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Karyotype stability and predictors of chromosome number variation in sedges: A study in *Carex* section *Spirostachyae* (Cyperaceae)

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ABSTRACT

Previous work on holocentric chromosomes in the angiosperm genus *Carex* demonstrates that many of the traditional sections are marked by different ranges of chromosome number, suggesting phylogenetic autocorrelation. It has been hypothesized that shifting constraints on chromosome rearrangements may limit the potential for hybridization among lineages, promoting speciation. In this study, we evaluated alternative evolutionary models to test for such transitions in *Carex* section *Spirostachyae* as well as the relative effects of several plausible drivers of intraspecific chromosome diversity. Chromosome number variation in section *Spirostachyae* shows significant phylogenetic signal, but no evidence of clade-specific shifts in chromosome number distribution. This gradual model of chromosome evolution contrasts with the shifting equilibrium model previously identified in a younger section of the same genus, suggesting that section *Spirostachyae* may have a more slowly evolving karyotype. Chromosome number variance, on the other hand, exhibits low phylogenetic signal. Average time of coalescence rather than geographic range or chromosome number itself predicts chromosome number variance, demonstrating a previously unreported relationship between population history and cytogenetic variation.

1. Introduction

At approximately 2000 species worldwide (Reznicek, 1990), *Carex* (Cyperaceae, the sedges) is one of the largest angiosperm genera in the world and is a sizeable component of the Cyperales clade, which has undergone a rapid radiation (Magallón and Sanderson, 2001). Carices (*Carex*) exhibit remarkable chromosome diversity, ranging from $n = 6$ to $n = 62$ (Hipp et al., 2006; Roalson, 2008; Hipp et al., 2009), with many species exhibiting a range of 10 chromosome pairs or more (e.g., *C. laevigata*, $2n = 69–84$, Luceño and Castroviejo, 1991; *C. sachalinensis*, $2n = 60–84$, Tanaka, 1938; *C. scoparia*, $2n = 56–70$; *C. brevior*, $2n = 48–68$, Hipp, 2007).

Carex species have several special cytogenetic characteristics: (1) three of the four nuclei resulting from meiotic division degenerate, producing pseudomonads in lieu of functional tetrads; (2) post-reductional meiosis; and (3) holocentric chromosomes, which have a diffuse rather than localized centromere. Diffuse centromeres characterize all studied species of Cyperacae and its sister family Juncaceae (Greilhuber, 1995) as well as several unrelated angiosperm genera and scattered genera in the green algae, protists, bryophytes, nematodes, and insects of several orders (reviewed in Dernburg (2001), Mola and Papeschi (2006), and Hipp et al. (2009)).

Diffuse centromeres allow rapid evolution of chromosome rearrangements via fission (agmatoploidy; Malhehiros Gardé and Gardé, 1950; Davies, 1956b), fusion (simploidy; Luceño and Guerra, 1996), and translocations (Greilhuber, 1995). In chromosomes with localized centromeres, chromosome fragments that lack a centromere are unable to segregate normally, resulting in a loss of genetic material and potentially unviable gametes. In holocentric chromosomes, fragments are not lost at meiosis, and consequently changes in chromosome number may become stabilized through backcrossing or selfing, or even crossing among individuals that have undergone convergent rearrangements (see discussion of fragile chromosome sites in Luceño (1994) and Sutherland et al. (1998). Moreover, non-bivalent associations of holocentric chromosomes often segregate normally during meiosis, reducing the selective pressure against chromosome rearrangements (Faulkner, 1972; Luceño, 1993; Mola and Papeschi, 2006). Holocentry thus has the potential to reduce or eliminate the underdominance of chromosome rearrangements, allowing them to become fixed at a higher rate.

One of the outstanding questions in the evolution of sedges is what explains among-species differences in chromosome number distribution. Previous work at the interspecific level has demonstrated that chromosome number in at least some *Carex* clades exhibits minimal phylogenetic signal at fine scales, but

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clade-specific shifts in stationary distribution at broader phylogenetic scales (Hipp, 2007). This introduces a correlation between large-scale taxonomic groups (sections or subsections within the genus *Carex*) and chromosome number (reviewed in Wahl (1940)). Chromosome divergence has a demonstrated correlation with fitness of F_1 hybrids (reviewed in Hipp et al. (2009)) and seems likely to play a role in species diversity within the genus.

