CHROMOSOME NUMBER EVOLVES INDEPENDENTLY OF GENOME SIZE IN A CLADE WITH NONLOCALIZED CENTROMERES (*CAREX*: CYPERACEAE)

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The effects of chromosome rearrangement on genome size are poorly understood. While chromosome duplications and deletions have predictable effects on genome size, chromosome fusion, fission, and translocation do not. In this study, we investigate genome size and chromosome number evolution in 87 species of *Carex*, one of the most species-rich genera of flowering plants and one that has undergone an exceptionally high rate of chromosome rearrangement. Using phylogenetic generalized least-squares regression, we find that the correlation between chromosome number and genome size in the genus grades from flat or weakly positive at fine phylogenetic scales to weakly negative at deeper phylogenetic scales. The rate of chromosome evolution exhibits a significant increase within a species-rich clade that arose approximately 5 million years ago. Genome size evolution, however, demonstrates a nearly constant rate across the entire tree. We hypothesize that this decoupling of genome size from chromosome number helps explain the high lability of chromosome number in the genus, as it reduces indirect selection on chromosome number.

KEY WORDS: Agmatoploidy, chromosome evolution, holocentric chromosomes, karyotype, phylogenetic comparative methods.

Genome size in flowering plants is a significant predictor of seed mass, flowering time, and habitat (Grime and Mowforth 1982; Grotkopp et al. 2004; Beaulieu et al. 2007a,b). Genome size varies widely, from 1C = 60.8–1.24 × 10^5 megabases (mb) DNA in flowering plants (Bennetzen and Kellogg 1997; Soltis et al. 2003; Knight et al. 2005), providing ample variance for testing adaptive hypotheses. While large genomes have traditionally been assumed to be maladaptive (Lynch 2007; Grover and Wendel 2010), recent phylogenetic studies demonstrate both increases and de-

creases in flowering plant genome sizes (Wendel et al. 2002; Lysak et al. 2009; Enke et al. 2010; Yotoko et al. 2011). Recent studies also show weak support for the negative correlation between effective population size and genome size that would be expected under a scenario in which genome size inflation is enabled by drift in small populations (Whitney and Garland 2010; Whitney et al. 2010). This suggests that the mechanisms of genome size increase—for example, transposable element (TE) proliferation and polyploidy—and of genome size decrease—for

example, unequal recombination and indirect selection against genome size—may evolve neutrally and perhaps independently on the plant tree of life.

DNA content is often used as a proxy for ploidy in comparative studies of chromosome variation (e.g., Ceccarelli et al. 1995; Suda and Travnicek 2006; Suda et al. 2007), based on the assumption that DNA content will vary with chromosome number at fine phylogenetic scales when chromosome number increases are due to whole- or partial-genome duplications (although see Soltis et al. 2003 for a broader phylogenetic perspective). In organisms in which chromosome evolution is dominated by fission, fusion, and translocations—rearrangements that do not necessarily entail losses or gains of DNA—it is less clear what relationship should be expected between chromosome number and genome size, if any.

Organisms that lack localized centromeres (holocentric organisms; Luceño and Guerra 1996; Mola and Papeschi 2006) have the potential to undergo especially rapid chromosome rearrangements, as meiotic chromosome breakages produce chromosome fragments that all have the potential to segregate normally (Wahl 1940; Mola and Papeschi 2006). Holocentric chromosomes are known from six angiosperm clades, a few algae, several arthropod orders, and nematodes, including the model system *Caenorhabditis elegans* (Heilborn 1924; Godward 1954; King 1960; Flach 1966; Tanaka and Tanaka 1977; Hoshino 1981; Sheikh et al. 1995; Pazy 1997; Perez et al. 1997; Buchwitz et al. 1999; Nokkala et al. 2002; Guerra and García 2004; Wang and Porter 2004; Mola and Papeschi 2006). While the effects of chromosome evolution on speciation have been studied in numerous groups with centric chromosomes (e.g., White 1969; Baker and Bickham 1986; Rieseberg 2001; Kraaijeveld 2010), the role of chromosome rearrangements on lineage or genetic diversification in organisms with holocentric chromosomes has not been well studied (although see Kandul et al. 2007; Hipp et al. 2010).

Among holocentric organisms, the flowering plant genus *Carex* has been of particular interest to evolutionary biologists (Stebbins 1950; Stebbins 1971; Grant 1981; Bell 1982). At more than 2000 species worldwide (Reznicek 1990), *Carex* is the most diverse genus of angiosperms in the temperate zone and the second or third most diverse worldwide (Judd et al. 2007). It exhibits remarkable chromosomal diversity, with every number between $n = 6$ and $n = 48$ represented by at least one species (Davies 1956; Roalson et al. 2007; Roalson 2008). Polyploidy is rare in the genus, and chromosome number changes are predominantly due to fission, fusion, or translocations (reviewed in Hipp et al. 2009).

To date, three comparative studies of chromosome number and genome size have been conducted in order Cyperales, which contains the genus *Carex* (Nishikawa et al. 1984; Roalson et al. 2007; Zedek et al., 2010). The last of these studies was conducted in *Eleocharis*, a sedge genus that exhibits a high rate of polyploidy (Zedek et al., 2010); in that study, 1C genome size was reported

as ranging from 1C = 0.42 to 9.00 picogram (pg) DNA, with a strong positive correlation between chromosome number and genome size. The first two were dominated by *Carex* species and demonstrated a strong negative correlation between chromosome number and genome size, suggesting that the balance between genome inflation and shrinkage may evolve in response to chromosome number. However, they were conducted in a nonphylogenetic framework, raising questions as to whether the Type-I error rate on these regressions is biased (Rohlf 2006). At the same time, recent investigation at the intraspecific level (*C. scoparia*, $2n = 56$–70) demonstrates a flat relationship between chromosome number and genome size (Chung et al. 2011). These studies in combination suggest a gradient in the dynamics of genome size evolution: among recently diverged populations, we expect to find a flat relationship between chromosome number and genome size as a consequence of the mode of chromosome evolution in holocentric chromosomes, while at deeper phylogenetic scales, a negative relationship between chromosome number and genome size may result from selection on genome size as a function of chromosome number.

In this study, we investigate in a phylogenetic context the correlation between chromosome number and genome size in *Carex* subgenus *Vignea*, a clade of ca. 300 species (Ford et al. 2006), with a focus on *Carex* section *Ovales*, a predominantly New World clade of 90 species (Hipp et al. 2006, 2007). We also investigate whether transitions in the rate of chromosome number and genome size evolution occur on common branches of the sedge phylogeny as a way of ascertaining whether chromosome evolution and genome size evolve independently. Understanding these dynamics of chromosome evolution is the first step to understanding how chromosome evolution affects genetic and lineage diversification in one of the world's largest flowering plant genera.

