**Not all centromeres are equal, or are they?**

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**Abstract**

Despite a century of research, many fundamental aspects of chromosome number evolution remain a mystery. One example is the dynamics of fissions and fusions in clades with holocentric or monocentric chromosomes. Holocentric chromosomes have diffuse centromeres, where spindle fibers attach along the entire length of the chromosome, while monocentric chromosomes have a single, localized centromere. This difference in the arrangement has led to the hypothesis that species with holocentric chromosomes may tolerate fissions with less impact on fitness as compared to species with monocentric chromosomes. To test for differences in the rates of fissions and fusions, we analyzed data from 12,411 species of insects in both a taxonomic and phylogenetic framework. Insects exhibit a wide range of chromosome number and several large clades with monocentric or holocentric chromosomes making them ideal for analyzing differences in chromosome number evolution. We found that insect orders exhibit striking differences in rates of fissions, fusions, and polyploidy. Lepidoptera, a holocentric clade, exhibits some of the highest rates, while other holocentric clades exhibit some of the lowest rates. However, looking across all insects we found no evidence that holocentric clades have higher rates of fissions, fusions, or polyploidy than monocentric clades. Our results suggest that holocentricity alone does not lead to higher rates of chromosome number changes. Instead, we suggest that meiotic drive or population size may be contributing to differences among clades.

**Introduction**

Chromosome number stability is expected among lineages as shifts in chromosome number can lead to a decrease in fitness (Wilson *et al.* 1975a; White 1978a; Bush 1982). This stability in chromosome number is driven by the underdominance of chromosomal rearrangements (Escudero *et al.* 2016). Chromosome number has even been proposed as a possible driver of speciation (White 1978a) . However, theoretical work has suggested that a stasipatric model of speciation may be unlikely (Lande 1984). Furthermore, empirical evidence suggests that many chromosomal rearrangements may have little or no fitness effect (Sites Jr and Moritz 1987; Coyne *et al.* 1993). More recently structural rearrangements have been proposed as a mechanism of speciation without the requirement for underdominance (Rieseberg 2001; Faria and Navarro 2010; Guerrero and Kirkpatrick 2014). In light of the possible role of chromosomal change in speciation, identifying traits associated with increased rates of chromosomal rearrangements may explain patterns of extant diversity.

Unfortunately, the evolution of chromosome number has been recalcitrant to the formation of rules or generalizations that can explain variation in patterns and rates across large clades. However, within clades, fusions and fissions are two of the dominant forces in reshaping karyotypes (Lucek 2018). We use these terms (fusion and fission) for simplicity to describe chromosome number changes of plus or minus one. However, in reality, fusions decreasing chromosome number capture two different processes at the molecular level. First, Robertsonian translocations followed by the loss of the short arms can decrease chromosome number (Garagna *et al.* 1995). Second, the fusion of telomeres from two chromosomes followed by inactivation of one of the ancestral centromeres can occur as evidenced by the evolutionary history of human chromosome 2 (Miga 2017). In contrast, changes increasing chromosome number can occur through simple fissioning in the centromere region and the gaining of new telomeric sequences (Moretti and Sabato 1984; Garagna *et al.* 1995). Increases in chromosome number can also occur through polyploidy or aneuploidy. In the case of polyploidy, an offspring can be produced from an unreduced gamete increasing by one the number of copies of the genome (Harlan and deWet 1975). Likewise, aneuploidy can lead to the duplication of single chromosomes (Torres *et al.* 2008).

Because chromosomal rearrangements are thought to often be deleterious or underdominant they should be more likely to fix in populations with low effective population size or meiotic drive (White 1968). However, centromeric structure may modulate the fitness effects of fusions and fissions (Lukhtanov *et al.* 2018; Hill *et al.* 2019). In species with monocentric chromosomes fusions and fissions can lead to multiple or no centromeres along the length of a chromosome which leads to failed segregation (Mola and Papeschi 2006; Melters *et al.* 2012). In contrast in holocentric species, the centromeres are diffuse and spindle fibers attach along the entire length of the chromosome. In these species, fusions and fissions do not appear to disrupt proper segregation (Malheiros-Garde and Gardé 1950; Faulkner 1972; Cope 1985; Luceño and Guerra 1996). Therefore, holocentricity has the potential to reduce or eliminate selective pressure against chromosomal rearrangements. This should lead to higher rates of chromosome number evolution in clades with holocentric chromosomes relative to clades with monocentric chromosomes. However, studies of individual holocentric clades have been mixed with some clades showing great variation (Schneider *et al.* 2009; Escudero *et al.* 2012) and others being almost static (Panzera *et al.* 1996).