In this study, we utilize a densely sampled *Carex* group, sect. *Spirostachyae* (38 species), with substantial cytogenetic variability ($2n = 60–84$; $N = 204$ population-counts: Luceño and Castroviejo, 1993; Escudero et al., 2008a; Escudero and Luceño, 2009) to investigate four questions. First, we estimate the phylogenetic signal of chromosome number variance and chromosome number mean as a way of decomposing the phylogenetic and species-specific components of chromosome number variance. Second, we investigate whether transitions in mean chromosome number are associated with cladogenesis, testing alternative models of chromosome number evolution to evaluate whether the observed distribution of chromosome number can be explained by uniform evolutionary process or if a non-uniform process is necessary to explain the data. Third, we utilize simple, partial and multiple regression to quantify the relative contribution of mean chromosome number, mean coalescence depth of sequenced loci, geographic sampling range, and cytogenetic sampling intensity to the variance in chromosome number within species. Finally, we introduce a permutation test to evaluate the hypothesis that chromosome number variance is inherited asymmetrically by sister species as a consequence of founder effects in species that bud off of a main lineage.

2. Materials and methods

2.1. Sampling

Cytogenetic sampling included 204 population-counts representing 24 independent phylogenetic lineages, of which 21 correspond to named species (Appendix A). Each count analyzed in this study represents a distinct chromosome count found within a population (e.g., a population with 10 individuals of $2n = 72$ would provide one chromosome count; a population with 7 individuals of $2n = 72$ and 2 individuals of $2n = 74$ would provide two chromosome counts). Chromosome means and standard deviations were estimated for each species, and means were log-transformed for analysis. Sampling area for each species was defined by mapping sampling points (Appendix A) in Google Earth Pro and taking the area of the resulting polygon. Where only two chromosome observations were made, the area was taken to be a 1 km-wide rectangle terminating at the two sample points, and where only one chromosome count was made, the area was taken to be 1 km^2.

Molecular sampling included 93 accessions of 38 species of *Carex* sect. *Spirostachyae* (65 of 27 species of subsection *Elatae* and 28 of 11 species of subsect. *Spirostachyae*), three accessions of sect. *Ceratocystis*, one accession of sect. *Sylvatica*, and *C. acutiformis* and *C. rostrata* as outgroup, for a total of 99 accessions of combined ITS–5′*trn*K intron (Supplementary Table 1 (Table S1)). Sequences were downloaded from NCBI GenBank (Benson et al., 2008). For phylogenetic comparative analyses we assigned species to one of three groups: an "all-inclusive" sample of 24 lineages with at least one cytogenetic sample each; a "moderately inclusive" sample of 21 lineages with $\geqslant 2$ cytogenetic samples each; and a "restricted" sample of 17 lineages with $\geqslant 3$ cytogenetic samples. We will utilize these labels hereafter in the paper.

For molecular dating of clades, a total of 48 species were analyzed: 11 species representing the diversification of three main clades of tribe *Cariceae* (*Schoenoxiphium*, unispicate *Carex*, and *Vignea* clades; Waterway and Starr, 2007); 35 species representing the diversification of the subgenus *Carex* clade, 20 of these from sect. *Spirostachyae* (Escudero et al., 2008a; Escudero and Luceño, 2009); and two species as outgroup (*Eriophorum vaginatum* and *Scirpus polystachyus*) (Supplementary material 1). Sequences were downloaded from NCBI GenBank (Benson et al., 2008) (Supplementary material 1).

2.2. Analysis

2.2.1. Phylogenetic estimates and estimation of node depths

Phylogenetic trees with branches proportional to time were estimated using the uncorrelated log-normal relaxed clock model as implemented in BEAST v. 1.4.8 (Drummond and Rambaut, 2007). To estimate a phylogeny with one tip per in-group species, phylogeny was estimated using the combined ITS and *trn*K data using all in-group and outgroup taxa, then pruned back to one exemplar per lineage to generate the "all-inclusive", "moderately inclusive", and "restricted" samples (with 24, 21, and 17 lineages, respectively). The GTR+I+G substitution model was selected based on the Akaike Information Criterion (ΔAIC between two best models = 6.95) in MrModeltest 1.1b (Nylander, 2002). Analysis was conducted using several independent MCMC runs of 10,000,000 generations each to assess convergence, assuming the Yule tree prior with mean substitution rate set at 1.0.

To estimate average coalescent depth for each species (sensu Lahaye et al., 2008), we estimated phylogeny using ITS and *trn*K for the 21 in-group species for which at least two sequences were available per species (the three lineages with a single sequence each were excluded; Table S1). Because more sequences were obtained in species with a greater chromosome number spread and area of distribution, there is a potential ascertainment bias in the study, such that apparently deeper gene coalescence in species with greater chromosome number spread might be a product of sequencing intensity rather than actual species age. To correct for this, we analyzed the relationship between coalescence depth and chromosome number variance on both the full sample of sequences (2–5 accessions per species) and on a randomly-selected subsample of two sequences per species (Table S1).

