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

**Authors:** Sarah N. Ruckman12+, Michelle Jonika13+, and Heath Blackmon123

1Department of Biology, Texas A&M University, College Station, Texas, USA

2Ecology and Evolutionary Biology Interdisciplinary Program, Texas A&M University, College Station, Texas, USA

3Genetics Interdisciplinary Program, Texas A&M University, College Station, Texas, USA

+Authors contributed equally and are co-first authors order was decided based on an R script simulating a coin toss

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**Abstract**

Chromosome number is a central aspect of genome architecture that is persistent to changes in chromosomal rearrangement. While changes in chromosome number can trigger speciation and suppress recombination, they are crucial to the progress of adaptive evolution. Insects show a wide distribution of both type and number of chromosomes within their genomes and provide a good system to study chromosome number evolution. Despite a century of research, many fundamental aspects of chromosome number evolution remain a mystery. One example is the dynamics of fissions in holocentric and monocentric chromosomes. Holocentric chromosomes have centromeres that are diffuse and spindle fibers attach along the entire length of the chromosome, while monocentric chromosomes have a single, localized centromere. This difference in arrangement has led to the hypothesis that species with holocentric chromosomes can tolerate higher rates of fusions and fissions as compared to monocentric chromosomes, which may generate chromosomal fragments lacking centromeres. To test for differences in the rates of fusions and fissions, we analyzed data from 12,412 species of insects in both a taxonomic and phylogenetic framework. We found there was no major differences between orders in rates of chromosome evolution when polyploidy was included, however when polyploidy was excluded from our model, Lepidoptera had an increase in the rate of fissions and fusions.

**Introduction**

Chromosome number stability is expected among lineages as shifts in chromosome number can lead to a decrease in fitness. This stability in chromosome number is driven by underdominance of chromosomal rearrangements that cause speciation by reducing the fitness of heterozygotes and suppressed recombination when chromosomal rearrangements are neutral (Faria and Navarro 2010). 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 for simplicity to describe single chromosome number changes. However, in reality fusions decreasing chromosome number capture two different processes at the molecular level. First, Robertsonian translocations followed by the loss of nonessential DNA can decrease chromosome number, and second, fusion of telomeres from two chromosomes followed by inactivation of one of the centromeres (Miga 2017). In contrast, fissions increasing chromosome number can occur through fissions in the centromere region and the gaining of new telomeric sequences (Moretti and Sabato 1984; Garagna, et al. 1995).

The stability of chromosome number makes sense in light of heterozygote disadvantage associated with chromosomal rearrangements and as such they should only fix in a population if there is low effective population size. However, centromeric structure may modulate the fitness effect of fusions and fissions. Since holocentric centromeres are diffuse and spindle fibers attach along the entire length of the chromosome, it has been hypothesized that species with this type of centromere should have little difficulty segregating chromosomes that have experienced fusions or fissions (Malheiros-Garde and Gardé 1950; Greilhuber 1995; Luceño and Guerra 1996). Single chromosome fusion and fission events in holocentric chromosomes do not appear to be underdominant and fragments created during fissions of these chromosomes have been observed to segregate normally during meiosis (Faulkner 1972; Cope 1985). Therefore, holocentricity has the potential to reduce or eliminate selective pressure against and underdominance of chromosomal rearrangements. This could allow for a higher rate of fixation (Escudero, et al. 2012). Despite this prediction, the range of chromosome numbers in holocentric species does not appear remarkably different from those species with monocentric chromosomes. Although tolerance in fragmentation of chromosomes has been observed for some species with holocentric chromosomes, this evolution does not appear to lead to excessive ranges in chromosome number for many species (White 1977; Blackman 1980; Papeschi 1988, 1991; Brown, et al. 1992; Sunnucks, et al. 1996). An example of this is the order Lepidoptera, a group with holocentric chromosomes that contains large diversity in chromosome number (Wolf, et al. 1997). While a few species seem to be tolerant to chromosome rearrangements (Brown, et al. 1992; Robinson 2017), many species exhibit little variation in chromosome number (White 1977; Emmel, et al. 1995; Robinson 2017). Though these observations have been made for some orders, patterns of chromosome number evolution driven by centromere type across large clades have yet to be investigated.

Insects are incredibly speciose and account for much of the variation present in animal species (Mora, et al. 2011). In this study, we used chromosome number and centromere type trait data for insects to test whether holocentric chromosomes have a higher rate of fusions and fissions (Figure 1). Using chromosome data, centromere data and trees from previous studies, we fit a model of chromosome number evolution to our trait data using chromePLUS. This model of chromosome number evolution allows us to test the rate of chromosome number evolution in clades with holocentric and monocentric chromosomes to determine if there are significant differences. Our hypothesis is that clades with holocentric chromosomes will tolerate fusions because the centromere is diffuse across the entire length of the chromosome, therefore each chromosome fragment will be more likely to properly segregate during meiosis.

