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**Authors:**

Jeffery P. Demuth1 and Heath Blackmon2,3

*1Department of Biology, University of Texas at Arlington, Arlington, TX 76010 USA*

*2Department of Biology, Texas A&M University, College Station, TX 77843 USA*

*3coleoguy@gmail.com*

**Corresponding Author:**

Heath Blackmon

3258 TAMU

*Department of Biology*

*Texas A&M University*

*College Station, TX 77843 USA*

coleoguy@gmail.com

**Title:**

*Assessing the impact of key ecological and phenotypic transitions on the rate of karyotype evolution: drift drives the evolution of chromosome number.*

Heath Blackmon and Jeffery P. Demuth

**Abstract:**

Chromosomal mutations such as fusions and fissions are often thought to be deleterious, especially when at low frequency in a population (underdominant), and consequently are unlikely to become fixed. Yet, many models of chromosomal speciation ascribe an important role to chromosomal mutations. When effective population size (Ne) is small the efficacy of selection is weakened and the likelihood of fixing underdominant mutations by genetic drift is greater. Thus, it is possible that ecological and phenotypic transitions that modulate Ne facilitate fixation of chromosome changes, increasing the rate of karyotype evolution. We synthesize all available chromosome number data in Coleoptera, and estimate the impact of traits expected to change Ne on the rate of karyotype evolution in the family Carabidae and in 12 disparate genera from across Coleoptera. Our analysis indicates that in Carabidae, wingless clades have faster rates of karyotype evolution, and that genera with high rates of karyotype evolution exhibit multiple traits expected to reduce Ne, including sibmating, oligophagy, winglessness, and island endemism. This suggests that changes in chromosome number are likely fixed by genetic drift despite an initial fitness cost, and that Ne is small enough for chromosomal speciation models to be important drivers of speciation in many Coleopteran clades.

Understanding the evolutionary forces that underlie strong karyotype conservation in some groups (Boyes and Shewell 1975; White 1978) while others are more labile (Kandul et al. 2007; Carbone et al. 2014) has remained elusive despite over 50 years of work (but see: Blackmon and Demuth 2014; Ross et al. 2015; Blackmon et al. 2019). Historically, one of the key issues has been the relative importance of natural selection versus random genetic drift as the principal driver of change in chromosome number.

Hypotheses that propose an important role for positive selection to change chromosome number are easily allied with arguments for the costs and benefits of recombination (Muller 1932; Fisher 1958; Muller 1964; Hill and Robertson 1966; Nei 1967; Lewontin 1971; Felsenstein 1974). Particularly when recombination is limited to a maximum of one crossover per chromosomal arm per meiosis, selection for increased or decreased recombination is expected to propel corresponding changes in chromosome number. Additionally, selection has been argued to favor increased chromosome number in social insects because having more chromosomes increases the average relatedness within a colony - limiting the opportunity for kin-recognition based cheating (Sherman 1979; Templeton 1979) - while simultaneously allowing for high genotypic diversity among sibs which has been shown to benefit colony growth, efficiency, and pathogen resistance (Tarpy 2003). However, comparative analyses comparing solitary and social Hymenoptera have illustrated that the strength of this selection on chromosome number is likely weak (Ross et al. 2015)

Countering the general applicability of positive selection’s role as the agent of karyotype evolution, is the empirical observation that most changes in karyotype are either neutral or deleterious, with many being underdominant (i.e. deleterious when heterozygous)(Max 1995). Underdominance of karyotype changes results from difficulties encountered in meiosis where mismatched chromosomal types do not segregate properly. Such underdominant mutations are only expected to fix when natural selection is overcome by random genetic drift in small populations (Wright 1941; Lande 1979, 1985). Despite the difficulties of fixing underdominant mutations, attempts to ascribe a causal role for karyotype evolution in the speciation process have been abundant (Lewis 1966; White 1978; Bickham and Baker 1979; Grant 1981; Templeton 1981; Baker and Bickham 1986; Rieseberg 2001). Some models assume that karyotype changes themselves are neutral but facilitate diverging local adaptation by sheltering some genome regions from the homogenizing effects of gene flow and recombination. Other models posit that underdominant karyotype changes that are differentially resolved between isolated populations by random genetic drift, may then act directly as isolating barriers upon secondary contact (see Rieseberg 2001 for more detailed review of chromosomal speciation models).