Node ages in years were estimated using previously reported ITS clock calibrations for herbaceous angiosperms (Kay et al., 2006). The ITS1 + ITS2 matrix was analyzed in BEAST as described above, but with monophyly of section *Spirostachyae* and subsections *Spirostachyae* and *Elatae* constrained. For comparative purposes, node ages were also estimated in the previously studied *Carex* section *Ovales*, including both the crown age of the clade and the age of a known significant change in the dynamics of chromosome evolution (Hipp, 2007). Absolute age estimates reported in this paper integrate over uncertainty in the clock calibrations and in relative node depths estimated in substitutions per site by sampling both at random; age distributions are estimated based on 5000 subsamples drawn at random from both the node depth estimates in substitutions per base pair resulting from MCMC run conducted in BEAST and clock calibrations reported in Kay et al. (2006) (code available from AH).

2.2.2. Phylogenetic comparative analysis

We evaluated the mode of chromosome number evolution by evaluating the Brownian motion (BM) model against three tree rescalings proposed by Pagel (1999): (1) a *lambda*-tree, in which off-diagonal elements of the variance–covariance matrix are multiplied by a parameter λ; this parameter estimates a character's phylogenetic signal (Pagel, 1994, 1999) or phylogenetic heritability (Lynch, 1991; Housworth et al., 2004); (2) a *kappa*-tree, in which an exponent κ is applied to all branches of the phylogeny, such that branch lengths are all equal to 1 at $\kappa = 0$; this special case is often

referred to as the speciational or punctuated model; and (3) a *delta*-tree, in which an exponent δ is applied to node depths, such that character change is concentrated closer to the tips of the tree at $\delta > 1$ and closer to the base of the tree at $\delta < 1$. These three models plus the Brownian motion model (BM, BM + λ, BM + δ, BM + κ) were fitted on 1000 trees sub-sampled from the "all-inclusive" MCMC analysis, irrespective of the number of chromosome counts available for each species, with mean chromosome number as the character state. Because of the importance of phylogenetic signal to further analyses, λ was estimated for chromosome number mean and variance on the "all-inclusive", "moderately inclusive", and "restricted" samples. Analyses were conducted both without accounting for intraspecific variance and with intraspecific variance accounted for by adding the squared standard error (SEM) to the diagonal of the rescaled variance–covariance matrix; as these alternative analyses do not affect our conclusions, only analyses that do account for measurement error are reported in this paper, and additional analyses are reported in Supplemental tables. Parameter estimates are reported as mean ± phylogenetic standard error to account for phylogenetic uncertainty, and relative support estimated using Bayes information criterion (BIC) weights (Burnham and Anderson, 2002). Analyses were conducted in R using GEIGER (Harmon et al., 2008) and APE (Paradis et al., 2004).

To evaluate the hypothesis that low phylogenetic signal in chromosome number variance ($\lambda \ll 0.001$) is a consequence of asymmetric inheritance of chromosomal variance at speciation, we evaluated two test statistics. The first is the difference in standard deviation of chromosome number between each pair of sister species, which should be higher than expected at random if the asymmetric inheritance of chromosome variance persists to the present. The second is the correlation between the difference in standard deviation of chromosome number between each pair of sister species and the time since divergence of those species (i.e., phylogenetic depth of their most recent common ancestor). This correlation should be negative under asymmetric inheritance of chromosomal variance, assuming that chromosome variance increases asymptotically within lineages (Hipp, 2007). Tip states were permuted at random with respect to phylogeny to simulate the distribution of both test statistics. These are likely to be conservative tests, both because they are non-parametric and because they ignore all potential information at internal nodes. Given the low phylogenetic signal in the chromosome variance, it is not certain that the internal node states could be reconstructed reliably. Analyses were conducted in R (code available from AH), with the test statistic averaged over 1000 "all-inclusive" trees sub-sampled from the MCMC analysis and the test statistic distribution estimated by permuting tip states 999 times over each of those trees.

The probability of clade-specific transitions in chromosome number was assessed in an information theoretic framework, modeling transitions as shifts in the equilibrium value of a set of multiple-optimum Ornstein–Uhlenbeck models (Butler and King, 2004; Hipp, 2007; Hipp and Escudero, 2010). Twelve nodes were designated at which transitions in karyotype equilibria were permitted (Fig. 1), for a total of 2^{12} models on each of 100 "all-inclusive" trees sub-sampled from MCMC analysis. Assessing the 4096 models entailing all possible changes at these 12 nodes and summing information criterion weights over the models that entail a change at each node provides an estimate of the support for a change at each node. Support estimates were conditioned on the existence of each node by model-averaging only over trees that possess that node. Analyses were conducted in R using MATICCE (Hipp and Escudero, 2010) and OUCH (King and Butler, 2009).

