*. subg. *Ergaleium* with increased species sampling. Similarly, self-incompatible species of *Drosera* have a significantly higher (130×) rate of single chromosome loss, suggesting that selfing, in this case, is not necessary for the fixation of a new karyotype. Given that *D*. subg. *Ergaleium* is predominantly self-incompatible and the remaining subgenera of *Drosera* are predominantly self-compatible, it is difficult to tease apart the interaction between phylogeny versus mating systems in relation to the drastic rate variation.

The pattern of elevated single chromosome evolution rate, especially single chromosome loss among *Drosera* lineages, is robust to phylogenetic uncertainty, taxon sampling, and gene tree discordance. This pattern remains unchanged in rates estimated from the last 10 trees of the *rbc*L MCMC sampling. Despite differences in tree topology estimated from the nuclear ITS data set compared with the chloroplast *rbc*L data set, the rates estimated from the two loci are similar. More importantly, the results followed the same trend, except that using ITS resulted in wider credible intervals in rate estimates likely due to including only 18% instead of 23% of species. Since *Dionaea muscipula* (2*n* = 30 or 32; Rivadavia et al., 2003) and *Aldrovanda vesiculosa* (2*n* = 38; Rivadavia et al., 2003) have chromosome counts similar to that of *Drosera regia* (2*n* = 34; Supplementary Table S1.1), the uncertainty we observed in the placement of *D. regia* is unlikely to significantly change the rate estimations or ancestral chromosome state. Further taxon sampling may reveal that some single chromosome changes were inaccurately modeled as polyploidy events, especially in *D*. subg. *Ergaleium* where the posterior probabilities for the ancestral state reconstruction were mostly 0.1–0.5 at internal nodes (Figure 4). In addition, to limit parameter space, our modeling framework did not take 1.5× allopolyploidy events into consideration (e.g., *D. tokaiensis*, *D*. subg. *Drosera*; Nakamura & Ueda, 1991), which may inflate the rate estimates for single chromosome gain. The different modes of chromosome number changes among subgenera of *Drosera* are further supported by the pattern of within-species variation being primarily single chromosome changes in *D*. subg. *Ergaleium* in contrast to being primarily polyploidy series in *D*. subg. *Drosera*, and the lack of within-species variation in the two remaining subgenera (Figure 2). Therefore, considering the caveats of our taxon sampling and modeling approach, increasing the taxon sampling and using additional nuclear genes will likely narrow the credible intervals but unlikely lead to a different conclusion on the drastic difference in single chromosome changes among lineages within *Drosera*.

Potential drivers of chromosome evolution rate shift

Similar orders of magnitude differences in single chromosome number changes have also been documented among sedge and some insect lineages that have holocentric chromosomes (Escudero et al., 2014; Ruckman et al., 2020; Sylvester et al., 2020; Zenil-Ferguson et al., 2017). Holocentromeres have been associated with increased tolerance of chromosome