Materials and Methods
SAMPLING

Eighty seven of the approximately 300 species in *Carex* subgenus *Vignea* were sampled for DNA content using flow cytometry (FCM) (Appendix S1; see flow cytometry methods below). DNA sequences were obtained from NCBI GenBank or generated for this study (Appendix S2), and chromosome numbers were summarized from the literature or reported for the first time in this article (Appendix S3). Species sampled represent fourteen sections of the approximately 28 recognized in subgenus *Vignea* (Ford et al. 2006) and 43 of the ca. 90 species in *Carex* section *Ovales*, which is the largest strongly supported clade within subgenus *Vignea* (Hipp et al. 2006). We focused on the *Vignea* clade for purposes of concentrating our taxon sampling, as opposed to sampling across the entire genus of more than 2000 species. While the relatively

narrow phylogenetic scope of our study may decrease our power to detect a correlation between chromosome number and genome size across the genus, it increases our power to detect transitions in evolutionary rate at fine scales and to localize transitions on the *Carex* tree. All voucher specimens for DNA content, new chromosome numbers, and new DNA sequences were deposited at the Morton Arboretum herbarium (MOR). Sequence alignments and consensus tree are deposited in TreeBASE (S12042).

PHYLOGENETIC DATA AND ANALYSES

DNA was extracted from fresh, frozen, or silica-dried leaves using DNeasy kits (QIAGEN, Valencia, CA), and nrDNA regions (ITS and ETS) were amplified and sequenced using standard protocols (Hipp et al. 2006). Sequences were analyzed on an ABI 3730 in the Field Museum's Pritzker Laboratory (Chicago, IL). Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted under a relaxed molecular clock model in BEAST version 1.5.4 (Drummond and Rambaut 2007). Phylogenetic MCMC analyses were conducted under the GTR+I+Γ model, assuming an uncorrelated relaxed lognormal clock and Yule tree prior, sampling trees every 1000 generations for 10 million generations. The substitution model was selected based on Akaike information criterion (AIC) values calculated in MrModeltest 2.3 (Nylander 2004). The GTR+I+ Γ model was strongly supported (AIC weight = 0.7620). Analyses reached stationarity after 2 million generations in several independent analyses, as assessed by inspection of topologies and likelihoods in the posterior sample. The tree presented in this article (Fig. 1) is a majority-rule consensus of 8000 post-burn-in trees, with node depths on the consensus tree estimated as the average over trees possessing that node using TreeAnnotator version 1.5.4 (Drummond and Rambaut 2007). The posterior sample was rarefied to 200 trees by subsampling at even intervals for comparative analyses; this treeset is deposited at Dryad (doi:10.5061/dryad.1c1m4qc6). Most analyses reported in this article are presented as means followed by the 0.025 and 0.975 quantiles over the MCMC subset; hereafter in the article, these quantiles are referred to as the "phylogenetic uncertainty interval." (See discussion of model uncertainty in section Analysis of Chromosome Number and Genome Size below.) Because fossil data for *Carex* are poor and difficult to place accurately (see discussion in Egorova 1999; Escudero et al. 2010), clade ages were estimated in a previous study (Hipp et al. 2010) using a molecular clock calibration based on ITS1 and ITS2 data only. This dating uses previously published ITS calibrations for herbaceous plants (Kay et al. 2006) to estimate node ages. Node age means and confidence intervals (CIs) were estimated by drawing rates at random from absolute rates reported for herbaceous angiosperms (Kay et al. 2006) and node ages drawn at random from trees visited in the BEAST MCMC analysis (Drummond et al. 2006; Drummond and Rambaut 2007) using the morton project (http://r-forge.r-project.org/projects/morton/) in R version 2.6.2 (R Development Core Team 2004). While none of the angiosperm calibrations used (Kay et al. 2006) are sedges or graminoids, the range of calibrations included in this analysis produces a wider CI than we would expect to find with accurately placed fossil calibrations, and our interpretation of these ages is thus tentative.

CHROMOSOME NUMBER AND GENOME SIZE

Chromosome numberswere summarized from literature (Appendix S3; Hipp 2007; Roalson 2008) or counted for this article using methods described in previous studies (Hipp et al. 2010; Chung et al. 2011). In this study, genome size (GS, 1C DNA content) refers to the amount of DNA in the unreplicated gametic nucleus (Greilhuber et al. 2005). Throughout the article, we interpret DNA content as genome size, based on the assumption that there have been no recent polyploidy events among the species identified (see discussions in Hipp et al. 2009 and references therein). 1C values were estimated from total somatic DNA content using FCM following standard protocols, with modifications for *Carex* as described previously (Chung et al. 2011). Briefly, young, fresh leaves (15–20 mg) were prepared in a De Laat buffer (de Laat and Blaas 1984, as modified in Kron and Husband 2009) following Doležel et al. (2007). An internal size standard of *Raphanus sativus* "Saxa" (1C = 0.555 pg DNA; Doležel et al. 1992; Doležel and Bartoš 2005) was used for all analyses, and propidium iodide (50 μg/mL) stained nuclei were analyzed on a BD LSRII flow cytometer (BD Biosciences, San Jose, CA). All analyses presented in this study were conducted on log-transformed data, following previous work in the genus (Hipp 2007; Escudero et al. 2010) and based on the expectation that increases in chromosome number and genome size will be proportional to total chromosome number and genome size, respectively. Appendix S1 presents voucher specimens, means and standard deviations of genome size, coefficient of variation (CV), and relative standard error (RSE) values. Individual genome size datapoints used to generate these summary data are archived as a separate table in Dryad (doi:10.5061/dryad.1c1m4qc6).

ANALYSIS OF CHROMOSOME NUMBER AND GENOME SIZE

Chromosome data were analyzed as the mean of chromosome number reported in the literature or new for this study, weighted by the number of populations for which each count was made, where population numbers were available (Appendix S3). Standard error for each count was estimated using population as the sampling unit. Genome size was estimated as the mean of individual counts for each individual, and the mean of individuals for each species. Standard error for each genome size estimate was estimated using individuals as the sampling unit. Variables were

Figure 1. Bayesian majority-rule consensus tree with chromosome number (2*n*) and DNA content (1C). Consensus tree is based on 8000 post-burn-in trees visited in a single MCMC relaxed-clock phylogenetic analysis of combined nuclear ribosomal DNA data. The thickest branches represent posterior probabilities ≥0.95, intermediate braches represent posterior probabilities ≥0.7, and thinnest branches have posterior probabilities <0.7. Diploid chromosome number (filled circles, 2*n*) is displayed as the mean chromosome number for the taxon (large circles), weighted by the number of populations from which counts were reported, with one point for each count reported in the taxon (small circles). DNA content (open squares, 1C in picograms DNA) is displayed as the mean value ± the standard error of the mean (solid horizontal line).

rescaled by the standard deviation of the sample for regression, so that regression coefficients reported here are in estimated standard deviation units, although data are plotted in original units. Based on previous work on *Carex* section *Ovales* suggesting a shift in diversification rate at the base of section *Ovales* (Hipp et al. 2006), regressions were conducted on the entire dataset (87 taxa), section *Ovales* only (43 taxa) and *Carex* subgenus *Vignea* excluding section *Ovales* (44 taxa). This renders one of our analysis sets nonmonophyletic (*Vignea* excluding *Ovales* is paraphyletic to *Ovales*). However, for our analyses, we are most concerned with estimates of regression coefficients in a set of general linear models, using the branch lengths to estimate correlation structure of the error terms. In this case, there is no reason to expect paraphyly to bias the regression coefficients.