2.2.3. Regressions

Because interspecific chromosome number variance exhibits low phylogenetic signal ($\lambda \ll 0.001$), species-specific predictors of chromosome number variance were estimated using non-phylogenetic ordinary least squares regression, which is equivalent to using phylogenetic generalized least squares regression on trees rescaled to λ = zero. Four plausible predictors of interspecific chromosome number variance were analyzed using simple and multiple regression: (1) average coalescent depth (ACD) for each species based on ITS and 5′*trn*K intron data, as a proxy for species age or genetic variation; (2) the geographic range (GR) enclosed by populations sampled for chromosome variance; (3) chromosome number mean ($2n$), as changes in chromosome number may be associated with changes in the rate of fission and fusion (Imai et al., 1986; Hipp, 2007); and (4) the number of cytogenetic (NCYT) or molecular populations (NSEQ) sampled. Additionally, we used multiple and partial regression to investigate whether two estimates of sampling intensity—number of cytogenetic samples and number of individuals sequenced—covary significantly with our predictors of chromosome variance, and whether holding sampling intensity constant affects our conclusions. All analyses and randomizations were conducted in R using the BASE and STATS packages. Nested ANOVA (conducted in STATISTICA 6.0; StatSoft, Tulsa, USA) was used to characterize cytogenetic variance among clades (subsect. *Spirostachyae* and subsect. *Elatae*), among species within clades, and among populations within species.

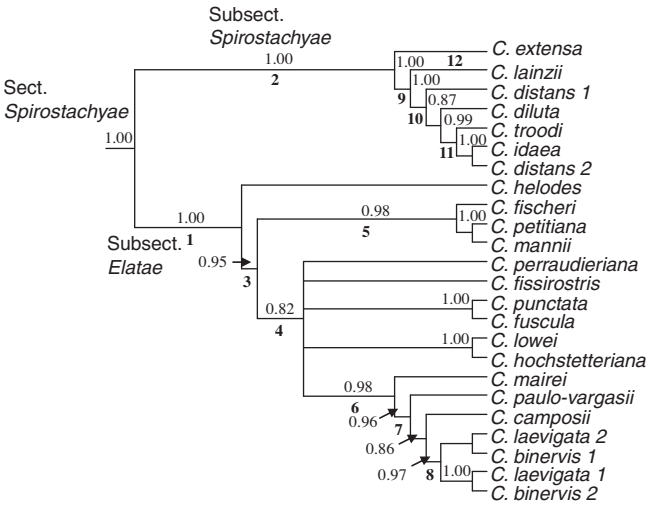

Fig. 1A. BEAST consensus tree with 24 tips (all-inclusive sampling). Posterior clade probabilities from MrBayes are shown above branches ($\geqslant$0.80). Node labels are shown below branches.

3. Results

3.1. Phylogeny and node ages

The New World clade of *Carex* section *Ovales* (Hipp, 2007) exhibits a significant shift in dynamics of chromosome evolution at 2.18 mya (0.95 confidence interval: 0.770–5.04 mya), and origin of the New World clade dates to 4.79 (1.77–10.7) mya. In contrast, the crown of section *Spirostachyae* dates to 13.8 (5.25–30.0) mya, of subsection *Elatae* to 9.90 (3.74–22.0) mya, and of subsection *Spirostachyae* to 10.95 (4.16–24.5) mya.

3.2. Phylogenetic comparative analysis

Analysis of alternative tree rescalings suggests that the constant-variance (Brownian motion) model cannot be rejected for the evolution of chromosome mean (Tables 1 and S2). While estimates of tree rescaling parameters (λ = 0.744, κ = 0.599, δ = 2.369; Tables 1 and