Given that effective population size (Ne) governs the efficacy of natural selection in relation to random genetic drift, one way to distinguish among these two evolutionary forces is to see whether factors associated with differences in Ne are also associated with differences in the rate of karyotype evolution and or the direction of change in chromosome number. More specifically, if karyotype changes are neutral, then their fixation rate should be unrelated to Ne.  However, if karyotype changes are deleterious, then species with small Ne should have faster rates of chromosome evolution as more changes are fixed by drift. In contrast, if selection plays a broad role then we expect species with larger Ne to have faster rates. Furthermore, unlike random genetic drift, which is expected to affect the rate but not the direction of karyotype evolution, selection should be associated with a directional change (i.e. rate changes due to selection consistently cause all species within a lineage to go up or down).

Irrespective of whether selection or random genetic drift is responsible for fixation of new karyotypes, recent analysis of gibbon genomes suggests that an elevated rate of karyotype evolution may also be a signature of elevated chromosomal mutation rate. In the gibbon lineage, a transposable element that preferentially inserts into chromosome segregation genes may be responsible for increased rates of chromosomal rearrangements, thereby helping explain why the karyotypes of these small apes have diversified to a range of 2n=38 - 52 in the past 4 - 6 million years (Carbone et al. 2014) while the rest of the ape lineage varies by only a single autosomal fusion (2n=48 - 46) over more than twice that time (Stanyon et al. 2008; Locke et al. 2011). If increased mutational input is the primary cause of faster karyotype evolution, rather than selection or drift, then increases in the rate of change are not expected to be directional or associated with Ne.

Efforts to relate Ne to karyotype evolution typically use ecological and phenotypic traits as proxies for expected differences in Ne. For instance, all else being equal, winged species should have larger Ne than wingless species because flight increases dispersal distances (*Ne = 4πσ2δ,* where *σ2* is a measure of dispersal distance;(Wright 1946). Other traits that have been used as proxies for Ne include: mating system (inbreeding vs. outbreeding), geographic distribution (island vs. continental), and feeding type (restricted vs varied diet). The distribution of these traits has then been compared to the rate of karyotype change as estimated by scaling the variance in chromosome number to a fossil date for the taxonomic group of interest (Wilson et al. 1975; Bush et al. 1977; Bengtsson 1980; Imai et al. 1983; Larson et al. 1984; Petitpierre 1987; Olmo 2005). These earlier studies suggest that taxa inferred to have highly structured populations also have faster rates of karyotype evolution. In some cases there is also a negative correlation between the estimated rate of karyotype evolution and allozyme heterozygosity levels further supporting the hypothesis that random genetic drift in small populations drives increased rates of chromosome change (Coyne 1984). However, previous work has been limited by not incorporating phylogenies or evolutionary models for chromosome change and using comparisons between highly divergent clades where the underlying mutation rates may be different (i.e. across all vertebrates).

The present study expands previous efforts to understand Ne’s effect on karyotype evolution in two ways. First, we incorporate ecological and phenotypic proxies for Ne into our comprehensive database of Coleoptera karyotypes (Blackmon and Demuth 2014). Second, we employ a modern statistical phylogenetic framework to model the relationship between Ne and chromosome evolution. Coleoptera are an excellent group to study the effect of Ne on chromosome evolution because they exhibit variation in all four types of traits traditionally used as proxies for Ne (Crowson 1981), there is a good phylogenetic scaffold, and the database of karyotypes is extensive (4,797 species).

We show that lineages that have multiple traits associated with reduced Ne (loss of wings, inbreeding, island endemism, restricted feeding) also have significantly faster rates of karyotype evolution. Our findings suggest that random genetic drift is the predominant driver of fast karyotype evolution, as predicted if changes to chromosome number are typically underdominant or mildly deleterious.

Material & Methods:

*Data Collection*

We compiled all available karyotypes from the Coleoptera Karyotype Database (www.uta.edu/karyodb). Coleoptera karyotypes rarely include banding data and are normally reported as the meioformula, consisting of the number of autosomes plus the sex chromosome complement of the male. For these reasons we use the male diploid number as a surrogate for the karyotype and for the remainder of the paper we refer to this as the chromosome number, we describe the rate of change in chromosome number as the rate of karyotype evolution. In 19 cases where multiple values were reported for a species, the mean value was used. Since some studies have shown that chromosome number is not normally distributed (Mank and Avise 2006), we conducted analyses with both the raw chromosome numbers as well as log transformed values, but we found that this does not change our conclusions and therefore we present results based on untransformed data.

