Chromosome Size as a Universal Predictor of Recombination Rate: Insights from Holocentric and Monocentric Systems

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Abstract

Recombination is a fundamental evolutionary process essential for generating genetic diversity, facilitating adaptation, and driving speciation. However, direct measurement of recombination rate remains challenging, as standard methods—such as chiasma counts or genetic linkage maps—are labor-intensive and often infeasible for non-model species. In this study, we identify chromosome number and mean chromosome size as practical proxies for genome-wide recombination rate by analyzing genetic map data from 69 insect species and supplementary analyses of 157 monocentric flowering plants. Using both ordinary and phylogenetically informed Bayesian regression models, we show that recombination rate increases with chromosome number and decreases with mean chromosome size. Crucially, mean chromosome size is a significantly better predictor, particularly in holocentric species. This insight enables recombination rate estimation in thousands of species with known genome and chromosome sizes, thereby allowing hypothesis testing at scales previously unattainable. We also confirm the long-standing hypothesis that monocentric species have nearly twice as many crossovers per chromosome as holocentric species, reflecting structural constraints imposed by diffuse centromeres. Building on these results, we present predictive models applicable to poorly studied holocentric plants. Overall, our study highlights the pivotal role of chromosome architecture in recombination evolution and provides an accessible framework for evolutionary genomic research across diverse lineages.

Keywords: Recombination rate, Chromosome size, Chromosome number, Crossover frequency, Holocentric chromosomes, Monocentric chromosomes, Evolutionary genomics, Meiotic recombination, Phylogenetic comparative methods, Genome architecture

Article summary: Why do some species have more genetic shuffling than others? We investigated 69 insect species with detailed genetic maps and found that mean chromosome size is the best predictor of genome-wide recombination rate, not the number of chromosomes. This means we can now estimate recombination rates even in species where genetic maps are unavailable, using only genome size and chromosome number. We also show that chromosome structure matters—species with a single centromere per chromosome (monocentric) have nearly double the recombination compared to those with diffuse centromeres (holocentric). These insights open new doors for studying evolution across many organisms.

Introduction

The key evolutionary implications of recombination are twofold: recombination can facilitate adaptation across environments by generating and maintaining genetic variation, or it can break up co-adapted allele combinations, potentially disrupting current optimal gene combinations (Ritz et al., 2017; Stapley et al., 2017a). Recombination also plays a crucial role in speciation as the formation of new species often involves a progressive reduction in genetic exchange in specific genomic regions that act as barriers to gene flow (Butlin, 2005). In this context, a lot of attention has been given to the evolutionary advantage of sex, which promotes recombination. However, the reason why average genome-wide recombination also varies between sexually reproducing organisms is less understood (Stapley et al., 2017a). In sexual eukaryotes, genome-wide recombination rate is determined by the number of crossovers (COs; i.e., chiasmata) per meiosis and by random meiotic segregation of homologs (Stapley et al., 2017b; Otto & Payseur, 2019; Hofstatter et al., 2021). Because estimating recombination rates with standard approaches, such as counting chiasmata or establishing linkage maps (Stapley et al. 2017b), demands time-consuming analysis and is typically impractical for non-model species, it remains challenging to investigate recombination rate-related hypotheses at a macroevolutionary scale across species and environments.

Since at least one CO per chromosome is usually required for proper segregation during meiosis (a phenomenon known as CO assurance; Hunter, 2015) and the formation of additional COss is tightly regulated by CO interference (a mechanism that prevents COs from occurring too closely together; Hunter, 2015), the number of COs per chromosome is maintained within a narrow range (Lian et al., 2023). Therefore, chromosome number is expected to predict genome-wide recombination rate across species (Stapley et al., 2017b). Having more chromosomes also increases the potential for diverse combinations during the assortment of chromosomes in gametes. However, variation in genome size can obscure this relationship.

All else being equal, the necessity of at least one CO per chromosome results in proportionally higher recombination rates in shorter chromosomes (Lynch et al., 2011; Tigano et al., 2022). Heterochromatin is moreover associated with reduced recombination, for instance, in pericentromeric regions, which often accumulate transposable elements and display suppressed CO activity (Kent et al., 2017). Consequently, larger chromosomes, which tend to contain proportionally larger heterochromatic regions (Leitch et al., 2023), may experience a lower rate of recombination per unit length compared to smaller chromosomes. This dynamic could explain the commonly observed negative association between recombination rate and chromosome size of individual chromosomes inside a single karyotype (Kaback et al., 1992; Jensen-Seaman et al., 2004; Backström et al., 2010; Martin et al., 2019; Brazier & Glémin, 2022; Shipilina et al., 2022; Näsvall et al., 2023; Palahí I Torres et al., 2023), which appears to translate to a negative relationship between mean chromosome size and average genome-wide recombination rate (Lynch et al., 2011; Brazier & Glémin, 2022). Thus, chromosome counts and mean chromosome size can potentially be used as proxies for genome-wide recombination rate comparison across a large number of species.

In eukaryotes, two predominant types of chromosomes have evolved that differ regarding their centromere organization, i.e. monocentric and holocentric chromosomes. Importantly, the accuracy with which chromosome number and mean chromosome size predict genome-wide recombination rate is expected to differ between these two types of chromosome organization. For species with monocentric chromosomes, where kinetochores and chromatid cohesion are restricted to a single centromeric region, the number of COs per chromosome mostly varies between one to four (Crismani & Mercier, 2012; Mercier et al., 2015; Blary & Jenczewski, 2019; Otto & Payseur, 2019; Brazier & Glémin, 2022; Lian et al., 2023), but higher numbers have also been reported (Jones & Franklin, 2006; Mancera et al., 2008; Brazier & Glémin, 2022; Lian et al., 2023). In contrast, species with holocentric chromosomes, where kinetochores are maintained along the entire chromosome length (Bureš et al., 2013; Mandrioli & Manicardi, 2020; Hofstatter et al., 2021) cannot rely on the two-step loss of cohesion during meiosis and restrict the CO frequency per homolog to one or two chiasmata at maximum (Schvarzstein et al., 2010; Metlers et al., 2012; Cuacos et al., 2015; Marques & Pedrosa-Harand, 2016; Mandrioli & Manicardi, 2020; Castellani et al., 2024). Such decreased recombination has been shown for both holocentric plants (e.g., Nordenskiöld, 1962; Cabral et al., 2014; Heckmann et al., 2014; Hofstatter et al., 2021; Castellani et al., 2024) and animals (e.g., Barnes et al., 1995; Nokkala et al., 2004; Viera et al., 2009; Lukhtanov et al., 2018). Another difference between holocentric and monocentric species is the composition of chromatin. In holocentric species, chromatin is often not compartmentalized and shows no obvious euchromatic or heterochromatic clusters as in monocentric species (Mandrioli & Manicardi, 2012; Heckmann et al., 2013; Hofstatter et al., 2022; Mata-Sucre et al., 2024) with homogeneous TEs distributions along chromosomes (Hofstatter et al. 2022; Shipilina et al., 2022; Mata-Sucre et al., 2024; Castellani et al., 2024). This could potentially result in a more uniform – yet still telomere-biased – distribution of recombination rates along holocentric chromosomes compared to monocentric (Haenel et al., 2018; Palahí i Torres et al., 2022; Hofstatter et al., 2022; Shipilina et al., 2022; Castellani et al., 2024). Furthermore, due to their extended kinetochore and the attachment of spindle microtubules along their entire length, holocentric species are more likely to tolerate chromosomal fissions and fusions that have negligible fitness costs (Bureš et al., 2013; Jankowska et al., 2015; Zedek & Bureš, 2019; Mandrioli & Manicardi, 2020; Lucek et al., 2022). Therefore, holocentric species are expected to more readily change chromosome numbers and size and, as a consequence, recombination rates in response to selection compared to monocentric species (Bell, 1982; Escudero et al., 2012; Bureš et al., 2013; Escudero et al. 2013; Bureš & Zedek, 2014). Thus, a higher recombination rate in monocentric species is usually expected to be reached by increasing the number of COs per chromosome (Burt, 2000; Stapley et al., 2017b), whereas in holocentric species, a higher recombination rate is predicted to primarily result from increasing chromosome numbers (and thereby decreasing their size).