If holocentric clades have higher rates of chromosome evolution, we might expect holocentric species to exhibit higher chromosome number. Anecdotal evidence does seem to suggest that some of the highest chromosome numbers observed are in clades with holocentric chromosomes. For instance, in insects, the highest chromosome numbers are observed in the holocentric order, Lepidoptera (Blackmon *et al.* 2017). However, initial analyses have found no significant difference in chromosome number among holocentric and monocentric clades of insects (Blackmon *et al.* 2017). This previous study was limited to an order level analysis and looked only for an absolute difference in chromosome number between monocentric and holocentric clades. A stronger test of the impact of holocentricity would be to investigate the rates of fusions, fissions, and polyploidy in clades with holocentric and monocentric chromosomes.

In this study, we used chromosome number and centromere type for 599 insects to test whether clades with holocentric chromosomes have a higher rate of fusions and fissions than clades with monocentric chromosomes (Figure 1). We chose to use insects because they have multiple clades with monocentric and holocentric chromosomes, are incredibly speciose, and exhibit striking diversity in chromosome number (Cook 2000; Mora *et al.* 2011; Ross *et al.* 2015; Blackmon *et al.* 2017; Vershinina and Lukhtanov 2017). We hypothesized that clades with holocentric chromosomes should exhibit higher rates of fusions and fissions since these mutations should be less costly in these clades.

**Methods**

*Data collection:* We downloaded all available chromosome data for insects from a prior study (Blackmon *et al.* 2017). This dataset is composed of 12,411 species comprising 376 families and 3,872 genera. The minimum haploid chromosome number is 2 while the maximum haploid chromosome number is 141. There are 3,465 species with holocentric chromosomes and 8,946 species with monocentric chromosomes. This paper also included classification for each order into either monocentric or holocentric. From this dataset, we extracted the homogametic haploid chromosome number for each of the species. We used genus level phylogenies from a previous study that contained 1,726 tips (Church *et al.* 2019). These trees were built using one of two backbones trees from previous studies (Misof *et al.* 2014; Rainford *et al.* 2014). We downloaded two posterior distributions based on these backbone trees. These trees were used for all downstream comparative analyses. Our trait data set had an overlap of 599 genera with the phylogenetic data (Figure 1). In cases where we had multiple chromosome number records for genus, we retained all values and sampled from them as described below. As a first test of the differences between holocentric and monocentric clades we fit an ANOVA and a phylogenetic ANOVA to determine whether there were any differences in mean chromosome number among holocentric and monocentric clades (Harmon *et al.* 2008).

*Comparative analyses:* We fit a model of chromosome evolution on each tree from the posterior distribution. This model can accommodate up to eight rates: rate of chromosome number increase (fissions γ), rate of chromosome number decrease (fusions δ), rate of whole genome duplication (polyploidy ρ), each of these is estimated separately for clades with holocentric and monocentric chromosomes leading to six chromosomal rate parameters. The final two parameters describe the transition to and from monocentric and holocentric (qMH and qHM). This model was specified using the R package chromePlus (Blackmon *et al.* 2019) and was fit using a Bayesian approach in the R package diversitree (FitzJohn 2012).