Lack of overlap in the available data for karyotype, phylogeny, and Ne related traits resulted in our analysis being subdivided into a family level analysis of Carabidae using presence absence of wings, and a sparser but more phylogenetically diverse analysis of 12 genera using multiple Ne influencing traits. Data for the presence of wings in Carabidae was taken from a previous compilation of natural history data (Larochelle and Lariviere 2003). Species reported as being polymorphic for wings were scored as having equal probability of being either winged or wingless. If wing data was not present for a species in the karyotype and phylogenetic datasets, but other species in the genus were reported, the species was assigned a probability reflecting available data for the genus. For instance, in the genus *Calathus* 64% of species were reported as wingless, so any *Calathus* species not in the trait dataset were assigned a 64% probability of being wingless and 36% probability of being winged.

For each of the 12 genera included in our genus level rate estimates we performed literature searches to score them for the following traits: winged vs. wingless inbreeding vs. outbreeding, island vs. continental distributions, and oligophagy vs. polyphagy. We use the number of times high or low Ne traits occur in each group to classify them into high, medium, or low expected Ne classes (Table 1). To account for phylogenetic uncertainty in our comparative analysis we used 100 trees from the posterior distribution produced in an earlier study (Blackmon and Demuth 2014). Briefly, these trees are based on an analysis of seven genes (16s, 18s, 28s, COI, elongation factor 1, arginine kinase, and wingless) across 1042 taxa in BEAST (v1.7.5; (Drummond and Rambaut 2007; Suchard and Rambaut 2009)). We assumed a lognormal relaxed clock and used normal distributions to place priors on the age of seven nodes; ages were based on previous estimates (McKenna and Farrell 2009).

*Phylogenetic Model Based Analyses*

To test whether chromosome number is under selection to increase or decrease, we first tested whether the groups in our datasets had significantly different absolute number of chromosomes as would be expected for instance if small population size selected for more chromosomes. For the Carabidae data we calculated phylogenetically independent contrasts for both chromosome number and the probability that a clade was winged using the R package APE (Paradis 2011). We then computed Pearson’s correlation coefficient to test for a correlation between chromosome number and probability of being winged. For the genus level data we used the R package GEIGER (Harmon et al. 2008) to compute a phylogenetically corrected ANOVA that tests whether our three Ne classes have significantly different chromosome numbers. We used 1000 simulations to assess significance of the F-statistic (Garland et al. 1993)

Next, for the family and genus level data we used censored rate tests based on Brownian motion models to determine whether the data indicate multiple rates of karyotype evolution as implemented in the R package Phytools (O'Meara et al. 2006; Revell 2012). We compare a model where the continuous trait (chromosome number) evolves at a single rate on all branches, to a model where each discrete state has an independent rate of chromosome number evolution. In the analysis of Carabidae our discrete states were winged and wingless and in the analysis of genera our states were low, medium and high Ne. Conducting the censored rate test required reconstruction of the history of the discrete state across the phylogenies. In the analysis of Carabidae, since wing loss is widely accepted as a derived state within Coleoptera we fixed the root state of the tree as winged (Grimaldi and Engel 2005). For the genus level analyses we fixed the root of the tree as high Ne since the last common ancestor of the included genera is expected to have all high Ne traits. (Grimaldi and Engel 2005). We used an all rates differ Mk model (allows for rates to be different into and out of each state) to estimate the parameters of the transition rate matrix, and used stochastic mapping to assign a state along all branches in the tree. To account for uncertainty in phylogenetic inference and ancestral state reconstruction we performed five stochastic mappings on each of our 100 trees. Since previous work has shown that different rates of chromosome evolution may be occurring in the two major suborders of beetles we analyzed the genera in each suborder separately (Blackmon and Demuth 2014). To explore whether the result of censored rate tests were being driven by exceptional rates in a single clade we also independently estimated the rate of karyotype evolution in each of the 12 genera. The R packages Geiger version 2.03 and Phytools version 0.4-21 were used to reconstruct ancestral states and fit models of chromosome number evolution (Harmon et al. 2008; Revell 2012).