Although chromosome numbers and mean chromosome size have been used as proxies for genome-wide recombination rate inference in both monocentric (Pessia et al., 2012; Carta et al., 2018) and holocentric species (Bell, 1982; Escudero et al., 2012; Escudero et al., 2013; Spalink et al., 2018; Mackintosh et al., 2019; Márquez- Corro et al., 2021; Elliott et al., 2022), the assumption that these two genomic traits accurately represent recombination rates should be critically assessed. A system encompassing multiple holocentric and monocentric lineages, for which chromosome numbers, genome sizes, and linkage maps or properly counted chiasmata are available to estimate genome-wide recombination rates is

required to address these questions. Insects may constitute such a group (Figure 1), where holocentric chromosomes have independently evolved in the orders Lepidoptera, Trichoptera, Hemiptera, Thysanoptera, Psocodea, Zoraptera, Dermaptera, and Odonata, whereas monocentric chromosomes are more broadly found, for example in Diptera, Coleoptera, Hymenoptera, Orthoptera, or Blattodea (Melters et al., 2012; Zedek & Bureš, 2019; Mandrioli & Manicardi, 2020; Marques & Drinnenberg, 2025). Moreover, chromosome number and size, genome size information, and linkage maps are available for a reasonable amount of species of both holocentric and monocentric insect orders.

By analyzing recombination data from insect species with well-characterized genetic maps (or counted chiasmata), we aimed to assess the suitability of chromosome numbers and mean chromosome size as predictors of genome-wide recombination rate and test the hypothesis that these predictors are more accurate in holocentric than in monocentric species. Specifically, we expect that (1) the average number of COs per chromosome is higher in monocentric species than in holocentric ones, which is the key assumption stemming from the structural differences between holocentric and monocentric chromosomes (see above), and (2) chromosome number and size predict genome-wide recombination rate better in holocentric than monocentric species. To assess the broader applicability of our results beyond insects, we evaluated the accuracy of chromosome number and mean chromosome size as predictors of recombination rate in flowering plants with monocentric chromosomes. Holocentric species could not be meaningfully included, as only two holocentric plant species currently have established genetic maps (Escudero et al., 2018; Castellani et al., 2024), but we made a prediction of holocentric plants based on the results we obtained from insects and monocentric plants.

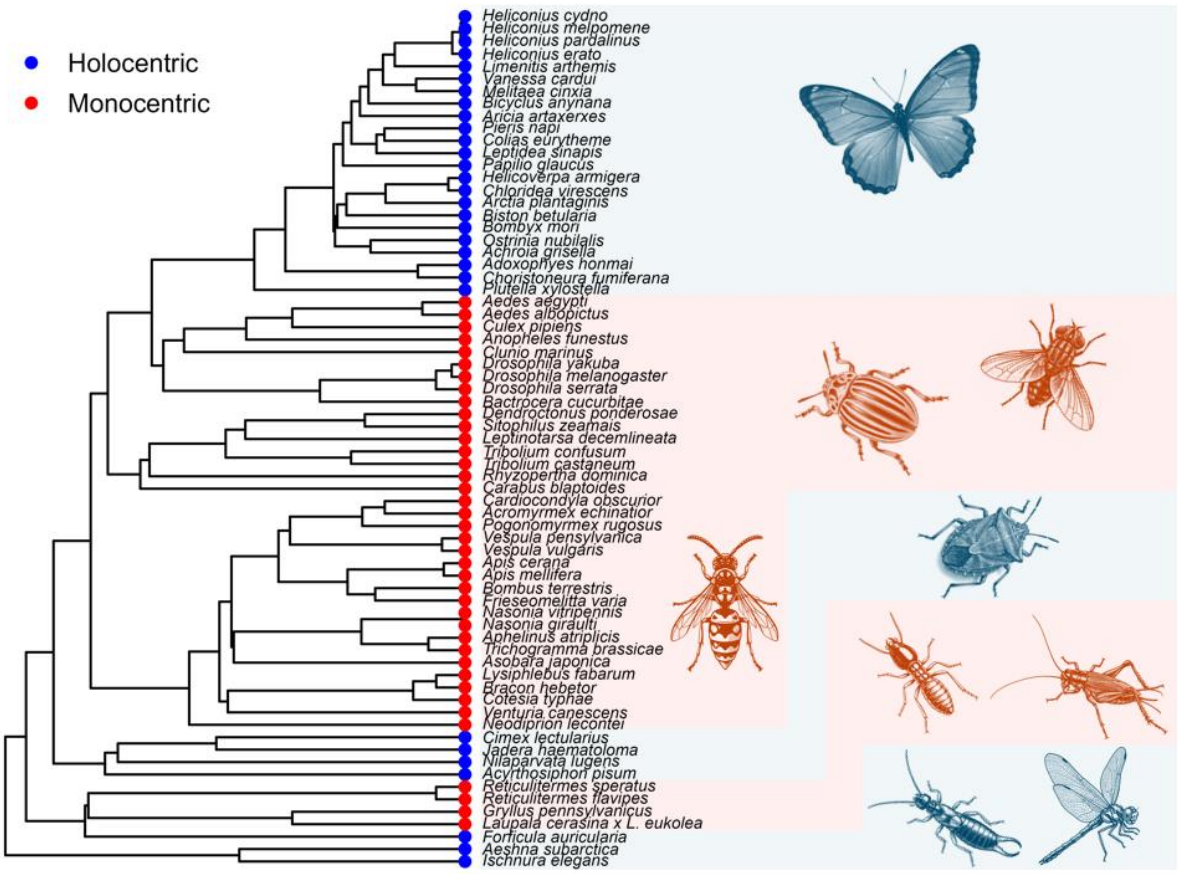

Figure 1. Phylogenetic tree of 30 holocentric and 39 monocentric species included in our study. Holocentric species include butterflies (Lepidoptera), true bugs (Hemiptera), earwigs (Dermaptera), and dragonflies and damselflies (Odonata). Monocentric species include flies (Diptera), beetles (Coleoptera), sawflies, wasps, bees, and ants (Hymenoptera), termites (Blattodea), and crickets (Orthoptera).