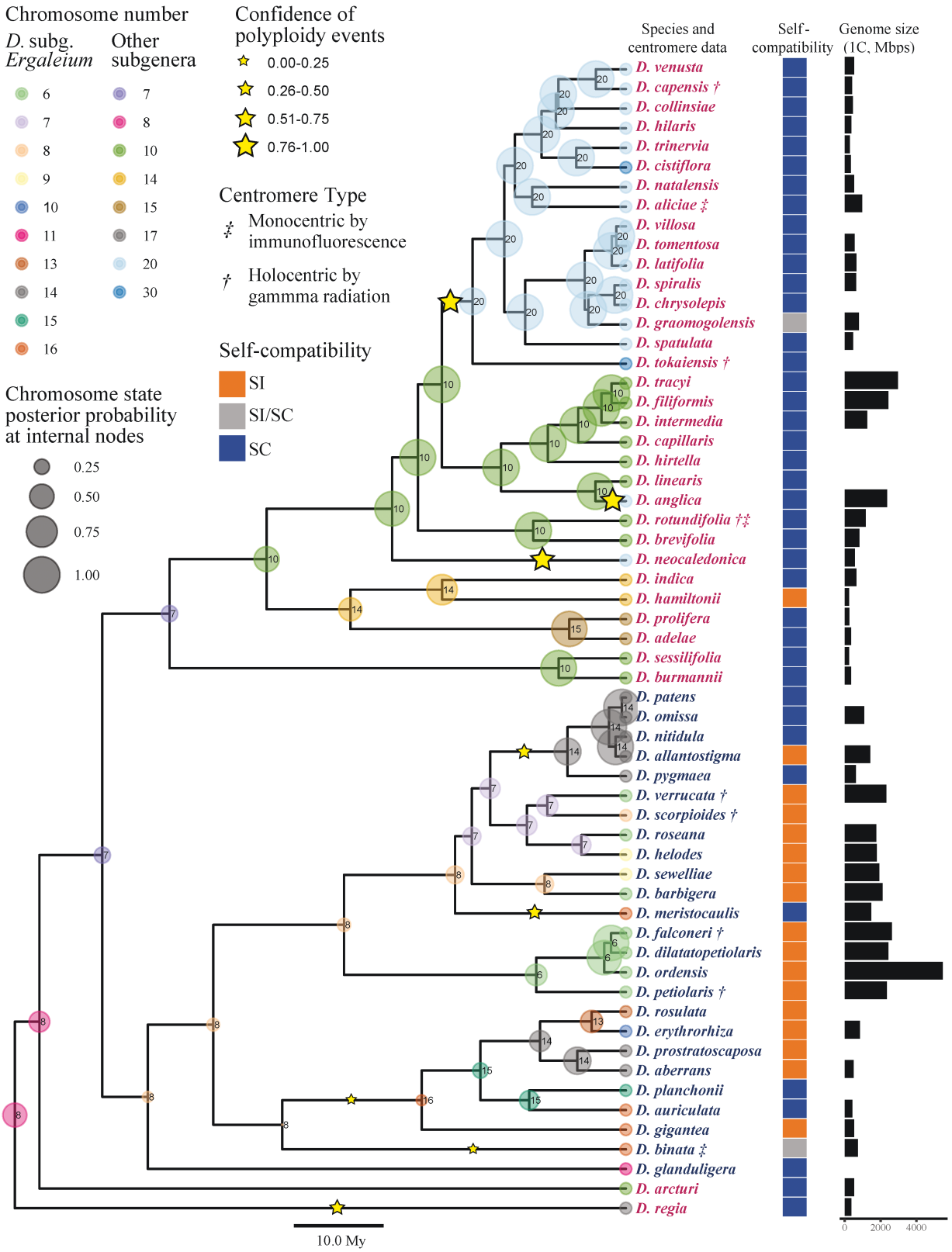

Figure 4. Ancestral state reconstruction of chromosome number evolution using model H1 in RevBayes with the binary state and chromosome state jointly estimated. In addition to having higher rates of single chromosome change, *Drosera* subg. *Ergaleium* (species with names in blue) have more species that are self-incompatible than the other three subgenera (species name in red). Lineages with a chromosome doubling (yellow star) tend to have smaller genome sizes than their sister lineages in our sampling. The size of the polyploidy star is scaled based on the posterior probability for the chromosome state at the previous node. Species with experimental evidence for their centromere type are distributed across the genus, but results from gamma-radiation versus immunofluorescence disagree about the type of centromere in *Drosera*, or even within the same species as in the case of *D. rotundifolia*.

fission (Cuacos et al., 2015; Jankowska et al., 2015; Ruckman et al., 2020) as the resulting chromosome fragments with centromeres can pair and segregate properly even in heterozygous individuals (Jankowska et al., 2015; Luceño & Guerra, 1996; Ruckman et al., 2020). Experimental investigation of centromere type in *Drosera* has been limited to a small number of species (Figure 4; Supplementary Table S3.3) using indirect methods, primarily response to gamma-radiation-induced breakages and the distribution of a histone commonly associated with the centromeric or pericentric region. Each of these methods supported all investigated species having the same centromere type. Therefore, so far, no evidence supports the presence of holocentromeres as the cause of the heterogeneity in chromosome evolution rate in *Drosera*, and more direct experimental investigations into the centromere type are needed. A similar lack of association between holocentric chromosomes and heterogeneity in chromosome evolution rates has also been documented across 22 orders of insects (Ruckman et al., 2020).