The effects of chromosome number on genome size were estimated using phylogenetic linear regression, with variances assumed to be equal and covariances estimated from the phylogeny (reviewed in Felsenstein 2004, chap. 23; see also Rohlf 2006; Revell 2010). Regression models included Pagel's λ, a rescaling of the covariance matrix that estimates the phylogenetic dependence in the regression residuals (Pagel 1999). Estimating λ jointly as a part of phylogenetic regression has been shown to minimize variance in regression coefficient estimates (Revell 2010). Models were fitted by maximizing the restricted log likelihood (REML), which yields minimum-bias estimators for regression parameters under most conditions (Appendix 1 of Ives et al. 2007). All regressions were performed on 200 trees evenly subsampled from one BEAST MCMC phylogenetic analysis, with Pagel's (1999) λ optimized separately on each tree. Most parameter estimates in this study are presented as means over the tree sample, followed by the 95% phylogenetic uncertainty interval. For the model parameter of greatest interest in our study, the regression slope (β_1), we approximate a CI that integrates over model uncertainty and phylogenetic uncertainty. Model uncertainty for each regression on each tree is calculated as $\beta_1 \pm 1.96$ the standard error of β_1. To integrate over model uncertainty and phylogenetic uncertainty, we calculate this interval for all 200 trees in the MCMC subsample, then report the mean value of β_1 over trees followed by the 0.025 and 0.975 quantiles for the resulting upper and lower bounds of the model confidence over all 200 trees from the MCMC subsample. The percent of variance in genome size that is explained by chromosome number was estimated using R^2, calculated following Judge et al. (1985), formula 2.3.16. Significance of the model fit was assessed in two ways: as the *P*-value reported under REML estimation, and using small-sample Akaike information criterion (AIC*c*) weights to compare the regression model fit against a model in which there is no predictor (Burnham and Anderson 2002). Comparing these models, AIC*c* weight represents the relative support for chromosome number being a predictor of genome size; AIC*c* weights > 0.5 provide stronger

support for the three-parameter predictive model (of form $Y = mX + b + \varepsilon$) than for the two-parameter nonpredictive model (of form $Y = b + \varepsilon$). The AIC*c* weights were based on maximum likelihood (ML) estimates following standard formulas (Pagel 1999; O'Meara et al. 2006; Revell 2010), with Pagel's λ estimated jointly with regression coefficients separately for each tree in the MCMC analysis. Outliers—here interpreted as datapoints that have disproportionately large effect on the regression—were detected using a generalization of Cook's (1977) distance (hereafter Cook's D_i, following Cook's nomenclature), an estimate of the effect of each datapoint on the estimate of regression coefficients (β) in least-squares linear regression. The generalized Cook's D_i considers how the deletion of the *i*th observation influences the model while incorporating phylogenetic relatedness. Cook's D_i was calculated using an R script written and provided by J. Beaulieu (pers. comm.).

In a previous study (Hipp 2007), we had identified an increase in the rate of chromosome evolution at the base of the eastern North American clade of *Carex* section *Ovales* (Fig. 1, "Eastern North American clade"). In the current study, we hypothesized a shift in the rate of evolution at the base of section *Ovales* as a whole, which has been identified previously as having a high rate of lineage diversification (Hipp et al. 2006; Escudero et al. 2012), as well as at the base of the eastern North American clade. We evaluated this hypothesis using two approaches, both based on a rescaling of branch lengths on the original tree and estimation of the likelihood of the rescaled branches given the character data at the tips (O'Meara et al. 2006). The first approach is a reversible-jump MCMC (rjMCMC) method that does not condition on either the placement or number of rate changes on the tree (as implemented in the AUTEUR package; Eastman et al. 2011). The rjMCMC chain traverses a parameter space that varies in the placement of rate-change breakpoints on the phylogeny and the rates of tree partitions separated by those breakpoints. The posterior distribution thus samples from a distribution of rates for each branch that integrates over uncertainty in the number and position of rate-change breakpoints (model uncertainty) as well as uncertainty in the rates themselves (parameter uncertainty). We summarize the analysis by color coding each branch according to the model-averaged rate estimate for that branch, where the model-averaged rate estimate is the average over post-burn-in portions of the rjMCMC run; and by indicating for each node the posterior probability of a shift having occurred at that node (Fig. 4). Standard rjMCMC methods are used for the proposal mechanism and acceptance ratio (Green 1995; Huelsenbeck et al. 2004; Eastman et al. 2011), and the AUTEUR package defaults were accepted for all analysis parameters. Three independent runs of 4,000,000 generations each were conducted on the single consensus tree from the relaxed-clock MCMC phylogenetic analysis and assessed visually for convergence. The first 1,000,000

generations of each run were discarded as burn-in. Statistics reported are averaged over the post-burn-in portions of the three runs, pooled.

The other approach we took was to evaluate our previous hypotheses in a likelihood framework (O'Meara et al. 2006), in which we tested the hypothesis of no rate change relative to hypotheses of rate change at the base of *Carex* section *Ovales*, which we hypothesized would exhibit a higher rate of chromosome evolution associated with high lineage diversity; as well as at the base of a predominantly eastern North American clade embedded within *Ovales* (Fig. 1, "Predominantly eastern North American clade") identified as a shift point in the rjMCMC analysis described above. The inclusion of this latter clade allowed us to evaluate whether the rate change identified using rjMCMC on a single tree is robust to uncertainty in branch length reconstructions. In a follow-up analysis, we compared the relative support for a shift occurring at the base of the eastern North American clade identified as being a point at which the dynamics of chromosome evolution change within section *Ovales* (Hipp 2007) versus the predominantly eastern North American clade identified by the rjMCMC analysis in the current study to assess whether the Bayesian (rjMCMC) and information-theoretic (AICc) approaches are congruent in their isolation of a shift in rate to a particular branch, and how branch length variation among MCMC trees affects localization of a rate change between adjacent branches. We used O'Meara's noncensored approach by first assigning all branches to a tree partition. The rate of trait evolution in any tree partition other than the partition that holds the root of the tree was allowed to vary by multiplying all branches in each tree partition except for the root partition by a scalar and estimating the likelihood of the rescaled tree using generalized least squares (GLS; formulas follow O'Meara et al. 2006). Scalars were optimized for each tree partition by maximizing the log likelihood. On each of 200 trees subsampled from the relaxed-clock MCMC phylogenetic analysis (not to be confused with the rjMCMC analysis of character evolution), four models were compared: the single-rate ($K = 2$ parameters) model; a two-rate model ($K = 3$) in which the rate of evolution in *Ovales* differs from the remainder of the tree; a two-rate model ($K = 3$) in which the rate of evolution of the predominantly eastern North American clade embedded within *Ovales* differs from the remainder of the tree; and a three-rate model ($K = 4$) in which the rate of evolution shifts at the base of *Ovales* and at the base of the predominantly Eastern North American clade. Models were compared using AICc weights to estimate the model-averaged change in rate at the base of the *Ovales* clade for each tree in the relaxed-clock MCMC subsample. Model-averaged rates were estimated by averaging the relative rate estimated for each branch under all four models, weighted by the AICc weight for those models. Model support and model-averaged rates were estimated for the