Materials and Methods

Data Collection

We compiled genetic map length (67 species) and chiasmata count (2 species) data from the literature for a range of insect species, spanning both holocentric and monocentric species (Supplementary Table S1). Genome size and chromosome count information were obtained from the NCBI Genome database (Sayers et al., 2022), the Animal Genome Size Database (Gregory, 2025), and additional publications (Supplementary Table S1). We defined the recombination rate as the haploid genetic map length divided by the 1C genome size (Stapley et al., 2017b). In the case of the holocentric odonata *Aeshna subarctica* and the earwig *Forficula auricularia*, we calculated the genetic map length from the number of chiasmata as 50 × average number of chiasmata per meiosis because 50 centiMorgans (cM) correspond to an average of one CO per meiosis (Anderson et al., 2004; Chuang & Smith, 2023). We estimated the mean number of COs per chromosome by dividing the genetic map length by 50 divided by the chromosome count. The final dataset included the haploid chromosome number (n), mean chromosome size (Mbp), the recombination rate (cM/Mbp), the mean number of COs per chromosome, and the chromosome type

(holocentric or monocentric) (Supplementary Table S1). The phylogenetic tree (Figure 1) used in the following analyses was based on the TimeTree (Kumar et al., 2022). If a species was not available from TimeTree, we used the tip of its most closely related relative, which was renamed, or added manually based on published phylogenies.

Recombination rate predicted by chromosome number and mean chromosome size

Recombination rate (cM/Mb) and the average number of COs per chromosome (COperChr) were analyzed within a Bayesian framework and through comparative phylogenetic methods to account for the data's distributional properties and the non-independence among species employing the *brms* R package (Bürkner, 2017; 2018; 2021).

Because recombination rates are strictly positive and right- skewed, different modeling strategies were employed depending on the focal chromosomal predictor. For chromosome number as a predictor, we observed that heteroscedasticity persisted even after log-transforming recombination rate. Therefore, recombination rate was modeled on its original scale using a Gamma regression with a log link. This approach accommodates the positive nature of the data and the residual heteroscedasticity, permitting a multiplicative interpretation of regression coefficients. A model with interaction effects was initially specified as *recombination rate~chromosome number×chromosome type*, where chromosome type distinguishes holocentric from monocentric species. If the 95% credible interval for the interaction term overlapped zero, a simpler additive formulation was adopted.

For analyses involving chromosome size as a predictor, both recombination rate and chromosome size were log-transformed. This transformation effectively stabilized the variance and linearized the multiplicative relationship, allowing us to apply a Gaussian model to the log–log data: *log(recombination rate)~log(chromosome size)×chromosome type*. As before, evidence for interaction was evaluated via the 95% credible intervals, with additive models used if the interaction term was not supported.

For both sets of analyses, models were estimated using four Markov chains with total iterations ranging from 4,000 to 100,000 (including 100 to 5,000 warmup iterations) as needed. In Gamma models, mildly informative priors (normal(0,2)) were placed on regression coefficients, and a gamma(2,0.1) prior was applied to the shape parameter; analogous priors were used for Gaussian models on log-transformed data. Sampling convergence and model diagnostics were evaluated with R-hat statistics, trace plots, and effective sample sizes. To account for shared evolutionary history, phylogenetically informed versions of these models were implemented by including a random intercept of the form

(1|gr(phylo, cov = A)), where the covariance matrix A was derived from the species tree using the vcv function in the R package ape (Paradis & Schliep 2019).

Residual Analysis for Predictive Accuracy

To further evaluate model performance, we conducted a residual analysis comparing the predictive accuracy of recombination rate from chromosome number and mean chromosome size. For the Gamma regression model using chromosome number as the predictor, posterior expected predictions were obtained on the original scale and residuals were calculated as the difference between the observed recombination rate and the predicted value. For the Gaussian model based on log- transformed recombination rate and mean chromosome size, predictions were first obtained on the log scale and then back- transformed using the exponential function to yield estimates on the original scale; residuals were computed analogously. We quantified model performance using the root mean squared error (RMSE) and mean absolute error (MAE). Furthermore, absolute residuals of holocentric versus monocentric species were compared using Wilcoxon rank- sum tests to determine whether the models predicted recombination rate more accurately in one group than the other. This analysis allowed us to compare both the overall predictive precision of the two chromosomal predictors and the relative fit across chromosome types.

Assessing the relation of crossover number per chromosome to chromosome type

To test whether the average number of COs per chromosome differed between holocentric and monocentric species, we pursued two complementary approaches. Given that COs per chromosome is strictly positive, we first modeled it using a Gamma regression with a log link and used chromosome type as the main predictor. The same priors as in the recombination rate analyses (normal(0,2) for regression coefficients and gamma(2,0.1) for the shape parameter) were applied. Phylogenetic non-independence was incorporated by including a random intercept specified as (1|gr(phylo, cov = A)).

To further evaluate the evolutionary dynamics underlying variation in COs per chromosome between holocentric and monocentric species, we employed an OUwie-based approach (Beaulieu et al., 2012). A SIMMAP tree was constructed by mapping the discrete character (chromosome type) onto the species' phylogeny using an equal rates (ER) model. To account for uncertainty in ancestral state reconstructions, we generated a SIMMAP tree based on Drinnenberg & Marques (2025). A suite of OUwie models (BM1, BMS, OU1, OUM, OUMV, OUMA, and OUMVA) was fitted to COs per chromosome. Model selection was based on weighted AIC computed across replicates, thereby identifying the best-fitting evolutionary model while explicitly incorporating uncertainty in the character history. Once

the best model(s) was selected, we ran 100 bootstrap replicates using the OUwie.boot function in OUwie to obtain empirical estimates and 95% confidence intervals for the evolutionary optimum (θ).

Evaluation of chromosome number and mean chromosome size predictors in angiosperms

To assess the broader applicability of our results beyond insects, we evaluated the accuracy of chromosome number and mean chromosome size as predictors of recombination rate in flowering plants with monocentric chromosomes. Holocentric species could not be meaningfully included, as only two holocentric plant species currently have genetic maps (Escudero et al., 2018; Castellani et al., 2024). To this end, we obtained data on recombination rates, genome sizes, and chromosome numbers from Stapley et al. (2017b) and randomly selected one phylogenetic tree from a set of 100 angiosperm phylogenies provided by Forest (2023). The dataset and phylogenetic tree overlapped with 157 angiosperm species (Supplementary Table S2), which we used in the regression models and residual analyses as described above for insects.

