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NONUNIFORM PROCESSES OF CHROMOSOME EVOLUTION IN SEDGES (*CAREX*: CYPERACEAE)

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Holocentric chromosomes—chromosomes that lack localized centromeres—occur in numerous unrelated clades of insects, flatworms, and angiosperms. Chromosome number changes in such organisms often result from fission and fusion events rather than polyploidy. In this study, I test the hypothesis that chromosome number evolves according to a uniform process in *Carex* section *Ovales* (Cyperaceae), the largest New World section of an angiosperm genus renowned for its chromosomal variability and species richness. I evaluate alternative models of chromosome evolution that allow for shifts in both stochastic and deterministic evolutionary processes and that quantify the rate of evolution and heritability/phylogenetic dependence of chromosome number. Estimates of Ornstein–Uhlenbeck model parameters and tree-scaling parameters in a generalized least squares framework demonstrate that (1) chromosome numbers evolve rapidly toward clade-specific stationary distributions that cannot be explained by constant variance (Brownian motion) evolutionary models, (2) chromosome evolution in the section is rapid and exhibits little phylogenetic inertia, and (3) explaining the phylogenetic pattern of chromosome numbers in the section entails inferring a shift in evolutionary dynamics at the root of a derived clade. The finding that chromosome evolution is not a uniform process in sedges provides a novel example of karyotypic orthoselection in an organism with holocentric chromosomes.

KEY WORDS: Brownian motion model, chromosome evolution, holocentric chromosomes, karyotypic orthoselection, Ornstein–Uhlenbeck models, phylogenetic dependence, phylogenetic generalized least squares (GLS).

Sedges (*Carex*: Cyperaceae), one of the largest genera of angiosperms with more than 2000 species worldwide (Reznicek 1990), exhibit a relatively high rate of species diversification (Magallon and Sanderson 2001). The genus also exhibits an exceptional agmatoploid chromosome series ranging from $n = 6$ to $n = 66$ (Tanaka 1949). Every chromosome number from $n = 6$ to $n = 47$ is represented by at least one species (Roalson 2006). Chromosome number varies within species as well: some taxa exhibit a wider range in chromosome number than even the highly variable house mouse (Nachman and Searle 1995). Chromosome evolution in the genus proceeds almost exclusively by fission and fusion, without duplication of chromosomes (Hipp et al., in press). Unlike organisms in which karyotype evolution proceeds by Robertsonian rearrangements, however, sedges have holocentric chromosomes, meaning that the spindle fibers attach along the entire length of the chromosome arms instead of at localized centromeres (Håkansson 1954).

Holocentric chromosomes occur throughout the Cyperaceae (sedges) and their sister family Juncaceae (rushes) as well as in four other angiosperm genera (*Cuscuta* subgenus *Cuscuta*, *Drosera*, *Chionographis*, and *Myristica fragrans*; Flach 1966; Tanaka and Tanaka 1977; Pazy and Plitmann 1994; Sheikh et al. 1995; Guerra and García 2004); nematodes, including *Caenorhabditis elegans* (Buchwitz et al. 1999); and insects of several orders, including Lepidoptera, Heteroptera, and Odonata (Perez et al. 2000; Nokkala et al. 2002; Wang and Porter 2004). In these phylogenetically disparate organisms, chromosome fragments that arise by breakages during meiosis segregate normally and have

the potential to be inherited in Mendelian fashion (Faulkner 1972; Luceño 1993). As a consequence, breakages generally result in viable gametes with agmatoploid numbers that may become stabilized through backcrossing or selfing (although see Dernburg 2001 regarding decreased fitness of *C. elegans* structural heterozygotes).

The potential for rapid karyotype diversification both within and among species is manifest in *Carex*, with counts in some species spanning 10 chromosome pairs (Roalson 2006; Hipp et al., in press). Chromosome rearrangements in sedges have been shown to be weakly underdominant (Whitkus 1988), and the observed intraspecific karyotype variation within the genus may consequently be due in part to inadequate taxonomic study (i.e., the various chromosome races within some species may in fact be separate species). However, ecologically and morphologically coherent taxa often show variation in chromosome number between populations (e.g., Naczi et al. 1998; Rothrock and Reznicek 2001), suggesting that the high degree of variation in chromosome number represents real intraspecific variation.

Previous research on a densely sampled clade of North American sedges, *Carex* section *Ovales*, has demonstrated that there is a significantly higher chromosome count in the western North American grade than in the eastern North American clade that arises from it (Hipp et al. 2006a). In this study, I test alternative models of chromosome number evolution in an information theoretic framework to evaluate whether the observed distribution of chromosome numbers can be explained by a uniform evolutionary process or whether shifts in evolutionary rate or equilibrium chromosome number are needed to explain the data. If the evolution of chromosome number within a clade of about 90 species cannot be explained by a uniform evolutionary model, then understanding the processes of chromosome evolution in sedges and other organisms with holocentric chromosomes will require equal attention to inferring models of chromosome evolution for individual clades and to characterizing and explaining phylogenetic shifts between those models.

Modeling the Evolution of Holocentric Chromosomes

A comprehensive reconstruction of karyotype evolution would entail reconstructing the history of individual duplications, fission and fusion events, and rearrangements that comprise the karyotype, much as reconstructing the evolution of DNA sequences entails reconstructing the history of individual mutations and rearrangements. Most sedges have extremely small chromosomes that lack obvious physical landmarks (Hoshino 1981), making such an approach to reconstructing karyotype evolution currently impractical. An alternative approach is to use chromosome number as a proxy for karyotype. Chromosome number evolution has been reconstructed as a categorical character in previous studies (e.g., Rockman and Rowell 2002; Kandul et al. 2004; Hipp et al. 2006a). However, the more or less independent fission and fusion events that lead to changes in chromosome number suggest that chromosome number in sedges may also be modeled using methods developed for additive quantitative traits, in which character state is determined by numerous "at least partially independent. . . factors of individually small effect" (Hansen 1997: 1343).

The evolution of a character that is appropriately modeled on short time frames as a normally distributed random variable may be reconstructed using a Brownian motion model. In the Brownian motion model, the change in character state from time t to time $t + dt$ is a function of σ—the strength of all stochastic influences on phenotype, such as drift and randomly shifting adaptive optima—and a normally distributed random variable $dB(t)$,

$$dX(t) = \sigma dB(t) \quad (1)$$

(from Butler and King 2004). This model underlies Felsenstein's (1985) method of independent contrasts. The causes of character evolution in this model are not distinguished from one another, but combined into the single term $dB(t)$, which is normally distributed with mean 0 and variance dt. In the case of chromosome number, the causes of character evolution are, proximally, the more or less independent fission and fusion events that lead to chromosome number change. Distally, these causes include all factors that affect rates of chromosome fission and fusion in a nondirectional, nonstabilizing manner. The variance and covariance of data that evolve according to a Brownian motion process on a phylogenetic tree is described by a variance–covariance matrix based on the tree topology and branch lengths. The expected covariance in character state between any two species on a tree is a function of how recently they diverged from their most recent common ancestor.

A purely Brownian motion model will overestimate the correlation in character state among species if there is low phylogenetic heritability (H^2; Lynch 1991; Housworth et al. 2004) or phylogenetic dependence (λ; Pagel 1999; Freckleton et al. 2002), for example, as a consequence of strong selection or of evolution being disproportionately concentrated near the tips of the tree rather than in shared branches of the phylogeny. The phylogenetic mixed model (Lynch 1991) partitions character variance into two components, a heritable portion and a nonheritable portion, and estimates phylogenetic heritability as $H^2 = \sigma^2{}_a / \sigma^2$, where $\sigma^2{}_a$ is the variance of the heritable component of the phenotypic trait being measured, and σ^2 is the sum of the variance of the heritable component and the residual variance (i.e., that portion of the phenotypic variance that cannot be explained by phylogeny; Housworth et al. 2004). It is to be expected that for traits in which $\sigma^2{}_a << \sigma^2$, models incorporating H^2 will fit the data significantly better than the Brownian motion model. Pagel's

(1999) generalized least squares (GLS) method estimates a similar parameter, phylogenetic dependence (λ), a multiplier of the off-diagonal elements of the variance–covariance matrix (Freckleton 2002). For both H^2 and λ, a value of 0 indicates that trait evolution is not predicted by the phylogeny (under a Brownian motion model), whereas a value of 1 indicates that the observed distribution of character states is compatible with evolution according to a Brownian motion process. Freckleton et al. (2002) observe that Pagel's λ is similar to Lynch's (1991) H^2, and Housworth et al. (2004) argue that the two are mathematically equivalent. In the current study, I use λ exclusively.

A Brownian motion process may inadequately explain the evolution of a character due to the action of natural selection or other deterministic forces (Felsenstein 1985; Hansen 1997). The Ornstein–Uhlenbeck process can be used to model such forces through parameters for character state optimum (θ) and rate of evolution toward that optimum (α):

$$dX(t) = \alpha[\theta - X(t)]dt + \sigma dB(t) \quad (2)$$

(from Butler and King 2004). The only random variable associated with the model is $dB(t)$, so that the effect of selection toward θ is deterministic and the magnitude of its effect is estimated as α, whereas all other evolutionary forces are modeled as random and the magnitude of their cumulative effect is estimated as σ (Butler and King 2004). In this context, the evolution of a continuous character along a phylogenetic tree is modeled as an Ornstein–Uhlenbeck process in which selective regimes change with cladogenesis (Hansen and Martins 1996; Hansen 1997; Butler and King 2004). The expected covariance in character states between species is influenced by the period of time spent in each of i selective regimes, the collection of optimal states θ_i for all i selective regimes, and α, the rate of evolution toward θ_i, as well as by the phylogenetic effects described by the Brownian motion model.