200 trees subsampled from the MCMC phylogenetic analysis (the same trees used in GLS regressions). It is important to note that our taxon sampling represents approximately half of the species diversity of *Carex* section *Ovales*, but approximately one-quarter of subgenus *Vignea* outside of *Ovales*. This sampling bias will tend to decrease the mean node depth within *Ovales* and may bias our rate estimates upward in that clade through increased probability of detecting recent divergences in *Ovales* that are characterized by large karyotype or genome size differences. We consequently consider the analyses presented here a valid evaluation of the relative shifts in rate of genome size and chromosome evolution, but not an unbiased estimate of absolute changes in the rate of chromosome and genome size evolution on our phylogeny.

Analyses were conducted using the R packages ape (Paradis et al. 2004), geiger (Harmon et al. 2008), nlme (Pinheiro et al. 2009), morton (http://r-forge.r-project.org/projects/morton), and auteur (Eastman et al. 2011).

Results
PHYLOGENETIC RECONSTRUCTION

The phylogeny supports placement of *C. gibba* as sister to the rest of the subgenus, in agreement with a previous phylogeny of the group based on nrDNA data (Fig. 1; Ford et al. 2006). Phylogenetic relationships within the subgenus are well resolved, having sections *Glareosae*, *Stellulatae*, and *Ovales* as monophyletic, although the other sections fail to support traditional sectional classification. As found in previous studies, *C. illota* is excluded from the *Ovales* clade (Ford et al. 2006; Hipp et al. 2006). Dating of the *Ovales* clade based on ITS calibrations from previous studies (Kay et al. 2006) indicates that the crown age is approximately 4.79 million years (95% CI = 1.77–10.7 million years); this date for the group is also supported by calibration of a broader *Carex* phylogeny using fossil data (Escudero et al. 2012).

PATTERNS OF VARIATION AND PHYLOGENETIC SIGNAL

Chromosome numbers in *Carex* subgenus *Vignea* range from $2n = 46$ (*C. infirminervia* and *C. muehlenbergii*) to $2n = 104$ (*C. nubigena*), a 2.3-fold range of variation, with a mean of 64.9 ± 11.6 (SEM). Genome size ranges from 1C = 0.285 pg (*C. nubigena*) to 1C = 0.483 pg (*C. gibba*), a 1.7-fold range of variation, with a mean of 0.388 ± 0.042 pg DNA (Fig. 1). Within section *Ovales*, genome size ranges from 1C = 0.330 to 1C = 0.375 pg (a 1.34-fold range of variation) while diploid chromosome numbers range from $2n = 48$ (*C. brevior*) to $2n = 86$ (*C. macloviana*), a 1.79-fold range of variation (Fig. 1). Both genome size and chromosome number exhibit high phylogenetic signal

Table 1. Phylogenetic models of genome size (log 1C) regressed on chromosome number (log 2*n*). All parameters and statistics were estimated under restricted maximum likelihood (REML) except for AICc weight, which was based on log likelihoods calculated under ML. The standardized regression coefficient (β1) is presented as the mean estimate over trees, followed by a 95% confidence interval that integrates over both model uncertainty and phylogenetic uncertainty (see Methods). Estimates of Pagel's λ, R^2, *P*-value, and AICc weight are presented as the mean followed by 95% phylogenetic uncertainty interval over 200 trees subsampled from the MCMC phylogenetic analysis. AICc weight represents the evidential support for the model in which chromosome number predicts genome size (a model of form $Y = mX + b + \varepsilon$) relative to the model in which genome size is normally distributed with no predictor (a model of form $Y = b + \varepsilon$).

	β	*P*-value	R^2	Pagel's λ	AICc weight
Ovales	0.061 (−0.017, 0.135)	0.093 (0.043, 0.148)	0.069 (0.050, 0.094)	0.328 (0.017, 0.616)	0.593 (0.485, 0.737)
Non-*Ovales*	−0.211 (−0.403, −0.008)	0.014 (0.002, 0.046)	0.147 (0.091, 0.204)	0.862 (0.782, 0.933)	0.783 (0.599, 0.920)
All taxa	−0.021 (−0.134, 0.079)	0.617 (0.256, 0.963)	0.004 (0.000, 0.014)	0.965 (0.943, 0.984)	0.283 (0.250, 0.399)

in subgenus *Vignea* as a whole ($\lambda_{1C} = 0.951$, $\lambda_{2n} = 0.886$) and *Vignea* excluding section *Ovales* ($\lambda_{1C} = 0.950$, $\lambda_{2n} = 0.992$), but lower phylogenetic signal in section *Ovales* ($\lambda_{1C} = 0.314$, $\lambda_{2n} = 0.428$).

CORRELATION BETWEEN CHROMOSOME NUMBER AND GENOME SIZE

Phylogeneticregression demonstrates a weakly positive relationship between chromosome number and genome size in *Carex* section *Ovales* (GLS $\beta_1 = 0.061$, $R^2 = 0.069$, $P = 0.093$, AICc weight for the predictive model $= 0.593$; Table 1) and a significantly negative relationship in subgenus *Vignea* excluding *Ovales* (GLS $\beta_1 = -0.211$, $R^2 = 0.147$, $P = 0.014$, AICc weight for the predictive model $= 0.783$; Table 1, Fig. 2). Cook's D_i is less than 0.32 for all taxa except for *C. nubigena* ($D_i = 1.56$, phylogenetic uncertainty interval $= 0.940$, 2.31). Following Cook's (1977, 1982) guideline of interpreting $D_i > 1$ as indicating datapoints of disproportionately strong effect, we removed *C. nubigena* for a second round of GLS regressions. Analysis with this datapoint excluded suggests a nonsignificant relationship between chromosome number and genome size (GLS $\beta_1 = -0.035$, $R^2 = 0.006$, $P = 0.670$, AICc weight for the predictive model $= 0.251$). Although we have no reason to doubt the data for *C. nubigena*, we consider the disproportionately strong effect of this single datapoint to raise some questions regarding the hypothesis of a negative correlation between genome size and chromosome number for the taxa we analyzed.