Results

We obtained recombination rate, chromosome number, chromosome size, and the average number of COs per chromosome for 30 holocentric (1 Dermaptera, 4 Hemiptera, 23 Lepidoptera, and 2 Odonata) and 39 monocentric (2 Blattodea, 7 Coleoptera, 9 Diptera, 19 Hymenoptera, and 2 Orthoptera) insect species (Figure 1; Supplementary Table S1).

Differences in Crossover Number between Chromosome Types

We first assessed whether the average number of COs per chromosome (COperChr) differs between holocentric and monocentric insect species. Using a phylogenetically informed Bayesian Gamma regression model, we modeled COperChr as a function of chromosome type, incorporating species' relatedness via a random intercept (Figure 2A; Supplementary Table S3). In this analysis, the intercept for holocentric species was 0.01 (95% Credible Interval [CI]: –0.54 to 0.53), corresponding to an expected mean of exp(0.01) ≈ 1.01 COs per chromosome. The effect of chromosome type (with monocentric species coded as the focal level) was positive and significant (Estimate = 0.65; 95% CI: 0.10–1.21). This implies that, on average, monocentric species exhibit nearly double the number of crossovers per chromosome relative to holocentric species [exp(0.01 + 0.65) ≈ 1.93 COs per chromosome], supporting our prediction based on chromosomal architecture.

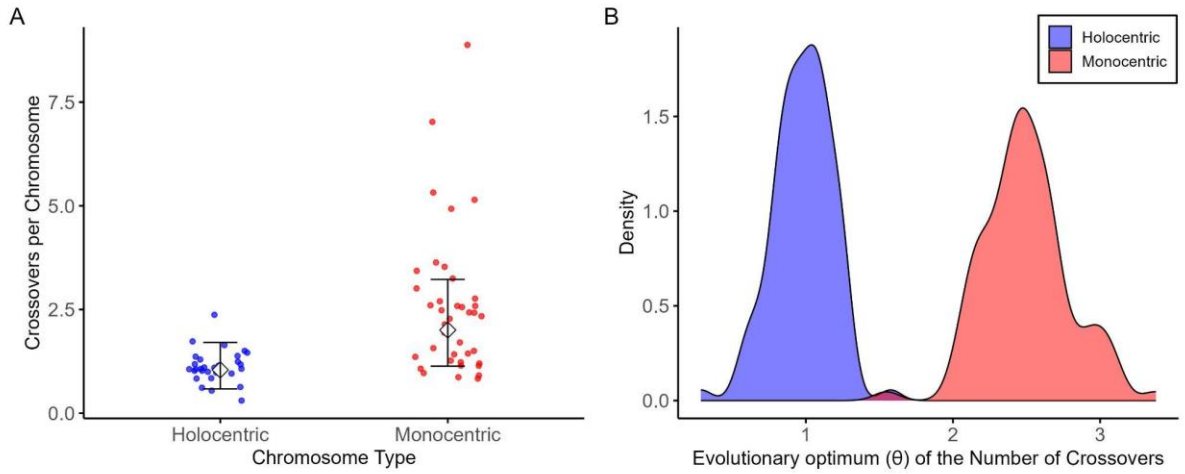

Figure 2. Number of crossovers in holocentric and monocentric insects. **A -** Observed COs per chromosome in holocentric (blue) and monocentric (red) species. Individual data points are jittered to display variation, and the overlaid symbols with error bars represent the group mean and 95% prediction credible intervals estimated from the model. **B -** Bootstrap density distributions (based on 100 replicates) of the evolutionary optimum (θ) for the number of COs estimated under the OUMVA model. The density for holocentric lineages (blue) and monocentric lineages (red) illustrate the uncertainty and differences in the adaptive optima between chromosome types.

To further explore the evolutionary dynamics underlying variation in CO numbers, we conducted an OUwie-based analysis. This approach allowed us to compare models that assume different evolutionary optima for holocentric versus monocentric lineages. The two best-fitting models were OUMVA and OUMV (Supplementary Table S3), which yielded almost identical results and estimated distinct adaptive optima (θ) for each chromosome type (Figure 2B; Supplementary Table S3). Therefore, here we report only the results from OUMVA model. Consistent with the regression analysis, the OUwie results indicated a lower optimum for holocentric species than monocentric ones. The optimum CO number for holocentric species was estimated at θ_h = 1.00 (95% CI: 0.59–1.27), whereas for monocentric species it was θ_m = 2.49 (95% CI: 2.05–3.08). Together, these results confirm that monocentric species maintain a significantly higher average number of COs per chromosome than holocentric species in insect orders, in line with the expectations derived from their distinct meiotic architectures.

Chromosome Number as a Predictor of Recombination Rate

Across the 69 analyzed insect species, genome- wide recombination rate was positively associated with haploid chromosome number (Figure 3; Supplementary Table S4). In a Bayesian Gamma regression that modeled recombination rate as a function of chromosome number and chromosome type (holocentric vs. monocentric), the estimated effect of chromosome number was positive and significant (β = 0.09; 95% Credible Interval [CI]: 0.07–0.12). Monocentric species exhibited higher baseline recombination rates than holocentric species (β = 1.65 95% CI: 1.16–2.13; Figure 3A). The interaction between chromosome number and chromosome type was not supported (β = 0.00; 95% CI: –0.05 to 0.06; Supplementary Table S4), indicating that the positive relationship between chromosome number and recombination rate does not differ appreciably between the two chromosomal architectures. Consistent results were obtained when accounting for phylogenetic non-independence, although the magnitude of the effect of chromosome number variation was somewhat reduced (β = 0.05; 95% CI: 0.01–0.08; Figure 3B; Supplementary Table S4). Our

results indicate that genome- wide recombination rates increase with haploid chromosome number, with monocentric species consistently exhibiting higher baseline rates than holocentric species, and this relationship remains robust even after accounting for phylogenetic relatedness.