It is worth noting that although the θ_i of Ornstein–Uhlenbeck models are typically interpreted as adaptive optima and α as the strength of selection (following Butler and King 2004) or the rate of adaptation (Hansen 1997), chromosome number per se is not likely to be adaptive in sedges. Stated another way, there is no reason to expect selective forces that influence the evolution of chromosome morphology and number—for example, meiotic drive, changes in cellular processes that affect meiosis and mitosis, and processes that underlie karyotypic orthoselection—will optimize organismal fitness (Sandler and Novitski 1957; Terzi 1972; Pardo-Manuel de Villena and Sapienza 2001; Kim et al. 2005). To the contrary, these processes are capable of driving chromosomal variants to fixation even if those variants are neutral, underdominant, or weakly maladaptive (Pardo-Manuel de Villena and Sapienza 2001; Kim et al. 2005). Consequently, I interpret θ_i in the current study as karyotypic equilibria rather than adaptive optima, on the grounds that if chromosome number evolution proceeds according to an Ornstein–Uhlenbeck process, chromosome number in each lineage will equilibrate at a stationary distribution with mean θ and variance determined by the relationship between α and σ, irrespective of the causes of chromosome evolution. Likewise, I interpret α as the rate of evolution toward the karyotypic equilibrium rather than as the rate of adaptation.

Methods

CHROMOSOME DATA: SOURCES AND CODING

Diploid chromosome numbers for all species in *Carex* section *Ovales* (Cyperaceae) are summarized from published sources (Appendix 1; Fig. 1). Additional unpublished counts were provided by P. E. Rothrock (Taylor University). Numbers are taken from mitotic counts or inferred from counts made at first interphase of meiosis, following the formula (1 × univalents) + (2 × bivalents) + ⋯ + (x × x-valents), where x indicates the number of chromosomes associated with one another at first meiotic interphase. Some individuals counted have odd inferred diploid ($2n$) counts, a common consequence of irregular meiotic pairing relationships in intraspecific hybrids (Whitkus 1988). An average chromosome number was calculated for each taxon by weighting diploid counts by the number of populations in which they were reported (Appendix 1). Weighted mean diploid chromosome counts were log transformed prior to analysis, based on the expectation that the rate and amount of chromosome number change by fission and fusion will be proportional to an individual's chromosome number. In trial analyses, log transformation improved model fit by over 200 log-likelihood units relative to untransformed data.

PHYLOGENY RECONSTRUCTION AND BRANCH LENGTH OPTIMIZATION

Phylogenies used in this study are from a previous study based on nuclear ribosomal DNA sequences (Hipp et al. 2006b). All sequences are deposited in GenBank (Hipp et al. 2006b), and the dataset and consensus Bayesian tree are deposited in TreeBase (study accession S1791, matrix accession M3273). Briefly, three nuclear ribosomal DNA partitions (the internal transcribed spacers ITS1 and ITS2, 5.8S, and a segment of the external transcribed spacer) were analyzed using Metropolis-coupled Markov chain Monte Carlo (MCMC) in MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001). Data partitions were modeled separately, all parameters except branch lengths and topology unlinked across data partitions. Three independent runs of four linked chains were each run for 5,000,000 generations, using the default priors and temperature parameter. Trees from the initial 1,000,000 generations (the "burn-in") were eliminated from the analysis. Comparative analyses presented in this study were conducted on (1)

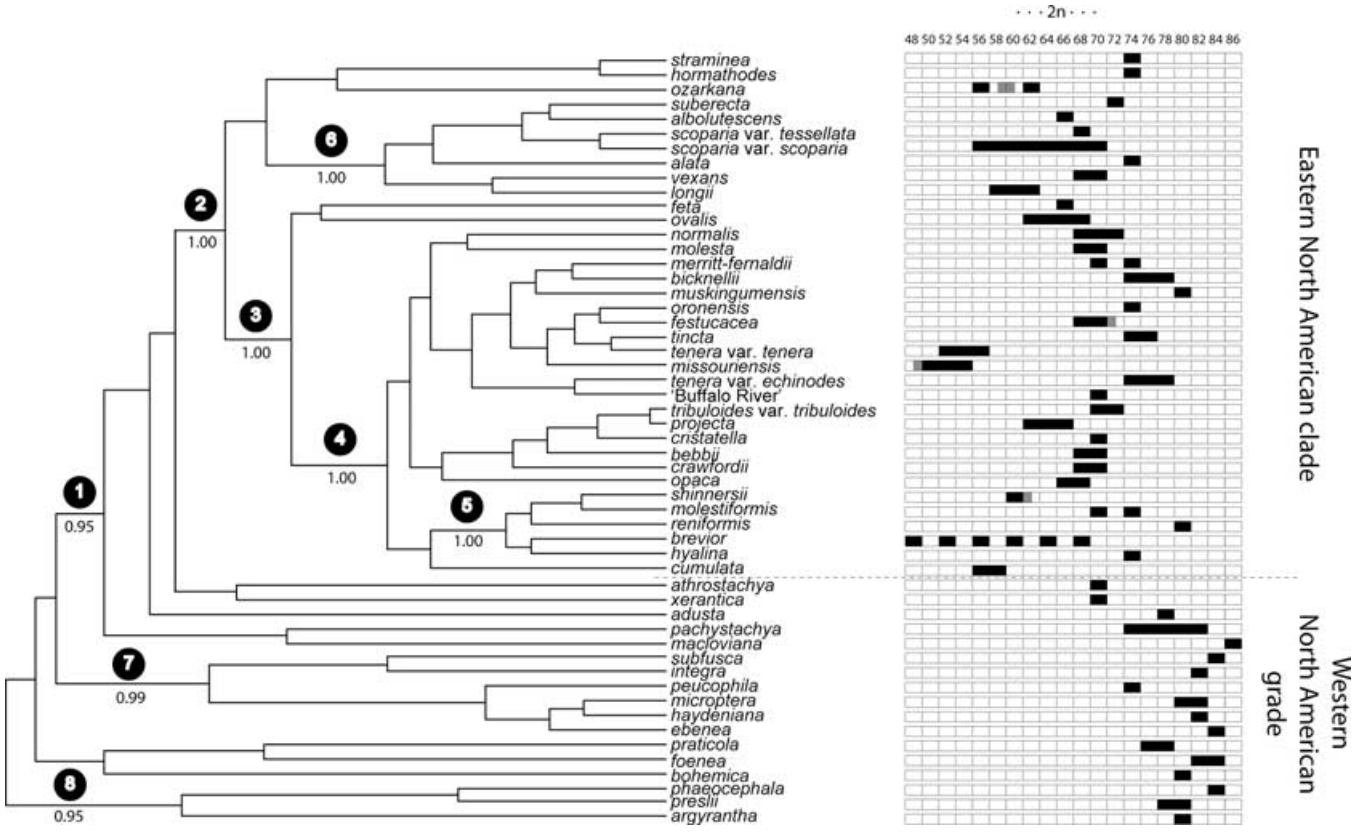

Figure 1. Bayesian consensus tree, ultrametric. The Bayesian consensus topology was generated from the last 4×10^6 generations of a 5×10^6 generation MCMC run using more than 75 taxa from the section (see Methods). Branch lengths were optimized using penalized likelihood, with smoothing parameter set at 0.1, and taxa for which chromosome data were not available were pruned after branch length optimization (see methods). Nodes included in the Ornstein–Uhlenbeck analysis are numbered; numbers below the branches are posterior probabilities estimated from runs with all taxa included. Chromosome numbers indicate euploid (black) and agmatoploid (gray) diploid counts as reported in the literature (Appendix 1).

the fully resolved tree that maximizes the posterior probability of each clade based on MCMC analysis, referred to in this article as the Bayesian consensus; and (2) a set of 100 trees subsampled at even intervals from the last 4,000,000 generations of MCMC—referred to in this article as, collectively, the Bayes subsample. The latter tree set was used to account for phylogenetic uncertainty.

Ultrametric trees (trees with branches scaled proportional to time rather than to estimated nucleotide substitutions) were generated using penalized likelihood (Sanderson 2002) in the *ape* package (Paradis et al. 2004) of R version 2.4.1 (R Development Core Team 2006). The smoothing parameter was optimized on the Bayesian consensus using cross-validation in r8s version 1.71 (http://ginger.ucdavis.edu/r8s/), which resulted in a smoothing parameter estimate of 0.1. Branch lengths were optimized on trees with all 81 sequenced taxa included. This taxon set includes 75 of the approximately 90 species recognized in the section and several infraspecies. Taxa for which chromosome counts are not known were pruned subsequent to branch-length optimization. Trial analyses were also conducted on a series of trees with branch lengths optimized under smoothing parameters ranging from 0.01 to 10,000, on ultrametric trees generated using nonparametric rate smoothing (Sanderson 1997) or under an assumption of a global molecular clock, on trees from which taxa were pruned before branch-length optimization, and on trees with branch lengths untransformed (nonultrametric). None of these treatments had an effect on the conclusions of this study and are consequently not reported. Overall tree length was scaled to 1.0 for all analyses.