*Scaled Variance Estimates*

Since the lack of overlap between species trait data and existing phylogenetic information causes a large reduction in the number of datapoints in our analysis, we also investigated whether estimating the rate of karyotype evolution without incorporating phylogenies is consistent with the phylogenetic model based approach above. We calculated time scaled coefficients of variation by first locating the oldest available fossil record for each genus of interest in the Paleobiology Database (http://paleodb.org). We then used the fossil ages to scale the coefficients of variation for chromosome number in each taxon (family or genus). To assess consistency between these “scaled variance” estimates and the phylogenetic model based rate estimates, we used a non-parametric correlation analysis (Kendall’s τ). The test was one-tailed since we expect either no significant correlation or a positive one. All tests were considered significant at p-value < 0.05.

Results:

*Data Collection*

We downloaded 4,537 records from the Coleoptera Karyotype Database (uta.edu/karyodb). This included data for all four extant suborders of Coleoptera. Two of these suborders are represented by only one and two karyotypes and thus we focused our analysis on the larger suborders of Adephaga and Polyphaga. These two suborders accounted for 1,224 and 3,310 karyotypes respectively. In Adephaga the number of autosomes ranged from 3 to 34 (mean =15.57±0.14), while in Polyphaga the range was from 1 to 32 (mean =10.63±0.06). Polyphaga exhibits a single mode of nine autosomes, accounting for 952 species or 29% of all Polyphaga records. Conversely, Adephaga is bimodal with concentrations at 11 and 18 autosomes accounting for 276 and 242 species or 23% and 20% respectively (Fig. 1).

*Selection on chromosome number*

If chromosome number comes under directional selection it could increase the rate of evolution in the lineage experiencing selection. However, unlike an increased rate due to random genetic drift, we expect change in response to be directional and result in a correlation between absolute chromosome number and traits we have associated with Ne. For instance, if wing loss consistently selects for increased chromosome number we expect wingless species to have more chromosomes on average. In Carabidae we find no evidence for a relationship between chromosome number and the probability that a species has wings (r= -0.039, t=-0.46, p-value = 0.65). Likewise, in the analysis of genera we found no relationship between Ne classes and chromosome number (F=4.06, p-value = 0.89). These results suggest that selection on chromosome number is not the primary influence on rates of karyotype evolution in these lineages.

*Phylogenetic Model Based Rate Estimates*

Karyotypes for 1065 Carabidae species were available, 136 of these were used in our comparative analysis because they were included in our phylogenetic tree and had data available on flight ability. The censored rate test supports the conclusion that chromosome number evolves at different rates in winged and wingless clades. The single rate model was rejected on all 500 stochastically mapped trees (max p-value < 0.01). Our analyses of Carabidae show that wingless lineages gain and lose chromosomes 6 times faster than their winged relatives. The mean estimate for the rate parameter σ2 in wingless clades was 9.15±0.41 while the mean for winged clades was only 1.51±0.08 (Fig. 2a).

For the analysis of genera we scored each clade for the presence or absence of traits thought to reduce Ne. This allowed us to assign each genus to a class based on expected Ne. Four genera posses none of the Ne reducing traits and we classify these as the high Ne class. Three possess only one of these traits and form the medium Ne class, and five genera (*Calathus*, *Chrysolina*, *Cytronus*, *Dendroctonus*, and *Timarcha*) posses two of the Ne reducing traits, and these form the low Ne class (Table 1).

The censored rate test supports the conclusion that chromosome number evolves at different rates in the different Ne classes. The single rate model was rejected for both datasets on all 500 stochastically mapped trees (max p-value < 0.01). The mean estimate for the rate parameter (σ2) was highest for the low Ne class in both suborders. In Polyphaga the low Ne class had the highest rate of chromosome evolution (0.624±0.098); the medium Ne class exhibited an intermediate rate (0.065±0.013) and the high Ne class had the slowest rate of chromosome evolution (0.017±0.001) (Fig. 2b). In Adephaga rates of karyotype evolution are typically much higher than in Polyphaga, but the low Ne class again had the highest rate of chromosome evolution (24.401±8.627); unexpectedly the medium Ne class exhibited the lowest rate (0.091±0.034) and the high Ne class had an intermediate rate (0.369±0.057) of chromosome evolution (Fig. 2c).