The relationship is flat when all data are analyzed (GLS $\beta_1 = -0.021$, $R^2 = 0.004$, $P = 0.617$, AICc weight for the predictive model $= 0.283$; Table 1). This contrasts with the strong support for a negative relationship reported in previous work (Nishikawa et al. 1984) and found in the same datasets reported here, when analyzed using ordinary (nonphylogenetic) least-squares (OLS) regression (for all taxa, OLS$\beta_1 = -0.404$ (−0.488, −0.321), $R^2 = 0.523$, $P = 2.62 \times 10^{-15}$; for *Vignea* excluding *Ovales*, OLS $\beta_1 = -0.374$ (−0.506, −0.242), $R^2 = 0.437$, $P = 1.02 \times 10^{-06}$). These results are weakly significant
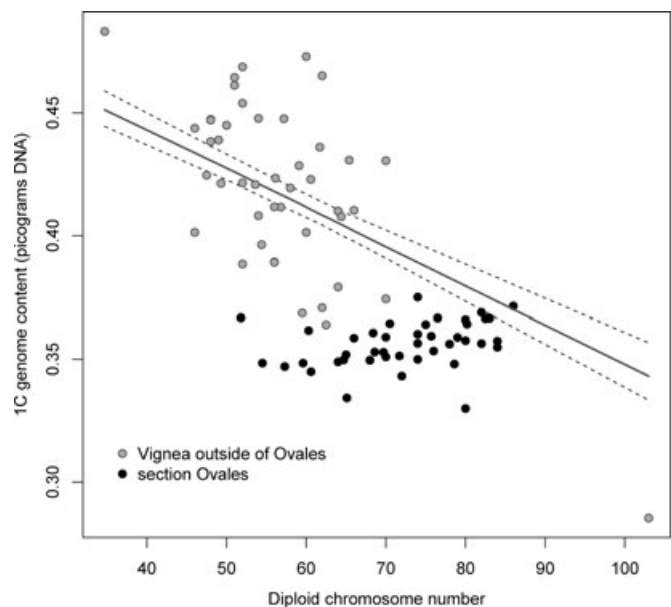

Figure 2. Generalized least-squares regressions of DNA content (1C) on chromosome number (2*n*) in section *Ovales* and subgenus *Vignea* excluding *Ovales*. Regressions were performed as described in the text, with Pagel's λ estimated separately for each tree. Regression line is significant at the 0.05 level (see text). Dashed lines are the phylogenetic uncertainty interval on the regression slope, based on analysis over 200 trees subsampled from the MCMC phylogenetic analysis.

even when the two most influential taxa (*C. gibba*, $D_i = 0.313$; and *C. nubigena*, $D_i = 1.56$) are excluded from the *Vignea*-excluding-*Ovales* analysis ($R^2 = 0.146$, $P = 0.013$). *Ovales* alone shows a weak positive correlation (OLS $\beta_1 = 0.067$ (0.003, 0.131), $R^2 = 0.099$, $P = 0.040$).

Assuming 980 mb DNA per 1 pg DNA (Bennett et al. 2000), phylogenetic regression coefficients for the raw data imply a loss of 3.11 mb DNA per additional chromosome pair in *Carex* subgenus *Vignea* outside of section *Ovales*. In both *Ovales* and subgenus *Vignea* outside of *Ovales*, average DNA content per chromosome decreases or remains flat with increasing

chromosome number, as expected if chromosome number increases are a function of chromosome fission rather than duplication (data not shown).

RATES OF EVOLUTION OF CHROMOSOME NUMBER AND GENOME SIZE

Three independent rjMCMC runs were conducted for each dataset (1C and 2*n*), and as replicate runs gave qualitatively similar results for each dataset, results were pooled across runs. Plots of log likelihood converged almost immediately for the chromosome data, and the first 25% of generations were excluded as burn-in by default. Log-likelihood plots for the genome size rjMCMC runs showed inconsistency across replicates, with one analysis of 4,000,000 generations reaching stationarity almost immediately at a mean log likelihood of 135.23, while the other two decreased continuously to a mean log likelihood <20. A longer run of 10,000,000 generations also reached stationarity almost immediately at a mean log likelihood of 136.13. Estimates of transitions in the rates of genome size were consistent across runs, which were consequently pooled for analysis.

The rjMCMC analysis of rate changes suggests that chromosome evolution undergoes a significant increase in rate within *Ovales* (Fig. 3), and that the location of that shift in rate is near the base of the predominantly eastern North American clade. However, identifying the precise location of a shift using the rjMCMC method is not always possible. The model-averaged rate of chromosome evolution increases 1.80-fold at the base of the predominantly eastern North American clade, but the model-averaged rate in the branches leading to the basal five taxa of this clade (all western North American taxa) is not significantly different than model-averaged rates over the remainder of phylogeny outside of the predominantly eastern North American clade ($P = 0.299$ based on 60,000 comparisons drawn at random from the posterior distributions for rates in the branches making up the predominantly eastern North American clade on one hand and the those making up the remainder of the tree outside of the eastern North American clade on the other). The model-averaged rate in the eastern North American clade itself, however, is significantly greater than the remainder of the tree ($P < 0.001$). In contrast, the model-averaged rate of genome size evolution is constant across nearly the entire phylogeny of *Carex* subgenus *Vignea*, with the only changes in rate occurring in isolated individuals (terminal branches) across the tree and between the two outlier taxa at the base of the tree, which exhibit a 6.90×10^7-fold higher rate of genome size evolution than the branches immediately descending from them (Fig. 3).

Using the hypothesis-testing framework introduced by O'Meara et al. (2006), the strongest supported models for the rate of chromosome evolution are the model that allows for a single shift in rate of chromosome evolution at the base of the predomi-

nantly eastern North American clade embedded in section *Ovales* (AIC*c* weight $= 0.728$ [phylogenetic uncertainty interval $= 0.670$, 0.750]); or two shifts, one at the base of section *Ovales* and one at the base of the predominantly eastern North American clade (AIC*c* weight $= 0.268$ [0.246, 0.325]). The most poorly supported models entail no change in rate (AIC*c* weight $= 3.44 \times 10^{-5}$ [3.02 $\times 10^{-29}, 2.20 \times 10^{-4}$]) or a shift only at the base of section *Ovales* (AIC*c* weight $= 0.005$ [$1.44 \times 10^{-8}, 3.30 \times 10^{-2}$]). All four models evaluated on the genome size data range between 0.173 and 0.336 AIC*c* weight, suggesting that no model is conclusive for genome size (AIC*c* weights: *Ovales*-change model $= 0.173$; no-change model $= 0.231$; three-rate model $= 0.259$; eastern North American change model $= 0.336$). The model-averaged change in rate of chromosome evolution in the basal branches of section *Ovales* is 0.996 times the background evolutionary rate averaged over MCMC trees (phylogenetic uncertainty interval $= 0.896$, 1.14), indicating stasis; the model-averaged change in rate within the predominantly eastern North American clade is 10.7 (3.71, 49.2) times the background rate. In contrast, the model-averaged change in rate of genome size evolution for the predominantly eastern North American clade is 3.62 (0.500, 24.2) times the background rate (Fig. 4). However, the distribution of shifts in rate of genome size evolution exhibits a long tail of large values over MCMC trees: the median change in rate of genome size evolution at the base of the predominantly eastern North American clade over MCMC trees is 1.13 times the background rate, while the median increase in the rate of chromosome evolution over the same trees is 6.93 times the background rate, suggesting stasis in genome size evolution but an increase in the rate of chromosome number evolution.