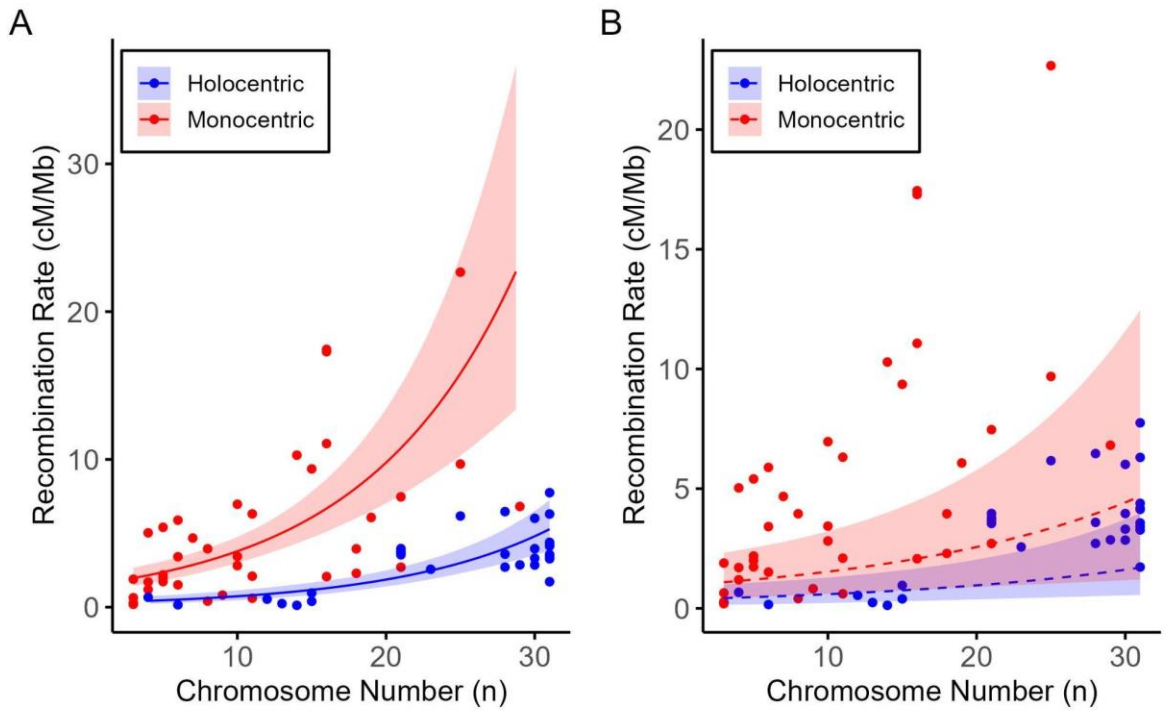

Figure 3. Relationships between chromosome number, recombination rate, and crossovers per chromosome in insects. **(A)** Relationship between recombination rate (cM/Mbp) and haploid chromosome number, showing holocentric (blue) and monocentric (red) species. Solid lines indicate model predictions and shaded ribbons represent 95% prediction credible intervals. **(B)** Relationship between recombination rate (cM/Mbp) and haploid chromosome number when phylogenetic relatedness is considered. Dashed lines indicate model predictions and shaded ribbons represent 95% prediction credible intervals.

Chromosome Size as a Predictor of Recombination Rate

A parallel set of analyses examined the effect of mean chromosome size on recombination rate by modeling log- transformed recombination rate against log- transformed chromosome size (Figure 4; Supplementary Table S5). In the non- phylogenetic additive model, mean chromosome size was a strong negative predictor of recombination rate (β = –1.08; 95% CI: –1.21 to –0.95; Figure 4A), indicating that species with smaller chromosomes tend to experience higher recombination rates per megabase. Although the interaction between log(chromosome size) and chromosome type was not significant (β = 0.10 95% CI: –0.16 to 0.36; Supplementary Table S5), monocentric species consistently maintained higher recombination rates than holocentric species at a given chromosome size (β = 0.74; 95% CI: 0.48–0.99; Figure 4A; Supplementary Table S5). Phylogenetic corrections did not alter these qualitative patterns, with log(chromosome size) remaining negatively associated with recombination rate (β = –0.93; 95% CI: –1.15 to –0.70) and the effect of chromosome type persisting (β = 0.64; 95% CI: 0.08–1.19) (Figure 4B; Supplementary Table S5). Our analyses indicate that smaller chromosome sizes predict higher recombination rates per megabase,

and that monocentric species consistently exhibit elevated rates compared to holocentric species—even after accounting for phylogenetic relationships.

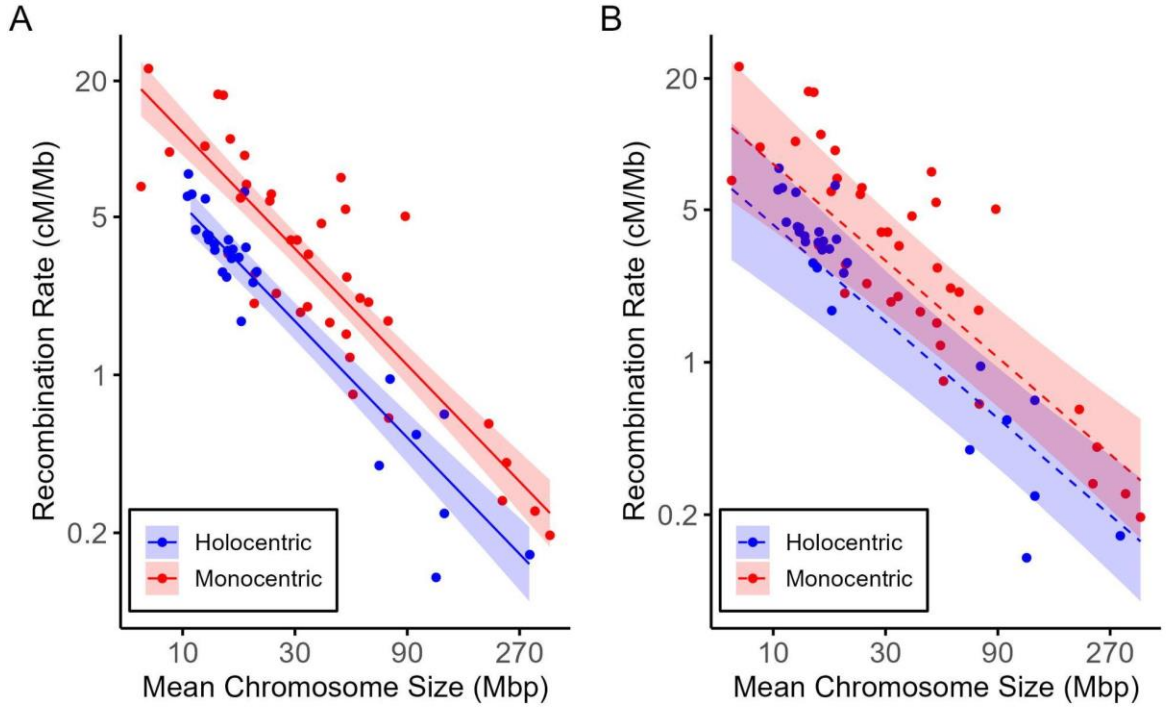

Figure 4. Relationships between mean chromosome size and recombination rate in insects. **(A)** Relationship between recombination rate (cM/Mbp) and chromosome number without considering phylogeny, showing holocentric (blue) and monocentric (red) species. Solid lines indicate model predictions and shaded ribbons represent 95% prediction credible intervals. **(B)** Relationship between recombination rate (cM/Mbp) and mean chromosome size when phylogenetic relatedness is considered. Dashed lines indicate model predictions and shaded ribbons represent 95% prediction credible intervals.

Residual Analyses: Assessing Predictive Accuracy

The Gamma regression model using chromosome number as the predictor yielded an RMSE of 3.73 and an MAE of 2.51, whereas the Gaussian model based on log- transformed recombination rate and mean chromosome size produced lower error metrics (RMSE = 2.75; MAE = 1.54). Following this, the model with mean chromosome size as a predictor shows lower absolute residuals than the model with chromosome number as a predictor (Figure 5). These results highlight that mean chromosome size is a more precise predictor of recombination rate than chromosome number when considering the overall dataset types.