Independent estimates for the rate parameter σ2 for karyotype evolution within each of the 12 genera examined provide insights into the impact of each genus on the results described above. The rate estimates for individual genera ranged from near zero in the genera *Diabrotica* and *Pimelia* to as high as 24.33 (95% CI = 12.17 - 48.66) in *Calathus* (Table 2). In each suborder the low Ne class genera exhibited the highest rates of karyotype evolution. Those genera in the high and medium Ne groups showed similarly slow rates.

*Estimates Based on Scaled Variance*

For each of the 12 genera included in our rate estimate inference, we searched PaleoDB for fossil records. Seven of our target genera had fossil data available; if multiple dates were available we recorded the age of the oldest available record. These ages ranged from a low of 7.24 million years for the genus *Chrysolina* to 150.8 million years for the genus *Cicindela* (Table 2). We calculated the scaled variance of chromosome number for each of the 7 genera by dividing the coefficient of variation for chromosome number by the fossil based age estimates (Table 2). These scaled variances ranged from 0.07 in *Cicindela* to 3.87 in the genus *Chrysolina.* Notably, we find that there is no correlation between the scaled variance based estimates of karyotype evolution and the phylogenetic model based rate estimates (σ2 parameter above) for the 7 genera with overlapping analyses (τ =0.143, p-value=0.773).

Discussion:

Our analyses clearly show that ecological and phenotypic transitions that are expected to reduce Ne are associated with increased rates of karyotype evolution and that this pattern holds independently of potential differences in mutation rate between lineages. The most likely explanation for this pattern is that chromosome number changes are predominantly deleterious while segregating and become fixed by random genetic drift. We do not find support for a directional trend in gain or loss of chromosome number in response to trait evolution that might indicate a signature of selection.

The effect of reduced Ne on accelerated rates of karyotype evolution is dramatic. In Carabidae wingless clades show a 6-fold increase in the rate of karyotype evolution. In Polyphagan genera the difference between low and high Ne genera is >26-fold and in Adephagan genera it is greater than 30-fold. A few notable examples serve to highlight this overall pattern in beetles. First, within the Polyphaga family Curculionidae, our study includes the closely related scolytid genera *Ips* and *Dendroctonus*. Our estimate for the mean rate of karyotype evolution in *Dendroctonus* 0.323 (95% CI = 0.150-0.697) was 3-fold higher than the mean rate estimated in *Ips* 0.117 (95% CI = 0.068-0.201). This marked difference matches expectations based on breeding behavior. *Dendroctonus* is an inbreeding genus producing biased sex ratios and practicing predirspersal sibmating (Grégoire 1988). Meanwhile *Ips* is an outbreeding genus where both males and females disperse, and neither sib-mating nor biased sex ratios have been documented (Kirkendall 1993). These characteristics should lead to smaller Ne in *Dendroctonus* and should allow changes in karyotype to fix more easily even if they are underdominant as theory predicts.

Second, three of the four highest rates of karyotype evolution were observed in wingless, oligophagous genera within the family Chrysomelidae: *Timarcha*, *Cyrtonus*, and *Chrysolina* (0.438, 1.279, 0.558 respectively). In all three genera the mean maximum likelihood estimate (MLE) for the rates of karyotype evolution were higher than the MLE of *Diabrotica* 0.008 (95% CI = 0.004-0.018)*,* a chrysomelid genus lacking any of the small Ne traits.

Finally, the genus *Calathus* provide perhaps the best example of compound effects of phenotype and ecological history on the tempo of karyotype evolution. First, many species in this genus are wingless and thus may be characterized by populations composed of small demes where fixation of karyotype changes should be more likely. However, the exceptionally high rate estimate for karyotype evolution in this genus 24.33 (95% CI = 12.17-48.66) is likely driven by two taxa; *Calathus* *abaxoides* and *C. ascendens* which have respectively the highest and lowest chromosome number in the genus. Interestingly, *C. abaxoides* and *C. ascendens* are 2 of 24 endemic, wingless, *Calathus* species on the Canary archipelago. Both species occur on the island of Tenerife which the genus has colonized in the last 12 million years (Emerson et al. 1999). These species likely experienced an initial population bottleneck during colonization, and the continued restriction to an island has led to a sustained lower Ne than species with continental distributions. While 17-19 autosomes is the norm for most species in this genus, *C. abaxoides* has increased to 27 autosomes while *C. ascendens* has decreased to 10 autosomes. The observation that both the lowest and highest chromosome number are the product of a single recently colonized island further suggests that drift in small populations is responsible for rapid karyotype evolution.