In a second analysis, comparing the relative support for a shift occurring at the base of the eastern North American clade identified previously as being a point at which the dynamics of chromosome evolution change within section *Ovales* (Hipp 2007) versus the predominantly eastern North American clade identified by the rjMCMC analysis in the current study, we find in fact that models allowing a shift in rate only in the eastern North American clade are more strongly supported (AIC*c* weight $= 0.747$ [0.408, 0.969]) than models allowing a shift in rate only at the base of the predominantly eastern North American clade (AIC*c* weight $= 0.070$ [0.000, 0.287]) or shifts in rate at the base of both the predominantly eastern North American clade and the eastern North American clade (AIC*c* weight $= 0.184$ [0.000, 0.320]; Fig. 5). We recover the same relative support for alternative hypotheses when we analyze just the single consensus tree used in the rjMCMC analysis (shift in rate at the base of the ENA clade only: AIC*c* weight $= 0.701$; shift in rate at the base of the predominantly ENA clade only: AIC*c* weight $= 0.019$). Moreover, in models in which a change is permitted at both nodes, the branches leading to the five western North American taxa of the
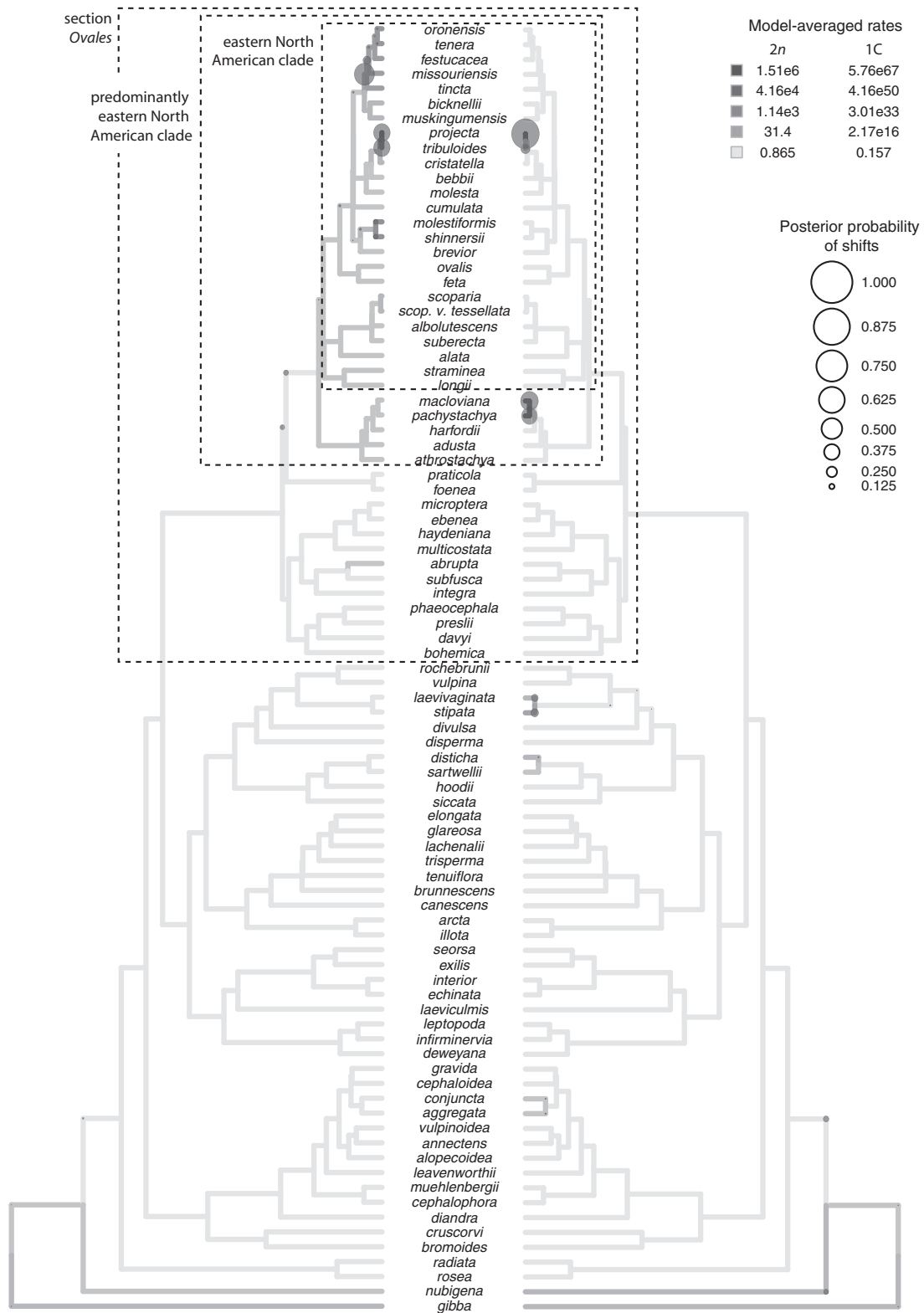
Figure 3. Reversible-jump MCMC analysis of shifts in rate of chromosome number and genome size evolution. Rate transition probabilities and model-averaged rates of chromosome (2*n*) and genome size (1C) evolution are averaged over three pooled rjMCMC runs of 4,000,000 generations each, sampled every 200 generations after the first 1,000,000 generations. Model averaging was conducted on a branch-by-branch basis. Relative sizes of the circles superimposed on each node represent the relative frequency with which a shift in evolutionary rate occurred at that node in the rjMCMC sample, and thus sample from the posterior probability distribution of evolutionary rate shifts.