For each of the 100 trees in a posterior distribution we randomly sampled tip states for genera with more than one record in our chromosome data set. By sampling across both datasets and trees from the posterior we are able to account for both phylogenetic and tips state uncertainty. For purposes of model fitting trees were rescaled to unit length; however, all rates reported in the paper have been back transformed to be in units of millions of years. The MCMC was initialized with parameter values drawn from a uniform distribution from 0 to 1. Preliminary analyses were conducted with a uniform prior and while most MCMC chains reached convergence quickly, a small fraction of runs would begin to sample very high rates that are biologically unrealistic. Given sufficient time we might expect these runs to eventually converge on the posterior distribution but this length of time can be large due to the relative flatness of the likelihood surface when rates are unrealistically high. To fix this problem, we added an exponential prior with a shape parameter of 0.5. This is a relatively uninformative prior but does favor lower rates avoiding the problem described above. With application of this prior we found that the vast majority of MCMC chains reach convergence in less than 10 generations. We repeated the MCMC with all 100 trees at 50 generations each. We removed the first twenty-five samples as our burnin for each run and combined the postburnin portion of the MCMC from each of the 100 trees. Because our central question is whether holocentric clades have higher rates than monocentric clades, we report our results in terms of a mean rate difference statistic, where the subscript x indicates the rate parameter. For example, for the rate fissions (γ), for each post-burnin sample we calculated as

In addition to estimating the magnitude of this statistic, we also reduced it to a simple test of the motivating hypothesis by comparing the 95% credible interval of (i.e. the 95% highest posterior density) with zero. If the entire 95% credible interval of is positive, we interpret it as support for a higher rate of chromosome evolution in holocentric clades. If the entire 95% credible interval of is negative, we interpret it as support for a higher rate of chromosome evolution in monocentric clades. Otherwise, we conclude there is not support for a significant rate difference in monocentric and holocentric clades. This approach also allows us to compare across all trees in the posterior distribution even if some trees exhibit on average higher or lower rates.

We repeated similar MCMC analyses as above for the analysis of orders, however, we ran the data under two different models. One with polyploidy and one without polyploidy included in the model and because each order is fixed for either holo- or monocentricity we only estimate one set of rate parameters in each clade. We only included orders with more than 20 tips in our phylogeny to insure a sufficient sample size for rate inference. This led to 10 orders level analyses; three of the included orders (Hemiptera, Lepidoptera, and Odonata) have holocentric chromosomes, and seven that have monocentric chromosomes. To compare rates among orders we compared the credible interval for each parameter among orders.

**Results**

*Alternative phylogenies:* The phylogenies used for this study were built using two different backbone trees that differ in the age of some clades. As such the rate estimates that are inferred using the phylogenies could be different. To determine if this variation impacted our results, we fit our full eight parameter model to both sets of phylogenies. As expected, we inferred slightly different rates depending on which posterior distribution we used. Rates were on average lower when using the posterior sampled based on the Rainford backbone than when using the posterior sampled based on the Misof backbone. To investigate the impact this has on our inference we calculated the statistic for the rate of fissions, fusions, and polyploidy comparing holocentric and monocentric species. We found that the statistics had nearly identical distributions (supplemental figure 1). Based on this finding for the remainder of the paper we present results based on our analysis of the Misof tree.

*Monocentric and Holocentric Rates:* Rates of chromosome evolution were estimated from the two insect trees with 599 genera. The eight parameters discussed in the methods were included in our model. These parameters included fusion, fission, polyploidy, and the transitions between monocentric and holocentric chromosomes. Overall, the data had a low variability in the posterior distribution generated by the MCMC (Figure 2). The rates were subtracted from one another to create the differences in rate parameters and graphed in Figure 2. The differences in rates of fusions (descending), fissions(ascending), and polyploidy did not show a difference between monocentric and holocentric chromosomes because all differences in the rates overlapped with zero (Figure 2). This indicates that there are no significant differences among rates of chromosome number evolution based on the type of chromosome.

*Orders Rates:* Rates of chromosome number were tested for each of the 10 orders. Three orders have holocentric chromosomes (Hemiptera, Lepidoptera, Odonata), while the other 7 have monocentric chromosomes (Blattodea, Coleoptera, Diptera, Hymenoptera, Isoptera, Neuroptera, and Phasmatodea). Our analysis of the data described with ascending (fissions) and descending (fusions) rates of chromosome number evolution alone showed a large variability between orders, with Phasmatodea and Lepidoptera having the largest variance (Figure 3a). Despite this variability, there is no pattern in chromosome number evolution associated with monocentric or holocentric orders. This indicates differences among orders, but no relationship between holocentricity and rates of chromosome number evolution.