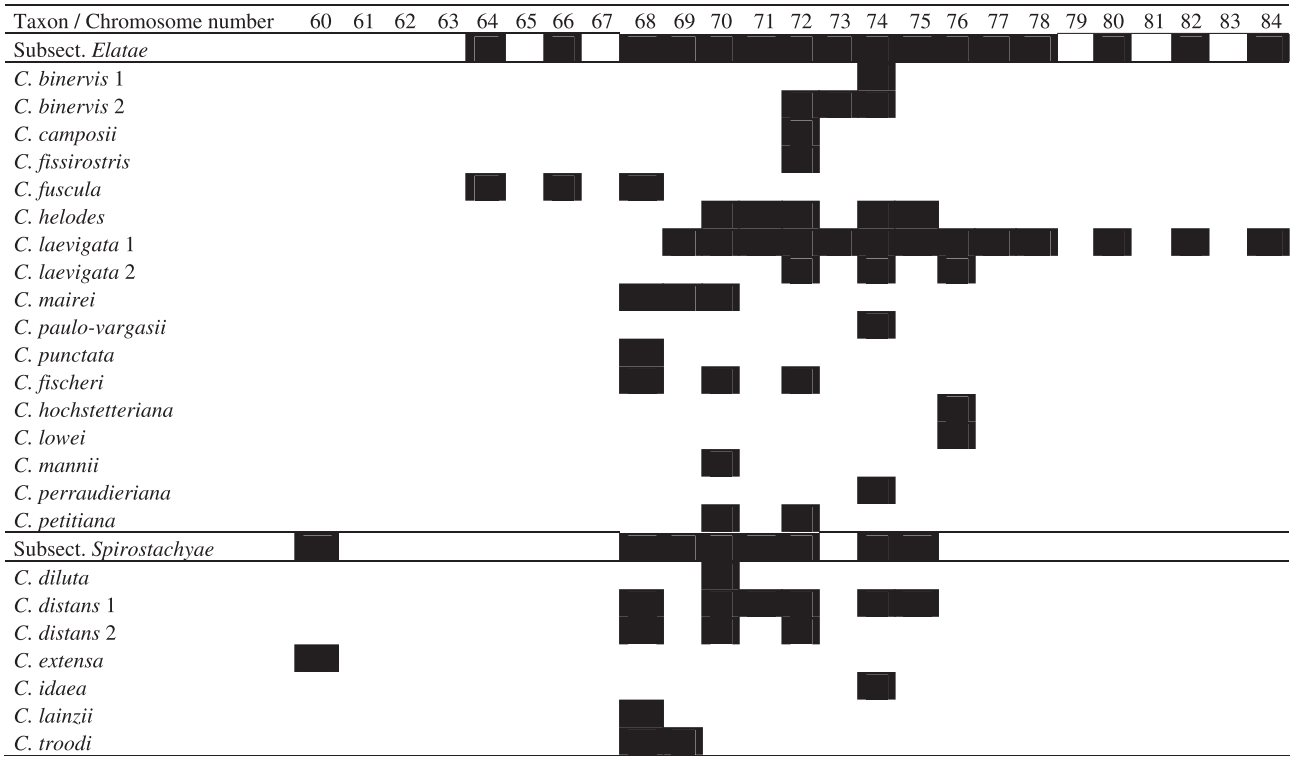

Fig. 1B. Number of chromosomes that are displayed by each different studied species or lineage as well as subsects. *Spirostachyae* and *Elatae*.

S2) suggest that chromosome number change is disproportionately concentrated near the tips of the phylogeny ($\delta > 1$) and that the mode of evolution is intermediate between a gradual and punctuational model ($1 > \kappa > 0$), support for the Brownian motion model is well above a standard 0.05 rejection threshold (BIC weight = 0.402, Tables 1 and S2). Phylogenetic signal for chromosome number is significantly higher than zero ($\lambda = 0.744$, Tables 1 and S2; BIC weight for the unconstrained model = 0.912 relative to a constrained model with $\lambda = 0$). Chromosome variance, on the other hand, exhibits no phylogenetic heritability ($\lambda = 1$ E−07 for "all-inclusive" taxon set), and the data strongly reject a constant-variance model (BIC weight $\ll 0.001$). Results were identical for the analyses of the "moderately inclusive" and "restricted" taxon set.

The permutation tests of asymmetric chromosomal variance are both non-significant, though the test statistics fall on the expected side of the permutation distribution. In the first test, the difference in variance between sister species is slightly but non-significantly greater than the mean of the null distribution (mean difference = 1.307 ± 0.0033, two-tailed $P = 0.2992 \pm 0.0023$), suggesting that sister species may be weakly divergent in chromosome number variance. In the second test, the correlation between the difference in variance and the time since sister species diverged is negative, as expected if one species inherits a disproportionately large component of the chromosomal variance and chromosome number variance accrues over time (Spearman's $\rho = -0.4497 \pm 0.0051$), but non-significant (two-tailed $P = 0.3542 \pm 0.0034$).

We tested the hypothesis that there are clade-specific transitions in chromosome number distribution by designating twelve nodes based on the criteria of posterior probability >0.95 and three or more tips in the clade (nodes labeled in Fig. 1). The only two exception are: (1) node 4, with support lower than 0.95; and (2) node 12, which was added because it leads to a single descendent (*Carex extensa*, $2n = 60$) that is an outlier in chromosome number within the study (Fig. 1; Appendix A). Support values estimated using cumulative small-sample Akaike information criterion (AIC*c*) and Bayes information criterion (BIC) weights are <0.50 for all nodes except the node leading to *C. extensa*, suggesting that clade-specific shifts in chromosome distribution do not play a significant role in the dynamics of chromosome evolution in this group (Table 2). Accordingly, a low percentage of the overall chromosome variance is attributable to among-clade differences: in a non-phylogenetic nested ANOVA, 87.4% of cytogenetic variance is apportioned among populations within species, 12.4% is apportioned among species within clades, and 0.20% is apportioned among clades.