section *Ovales*

Predominantly Eastern North American clade
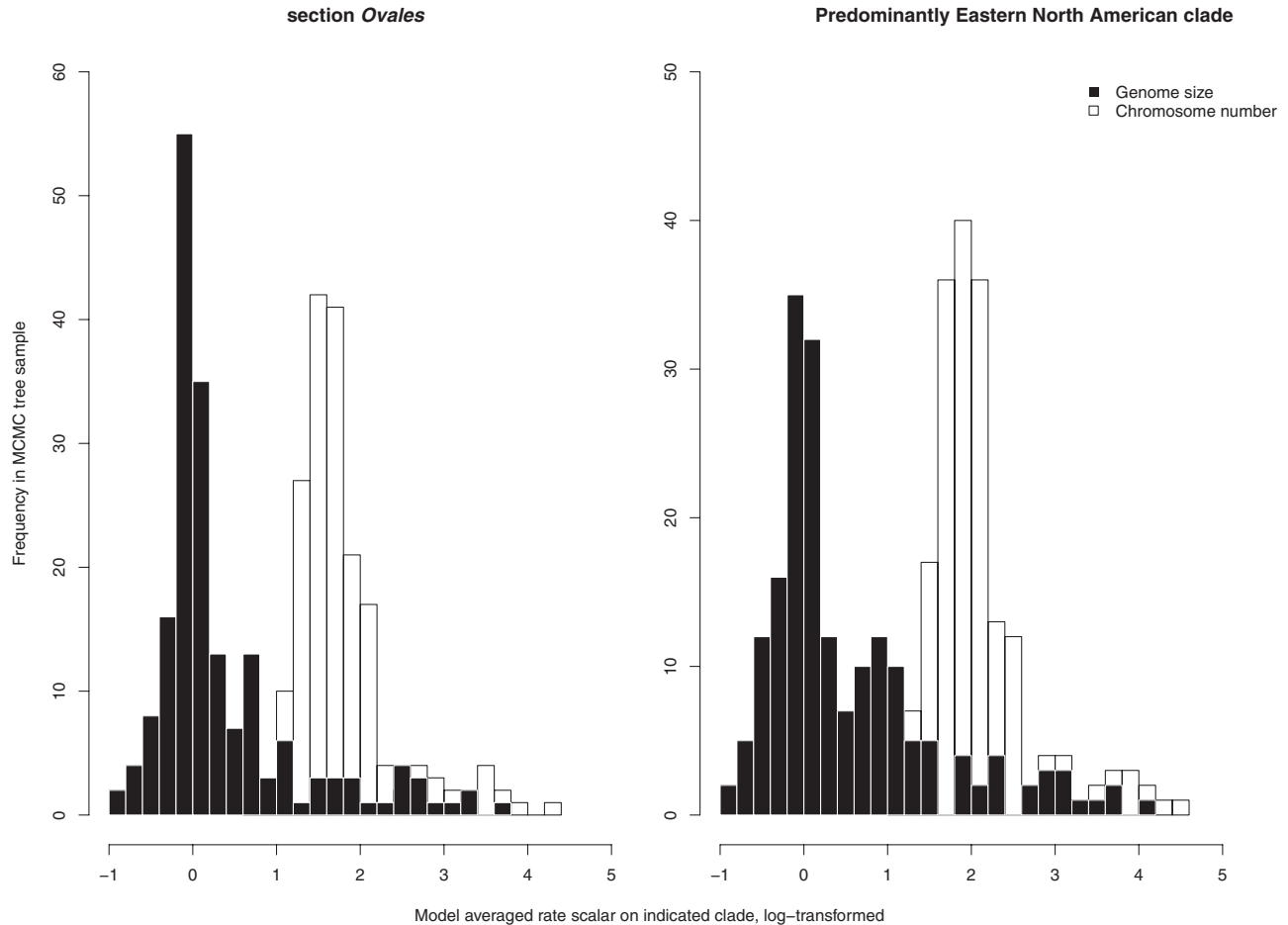
Figure 4. Histogram of model-averaged rate shifts at the base of the *Carex* section *Ovales* and the predominantly eastern North American clade within section *Ovales*, as estimated for trees subsampled from the MCMC phylogenetic analysis. Rate shifts were model averaged over the four models analyzed, using AICc weights to weight the rates inferred for each tree partition under each model. Rate shifts are log transformed so that values > 0 indicate an increase in the rate of evolution, values < 0 indicate a decrease. Trees that do not include the predominantly eastern North American clade (14 out of 200) were excluded from analysis.

predominantly eastern North American clade exhibit a rate of chromosome evolution nearly identical to the remainder of subgenus *Vignea* (relative rate = 1.064 [0.603, 2.198]), while the eastern North American clade itself exhibits a substantial increase in rate (relative rate = 13.613 [4.421, 59.069]). This finding is congruent with the rjMCMC analysis in identifying the eastern North American clade as exhibiting a significantly higher rate of chromosome evolution than the remainder of the phylogeny.

Discussion

Our study demonstrates that transitions in the rate and distribution of chromosome number are decoupled from genome size evolution. More precisely, the low variance in genome size in a clade that exhibits rapid changes in chromosome number (*Carex* section *Ovales*), along with a flat to negative correlation between chromosome number and genome size, demonstrates that changes in chromosome number are not accompanied by immediate shifts in genome size. The only two comparative studies of chromosome

number and genome size that we are aware of in organisms with holocentric chromosomes outside of sedges and their allies are in dragonflies (insect order Odonata; Ardila-Garcia and Gregory 2009), in which genome size was reported as ranging from 1C = 0.41 to 2.36 pg DNA, and significantly positively correlated with chromosome number in nonphylogenetic correlations; and two species estimated in *Cuscuta* subgenus *Cuscuta*, in which genome size estimates are 1C = 1.07 and 3.87 pg DNA for species with chromosome numbers of 2*n* = 14 and 42, respectively (McNeal et al. 2007). These findings, along with the demonstration of a positive correlation between chromosome number and genome size in the holocentric sedge genus *Eleocharis* (Zedek et al. 2010), contrast with our findings in *Carex*, demonstrating that chromosome evolution in *Carex* is dominated by rearrangements that have little if any effect on genome size.