With the addition of polyploidy into the model, the variance among rates of chromosome number evolution is reduced among the orders (Figure 3b). Despite this, the descending rate was higher in Blattodea, Isoptera, and Phasmatodea. This increase in rates is representative of some, but not all monocentric orders. The order Lepidoptera shows a decrease in ascending and descending rates of chromosome number evolution with the addition of polyploidy into the model. This is most likely driven by the large variation in chromosome number associated with the order.

**Discussion**

Lepidoptera have long been recognized as exhibiting striking variation in chromosome number (Robinson 1971). The hypothesis that holocentricity may allow for rapid changes in chromosome number has been widely viewed as an explanation for the extreme distribution of chromosome number in Lepidoptera (Lukhtanov *et al.* 2018; Hill *et al.* 2019). Our results find little support for this hypothesis. Looking across insects we find that rate estimates for all holocentric and all monocentric clades are very near equal. In fact, when we investigated rates of chromosome number evolution within orders, we found that clades with holocentric chromosomes exhibited both some of the highest and lowest rates observed in insects. We propose that the variation in chromosome number within Lepidoptera is likely better explained by other traits that can impact the rate of chromosome number evolution.

Meiotic drive is one possible driver of changes in chromosome number. We have recently shown that meiotic drive in mammals likely explains variation in rates of chromosome evolution and the distribution of chromosome morphologies (Blackmon *et al.* 2019). Work in mice has shown that meiotic drive is based on the strength of centromeres, where strength is characterized by the ability to express kinetochore proteins and interact with spindle fibers (Chmátal *et al.* 2014). In this system, a fusion with the same centromere strength was shown to be either favored or disfavored depending on the genetic background that it was segregating within. In holocentric chromosomes, since they have a diffuse centromere, meiotic drive is thought to be less likely since multiple sequences must be favored simultaneously to have a strong impact on segregation (Gassmann *et al.* 2012). Therefore, meiotic drive could potentially either increase or decrease rates of chromosome number evolution. This might suggest that in monocentric clades more extreme rate variation would be expected due to the presence or absence of meiotic drive. We note however that we observe roughly equal amounts of variation in our comparison of rates in insect orders.

While fissions and fusions can make small changes to chromosome number, polyploidy events have the potential to lead to large increases in chromosome number much more rapidly. Recent analyses of transcriptome data suggest that insects including Lepidoptera have had multiple large scale genome duplications (Li *et al.* 2018; Li *et al.* 2019; but see Nakatani and McLysaght 2019). Even a small number of polyploidy events depending on their distribution in the tree could lead to much higher variance in chromosome number for a clade. The application of probabilistic models that include polyploidy as a parameter are particularly important if the goal is to understand whether or not fissions and fusions are occurring at different rates among clades (Mayrose *et al.* 2010; Glick and Mayrose 2014; Zenil‐Ferguson *et al.* 2017; Blackmon *et al.* 2019).

Likewise, analyses within any one clade are difficult to interpret for instance the Reduviidae are a group of holocentric hemipterans as such we would predict that they should have higher rates of chromosome evolution. Surveys of this group show that they have very little variation in chromosome number (Poggio *et al.* 2007). However, without a closely related clade with monocentric chromosomes, it is difficult to weight the evidence against the traditional hypothesis for increased rates in holocentric clades. Furthermore, comparisons among studies is also difficult because rates are directly affected by divergence time estimates. We would argue that comparisons of rates are only informative in cases where a single phylogeny with a consistent approach to dating has been applied to both clades with holocentric and monocentric chromosomes.