When considering chromosome type, for the model using chromosome number as the predictor, holocentric species had significantly lower residuals (mean = 1.35, median = 1.24) than monocentric species (mean = 3.40, median = 2.73; Wilcoxon test: W = 331, p = 0.0018; Figure 5), indicating more accurate predictions. Similarly, when predicting log- transformed recombination rate from log- transformed chromosome size, holocentric species again showed lower residuals (mean = 0.60, median = 0.31) compared to monocentric species (mean = 2.27, median = 1.14; W = 304, p = 0.0005; Figure 5). These analyses demonstrate

that the predictive accuracy of recombination rate from both chromosome number and size is higher in holocentric than in monocentric species.

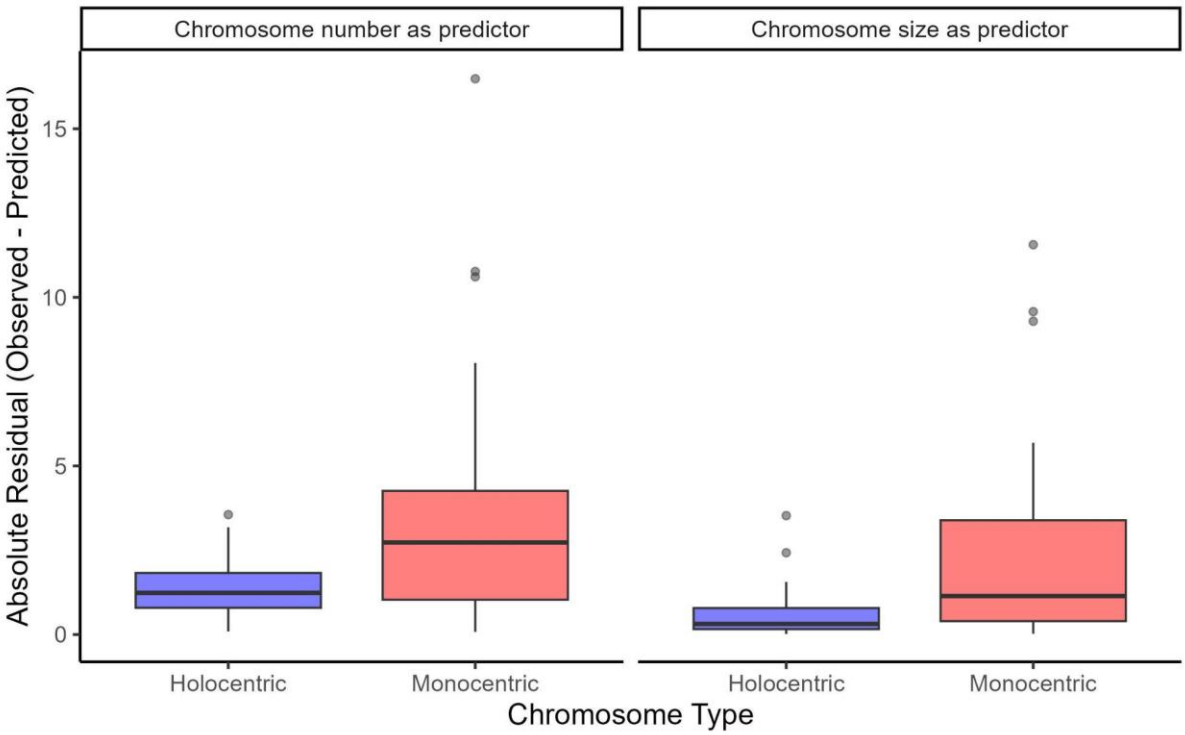

Figure 5. Absolute residuals from the non-phylogenetic recombination rate models, grouped by predictor (chromosome number or mean chromosome size) and chromosome type (holocentric or monocentric). The residuals from the model with chromosome size as the predictor were back-transformed from the log scale to make them comparable with residuals from the model with chromosome number as the predictor (see Methods for details).

Evaluation of Chromosome Number and Mean Chromosome Size as Predictors in Angiosperms

Regression analyses of 157 angiosperm species with monocentric chromosomes revealed that recombination rate is positively associated with chromosome number (Supplementary Figure S1A,B; Supplementary Table S6). In the non- phylogenetic Gamma model, the estimated coefficient for chromosome number was 0.03 (95% CI: 0.01–0.05). When accounting for phylogenetic non- independence, the chromosome number coefficient was 0.02 (95% CI: 0.00–0.04). The Gaussian model predicting log- transformed recombination rate from log- transformed chromosome size (Supplementary Figure S1C,D; Supplementary Table S7) produced a coefficient for log mean chromosome size of –0.90 (95% CI: –0.96 to –0.83). With phylogenetic corrections, the coefficient remained similar at –0.91 (95% CI: –0.98 to –0.83).

Residual analyses further demonstrated that the model based on mean chromosome size yielded lower error metrics than the model based on chromosome number (Supplementary Figure S2). Specifically, the Gamma model (chromosome number predictor) had an RMSE of 1.46 and an MAE of 1.12, with absolute residuals averaging 1.12 (median = 0.98). In contrast, the log- transformed model (mean chromosome size predictor) produced an RMSE

of 0.92 and an MAE of 0.63, with absolute residuals averaging 0.63 (median = 0.44). These results indicate that mean chromosome size is a more precise predictor of recombination rate than chromosome number in angiosperms.

Discussion

The evolution of recombination rates plays a pivotal role in speciation, balancing the benefits of adaptability with the risks of disrupting established genetic combinations (Ritz et al., 2017; Stapley et al., 2017a). High recombination rates, while fostering evolutionary potential by generating novel allele combinations for adaptation to diverse environments (Wang et al., 2019) or for highly competitive communities (Bell, 1992), can also dismantle favorable co-adapted gene complexes (Ritz et al., 2017). Conversely, lower recombination rates may provide greater reproductive assurance by preserving existing adaptive combinations but at the cost of reduced evolutionary flexibility. This trade-off is particularly evident when considering the factors influencing recombination.

Here, we show that the number of COs per chromosome is predominantly higher and more variable in monocentric insects compared to holocentric ones (Figure 2) which provides support for a long-standing hypothesis based on karyological observations suggesting that holocentric chromosomes typically exhibit more stringent constraints on chiasma formation (White, 1973; Nokkala et al., 2004; Gokhman & Kuznetsova, 2006; Schvarzstein et al., 2010; Metlers et al., 2012; Bureš et al., 2013; Cabral et al., 2014). Mechanistically, these differences can be attributed to the centromere structure in holocentric species, which complicates the two-step loss of cohesion that is typical for monocentric species and often leads to one or at most two chiasmata per homolog pair (Schvarzstein et al., 2010; Marques & Pedrosa-Harand, 2016; Hofstatter et al., 2021). More than two chiasmata appear to be either absent or non-viable in holocentric species studied so far (Nokkala et al., 2004; Hollis et al., 2020; Castellani et al., 2024). In contrast, monocentric chromosomes can achieve more COs per homolog pair (even over 10) and apparently do so across a range of species (Jones & Franklin, 2006; Fernandes et al., 2018; Otto & Payseur, 2019; Lian et al., 2023).