*The Role of Mutation*

Little is known about mutation rate variation in beetles, however we recently hypothesized that differences in the mechanisms of meiosis may provide a mutational basis for differences in the rate of sex chromosome turnover between the two main beetle suborders Polyphaga and Adephaga (Blackmon and Demuth 2014). The present analysis demonstrates that two suborders also have very different overall rates of chromosome evolution (note the difference in scales between figures 2b and 2c) that are consistent with our findings on sex chromosome rates. It is noteworthy however, that despite a >10-fold difference in the “background” rate of karyotype evolution, the pattern where small Ne is associated with relatively rapid karyotype evolution holds within both suborders. Thus, while mutation rate may be a major factor driving the baseline rate of karyotype evolution, our analysis suggests that within a given mutational context, most changes are at least mildly deleterious and become fixed by random genetic drift.

*Comparison with Previous Work*

While our findings are in accord with earlier studies relating Ne to variation in chromosome number, our phylogenetic model based rate estimates are not correlated with time-scaled variance estimates derived similarly to previous work. This inconsistency is worth noting because the scarcity of reliable phylogenies is a limiting factor to conducting analyses in other groups. The lack of consistency between approaches highlights the risk inherent in ignoring the pattern of chromosome evolution over the phylogeny. Theoretically, a scaled variance method could work. However its accuracy will be limited by the extent to which the ages estimated for the groups are both accurate and correlated with the total phylogenetic branch lengths relating the focal taxa. These requirements are unlikely to be met particularly in groups that have relatively incomplete and highly heterogeneous fossil records such as insects. Methods not using a phylogeny will also be misled when the number of records is insufficient to capture the true variance of the groups being studied. The variance in chromosome number across families of Coleoptera can be partly explained by the number of records available (Pearson’s correlation coefficient between family variance and number of records =0.41, p-value=0.008). This suggests that some families have not been sampled sufficiently to capture the true variance of extant species. Applying an evolutionary model for karyotype evolution using a time scaled phylogeny eliminates these issues.

Conclusion

There are almost certainly many individual cases where selection has driven a change in karyotype. For instance it has it has often been suggested that eusocial hymenoptera may through selection for increased recombination have evolved higher numbers of chromosomes. However, when chromosome numbers of solitary and eusocial hymenoptera were analyzed in a comparative framework the results suggested that selection for increased chromosome number is variable across eusocial hymenoptera (Ross et al. 2014). Our results in concert with previous work suggest that most chromosome changes are deleterious (at least while segregating) and that chromosome evolution is largely governed by random genetic drift in small populations. The association we find between factors influencing Ne and evolutionary rate also puts bounds on the selection coefficient of mutations, suggesting that many changes are likely to be only mildly deleterious; otherwise reduced Ne due to ecological and phenotypic transitions in Coleoptera would not be sufficient to drive significant increases in the number of chromosome changes that are fixed by random genetic drift. Our findings also indicate that the distribution of fitness effects of karyotype change is independent of the mutation rate.

More broadly, our work suggests that when species evolve traits, or inhabit locations that restrict population size, the rate of change in chromosome number increases often by orders of magnitude relative to closely related species. By increasing the fixation rate for karyotype changes, speciation mechanisms requiring genome rearrangements become more likely. This should be true for models that assume underdominance of karyotype mutations such as described by White (1978) and more recent models that assume karyotype changes to be neutral such as described by Riesberg (2001). Traditionally chromosomal rearrangements are thought to be more likely to contribute to speciation in plants than animals; possibly due to gene expression in pollen or lack of differentiated sex chromosomes in most plants (reviewed in Rieseberg 2001). Chromosomal speciation has also been suggested to be more likely in mammals than invertebrates due to differences in meiosis (Coyne and Orr 2004). However, given that our results suggest most karyotype changes in beetles are deleterious, models that invoke karyotypic changes acting directly as reproductive barriers seem more widely plausible than they have been considered recently. Unfortunately, the course nature of our karyotype data limits our analysis to mutations such as fusions and fissions that change the number of chromosomes. Future work incorporating genomic data would be useful to determine whether other types of mutations such as inversions, and translocations also reflect a similar pattern.