3.3. Predictors of intraspecific chromosome variation

Variables included in regressions are shown in Table 3. The simple regression of average coalescent depth (ACD) on chromosome number standard deviation (SD) is significant whether taxa are filtered by number of sequences ($\geqslant 2$ sequences, $\geqslant 1$ cytogenetic

Table 1
Four models of evolution of chromosome mean. The log-likelihood of each model, the value of the third parameter (λ, κ, or δ), and the Bayes information criterion (BIC) weight are reported for each model as the mean over 1000 trees visited in the MCMC analysis ± phylogenetic standard error. Chromosome variance was treated as measurement error and accounted for by adding the squared SEM for each species of the diagonal of the rescaled variance–covariance matrix.

Model	Log-likelihood	σ^2	K	3rd parameter	BIC weight
Brownian motion	16.832 ± 0.028	0.266 ± 0.002	2	–	0.402 ± 0.004
Brownian motion + λ	18.143 ± 0.008	0.157 ± 0.001	3	0.744 ± 0.003	0.302 ± 0.004
Brownian motion + κ	17.454 ± 0.013	0.046 ± 0.002	3	0.599 ± 0.006	0.144 ± 0.001
Brownian motion + δ	17.514 ± 0.016	0.159 ± 0.001	3	2.688 ± 0.015	0.152 ± 0.001

Table 2
Relative support for a shift in distribution of chromosome mean at each of 12 selected nodes. Nodes were selected based on their phylogenetic support (⩾0.95 posterior probability) and number of taxa descendent from them (⩾3), with the exception of node 4 which has a node support lower than 0.95 and node 12 which has a single descendent, *Carex extensa*, and was included because *C. extensa* is an outlier in chromosome number (see Section 3). Support estimates are conditioned on the existence of each node by model-averaging only over trees that possess that node. *Abbreviations:* BIC, Bayes information criterion; AIC, Akaike information criterion; AIC*c*, small-sample Akaike information criterion. All information criteria and weights were calculated according to Burnham and Anderson (2002).

	Node at which chromosome distribution shift was tested											
	1	2	3	4	5	6	7	8	9	10	11	12
Cumulative AIC weight	0.403	0.403	0.182	0.112	0.378	0.348	0.180	0.524	0.515	0.382	0.281	0.694
Cumulative AIC*c* weight	0.261	0.261	0.088	0.067	0.190	0.197	0.132	0.357	0.354	0.179	0.127	0.751
Cumulative BIC weight	0.310	0.310	0.118	0.083	0.254	0.247	0.149	0.414	0.412	0.248	0.178	0.727

sample, $N = 21$: $r^2 = 0.281$, $P = 0.013$) or by number of sequences and cytogenetic samples (⩾2 sequences, ⩾2 cytogenetic samples, $N = 19$: $r^2 = 0.248$, $P = 0.030$). While there is a strong correlation between ACD and the number of sequences obtained per taxon (NSEQ; ⩾2 sequences, ⩾1 cytogenetic sample, $N = 21$: $r^2 = 0.249$, $P = 0.021$), ACD still explains more of the variance in SD in multiple regression, though neither predictor is significant on its own in the multiple regression due to collinearity of ACD and NSEQ (⩾2 sequences, ⩾1 cytogenetic sample, $N = 21$, standardized coefficients: ACD = 0.411, $P = 0.083$; NSEQ = 0.239, $P = 0.299$; multiple $R^2 = 0.324$, $P = 0.029$). Partial regression of SD on ACD, holding NSEQ constant, also explains more of the variance in SD (⩾2 sequences, ⩾1 cytogenetic sample, $N = 21$: $r^2_{xy.z} = 0.158$, $P = 0.074$) than the partial regression of SD on NSEQ, holding ACD constant ($r^2_{xy.z} = 0.015$, $P = 0.599$). Moreover, the simple regression of SD on ACD is significant even after rarifying our sample to two randomly-selected sequences per species ($r^2 = 0.236$, $P = 0.035$).