In accordance with the tight regulation of the number of COs per chromosome via CO assurance and CO interference (Hunter, 2015), we identified both chromosome number and mean chromosome size as good predictors of genome-wide recombination rate in insects when phylogenetic relatedness is not taken into account (Figures 3A and 4A). However, once phylogeny is considered, the chromosome number does not perform as well as the mean chromosome size (Figures 3B and 4B). Our residual analyses further indicate that mean chromosome size is a more precise predictor of genome- wide recombination rate than chromosome number, even in the non-phylogenetic approach (Figure 5). It is likely that the number of chromosomes captures larger differences in recombination rates among higher taxonomic units (e.g., orders or families), which also differ in chromosome numbers. In contrast, within genera or families—where closely related species often differ little or not at all in chromosome number, as e.g. in Lepidoptera (de Vos et al., 2020)—more subtle differences in recombination rate related to chromosome size are not effectively captured by chromosome number. Moreover, polyploidization multiplies chromosome number and likely has little effect on genome- wide recombination rates (Ross- Ibarra, 2007; Tiley & Burleigh,

2015; Stapley et al., 2017b), although autopolyploids may reduce the CO number per chromosome to avoid multivalent formation (Bomblies et al., 2016). This may further undermine the predictive accuracy of chromosome numbers. In contrast, mean chromosome size effectively controls the multiplicative effects of (neo)polyploidy on chromosome number (Bureš et al., 2024). The superior model fit observed for holocentric species across both predictors (Figure 5) further suggests that the strict limitations on CO numbers—imposed by the constraints of their kinetochore architecture—result in more predictable recombination patterns than those seen in monocentric species. Together, these results indicate that mean chromosome size is generally a better proxy for recombination rate and that predictions are more accurate in holocentric than in monocentric systems.

While our findings provide significant support for chromosome number and mean chromosome size being valid proxies for recombination rate, particularly in holocentric insects, it is important to acknowledge the limitations of our study. Recombination varies along a genome and is undoubtedly influenced by many factors beyond chromosome number and mean chromosome size, such as structural variation, transposable element activity, chromatin structure, and specific meiotic genes (Johnston, 2024). Although the broad taxonomic sampling across insects is valuable, extrapolating these findings to other monocentric and holocentric animals — and especially to plants — should be done with caution. On the other hand, our analyses encompassing 157 monocentric species of flowering plants showed very similar results (Supplementary Figures S1, S2) to our findings in insects (Figures 3, 4, and 5), suggesting their general applicability. Building on results from insects and monocentric plants, we predicted how genome-wide recombination rate should relate to mean chromosome size in holocentric plants (Figure 6). Notably, *Rhynchospora breviuscula* (Castellani et al., 2024) closely follows the predicted relationship, whereas *Carex scoparia* (Escudero et al., 2018) deviates from it (Figure 6). This deviation may reflect random variation, lineage-specific biological factors, lack of a reference genome assembly, or systematic differences in recombination dynamics between holocentric and monocentric plants. Notably, while our insect analyses support a shared slope between chromosome types (Figure 4; Supplementary Table S5), it remains possible that the relationship between chromosome size and recombination rate differs more fundamentally between monocentric and holocentric plants. However, with data currently limited to just two holocentric plant species, we cannot determine whether the observed deviation is meaningful or incidental. Additional data from diverse holocentric plant lineages will be essential to assess the robustness and generality of our predictions.

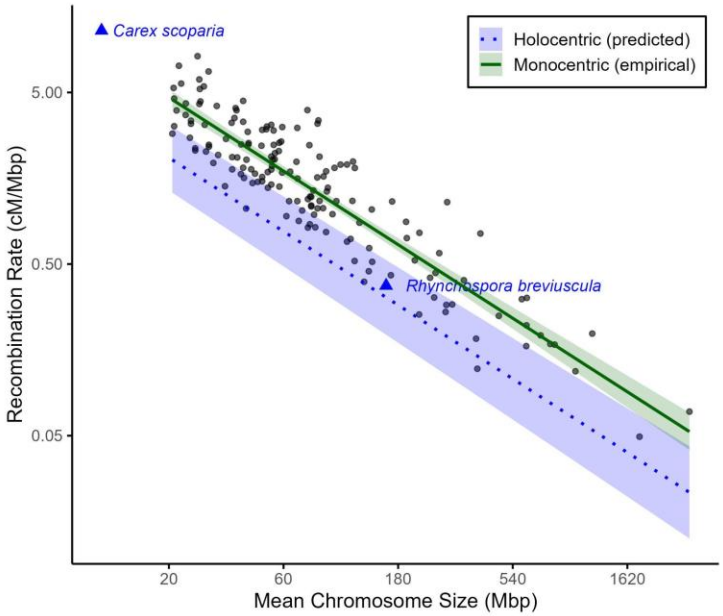

Figure 6. Relationship between mean chromosome size (Mbp) and recombination rate (cM/Mbp) in plants. Black points represent empirical data for monocentric plants. The dark green solid line and shaded area indicate the regression fit and 95% confidence intervals, respectively, for monocentric plants. The blue dotted line and shaded area represent the predicted regression and 95% confidence intervals for holocentric plants, derived by adjusting the monocentric plant intercept using the intercept difference between monocentric and holocentric insect species. Specifically, the intercept for holocentric plants was calculated by subtracting the insect-derived intercept difference (0.80 ± 0.14 SE) from the monocentric plant intercept (4.23 ± 0.15 SE), yielding a holocentric intercept of 3.43 ± 0.205 SE. The slope for holocentric plants was assumed identical to monocentric plants (-0.90 ± 0.03 SE). Blue triangles show the only two plant species with holocentric chromosomes for which both recombination rate and mean chromosome size are known: *Carex scoparia* (Escudero et al., 2018) and *Rhynchospora breviuscula* (Castellani et al., 2024).

Our demonstration of the links among CO number, mean chromosome size, chromosome type, and recombination rate provides a valuable framework for future large-scale evolutionary studies aimed at unraveling the evolution of recombination and its role in adaptation and speciation across the Tree of Life. In light of our findings, it is possible that previous studies on the holocentric plant genus *Carex* (Bell, 1982; Escudero et al., 2012; Escudero et al., 2013; Spalink et al., 2018; Márquez- Corro et al., 2021) and monocentric angiosperms (Carta et al., 2018) may have suffered from reduced power by using chromosome number rather than mean chromosome size as a proxy for recombination rate. However, chromosome numbers are available for many more species because the mean chromosome size requires knowledge of both chromosome number and genome size. For instance, in flowering plants, chromosome numbers are available for ca. 60,000 species (Rice et al., 2015) while mean chromosome size is for only about 14,000 species (Bureš et al., 2024). Therefore, continuing measurements of genome size (e.g., using flow cytometry) and more initiatives like the Darwin Tree of Life (Darwin Tree of Life Project Consortium, 2022) that generate chromosome-resolved reference genomes for thousands of species will allow us to obtain mean chromosome size data and chromosome numbers at an unprecedented scale.