TESTING ALTERNATIVE MODELS OF CHROMOSOME NUMBER EVOLUTION

Two classes of generalized least squares (GLS) models were evaluated in the current study: a wide range of alternative Ornstein–Uhlenbeck models (using the methods of Butler and King 2004 as implemented in *ouch* 1.1–2), and two of Pagel's (1999) tree scaling parameters, lambda (λ) and kappa (κ). Point estimates of model parameters were obtained using maximum likelihood on the Bayesian consensus, and confidence intervals were estimated using parametric bootstrapping (10,000 replicates) for the Ornstein–Uhlenbeck models or MCMC (1×10^6 generations following at

100,000-generation burn-in) for Pagel's scaling parameters. Confidence intervals were estimated on the Bayes subsample when possible, to incorporate phylogenetic uncertainty (e.g., Table 2), and on the Bayesian consensus when analyses entailed shifts at nodes not present in all trees of the Bayes subsample (e.g., Appendix 2). Small-sample Akaike information criterion (AIC_c) and Bayes information criterion (BIC) are used to evaluate support for alternative models and model parameters (Burnham and Anderson 2002). The two measures are superficially similar—$AIC_c = -2\ \mathrm{lnL} + 2K\ (N / [N–K–1])$; $\mathrm{BIC} = -2\ \mathrm{lnL} + K \cdot \ln(N)$); L = model likelihood, N = sample size, K = number of free parameters in the model—but estimate different quantities: AIC_c was derived as an unbiased estimator of relative Kullback–Leibler information—the "distance" between models—whereas BIC (= Schwarz's information criterion) was derived as a dimension-consistent estimator of the posterior probability that a given model is the correct (i.e., generating) model (Burnham and Anderson 2002: 286 ff.).

Ornstein–Uhlenbeck analyses were conducted on a large set of models, designating eight nodes at which karyotypic equilibria were allowed to change (Fig. 1), for a total of 2^8 models ranging from one to nine different values for θ_i. Evaluating such a large number of models is not generally recommended (Burnham and Anderson 2002), but doing so was necessary to test the relative support for a shift in evolutionary dynamics at the root of the eastern North American clade. Seven nodes were chosen based on the criteria that they were supported at phylogenetic posterior probability ≥ 0.95 and had at least five descendents. One node with only three descendents (Node 8) was added to increase representation of western North American nodes in the analysis. Allowing changes in model parameters only at strongly supported nodes makes it impossible to detect shifts in evolutionary dynamics at poorly supported nodes that may nonetheless represent real branching events. However, it is not clear how one should interpret a strongly supported shift in evolutionary dynamics at a node that may not represent a real branching event. Nodes supported at $< 95\%$ posterior probability were consequently not considered in this study. Intraspecific variance was incorporated into analysis by adding estimated measurement error (on log-transformed data) to the diagonal of the variance–covariance matrix (Martins and Hansen 1997). Because only a single count was available for many taxa, the squared standard error of the mean was estimated for each taxon as the mean variance for all taxa for which counts were available from ≥ 3 populations divided by sample size for each taxon separately. Accounting for intraspecific variance had minimal effect on model likelihoods and parameter estimates. BIC and AIC_c weights (w_i) were summed over sets of two, five, 17, and 257 models (Ornstein–Uhlenbeck and Brownian motion) to evaluate whether estimates of the support for changes in evolutionary process at specific nodes are sensitive to the number of models analyzed.

Additional Brownian motion assumptions regarding covariance among species and rate constancy within each subtree were evaluated by estimating two tree scaling parameters using the GLS method of Pagel (1997, 1999) as implemented in CONTINUOUS (http://www.evolution.reading.ac.uk). In this method, λ scales the off-diagonal elements of the variance–covariance matrix. Downweighting the off-diagonal elements of the variance–covariance matrix ($\lambda < 1$) has the effect of decreasing the estimated covariance among individuals relative to the variance–covariance structure predicted by the phylogeny under a Brownian motion model. Given the importance of this parameter and its similar effects to α of the Ornstein–Uhlenbeck models (Housworth et al. 2004), the effect of estimating λ and α jointly was evaluated in a set of analyses by incorporating λ into the variance–covariance matrix prior to optimizing α in *ouch*. A second parameter, kappa (κ), scales branch lengths exponentially, and its maximum-likelihood value consequently estimates the relationship between individual branch lengths and the amount of change in a character occurring on branches of that length. At $\kappa = 0$, the amount of evolutionary change along a path is a function of the number of branching events along that path rather than of the branch lengths in the unscaled tree. Conversely, $\kappa > 1$ models a scenario in which evolutionary change occurs at a disproportionately high rate on longer branches. A third scaling parameter, δ, is typically interpreted in light of the variance in total path-length on nonultrametric trees and is not employed in the current study.

Models were fit to the entire phylogeny ("whole-tree" tests) and to each subtree separately ("censored" tests) by deleting the branch that connects the two and resolving the western North American grade as monophyletic, analogous to the censored likelihood approach of O'Meara et al. (2006) for testing evolutionary rates in subtrees. Log-likelihoods for the subtrees were summed and information criteria were calculated using the summed number of parameters. The results are compared with the whole-tree tests to assess what shifts in evolutionary processes are needed to explain the observed differences in chromosome number between the eastern and western North American taxa.

Results

CHROMOSOME NUMBERS

The mean of diploid chromosome counts for the eastern North American taxa, with each taxon accorded equal weight, is 68.38 $\pm$ 1.118 [SEM] (N = 39 taxa, 36 of which are included in the phylogenetic analyses used for this study; populations counted per taxon = 5.61 $\pm$ 1.20; Figs. 1 and 2; Appendix 1). The mean of diploid chromosome counts for the western North American taxa is 79.23 $\pm$ 1.261 (N = 17 taxa, all of which are included in this study; populations counted per taxon = 5.18 $\pm$ 1.67).

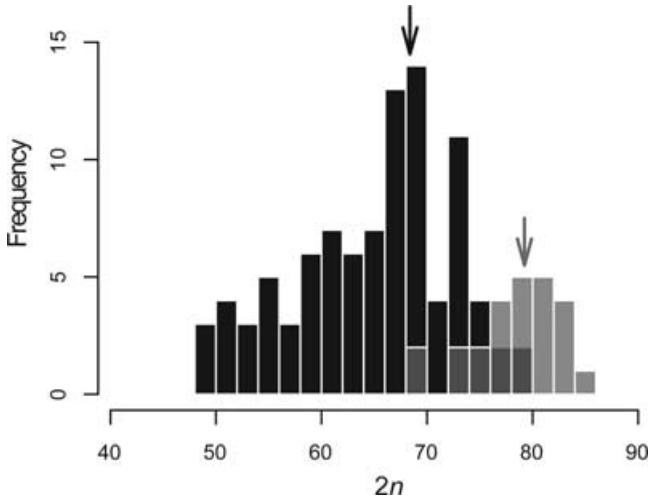

Figure 2. Histogram of unweighted diploid counts by geographic area. Each chromosome count in Appendix A was included once per species and histograms generated separately for the eastern North American taxa (black) and the western North American taxa (gray). Arrows indicate the weighted mean of diploid counts for the eastern and western North American taxa ($2n$ = 68.38 and 79.23, respectively).

WHOLE-TREE ORNSTEIN–UHLENBECK MODELS

Analysis of whole-tree Ornstein–Uhlenbeck models most strongly supports a shift in karyotypic equilibrium at the root node of the eastern North American clade (Node 2, cumulative BIC w_i = 0.874–0.894; Table 1) and moderately supports a change along a branch midway between the root and the eastern North American clade (Node 1, cumulative BIC w_i = 0.336–0.346). There is little evidence for a change in karyotypic equilibrium at all other nodes investigated (BIC $w_i < 0.15$). The Brownian motion model is the most poorly supported model regardless of the number of models evaluated (Table 1; Appendix 2). When only the single-θ Ornstein–Uhlenbeck model is tested along with the Brownian motion model, BIC weight for the Brownian motion model is 0.016 (Table 1). When models are included that allow at least one change in karyotypic equilibrium (Tables 1 and 2; Appendix 2), the Brownian motion model and the single-θ Ornstein–Uhlenbeck model together are supported at cumulative BIC weight < 0.0001.

The parameter for rate of evolution (α) toward θ_i was optimized over the interval 0.001–20 and was found to be at the maximum for all multiple-θ Ornstein–Uhlenbeck models, although parametric bootstrapping suggests considerable uncertainty in the estimates of α (Appendix 2). Increasing the interval over which α was optimized had very little effect on model likelihoods and no effect on model support, because the model likelihood is relatively flat at $\alpha > 20$ (Fig. 3). When intraspecific variance / measurement uncertainty is not modeled, model likelihoods become asymptotic at high α (plot not shown), but incorporating intraspecific variance depresses alpha estimates. Because the maximum-likelihood estimate of α is constrained by the upper bound of the search interval, confidence intervals based on parametric bootstrapping at α = 20 may be biased toward lower values of α. However, this potential bias does not affect the conclusions of this study. In all models incorporating a shift in karyotypic equilibrium, the lower end of the 95% confidence interval corresponds to $\alpha > 3.86$ (i.e.,

Table 1. Bayes information criterion (BIC) weights summed over nodes at which karyotypic equilibria (θ_i) are permitted to change. The sum of weights for a given node indicates the support across models for a change in θ at that node. All nodes are present in half of the models tested, as the candidate models include all possible combinations of change nodes (thus, the nodes are essentially binary, turned "on" or "off"). The cumulative weight for each node is relatively little changed by the number of models tested.