Elevated rates of single chromosome evolution can be due to increased rates of polyploidy and subsequent rediploidization (Mandáková & Lysak, 2018). However, we did not find evidence for different rates of polyploidy among lineages in *Drosera*. Instead, we found evidence for repeated genome downsizing after polyploidy across the entire genus. Of the eight polyploidy lineages inferred, the most recent has a genome size close to double that of the sister lineage, while the remaining seven more ancient polyploid lineages have similar or, in six cases, smaller genome sizes than their closely related diploid lineages (Figure 4; Supplementary Table S3.1; Veleba et al., 2017). Similarly, single chromosome number changesare not limited to polyploid lineages. Therefore, our analysis did not recover any evidence for post-polyploidy diploidization being associated with increased single chromosome number changes in the genus.

A newly formed karyotype may be eliminated due to drift or selection against heterozygous individuals (Husband et al., 2013). Species that can self-pollinate or have other reproductive assurances such as clonal propagation may alleviate these issues by avoiding producing individuals with heterozygous karyotypes (Husband et al., 2013; Spoelhof, Keeffe, et al., 2020; Van Drunen & Husband, 2019). While a perennial life history and clonal propagation are common across *Drosera* (Fleischmann et al., 2018), contrary to expectation, a higher percentage of species studied in *D.* subg. *Ergaleium* are self-incompatible (Supplementary Figure S4; Supplementary Table S3), and self-incompatible species have a higher rate of single chromosome decrease than self-compatible species (Supplementary Figure S4; Supplementary Table S4). This counterintuitive pattern may be explained by meiotic drive, through which chromosomes with larger centromeres in heterozygous individuals are preferentially inherited (Blackmon et al., 2019; Bureš & Zedek, 2014; Veleba et al., 2017). When a chromosome fusion occurs, self-incompatible species are more likely to produce heterozygous karyotypes, providing the opportunity for selection on chromosome size. A second potential explanation for increased single chromosome evolution in self-incompatibility may be that sexual reproduction, especially outcrossing, is important for the long-term maintenance of genetic diversity after the bottleneck when a new karyotype forms (Spoelhof, Keeffe, et al., 2020). These intriguing potential mechanisms underlying new karyotype establishment await future intraspecific investigations on population size, spatial distribution, and meiotic drive (Blackmon et al., 2019; Bureš & Zedek, 2014; Griswold, 2021; Reed et al., 2013; Ruckman et al., 2020; Spoelhof, Soltis, et al., 2020).

Future research should explore both the cytological and molecular evidence for polyploidy and single chromosome number changes. Synteny and cytological comparison between species with elevated single chromosome number change would provide evidence for the type of structural changes that occurred (Figure 1). To further understand the mechanism driving single chromosome changes, evidence for meiotic drive can be evaluated by quantifying the differential inheritance of chromosomes in heterozygous individuals. The evolutionary impact of chromosomal changes should be evaluated by quantifying the fitness of offspring and testing how chromosomal changes affect the linkage of genes that underlie local adaptation.

Conclusion

In this study, we found highly elevated rates in single chromosome evolution but not polyploidy in *Drosera* subg. *Ergaleium* compared with the rest of the genus. This pattern is robust to taxon sampling and the phylogeny used and is not an artifact of errors or clade age. In addition to the 6-fold and 358-fold higher rates of gain and loss compared with other subgenera, respectively, *D.* subg. *Ergaleium* harbors a much higher percentage of self-incompatible species. More broadly, our findings suggest that factors other than holocentromeres and genome downsizing after polyploidy impact the rate of single chromosome number evolution. Because chromosome number change is a key driver of speciation, future work to tease apart the natural history and molecular mechanisms underlying lineages with highly elevated rates of chromosome number change would further our understanding of evolution at both the macro- and microevolutionary scales.

Supplementary material

Supplementary material is available online at *Evolution* (https://academic.oup.com/evolut)

Data availability

All data and scripts are archived and available at https://doi.org/10.5281/zenodo.6081366.

Author contributions

R.M. designed and led the work. T.K., A.F., and A.C. contributed to literature search and verified taxonomy. R.M. and R.Z.F. analyzed the data. R.M. and Y.Y. led the writing. All authors provided feedback on the manuscript and approved the final version.

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