**Methods**

We downloaded all available chromosome data for insects from a prior study (Blackmon, et al. 2017). This dataset is composed of 12,412 species comprising 376 families and 3,872 genera. The minimum haploid chromosome number is 2 while the maximum chromosome number is 141. There are 3,465 species with holocentric chromosomes and 8,946 species with monocentric chromosomes. For this dataset we collected the haploid chromosome number for each of the species. We additionally have obtained trees from a previous study that can be used for comparative analyses (Church, et al. 2019). We have downloaded two sets of phylogenetic trees based on different backbone trees that will be used for our comparative analysis. Each phylogeny includes 1,726 genera and contains a sample of 100 trees from a posterior distribution. Both of these distributions of trees are to the genera-level and matching this data to our trait dataset we have an overlap of 599 tips (Figure 1). We are fitting our model on each tree from the posterior distribution and we randomly sample trait data when more than one species is available for a genus. This approach allows us to account for uncertainty in the phylogeny and tip states.

Using the trait data and the posterior distribution trees, we implemented a chromosome number evolution model using chromePlus (Blackmon, et al. 2019). This model allows us to determine if the rate of chromosome number evolution is significantly different in clades with holocentric and monocentric chromosomes. We obtained estimates of six parameters: rates of chromosome number increase, fissions, (γ1 and γ2), rates of chromosome number decrease, fusions, (δ1 and δ2), and rates of change in karyotype state, monocentric vs. holocentric (*q*12 and *q*21). We then used an uninformative, unbounded improper prior that assumed that all non-negative values are equally likely for all of the parameters. The Markov Chain Monte Carlo (MCMC) was initialized with parameter values drawn from a uniform distribution from 0 to 8, which is broad but biologically reasonable. Preliminary analysis indicated that MCMC chains reached convergence, however some were sampling non-biologically relevant regions of parameter space. To fix this problem, we added a prior that drew from an exponential distribution with a shape parameter of 0.5. This prior tightened our sampled parameter space and ensured that values that were outside of a biologically relevant region were penalized. 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. We then calculated the Δ*r* statistic or the mean rate difference. For each post-burnin sample we calculated Δ*r* as

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We repeated similar analysis as above for the analysis of orders, however we ran the data twice. Once with polyploidy and once without polyploidy included in the model. We only included orders with more than 20 genera in the analysis. This was to ensure that we had a large enough sample size as well as enough time along the branches to determine the rates of chromosome evolution. In total, we used ten orders for our analysis. We used the same prior as above from an exponential distribution with a shape parameter of 0.5 and initialized our MCMC with parameter values drawn from a uniform distribution from either 0-2 or 3 depending on if we were including polyploidy in our analysis. 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.

**Results**

*Analysis of Monocentric and Holocentric Species*

This analysis is still underway and should be completed by next week. Due to the size of the transition rate matrix sampling from the posterior distribution and even simple calculations of the likelihood for a given parameter set is computationally expensive and takes a long time.

*Analysis of Orders*

We tested the rates of chromosome evolution including polyploidy for the ten orders that contained more than 20 samples for analysis. The range in rates was relatively low and there were no trends in the data (Figure 2a). The addition of polyploidy in the model decreased the variation of all rates of chromosome evolution. The fusion rate, however, was higher in Blattodea, Isoptera, and Phasmatodea, but this does not include all of the monocentric species. The holocentric orders had similar rates to all other orders.

Next, we tested the rates of chromosome evolution without polyploidy for the ten orders. There was more variability in this data, however, there were still no clear trends in the data (Figure 2b). Lepidoptera, however, had an increase in both the fission and fusion rates as compared to the rates with polyploidy. This is most likely driven by large variation in chromosome number associated with the order. The other species that are also holocentric do not show an increase in fission and fusion rates like Lepidoptera.

**Discussion**

**Figures:**



Figure 1: **Phylogeny of type of centromeres and chromosome number.** This order-level phylogeny was created from the backbone of Misof et al. 2014 (Misof, et al. 2014). The black branches represent orders with monocentric chromosomes and the gray branches represent orders with holocentric chromosomes. The colored circles around the phylogeny represents the log-transformed values of the haploid chromosome number whose true range is 2-84.



Figure 2: **Rate of chromosome evolution**. Ascending rates represent the rate of fissions, descending rates represent the rate of fusions, and pol rates represent the rate of polyploidy. (A) Modelling 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. (B) Modelling 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.

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