Node at which θ may change	Number of taxa in clade	Cumulative BIC weight (2 models)[1]	Cumulative BIC weight (5 models)[2]	Cumulative BIC weight (17 models)[3]	Cumulative BIC weight (257 models)[4]
1	41	na	0.33780	0.34592	0.33602
2	36	n/a	0.89382	0.88241	0.87351
3	26	na	na	0.13140	0.14038
4	24	na	na	0.13144	0.13270
5	5	na	na	na	0.12347
6	7	na	na	na	0.13494
7	6	na	na	na	0.14976
8	3	na	na	na	0.13379
None[1]	na	1.00000	3.2210×10^{-05}	2.4342×10^{-05}	1.3×10^{-05}
Brownian motion	na	0.01647	5.3064×10^{-07}	4.0102×10^{-07}	2.2506×10^{-07}

[1] Brownian motion and Ornstein–Uhlenbeck model with a single θ. [2] Brownian motion, Ornstein–Uhlenbeck model with a single θ, and Ornstein–Uhlenbeck model entailing changes in θ at nodes one and two. [3] Brownian motion, Ornstein–Uhlenbeck model with a single θ, and Ornstein–Uhlenbeck model entailing changes in θ at nodes one through four. [4] Brownian motion model and all Ornstein–Uhlenbeck models entailing changes in θ at nodes one through eight.

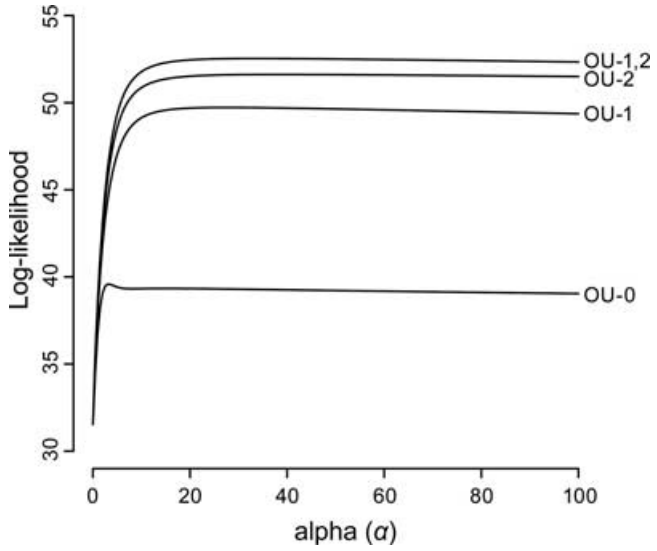

Figure 3. Natural log of model likelihood plotted against α for the four Ornstein–Uhlenbeck models defined by nodes 1 and 2. Likelihood of each model over α = 0 to α = 100 was calculated using the "badness" function in *ouch*. Model abbreviations denote the nodes at which θ is permitted to change.

"adaptive half-life" sensu Hansen 1997 ($t_{1/2} = \ln(2)/\alpha$) < 0.180; Appendix 2). The depth of the eastern North American clade in the Bayesian consensus tree scaled to a total length of 1.0 is 0.6664, which implies that even under the lowest α estimates, the eastern North American taxa have evolved $1–(0.5^{[0.6664/0.180]}) = 92.3\%$ of the distance from the chromosome number of the population ancestral to the eastern North American clade toward the eastern North American karyotypic equilibrium. These large values of α explain why estimates of θ_0, the character state estimated for the root of the tree, are biologically implausible ($2n < 2$) under all Ornstein–Uhlenbeck models investigated: the effect of phylogeny on the variance–covariance structure of a phenotype decreases as the rate of evolution toward a lineage-specific equilibrium increases, so that θ_0, as well as θ_i that are restricted to internal branches, cannot be estimated accurately at large values of α (Butler and King 2004; Verdu and Gleiser 2006).

WHOLE-TREE AND CENSORED MODELS

When the eastern North American subtree is evaluated alone, the Brownian motion + λ model (BIC $w_i = 0.854 \pm 0.0034$) and single-θ Ornstein–Uhlenbeck model (BIC $w_i = 0.139 \pm 0.0024$) are strongly supported over the Brownian motion + κ model (BIC $w_i = 0.0017 \pm 0.0005$) and unmodified Brownian motion model (BIC $w_i = 0.0056 \pm 0.0017$) on all trees in the Bayes subsample (Table 3). Estimated phylogenetic dependence ($\lambda = 0$ [0 ± 0 over the Bayes subsample]) and rate of evolution toward a karyotypic equilibrium ($\alpha = 20$ [16.599 ± 0.0095 over the Bayes subsample]) demonstrate that phylogeny has essentially no effect on the evolution of chromosome number within the clade. At the same time, low support for the Brownian motion + κ model suggests that the low phylogenetic inertia is not a consequence of punctuational evolution or, conversely, of a disproportionate amount of evolution occurring on longer branches. Although the phylogenetic dependence parameter (λ) substantially improves the fit of the Brownian motion model to the data, estimating λ within the Ornstein–Uhlenbeck framework—that is, optimizing the likelihood of an Ornstein–Uhlenbeck model for both λ and α—has negligible effect on model likelihoods (Fig. 4). In the western North American subtree, the high point estimate of phylogenetic dependence ($\lambda = 1.100$ [0.865 ± 0.0302 over the Bayes subsample]) is essentially that of an unmodified Brownian motion model, and the Brownian motion model is the best-supported model (BIC $w_i = 0.565 \pm 0.0075$).

Under all censored models evaluated, the stochastic rate of chromosome evolution (σ) in the eastern North American subtree is higher than that of the western North American subtree (model-averaging over censored models, $\Delta\sigma^2 = 0.0159$; Table 2). Only one whole-tree model compares favorably with the censored models in explaining the data: the dual-θ Ornstein–Uhlenbeck model ("OU-2") has relatively high support (BIC $w_i = 0.298$; Table 2) and is comparable in support to the censored Brownian motion + λ model (BIC $w_i = 0.679$; ΔBIC = 1.646; Table 2). Counting the OU-2 model with the censored models, the cumulative BIC weight for all models that specify a shift in at least one model parameter at Node 2 is 0.989 (Table 2); this estimate of the posterior probability of a shift in parameters at Node 2 is similar to the estimate when only whole-tree Ornstein–Uhlenbeck models are considered (cumulative BIC $w_i = 0.874$–0.894; Table 1). Between models that specify a shift in evolutionary dynamics at the base of the eastern North American clade and similar models that do not, the former explain the data significantly better.

Discussion

MODEL SUPPORT AND EVOLUTIONARY DYNAMICS OF CHROMOSOME EVOLUTION

The major findings of this study are that (1) chromosome evolution is rapid and relatively unconstrained by phylogeny, at least in the eastern North American clade; (2) the origin of the eastern North American clade is associated with a significant increase in the rate of chromosome evolution; and (3) chromosome number evolves toward divergent karyotypic equilibria in different clades. These findings suggest that cladogenetic shifts in evolutionary dynamics may play as important a role in chromosome evolution as the gradual evolution of chromosome number predicted by the processes of fission and fusion that drive chromosome evolution in *Carex*.

The whole-tree Brownian motion model explains the data more poorly than any other model evaluated in this study (BIC weight = 2.33×10^{-7}; Table 2). The censored Brownian motion

Table 2. Models evaluated on complete phylogeny ("whole-tree" models) and on the eastern and western North American subtrees separately ("censored" models). Point estimates were obtained by maximum likelihood on the Bayesian consensus; 95% confidence intervals (in parentheses) were inferred over the Bayes subsample using Markov chain Monte Carlo (for Brownian motion + κ and Brownian motion + λ models; 1,000,000 generations following a 100,000-generation burn-in, sampling trees at random for each MCMC iteration) or parametric bootstrapping (for the Ornstein–Uhlenbeck and unmodified Brownian motion models; 100 parametric bootstrap replicates simulated on each of the 100 trees in the Bayes subsample). All θ are presented both as inferred directly from the log-transformed data and, in brackets, as e^{θ}.