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**Figure 1.** Haploid number of autosomes in the two major suborders of Coleoptera. The height of the bars reflect the number of records indicating a species with that haploid number and is based on 4,534 records available from www.uta.edu/karyodb.

**Figure 2.** Phylogenetic based estimates of the rate of chromosome number evolution.Rates are the maximum likelihood estimates across 500 stochastically mapped trees. a) Estimates for the family Carabidae under a two-rate Brownian motion model. This dataset included 136 species scored as winged or wingless. b) Estimates under a three-rate Brownian motion model for the suborder Polyphaga with seven genera totaling 149 species. c) Estimates under a three-rate Brownian motion model for the suborder Adephaga with five genera totaling 112 species.

**Table 1. Distribution of traits likely to effect population size.** Traits were scored based only on species included in the analysis and the majority state is reported below. Expected Ne is categorized by the number of traits expected to reduce Ne. High, medium, and low Ne categories were assigned if a clade had zero, one, or two Ne reducing traits respectively. A (+) indicates the high Ne version of a trait and a (-) indicates the low Ne version of a trait.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Genus | Breeding | Feeding | Distribution | Wings | Expected Ne |
| Adephaga | *Bembidion* | + | + | + | + | high |
| *Calathus* | + | + | - | - | low |
| *Cicindela* | + | + | + | + | high |
| *Harpalus* | + | + | + | + | high |
| *Pterostichus* | + | + | + | - | medium |
| Polyphaga | *Chrysolina* | + | - | + | - | low |
| *Cyrtonus* | + | - | + | - | low |
| *Dendroctonus* | - | - | + | + | low |
| *Diabrotica* | + | + | + | + | high |
| *Ips* | + | - | + | + | medium |
| *Pimelia* | + | + | + | - | medium |
| *Timarcha* | + | - | + | - | low |

**Table 2. Genus level phylogenetic and scaled variance based estimates of rate of chromosome evolution.** Dashes indicate those taxa for which reliable fossil dates where unavailable and where not included in comparison of approaches.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clade | NMR1 | MR2 | 95% CI3 | NSCV4 | CV5 | Age | SCV6 | Fossil Reference |
| *Bembidion* | 40 | 0.243 | 0.157 - 0.376 | 230 | 0.06 | 48.6 | 0.12 | (Arillo and Ortuno 1997) |
| *Calathus* | 16 | 24.33 | 12.16 - 48.66 | 36 | 0.12 | 28.4 | 0.42 | (Martynov 1929) |
| *Cicindela* | 27 | 0.462 | 0.271 - 0.788 | 83 | 0.10 | 150.8 | 0.07 | (Weyenbergh 1869) |
| *Harpalus* | 14 | 0.515 | 0.246 - 1.082 | 31 | 0.05 | 65.5 | 0.08 | (Birket-Smith 1977) |
| *Pterostichus* | 15 | 0.088 | 0.043 - 0.180 | 58 | 0.09 | 37.2 | 0.24 | (Wickham 1910) |
| *Chrysolina* | 26 | 0.558 | 0.324 - 0.961 | 64 | 0.28 | 7.24 | 3.87 | (Hopkins et al. 1971) |
| *Dendroctonus* | 13 | 0.323 | 0.150 - 0.697 | 16 | 0.32 | 46.2 | 0.69 | (Labandeira et al. 2001) |
| *Cyrtonus* | 13 | 1.279 | 0.593 - 2.758 | - | - | - | - | - |
| *Diabrotica* | 12 | 0.008 | 0.004 - 0.018 | - | - | - | - | - |
| *Ips* | 26 | 0.117 | 0.068 - 0.201 | - | - | - | - | - |
| *Pimelia* | 29 | 0.002 | 0.001 - 0.004 | - | - | - | - | - |
| *Timarcha* | 30 | 0.438 | 0.264 - 0.726 | - | - | - | - | - |

1 number of taxa used in phylogenetic model based estimate of rates

2 mean rate of chromosome change (phylogenetic approach)

3 95% confidence interval (phylogenetic approach)

4 number of taxa used in calculation of scaled variance

5 coefficient of variation.

6 scaled CV = CV/Age/100MY

Snippets

However, recent analysis of the Gibbon genome suggests that mutation rate may also be an important factor determining the rate of karyotype evolution (Carbone et al. 2014).