While holocentric chromosomes appear to offer certain evolutionary advantages, such as the ability to more readily tolerate increased rates of chromosomal fissions and fusions without necessarily reducing fitness (Zedek & Bureš, 2019; Lucek et al., 2022; Zedek et al., 2022)—allowing for rapid karyotypic evolution and potentially increased genetic diversity through altered recombination rates and linkage groups—the prevalence of monocentric

chromosomes in the vast majority (80-85%) of eukaryotes (Márquez-Corro et al., 2018) and comparable rates of chromosome number evolution in monocentric and holocentric insects (Ruckman et al., 2020) suggest a different story. This apparent paradox may be explained by the spatial separation of chromosome segregation and recombination in monocentric chromosomes. In monocentric species, these two crucial meiotic processes are functionally and physically distinct. Holocentric chromosomes, by contrast, must manage both segregation and recombination across their diffuse centromere structure. This constraint likely necessitates a reduction in the number of chiasmata per chromosome to ensure proper segregation, as multiple chiasmata in holocentric species can lead to meiotic errors (Nokkala et al., 2004; Hollis et al., 2020). However, some holocentric insects and plants have also evolved non-canonical meiosis to solve these problems (Melters et al., 2012; Lukhtanov et al., 2018). Our findings, demonstrating a significantly higher number of chiasmata per homolog in monocentric species compared to holocentric species, directly support this explanation and help to resolve the paradox. The increased chiasma frequency in monocentrics likely contributes to their greater evolutionary success by allowing for higher levels of genetic recombination. Thus, while holocentric species may offer flexibility in karyotypic evolution, the inherent trade-off between recombination rate specifically for chiasma formation and proper segregation, might ultimately limit their overall evolutionary success compared to the more specialized and efficient mechanisms found in monocentric chromosomes. In summary, our findings reveal a fundamental difference in how insect recombination is regulated. While monocentric species rely on increased CO rates, holocentric species appear to compensate through changes in chromosome number. This stark contrast underscores centromere architecture's critical role in shaping insect genomes' evolutionary trajectory.

Data availability statement

All the data used in this article are available in supplementary materials online on the publisher’s website.

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Author contributions

FZ conceived the study and performed analyses. FZ, PB, TLE, ME, KL, and AM wrote the manuscript.

Conflicts of interests

None declared.

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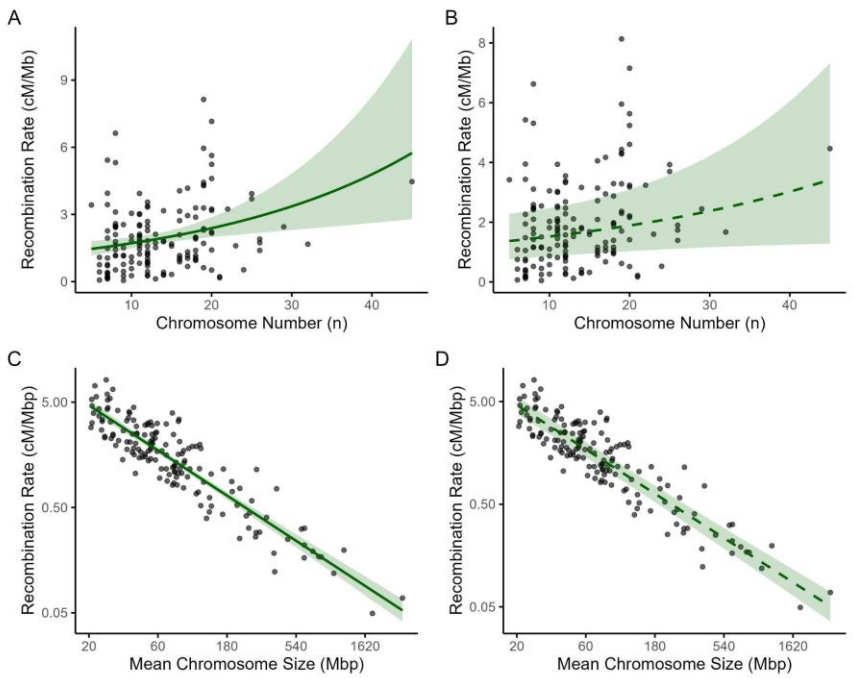
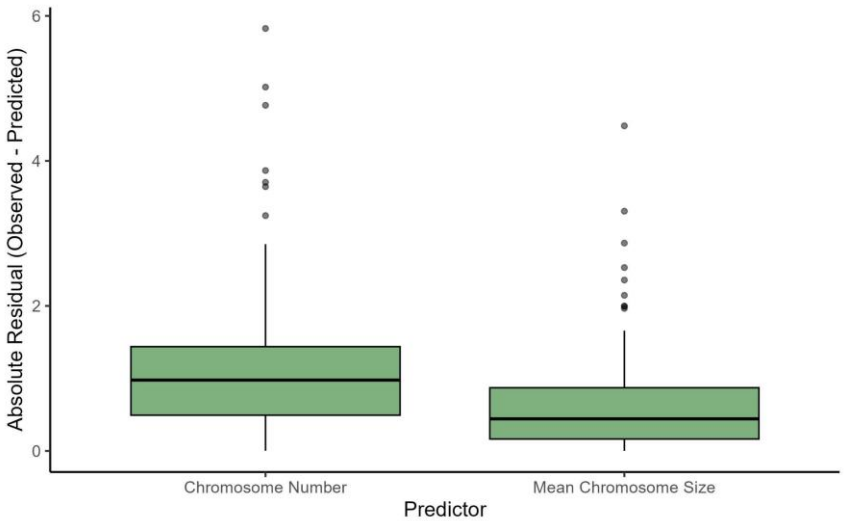
Supplementary Figures



Supplementary Figure S1. Relationships between chromosome number, mean chromosome size, and recombination rate in flowering plants with monocentric chromosomes. (A) Relationship between recombination rate (cM/Mbp) and haploid chromosome number. (B) Relationship between recombination rate (cM/Mbp) and haploid chromosome number when phylogenetic relatedness is considered. (C) Relationship between recombination rate (cM/Mbp) and mean chromosome size (both variables are on a logarithmic scale). (D) Relationship between recombination rate (cM/Mbp) and mean chromosome size when phylogenetic relatedness is considered (both variables are on a logarithmic scale). Solid and dashed lines indicate model predictions and shaded ribbons represent 95% prediction credible intervals.



Supplementary Figure S2. Absolute residuals from the non-phylogenetic recombination rate models using chromosome number and mean chromosome size as predictors in flowering plants with monocentric chromosomes. Residuals from the model with chromosome size were back-transformed from the log scale to enable direct comparison with those from the chromosome number model.