Censored tests	lnL	K	AICc	BIC	AICc weight	BIC weight	σ^2 ENA	σ^2 WNA	θ_0 ENA	θ_0 WNA	Additional parameters by subtree ENA	Additional parameters by subtree WNA	ENA	WNA
Brownian motion + λ	54.327	6	−94.828	−84.832	0.786	0.679	0.01569	0.00594	4.220 [68.0]	4.369 [79.0]	λ = 0	λ = 1.100	–	–
							(0.013–0.025)	(0.005–0.007)	(4.154–4.274)	(4.317–4.413)	(0.006–0.730)	(0.041–0.981)		
Single-optimum O-U	54.149	8	−89.024	−76.535	0.043	1.07E-02	0.40697	0.00640	6.876E-06 [1.0]	5.240 [188.7]	$\theta_1 = 4.219$	$\theta_1 = 1.736$	α = 20	α = 0.286
('OU-0')							(0.055–0.552)	(0.003–0.155)	(1.131E-07–0.539)	(8.972E-09–5.241)	(4.188–4.633)	(0.004–5.264)	(2.944–20)	(0.001–20)
Brownian motion	43.956	4	−79.079	−72.031	2.99E-04	1.13E-03	0.05985	0.005513	4.203 [66.9]	4.369 [79.0]	–	–	–	–
							(0.027–0.130)	(0.0021–0.0094)	(4.036–4.383)	(4.312–4.415)				
Brownian motion + κ	45.698	6	−77.570	−67.574	1.40E-04	1.21E-04	0.01449	0.002645	4.192 [66.1]	4.379 [79.7]	κ = 0.345	κ = 0.349	–	–
							(0.009–0.152)	(0.002–0.047)	(4.075–4.335)	(4.309–4.430)	(0.149–1.971)	(0.068–4.069)	–	–

Whole-tree tests	**lnL**	***K***	**AICc**	**BIC**	**AICc weight**	**BIC weight**	**σ^2**	**θ_0**	**Additional parameters, whole tree**			
Dual-optimum O-U	51.519	5	−91.761	−83.187	0.170	0.298	0.33118	1.771E-8 [1.00]	α = 20	$\theta_1 = 4.370$ [79.1]	$\theta_2 = 4.219$ [68.0]	–
('OU-2')							(0.02809–0.58811)	(1.740E-9–5.252)	(0.77907–20)	(3.379–4.795)	(3.224–4.857)	
Brownian motion + λ	44.274	3	−82.058	−76.637	1.33E-03	1.13E-02	0.01286	4.331 [76.05]	–	λ = 0.372	–	–
							(0.012–0.019)	(4.253–4.397)		(0.164–0.832)		
Brownian motion + κ	39.755	3	−73.020	−67.599	1.44E-05	1.23E-04	0.00529	4.383 [80.08]	–	κ = 0	–	–
							(0.005–0.034)	(4.200–4.480)		(0.016–1.208)		
Single-optimum O-U												
('OU-0')	39.586	4	−70.339	−63.291	3.78E-06	1.43E-05	0.09495	0.186 [1.20]	α = 3.218	$\theta_1 = 4.470$ [87.3]	–	–
							(0.0283–0.5967)	(8.772E-9–4.3061)	(0.6723–20)	(4.120–5.178)		
Brownian motion	31.500	2	−58.760	−55.059	1.16E-08	2.33E-07	0.04286	4.351 [77.57]	–	–	–	–
							(0.0185–0.0640)	(4.213–4.467)				

Table 3. Model comparisons conducted on subtrees over Bayes subsample. Parameter values, log-likelihoods, BIC and BIC weights are averaged over the 100-tree Bayes subsample (± standard error).

ENA subtree	lnL	K	BIC	BIC weight	σ^2	θ_0	λ	κ	α	θ_1
Brownian motion + λ	31.521 ± 1.452E-07	3	−52.292 ± 2.904E-7	0.854 ± 3.394E-3	2.976E-4 ± 4.301E-8	4.220 ± 6.249E-16	0 ± 0	–	–	–
Single-optimum O-U ('OU-0')	31.488 ± 1.872E-2	4	−48.642 ± 0.375	0.139 ± 2.371E-3	0.328 ± 9.162E-5	9.167E-4 ± 3.848E-4	–	–	16.599 ± 0.490	4.220 ± 3.517E-4
Brownian motion	21.199 ± 0.358	2	−35.231 ± 0.716	5.588E-3 ± 1.663E-3	3.712E-2 ± 5.823E-6	4.209 ± 3.924E-4	–	–	–	–
Brownian motion + κ	23.779 ± 0.165	3	−36.807 ± 0.329	1.653E-3 ± 5.270E-4	4.000E-4 ± 2.170E-6	4.200 ± 7.224E-4	–	0.427 ± 3.245E-2	–	–
WNA subtree	lnL	*K*	BIC	**BIC weight**	σ^2	θ_0	λ	κ	α	θ_1
Brownian motion + λ	22.610 ± 4.311E-2	3	−36.721 ± 8.578E-2	0.156 ± 3.550E-3	4.532E-5 ± 4.582E-9	4.364 ± 5.074E-4	0.865 ± 3.020E-2	–	–	–
Single-optimum O-U ('OU-0')	22.633 ± 3.822E-2	4	−33.932 ± 7.644E-2	3.898E-2 ± 1.056E-3	8.558E-3 ± 1.533E-5	4.107 ± 0.133	–	–	0.765 ± 0.207	2.571 ± 0.199
	22.490 ± 5.124E-2	2	−39.314 ± 0.103	0.565 ± 7.519E-3	5.379E-3 ± 1.669E-7	4.364 ± 5.274E-4	–	–	–	–
Brownian motion + κ	23.003 ± 6.458E-2	3	−37.507 ± 0.129	0.240 ± 8.984E-3	4.413E-5 ± 5.322E-7	4.371 ± 1.060E-3	–	0.716 ± 7.511E-2	–	–

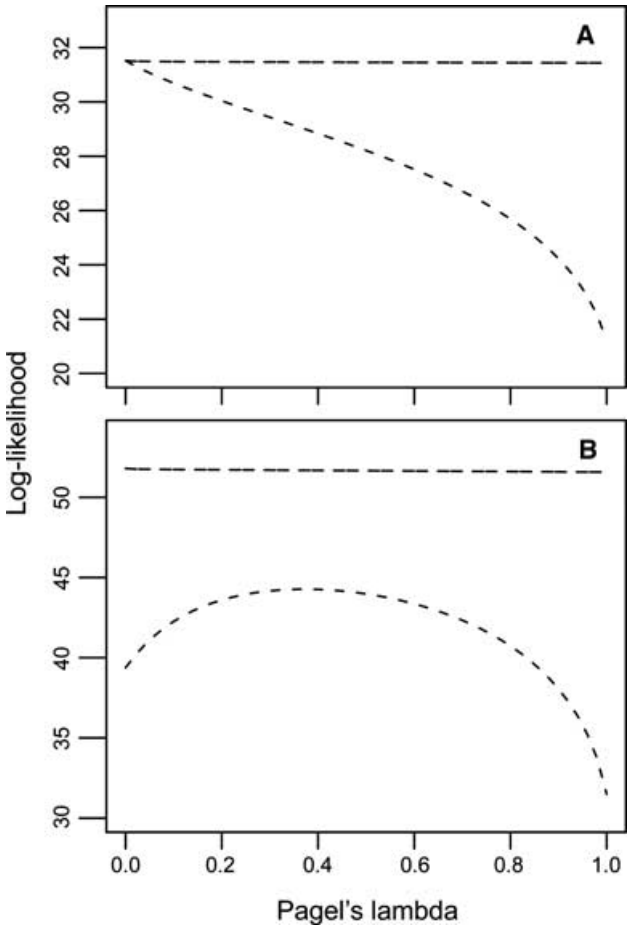

Figure 4. Effects of incorporating Pagel's lambda (λ) into the Brownian motion and Ornstein–Uhlenbeck models. In both plots, the single-dashed (lower) line indicates the Brownian motion + λ model, and the double-dash (upper) line indicates the Ornstein–Uhlenbeck + λ model. (A) Eastern North American Bayesian consensus subtree; double-dash line represents the single-θ Ornstein–Uhlenbeck ("OU-0") + λ model. (B) Whole tree, Bayesian consensus; double-dash line represents the dual-θ Ornstein–Uhlenbeck ("OU-2") + λ model. On both trees, λ has a strong effect on the Brownian motion model, but negligible effect on the Ornstein–Uhlenbeck model.

model, in which the model is applied to the two subtrees independently, fits the data considerably better but is still a poor fit relative to the other models evaluated (BIC weight = 1.1×10^{-3}; Table 2). In the censored Brownian motion model, evolutionary rate (σ) and root ancestral state (θ_0) are inferred independently in the two subtrees. Consequently, the censored Brownian motion model should explain the data well if chromosome evolution proceeded according to a Brownian motion process in the two subtrees, with a discontinuity in chromosome number simultaneous with a shift in rate at the base of the eastern North American clade. Such a case could result, for example, from a polyploid event giving rise to the ancestor of the eastern North American clade. Although there is a demonstrable increase in evolutionary rate at the base of the eastern North American clade, the poor fit of the censored Brownian motion model relative to the other models evaluated indicates that other factors play a role in the observed pattern.

The two models with strongest support (censored Brownian motion + λ and whole-tree "OU-2," cumulative BIC weight = 0.977; Table 2) incorporate a change in evolutionary dynamics at the base of the eastern North American clade along with parameters that allow for evolutionary trends not predicted by the phylogeny under a Brownian motion model. The single parameter that most improves the model fit over the Brownian motion model is the phylogenetic dependence parameter (λ). Addition of λ to the whole-tree Brownian motion model increases the BIC weight from 2.33×10^{-7} to 1.13×10^{-2}, and the censored Brownian motion + λ model is the best fit of the models tested (BIC weight = 0.679). This reflects the fact that phylogenetic dependence differs greatly between the subtrees: the point estimate of λ, which scales from zero if patterns of covariance are not at all predicted by tree to one if patterns of covariance are perfectly predicted by the tree assuming a Brownian motion model, is zero for the eastern clade and 1.100 for the western subtree (Table 2). Interpreting λ in terms of Lynch's H^2, the phylogenetically heritable component (σ^2_a) of chromosome number variance in the eastern North American clade is negligible relative to the total variance (σ^2). In the western North American subtree, incorporating λ adds little to the explanatory power of the Brownian motion model (Tables 2 and 3). However, the power to reject Brownian motion model assumptions is typically low for small sample sizes (Housworth et al. 2002), and the minimal difference in likelihood for alternative models evaluated on the western North American subtree (Table 3) suggests that sample size in this subtree may be too low for accurate parameter estimation in more complex models. More complete sampling in the western North American subtree will likely be needed to accurately estimate model parameter differences between the subtrees.