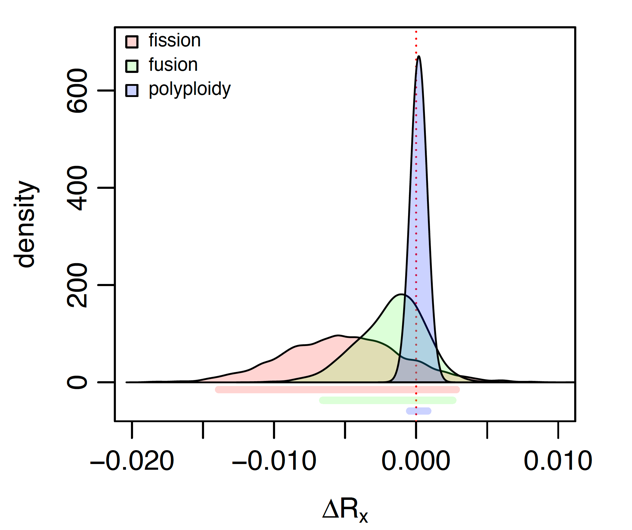
One potentially important cause of variation in rates of chromosome evolution is population size. This idea has its origins in the development of models of chromosomal speciation (White 1978b). White proposed that most chromosomal rearrangements were underdominant and would be more likely to fix in small demes due to drift, and that these changes could then act as reproductive barriers when demes expanded their range and came into secondary contact (White *et al.* 1969). This model of speciation likely is not representative of most diversity and has been shown to be unlikely under a range of potential parameter values (Lande 1984). However, White’s ideas led to an intense focus on predictors of chromosomal variation (Wilson *et al.* 1975b; Bush *et al.* 1977; Bengtsson 1980; Imai *et al.* 1983; Coyne 1984; Larson *et al.* 1984; Petitpierre 1987). Many of these studies suggest that species or clades with small population sizes have higher rates of chromosome evolution. Unfortunately, these were all completed prior to the robust development of comparative methods than can be applied to the evolution of chromosome number across large clades and some compared highly divergent clades. Explicitly modeling the impact of population size on estimated rates of chromosome evolution within clades would be a significant advancement to our understanding of the determinant of rates of chromosome evolution.

Variation in chromosome number is highly heterogeneous across clades – some large clades are nearly static while other closely related clades show striking variation. This observation has been difficult to explain despite a century of investigation. We believe that the approach that we have used here modeling chromosome number and a possible explanatory variable simultaneously offer a way forward to finally answer the recalcitrant question of what causes variation in rates of chromosome number evolution.

**Figures:**



**Figure 1: Phylogeny of type of centromeres and chromosome number.** The black branches represent orders with monocentric chromosomes and the gray branches represent orders with holocentric chromosomes. The colored circles at the tips represent the haploid chromosome number. The color scale is log transformed to allow better visualization of variation in species with low chromosome number.



**Figure 2: Rates of chromosome evolution.** Each curve represents the posterior distribution of the statistic. Where is either fission, fusion, or polyploidy which is indicated by the color of the fill. Positive values of this statistic indicate that holocentric clades evolve more quickly while negative values indicate that monocentric clades evolve more quickly. Below the curves the three lines indicate the credible interval of each statistic. Since all overlap zero we infer no significant difference in rates of chromosome evolution in clades with holocentric and monocentric chromosomes.



**Figure 3:** **Rate of chromosome evolution based on order**. Ascending rates represent the rate of fissions, descending rates represent the rate of fusions, and pol rates represent the rate of polyploidy. (A) Modeling for the rates of chromosome evolution without polyploidy. The removal of polyploidy has kept most of the other species at about the same rates of chromosome evolution, expect for Lepidoptera. Lepidoptera has an increase in both fusion and fission rates. (B) Modeling for rates of chromosome evolution with polyploidy. The addition of polyploidy in the model has decreased the variation of all rates of chromosome evolution. There is no trend between holocentric and monocentric chromosome orders.

**Supplement**



Supplemental Figure 1. Comparison of inferences under alternative backbones. In each plot we show the statistic for the three parameters of interest in our model. We find that regardless of the backbone phylogeny the resulting statistic has a largely similar distribution. Black lines represent the statistic estimate using the Misof backbone while red lines represent the statistic estimate using the Rainford backbone.

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