The geographic range over which a species is sampled (GR) does not predict SD (⩾2 cytogenetic samples, $N = 21$: $r^2 = [-]0.004$, $P = 0.791$; ⩾3 cytogenetic samples, $N = 17$: $r^2 = [-]0.002$, $P = 0.854$) despite the fact that the number of chromosome counts made for a species (NCYT) strongly predicts both SD ($r^2 = 0.416$, $P = 0.002$) and GR ($r^2 = 0.265$, $P = 0.017$). In multiple regression of SD on NCYT and GR, GR has both a weaker effect than NCYT and an unexpected effect direction (standardized coefficients: GR = −0.369, $P = 0.070$; NCYT = 0.835, $P < 0.001$). Mean chromosome number ($2n$) itself does not predict SD (⩾2 cytogenetic samples, $N = 21$: $r^2 = [-]0.057$, $P = 0.869$; ⩾3 cytogenetic samples, $N = 17$: $r^2 = 0.016$, $P = 0.288$). In multiple regression of the four potential predictors of SD, the strongest effect was shown by sampling effort (NCYT: standardized coefficient = 0.751, $P = 0.001$), the lowest by NSEQ (0.012, $P = 0.950$), and nearly equal effects by GR (−0.370, $P = 0.051$) and ACD (0.348, $P = 0.064$).

4. Discussion

This study presents two significant findings about the pattern of chromosome evolution in *Carex*: (1) high phylogenetic signal in chromosome number over a relatively deep phylogenetic timeframe (ca. 13.8 million years), with no evidence of a shift in stationary distribution as is characteristic of taxonomic shifts in the genus; and (2) low phylogenetic signal in chromosome number variance, associated with a correlation between average coalescent depth and chromosome number standard deviation. These findings demonstrate that while chromosome number exhibits high phylogenetic heritability (sensu Lynch, 1991) and the karyotype exhibits a relatively uniform mode of evolution in *Carex* section *Spirostachyae*, chromosomal variation is a species-specific property that may be best explained by the time to coalescence for genetic loci, which integrates over population size, substructure, and age. Moreover, the preliminary (but non-significant) evidence for asymmetrical inheritance of chromosome number variance presented in this study is compatible with a peripatric speciation scenario, which bears further investigation.

4.1. Phylogenetic signal and uniformity of chromosome evolution

While chromosome number change is disproportionately concentrated near the tips of the phylogeny ($\delta > 1$; Tables 1 and S2), chromosome number exhibits reasonably high phylogenetic signal ($\lambda = 0.744$ (with SEM) to 0.908 (SEM = 0), Tables 1 and S2). The only other phylogenetic study of chromosome evolution dynamics in *Carex* found relatively low phylogenetic signal in chromosome number and clade-specific stationary distributions in chromosome number (Hipp, 2007). Shifts in chromosome number distributions among taxonomic sections within *Carex* have long been recognized (Wahl, 1940), but the time frame for these shifts has not previously been documented. In the present study, the crown of section *Spirostachyae* is found to be approximately six times as old as the crown of the eastern North American clade of section *Ovales*, which was identified in the previous study as a significant transition point in chromosome number stationary distribution. While the inability to reject a constant-variance model may be related in part to sample size—the current study comprises fewer taxa ($N = 24$) than the larger clade in the earlier study (Hipp, 2007; $N = 36$)—the failure to detect a shift in stationary distribution among clades is unlikely to be. Approximately 90% of cytogenetic variance in *Spirostachyae* is apportioned among populations within species, while only 0.20% of cytogenetic variance is apportioned among the major clades within the section. This suggests the question of what influences the karyotypic stability that we see at the section level in section *Spirostachyae* in the face of such remarkable chromosome variation within species. The shifting equilibria observed in section *Ovales*, combined with the relative stasis observed in the present study, suggest that the chromosome diversity we see in sedges may be a consequence of both the near-neutrality of chromosome rearrangements and shifts in chromosomal equilibria associated with higher taxa.

4.2. Species-specific predictors of chromosomal diversity

Simple, partial, and multiple regressions conducted in this study all suggest that average coalescent depth explains chromosome number standard deviation, even when the effect of number of sequences sampled per species is accounted for. This is compatible with the hypothesis that the time to coalescence for chromosome rearrangements correlates with the time to coalescence for gene copies. Sampling range, on the other hand, does not explain chromosome variance. This remarkable finding is in keeping with observations that some widespread species are nearly invariant in chromosome number, while others show wide variance. Within section *Spirostachyae*, *Carex helodes*, a restricted endemic of the southwestern Iberian Peninsula and Morocco, displays high cytogenetic diversity ($2n = [70, 71]$ 72 [74, 75] and frequent meiotic irregularities), with relatively low levels of molecular diversity

Table 3
Variables included in regressions. *Abbreviations:* NCYT, number of individuals sampled for chromosome number; 2*n*, mean diploid chromosome number; SD, standard deviation of chromosome number; GR, geographic range of cytogenetic samples (see Section 2 for calculation); NSEQ, number of sequences obtained per taxon; ACD, average coalescent depth.