The dual-θ Ornstein–Uhlenbeck (OU-2) model explains the data relatively well (BIC weight = 0.298; Table 2) and suggests that chromosome number evolution is driven toward clade-specific karyotypic equilibria. With $\alpha = 20$, the OU-2 model is essentially free from phylogenetic effects; consequently, adding λ changes the model likelihood by less than 0.25 log-units (Fig. 4). Similarly, the single-θ Ornstein–Uhlenbeck model and the Brownian motion + λ model are within 0.15 log-likelihood units of each other on both the western and the eastern North American subtrees, (Table 3, Fig. 4). Although the likelihood of these two models is comparable, the dual-θ model is more plausible in that it does not entail partitioning the tree into independent subtrees. Moreover, there is a straightforward interpretation of α and θ, which is lacking for λ. The point estimate of variance ($\sigma^2 = 0.331$) reflects the

high interspecific variability in chromosome number within the section, whereas the high alpha estimate ($\alpha = 20$) models the observed pattern as rapid evolution toward a karyotypic equilibrium. Taken together, the censored Brownian motion + λ and whole-tree OU-2 models provide compatible perspectives on the process of chromosome evolution in sedges.

EVOLUTIONARY AND ECOLOGICAL IMPLICATIONS

Rearrangements in holocentric chromosomes, like Robertsonian rearrangements, often do not have an effect on the fitness of hybrids (Whitkus 1988; Searle 1993; Andersson et al. 2004; Basset et al. 2006; although see Hauffe and Searle 1998 for a counterexample). If differences between populations entail single fission or fusion events, chromosome pairing in F_1 interpopulation hybrids should result in heteromorphic trivalent chromosome associations at first meiotic interphase, with little or no barrier to recombination. Trivalents are frequently observed in wild-collected sedges (Wahl 1940; Faulkner 1972; Hoshino 1981), suggesting that single-rearrangement chromosome mutations are common in natural populations and that these likely account for much of the chromosomal variation within *Carex* populations. Chromosomal variants in sedges and other organisms with holocentric chromosomes might thus be expected to accumulate gradually, as approximately neutral mutations.

Contrary to this expectation, analyses presented in this article demonstrate that the population divergence that gave rise to the eastern North American clade in *Carex* section *Ovales* entailed a dramatic shift in the dynamics of chromosome evolution. There are at least three possible explanations for this finding: (1) chromosome number evolution among the species studied is directional, and the shift in chromosome number at the base of the eastern North American clade is not a shift in karyotypic equilibrium, but a snapshot of a continuous, directional shift in mean; (2) cladogenesis entails shifts in the adaptive value of different chromosome number ranges; or (3) cladogenesis entails nonadaptive changes in karyotypic equilibrium, such that chromosome number evolves toward divergent stationary distributions in different lineages without effects on the fitness of the offspring.

Directional evolution has been shown to be important in karyotype evolution in both empirical and theoretical studies (Imai et al. 1986; Rockman and Rowell 2002). Hansen (1997) described Ornstein–Uhlenbeck models in which the rate of adaptation (α) approaches zero and the evolutionary optimum (θ_1) is outside the range of observed data as reducing to a Brownian motion model with a trend. Although not all parameters in a directional model can be estimated if all taxa are extant (Hansen and Martins 1996; Hansen 1997; cf. Pagel 1999), a very high point estimate for alpha suggests that the karyotypic equilibrium (θ_1) is reached quickly, which is precisely the opposite of the expectation under a model of directional evolution. In the current study, the single-θ Ornstein–Uhlenbeck model fits the data poorly (BIC $w_i = 1.43 \times 10^{-5}$; Table 2), and the point estimate of evolutionary rate toward θ_1 ($\alpha = 3.2181$) corresponds to an adaptive half-life of $t_{1/2} = 0.215$. At this rate of evolution, the chromosome number of a species in the western North American clade is expected to have evolved 96% of the distance from the root value to the karyotypic equilibrium, making a gradual directional trend unlikely. Moreover, the low support for change at any nodes within the western North American and eastern North American subtrees gives stronger support to a shift in mean chromosome number at the base of the eastern North American clade than a gradual directional trend in chromosome number that would affect numerous nodes.

As mentioned in the Introduction, there is not an obvious reason to expect chromosome morphology per se to affect organismal fitness (Pardo-Manuel de Villena and Sapienza 2001). Across the genus *Carex*, no life history correlates of different chromosome races are evident, such as have been found in the marine snail *Nucella lapillus* (Haskell 1952; Bantock and Cockayne 1975; Pasco and Dixon 1994). Adaptive explanations for the findings presented in this study are not supported by evidence currently available.

Karyotypic orthoselection—recurrent establishment of the same type of chromosomal rearrangement in a given lineage (White 1973)—has the potential to select for divergent chromosome arrangements in different lineages without affecting organismal fitness (Terzi 1972; Peters 1982; Greenbaum et al. 1986; Bowers et al. 1998). Nonrandom chromosome segregation (meiotic drive), for example, can drive chromosomal mutations to rapid fixation due to asymmetrical segregation of chromosome complements and the inherent polarity of female meiosis (Pardo-Manuel de Villena and Sapienza 2001). Within sedges, male meiosis (microsporogenesis) is inherently polar, as it entails the degeneration of three of the four microspores that result from each meiosis (Brown and Lemmon 2000). Selection for different chromosome numbers in different clades could easily result from clade-specific rearrangements in the distribution of heterochromatin (and, consequently, spindle-fiber attachment sites; Nagaki et al. 2005). Alternatively, chromosome numbers may evolve toward stable equilibria if chromosome number increases are accompanied by increased rates of fusion and/or decreased rates of fission. Imai's (1986) minimum-interaction hypothesis suggests that this scenario may be plausible. Because of the "suspension-arch" organization of chromosomes that is widespread in eukaryotes, the rate of interchromosome contact (and, consequently, breakage) decreases as chromosome number increases. The analyses presented in this study demonstrate an increase in the rate of chromosome evolution at the base of the eastern North American clade concomitant with a decrease in karyotypic equilibrium. If the minimum-interaction theory accounts for chromosome-number equilibria, then the mere

change in rate of chromosome evolution demonstrated in this study might account for the observed change in karyotypic equilibrium.

IMPLICATIONS FOR SPECIATION IN TAXA WITH HOLOCENTRIC CHROMOSOMES

Chromosome evolution is a driving force in speciation in diverse groups of organisms (Stebbins 1950; White 1978; Grant 1981; Coyne and Orr 2004; Ayala and Coluzzi 2005) and appears to have played an important role in the speciation of Agrodiaetus butterflies, another group with holocentric chromosomes (Kandul et al. 2007). In *Carex,* differences in chromosome number involving more than a small number of rearrangements appear to reduce the ability of different populations to cross, whereas differences involving one or few rearrangements have minimal effects (Tanaka 1949; Faulkner 1973; Cayouette and Morisset 1985; Whitkus 1988; Hoshino et al. 1993). This suggests a Dobzhansky-type speciation scenario (Gavrilets 1997), in which chromosomal rearrangements do not preclude backcrossing to the parental populations but may reduce the fertility of hybrids between populations derived from the parent. Such a speciation scenario has been postulated for mammals that undergo extensive chromosome rearrangements by monobrachial centric fusions (Baker and Bickham 1986). Increased complexity of chromosome rearrangements in shrew hybrids has been shown to correlate with decreased interpopulational gene flow (Basset et al. 2006), further supporting this scenario.

Cladogenetic shifts in equilibrium chromosome number and the dynamics of chromosome evolution have the potential to play an important role in species diversification. Within sedge species, populations isolated from one another are thought to accumulate chromosomal rearrangements gradually (Luceño and Castroviejo 1991). Because structural heterozygotes in which numerous rearrangements overlap suffer nondisjunction or incomplete chromosome pairing (Hauffe and Searle 1998), chromosome evolution toward divergent karyotypic equilibria would reduce the frequency of back mutations that might otherwise allow recently diverged populations to cross. Thus, the phenomenon of species richness in sedges may be in part a consequence of two processes in chromosome evolution working jointly. Weak underdominance of chromosomal variants, combined with shifts in karyotypic equilibria that make reversals of chromosome rearrangements less probable, could account for the great diversity of sedges that we find today.