The apparent shift in the dynamics of chromosome evolution in *Carex* relative to the remainder of the Cyperaceae (Hipp et al. 2009; Escudero et al. 2012) mirrors a previous report from the
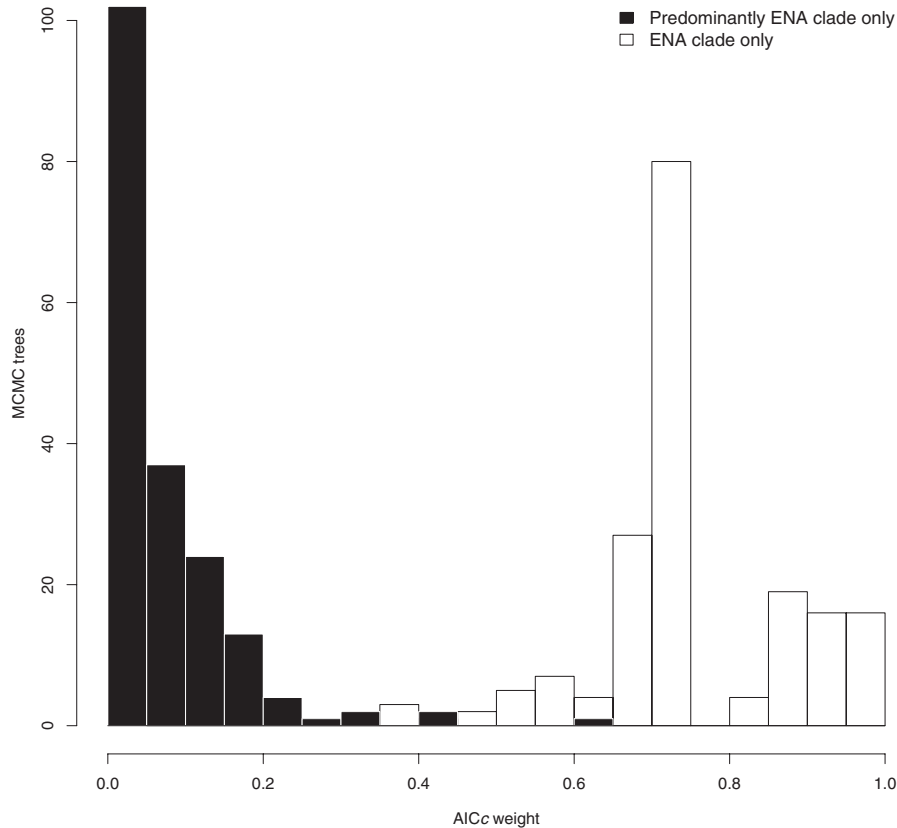
Figure 5. Relative support for a shift in the rate of chromosome evolution occurring along either of two adjacent branches, based on AICc model support. Relative support for models supporting a shift in the rate of evolution at the base of the predominantly eastern North American clade versus the eastern North American clade was estimated using AICc weights over trees subsampled from the MCMC phylogenetic analysis. Trees that do not include the predominantly eastern North American clade (14 out of 200) were excluded from analysis. Branch length heterogeneity in the MCMC subsample has little effect on relative support for these two alternative models of chromosome evolution.

butterfly family Lycaenidae, in which at least two clades exhibit abrupt increases in the rate of chromosome evolution relative to the remainder of the clade (Kandul et al. 2004). In that study, crown age of the chromosomally diverse genus *Agrodiaetus* was estimated at 2.51–3.85 million years, and chromosome numbers range from $2n = 20$ to 250 (Kandul et al. 2004). In comparison, *Carex* section *Ovales* has an estimated crown age of 4.79 million years and chromosome numbers ranging from $2n = 48$ to 86. It is clear from both of these studies that holocentry alone does not determine the dynamics or rate of chromosome evolution, as both demonstrate transitions from lower to higher rates of chromosome divergence within holocentric clades. Our study leaves open the important question of what drives transitions in the rate and dynamics of chromosome evolution.

Chromosome evolution by duplication or deletion of chromosomes has the potential to alter the balance of gene products, creating changes in phenotype that may come under strong selection (Osborn et al. 2003; Birchler and Veitia 2007, 2010). Chromosome rearrangements in *Carex* proceed at high rates without duplication or deletion of entire chromosomes (Wahl 1940; Hoshino 1981; Chung et al. 2011), and while chromosome rearrangements may be weakly underdominant (Faulkner 1973; Schmid 1982; Cayouette and Morisset 1985; Whitkus 1988a,b), there is no evidence that chromosome rearrangements per se affect individual fitness. This fact, combined with previous work demonstrating that chromosome evolution drives genetic divergence within and among species with holocentric chromosomes (Whitkus 1988a,b, 1992; Kandul et al. 2007; Hipp et al. 2010), suggests the potential for chromosome variability to evolve as a consequence of species selection (Rabosky and McCune 2010). That is, while chromosome rearrangements in sedges are expected to be neutral or perhaps weakly maladaptive at the individual level, chromosome variability may increase in frequency within clades if variability itself increases net diversification rate. While our sampling is not designed to test this hypothesis, it is compatible with our finding that the rate of chromosome evolution increases significantly within *Carex* section *Ovales*, one of the most diverse clades within the genus *Carex* (Hipp et al. 2006).

Genome size across *Carex* subgenus *Vignea* exhibits remarkable consistency of rate in comparison to the increase in rate of chromosome evolution within *Carex* section *Ovales* (Fig. 4). This might be expected if chromosome number changes in the genus are nearly neutral but genome size is under selective constraint. We might also see this result if chromosome number tracks a selective optimum that shifts more rapidly than the optimum for genome size. Microevolutionary studies (Narayan 1998; Šmarda et al. 2010) have demonstrated stabilizing selection on genome size. Ours is the first comparative dataset we are aware of to demonstrate that macroevolutionary transitions in genome size are dampened relative to chromosome number transitions.

One of the most tantalizing findings of this study is the possible shift from a flat relationship between chromosome number and genome size at fine phylogenetic scales (within *Carex* section *Ovales*, as well as within species; Chung et al. 2011) to a weakly negative relationship across the entire tree (excluding section *Ovales*). While the phylogenetic regression slopes become nonsignificant when the single datapoint of largest effect (*C. nubigena*) is removed, this outlier may nonetheless be biologically meaningful, and it certainly supports the negative relationship previously reported in nonphylogenetic studies (Nishikawa et al. 1984; Roalson et al. 2007). The importance of this datapoint cannot be evaluated outside of a broader study of the genus as a whole. Roalson et al. (2007) hypothesized that DNA digestion at exposed chromosome ends might result in a lower genome size in sedges with higher chromosome numbers. This hypothesis suggests that if transposon proliferation occurs at a constant rate and if chromosome ends are susceptible to degradation (e.g., if breakpoints are not associated with embedded telomeres; cf. Shampay et al. 1984), higher exposure to DNA degradation in organisms with higher chromosome numbers would push sedge species with higher chromosome numbers toward lower equilibrium genome size.

It remains to be seen whether our finding of a shift from a flat relationship at fine phylogenetic scales (within species and young clades) to negative correlation at deeper phylogenetic scales is borne out with further study. One of two scenarios might explain such a finding. First, an equilibrium relationship between genome downsizing and inflation might be reached gradually, with a phylogenetic lag time due to the rate of the processes underlying genome evolution. Alternatively, the equilibrium relationship might be relatively constant across the tree, but more dynamic chromosome rearrangements in some clades (e.g., section *Ovales*) may flatten the correlation between chromosome number and genome size. A recent study in *Eleocharis*, for example, suggested that shallow lineages have an increase in genome size and TE proliferation (Zedek et al. 2010), suggesting that shifts in the dynamics of TE evolution may occur on short time frames among closely related clades. As the dynamics of TEs in centromeres and telomeres may differ (DeBaryshe and Pardue 2011), understanding the distribution of telomeric and centromeric sequences in *Carex*, along with the dynamics of TE evolution, should provide important insights into how chromosome evolution and genome evolution are related in the genus.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Nuclear DNA content (1C) of individuals examined.
Appendix S2. ITS and ETS sequence data.
Appendix S3. Diploid number (2*n*) of individuals examined.

Supporting Information may be found in the online version of this article.

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