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Associate Editor: T. Hansen

Appendix 1. **Diploid chromosome counts reported for *Carex* section *Ovales.* Chromosome counts are summarized from the literature as described in the methods. Nomenclature follows Mastrogiusseppe et al. (2002). Counts that are underlined are the mean diploid count for each species, weighted by the number of populations from which each count was found. Counts reported in the literature but excluded from analysis are indicated with an asterisk. Unpublished counts by P.E. Rothrock (Taylor University) for *C. molestiformis, C. normalis, C. projecta, C. tenera, C. tincta,* and *C. scoparia* are included and indicated as unpublished in the citations column.**

Taxon	2n	Number of Populations	Citations
WNA			
adusta Boott	78.0		
	*64	–	Löve 1981, cited in Rothrock and Reznicek 1998 as unvouchered and problematic
	78	1	Rothrock and Reznicek 1998
argyrantha Tuckerman	80.0		
	*64	–	Löve 1981, cited in Rothrock and Reznicek 1998 as unvouchered and problematic
	80	4	Rothrock and Reznicek 1998; Wahl 1940
athrostachya Olney	68.0		
	68	1	Whitkus 1991
bohemica Schreb.	80.0		
	80	1	Löve 1981, cited in Whitkus 1991
ebenea Rydberg	84.0		
	84	1	Wahl 1940
foenea Willdenow	82.7		
	*64	–	Löve 1981, cited in Rothrock and Reznicek 1998 as unvouchered and problematic
	82	2	Rothrock and Reznicek 1998
	84	1	Rothrock and Reznicek 1998
haydeniana Olney	82.0		
	82	2	Whitkus and Packer 1984
integra MacKenzie	82.0		
	82	2	Whitkus 1991
macloviana D'Urville	86.0		
	86	15	Whitkus 1991 and citations therein
microptera Mackenzie	80.2		
	80	10	Whitkus and Packer 1984; Whitkus 1991
	82	1	Wahl 1940
	* 90	1	Clausen, Keck and Hiesey 1940 as *C. festivella;* excluded from analysis
pachystachya	78.6		
Chamisso ex Steudel	74	3	Whitkus and Packer 1984; Whitkus 1991
	76	4	Whitkus and Packer 1984; Whitkus 1991 and citations therein
	78	9	Whitkus and Packer 1984; Whitkus 1991
	80	1	Whitkus 1991
	82	8	Whitkus and Packer 1984; Whitkus 1991
peucophila Holm.	74		
	74	1	Beaman et al. 1962
phaeocephala Piper	84		
	84	2	Moore and Calder 1964
praticola Rydberg	76.5		
	*64	–	Löve 1981, cited in Rothrock and Reznicek 1998 as unvouchered and problematic
	76	10	Whitkus and Packer 1984; Whitkus 1991

Continued

Appendix 1. Continued.

Taxon	2n	Number of Populations	Citations
	78	3	Packer and Whitkus 1982; Rothrock and Reznicek 1998; Whitkus 1991
preslii Steudel	79.0		
	78	1	Moore and Calder 1964; approximate ("2n = ca. 78")
	80	1	Whitkus 1991; one count of 2n = 81 (40II + 1I) excluded as it appears to be an aberrant cell from an otherwise euploid individual
subfusca W. Boott	84.0		
	84	1	Whitkus 1991
xerantica L. H. Bailey	68.0		
	68	2	Löve 1981; Rothrock and Reznicek 1998
ENA			
alata Torrey	74.0		
	74	3	Rothrock and Reznicek 1996
albolutescens Schweinitz	66.0		
	66	2	Rothrock and Reznicek 1996
bebbii (L. H. Bailey) Olney	68.7		
	68	4	Löve 1981; Moore and Calder 1964; Wahl 1940
	70	2	Packer and Whitkus 1982; Whitkus 1991
bicknellii Britton	76.0		
	74	1	Tanaka 1942, cited in Rothrock and Reznicek 1996
	76	3	Löve 1981; Rothrock and Reznicek 2001
	78	1	Rothrock and Reznicek 2001
brevior (Dewey) Mackenzie	59.5		
	48	1	Rothrock and Reznicek 1998
	52	1	Rothrock and Reznicek 1998
	56	1	Rothrock and Reznicek 1998
	60	1	Rothrock and Reznicek 1998
	64	3	Rothrock and Reznicek 1998
	68	1	Löve 1981
"Buffalo River" (cf. *molesta*)	70		
	70	1	P. E. Rothrock, unpubl. data
crawfordii Fernald	68.2		
	*52	–	Mastrogiusseppe et al. 2002, but not in line with other published counts; excluded from analysis
	*ca. 66	–	Mastrogiusseppe et al. 2002, but not in line with other published counts; excluded from analysis
	68	10	Löve 1981; Moore and Calder 1964; Packer and Whitkus 1982; Whitkus 1991
	70	1	Moore and Calder 1964; approximate ("2n = ca. 70")
cristatella Britton	70.0		
	70	2	Löve 1981; Wahl 1940
cumulata (L. H. Bailey) Mackenzie	57.3		
	*36	–	Mastrogiusseppe et al. 2002, but apparently in error (for 56); excluded from analysis
	*38	–	Mastrogiusseppe et al. 2002, but apparently in error (for 58); excluded from analysis
	56	1	Löve 1981
	58	2	Rothrock and Reznicek 1996

Countinued

Appendix 1. Continued.

Taxon	2n	Number of Populations	Citations
festucacea Schkuhr ex Willdenow	69.7		
	68	1	Rothrock and Reznicek 1996
	70	1	Rothrock and Reznicek 1996
	71	1	Wahl 1940
feta L. H. Bailey	66.0		
	66	1	Rothrock and Reznicek 1998
hormathodes Fernald	74.0		
	74	3	Rothrock and Reznicek 1996; Wahl 1940
hyalina Boott	74.0		
	74	2	Rothrock and Reznicek 1998
longii Mackenzie	60.8		
	58	1	Rothrock and Reznicek 1998
	60	1	Rothrock and Reznicek 1996
	62	3	Rothrock and Reznicek 1996
merritt-fernaldii Mackenzie	72.0		
	*68	–	Löve 1981, cited in Rothrock and Reznicek 1998 as unvouchered and problematic
	70	1	Tanaka 1942, cited in Rothrock and Reznicek 1998
	74	1	Rothrock and Reznicek 1998
missouriensis P. E. Rothrock & Reznicek	51.8		
	49	1	Rothrock and Reznicek 2001
	50	1	Rothrock and Reznicek 2001
	51	2	Rothrock and Reznicek 2001
	52	2	Rothrock and Reznicek 2001
	53	1	Rothrock and Reznicek 2001
	54	2	Rothrock and Reznicek 2001
molesta Mackenzie ex Bright	68.6		
	68	5	Löve 1981; Rothrock and Reznicek 1998; Wahl 1940
	70	2	Rothrock and Reznicek 1998
molestiformis Reznicek & P. E. Rothrock	71.7		
	70	4	P. E. Rothrock, unpubl. data
	74	3	Reznicek and Rothrock 1997
muskingumensis Schweinitz	80.0		
	80	1	Rothrock and Reznicek 1998
normalis Mackenzie	69.1		
	68	1	Löve 1981
	68	3	Wahl 1940
	70	2	P. E. Rothrock, unpubl. data
	72	1	P. E. Rothrock, unpubl. data
opaca (F. J. Hermann) P. E. Rothrock & Reznicek	66.8		
	66	2	Rothrock and Reznicek 1996; Rothrock and Reznicek 2001
	67	2	Rothrock and Reznicek 2001
	68	1	Rothrock and Reznicek 2001
oronensis Fernald	74.0		
	74	2	Rothrock and Reznicek 1998
ovalis Goodenough	65.1		
	62	2	Whitkus 1991 and citations therein
	64	3	Whitkus 1991 and citations therein
	66	4	Whitkus 1991 and citations therein; Strid in Löve 1981

Countinued

Appendix 1. Continued.

Taxon	2n	Number of Populations	Citations
	68	2	Whitkus 1991 and citations therein
ozarkana P. E. Rothrock & Reznicek	59.0		
	56	1	Rothrock and Reznicek 1996
	59	1	Rothrock and Reznicek 1996
	62	1	Rothrock and Reznicek 1996
projecta Mackenzie	64.0		
	62	1	P. E. Rothrock, unpubl. data
	64	3	Löve 1981; P. E. Rothrock, unpubl. data; Wahl 1940
	66	1	P. E. Rothrock, unpubl. data
reniformis (L. H. Bailey) Small	80.0		
	80	3	Rothrock and Reznicek 1996
scoparia Schkuhr var. *scoparia*	65.5		
	56	1	Tanaka 1942, cited in Whitkus 1991
	58	2	Tanaka 1942, cited in Whitkus 1991; P. E. Rothrock, unpubl. data
	59	1	P. E. Rothrock, unpubl. data
	60	6	Löve 1981; Moore and Calder 1964; P. E. Rothrock, unpubl. data
	61	1	P. E. Rothrock, unpubl. data
	62	5	P. E. Rothrock, unpubl. data
	64	11	P. E. Rothrock, unpubl. data; Wahl 1940
	66	8	P. E. Rothrock, unpubl. data
	67	1	P. E. Rothrock, unpubl. data
	68	7	Moore and Calder 1964; P. E. Rothrock, unpubl. data; Whitkus 1981
	70	3	P. E. Rothrock, unpubl. data
scoparia Schkuhr var *tessellata* Fernald & Wiegand	68.0		P. E. Rothrock, unpubl. data
	68	4	P. E. Rothrock, unpubl. data
shinnersii P. E. Rothrock & Reznicek	60.3		
	60	2	Rothrock and Reznicek 2001
	61	1	Rothrock and Reznicek 2001
silicea Olney	75.0		
* placement uncertain; excluded from this study	74	1	Rothrock and Reznicek 1996
	76	1	Moore and Calder 1964
straminea Willdenow	74.0		
	74	4	Rothrock and Reznicek 1996; Wahl 1940
suberecta (Olney) Britton	72.0		
	72	2	Rothrock and Reznicek 1996
tenera Dewey var. *echinodes* Fernald	76.4		
	74	1	P. E. Rothrock, unpubl. data
	76	2	P. E. Rothrock, unpubl. data
	78	2	P. E. Rothrock, unpubl. data
tenera Dewey var. *tenera*	54.5		
	52	2	P. E. Rothrock, unpubl. data; Wahl 1940
	54	4	P. E. Rothrock, unpubl. data; Wahl 1940
	56	5	Löve 1981; P. E. Rothrock, unpubl. data; Wahl 1940
tetrastachya Scheele	64.8		

Countinued

Appendix 1. Continued.

Taxon	2n	Number of Populations	Citations
*sequences available only for ITS; excluded from this study	63	1	Rothrock and Reznicek 1998
	64	2	Rothrock and Reznicek 1998
	66	3	Rothrock and Reznicek 1998
tincta (Fernald) Fernald	75		
	74	1	P. E. Rothrock, unpubl. data
	76	1	Rothrock and Reznicek 1996
tribuloides Wahlenberg var. *sangamonensis* Clokey	70.0		
*sequences available only for ITS; excluded from this study	70	1	Rothrock and Reznicek 1996
tribuloides Wahlenberg var. *tribuloides*	70.5		
	70	3	Moore and Calder 1964; P. E. Rothrock, unpubl. data; Wahl 1940
	72	1	P. E. Rothrock, unpubl. data
vexans F. J. Hermann	69.3		
	68	1	Rothrock and Reznicek 1996
	69	1	Rothrock and Reznicek 1996
	70	2	Rothrock and Reznicek 1996

APPENDIX 2. Parameter estimates and support for each of 16 Ornstein–Uhlenbeck models and the Brownian motion model, analyses conducted on the Bayesian consensus tree. Ornstein–Uhlenbeck models evaluated entail all 16 combinations of nodes 1 through 4 (Fig. 1), the nodes investigated that give rise to the largest number of taxa. Model names (e.g., "OU-2,3") indicate nodes at which karyotypic equilibria (θ_i) are permitted to change. "OU-0" is the Ornstein–Uhlenbeck model with a single karyotypic equilibrium. All θ are presented both as inferred directly from the log-transformed data and (in brackets) as e^{θ}. Confidence intervals (95%) based on parametric bootstrapping on the Bayesian consensus tree are in parentheses.

							θ_i for branches descended from:					
model	lnL	K	alpha [α]	sigma^2 [σ^2]	theta.0 [θ_0]	Root	Node 1	Node 2	Node 3	Node 4	BIC	BIC w_i
OU-2	51.519	5	20.000	0.33118	1.771E-08 [1.000]	4.37037 [79.07288]	–	4.21948 [67.99812]	–	–	−83.187	0.500
			(4.002 – 20)	(0.06101 – 0.41849)	(1.762E-08–0.16166)	(4.32748–4.45303)		(4.18889–4.28760)				
OU-1,2	52.454	6	20.000	0.31970	2.666E-08 [1.000]	4.38989 [80.63155]	4.32360 [75.45980]	4.21948 [67.99812]	–	–	−81.086	0.175
			(4.139–20)	(0.05857–0.40293)	(2.649E-08–0.21305)	(4.33889–4.46865)	(4.24756–4.42365)	(4.18976–4.27936)				
OU-2,4	51.579	6	20.000	0.33043	2.639E-08 [1.000]	4.37037 [79.07288]	–	4.21201 [67.49206]	–	4.22332 [68.25973]	−79.337	0.073
			(4.641–20)	(0.06623–0.41278)	(2.627E-08–0.12606)	(4.32740–4.42959)		(4.16053–4.27671)		(4.18650–4.27654)		
OU-2,3	51.521	6	20.000	0.33115	2.640E-08 [1.000]	4.37037 [79.07288]	–	4.21789 [67.89008]	4.22011 [68.04097]	–	−79.220	0.069
			(4.495–20)	(0.06672–0.41579)	(2.627E-08–0.14603)	(4.32827–4.43112)		(4.16276–4.28637)	(4.18450–4.27205)			
OU-1,2,4	52.516	7	20.000	0.31895	3.535E-08 [1.000]	4.38989 [80.63155]	4.32360 [75.45980]	4.21201 [67.49206]	–	4.22332 [68.25973]	−77.240	0.026
			(4.872–20)	(0.06572–0.39608)	(3.515E-08–0.13366)	(4.34120–4.45191)	(4.24617–4.40958)	(4.16185–4.27243)		(4.18794–4.26974)		
OU-1,2,3	52.456	7	20.000	0.31967	3.535E-08 [1.000]	4.38989 [80.63155]	4.32360 [75.45980]	4.21789 [67.89008]	4.22011 [68.04097]	–	−77.120	0.024
			(4.789–20)	(0.06506–0.39709)	(3.516E-08–0.14420)	(4.34019–4.45249)	(4.24594–4.41527)	(4.16278–4.28329)	(4.18570–4.26455)			
OU-1	49.688	5	20.000	0.35486	1.777E-08 [1.000]	4.38989 [80.63155]	4.23238 [68.88097]	–	–	–	−79.525	0.080
			(3.869–20)	(0.06223–0.45154)	(1.767E-08–0.18674)	(4.33810–4.48284)	(4.20448–4.31510)					
OU-2,3,4	51.703	7	20.000	0.32889	3.503E-08 [1.000]	4.37037 [79.07288]	–	4.21789 [67.89008]	4.18260 [65.53603]	4.22333 [68.26041]	−75.613	0.011
			(4.995–20)	(0.06879–0.40835)	(3.478E-08–0.11614)	(4.32780–4.42549)		(4.16034–4.28208)	(4.05838–4.31606)	(4.18671–4.27096)		
OU-1,3	50.262	6	20.000	0.34726	2.651E-08 [1.000]	4.38989 [80.63155]	4.25311 [70.32378]	–	4.22011 [68.04097]	–	−76.703	0.020
			(4.477–20)	(0.06625–0.43412)	(2.637E-08–0.14793)	(4.33689–4.46138)	(4.20745–4.31649)		(4.18306–4.27623)			
OU-1,4	49.941	6	20.000	0.35149	2.650E-08 [1.000]	4.38989 [80.63155]	4.24483 [69.74390]	–	–	4.22331 [68.25905]	−76.061	0.014
			(4.431–20)	(0.06826–0.44072)	(2.637E-08–0.15591)	(4.33821–4.46235)	(4.20052–4.30597)			(4.18554–4.27861)		
OU-1,2,3,4	52.644	8	20.000	0.31741	4.398E-08 [1.000]	4.38989 [80.63155]	4.32360 [75.45980]	4.21789 [67.89008]	4.18260 [65.53603]	4.22333 [68.26041]	−73.526	3.99E-03
			(5.163–20)	(0.06724–0.38717)	(4.371E-08–0.12325)	(4.34024–4.44792)	(4.24492–4.40929)	(4.16375–4.27907)	(4.06277–4.30994)	(4.18743–4.26947)		
OU-1,3,4	50.435	7	20.000	0.34500	3.514E-08 [1.000]	4.38989 [80.63155]	4.25311 [70.32378]	–	4.18260 [65.53603]	4.22333 [68.26041]	−73.079	3.19E-03
			(4.638–20)	(0.06862–0.42372)	(3.489E-08–0.16749)	(4.33710–4.45692)	(4.20597–4.31114)		(4.05646–4.32042)	(4.18563–4.27425)		
OU-3	44.017	5	20.000	0.43954	1.759E-08 [1.000]	4.31384 [74.72689]	–	–	4.22011 [68.04097]	–	−68.183	2.76E-04
			(3.999–20)	(0.07989–0.55912)	(1.749E-08–0.16079)	(4.27451–4.39156)			(4.17896–4.29218)			
OU-3,4	44.154	6	20.000	0.43728	2.622E-08 [1.000]	4.31384 [74.72689]	–	–	4.18260 [65.53603]	4.22333 [68.26041]	−64.486	4.35E-05
			(4.420–20)	(0.08423–0.54447)	(2.596E-08–0.15676)	(4.27581–4.37271)			(4.03710–4.33960)	(4.18117–4.28406)		
OU-4	42.754	5	20.000	0.46101	1.758E-08 [1.000]	4.30479 [74.05366]	–	–	–	4.22330 [68.25837]	−65.656	7.81E-05
			(4.093–20)	(0.08705–0.58452)	(1.748E-08–0.14642)	(4.26631–4.37948)				(4.17877–4.28541)		
OU-0	39.586	4	3.21803	0.09495	0.18641 [1.20492]	4.46980 [87.33925]	–	–	–	–	−63.291	2.39E-05
			(1.294–20)	(0.04381–0.42258)	(8.918E-08–1.95218)	(4.26743–5.13098)						
Brownian motion	31.527	2	–	0.04196	4.35119 [77.57072]	–	–	–	–	–	−55.113	4.01E-07
				(0.02696–0.05903)	(4.21415–4.49612)							

Abbreviations: lnL = natural logarithm of the model likelihood; K = number of free parameters; θ_0 = diploid chromosome number inferred at the root of the tree; θ_i = karyotypic equilibrium; BIC = Bayes information criterion; BIC w_i = BIC weight for selected model.