Species	NCYT	2*n*	SD	GR	NSEQ	ACD
C. binervis 1	15	74.000	0.000	471,548.22	1	–
C. binervis 2	10	73.700	0.675	2,657.04	3	0.001666
C. camposii	3	72.000	0.000	169.00	2	0.002302
C. diluta	1	70.000	0.000	1.00	2	0.000385
C. distans 1	40	71.825	2.037	1.958,409.93	5	0.004130
C. distans 2	5	70.400	1.673	68,274.77	4	0.000925
C. extensa	11	60.000	0.000	2.262,496.88	2	0.000423
C. fissirostris	2	72.000	0.000	6.13	2	0.000430
C. fuscula	4	65.500	1.915	46,876.87	3	0.002206
C. helodes	14	72.286	1.490	29,492.52	2	0.001013
C. idaea	2	74.000	0.000	5.20	2	0.000716
C. laevigata 1	42	74.571	3.270	431,695.27	3	0.001963
C. laevigata 2	11	74.182	1.079	16,185.91	1	–
C. lainzii	1	68.000	0.000	1.00	2	0.000505
C. mairei	14	68.929	0.997	315,194.48	2	0.000518
C. paulo-vargasii	5	74.000	0.000	704.05	4	0.001020
C. punctata	7	68.000	0.000	1.029,448.89	2	0.000541
C. troodi	4	68.250	0.500	70.85	2	0.000459
C. fischeri	3	70.000	2.000	14,316.01	2	0.003544
C. hochstetteriana	1	76.000	0.000	1.00	1	–
C. lowei	2	76.000	0.000	10.74	2	0.000366
C. mannii	3	70.000	0.000	305.55	3	0.002137
C. perraudieriana	2	74.000	0.000	1.66	2	0.000500
C. petitiana	3	70.667	1.155	182,300.22	3	0.004985

(mutations: ITS = 1, *rps*16 = 1; AFLP: $H_T = 0.145$, $H_S = 0.097$; Escudero et al., 2008b). *C. extensa*, on the other hand, is a widespread species of European and Mediterranean coasts that displays no cytogenetic diversity ($2n = 60$) and even lower molecular diversity than *C. helodes* (mutations: ITS = 0, *trn*K = 1; AFLP: $H_T = 0.106$, $H_S = 0.039$; Escudero et al., 2010). This example shows a higher congruence between chromosome variance and genetic diversity than between chromosome variance and geographic range, consistent with the present study. As average coalescent depth is influenced by the complex interaction between species age, population history, and subpopulation structure, it is not surprising to find that it has the strongest effect on cytogenetic variance.

One of the interesting findings of this study is the lack of correlation between chromosome number and cytogenetic diversity, given previous arguments that changes in chromosome number should predict rates of chromosome breakage (e.g., the minimum interaction hypothesis, Imai et al., 1986). Our finding is line with other studies that have failed to find support for selective arguments regarding the relationship between chromosome number and rates of variation (e.g., Arévalo et al., 1994).

4.3. Speciation pattern in species with holocentric chromosomes

Chromosome evolution has been historically thought of as a powerful driving force in speciation in diverse groups of organisms (Stebbins, 1950; Grant, 1981). Previous work in *Carex* has demonstrated that meiotic irregularities and intraspecific karyotypic variance in the genus is common and, with a few notable exceptions, most likely due to fission and fusion (Wahl, 1940; Hoshino, 1981; Luceño and Guerra, 1996; Roalson, 2008; Hipp et al., 2009). Holocentric chromosome rearrangements are unlikely to be underdominant (Whitkus, 1988; Hoshino et al., 1993; Luceño, 1993; Mola and Papeschi, 2006) and are expected to accumulate gradually as nearly-neutral mutations. Our study is consistent with this expectation, as chromosome evolution *Carex* sect. *Spirostachyae* is rapid yet exhibits phylogenetic signal and a uniform process of chromosome evolution.

At the intraspecific level, intensely sampled *Carex* species rarely exhibit gaps in chromosome number (see Roalson (2008)). In the current study, although approximately 90% of cytogenetic variation is at intraspecific level, no chromosome-number discontinuities are found within species. Yet strong discontinuities can develop among *Carex* sections, abruptly at least in some cases (Wahl, 1940; Hipp, 2007). This is likewise compatible with the scenario that *Carex* species accumulate chromosome rearrangements gradually as a uniform process, but that changes in the dynamics of selection at the cellular level (e.g., Terzi, 1972) or non-random extinction of cytotypes (e.g., Sandler and Novitski, 1957; Pardo-Manuel de Villena and Sapienza, 2001) can generate the discontinuities observed at higher levels.

While differences in chromosome number involving one or few rearrangements have minimal effects, differences involving more than a small number of rearrangements may reduce the ability of different populations to cross (Tanaka, 1949; Faulkner, 1973; Cayouette and Morisset, 1985; Whitkus, 1988; Hoshino et al., 1993; Luceño and Castroviejo, 1991). No authors have revisited the question of whether chromosome number changes precede or follow population divergence in light of the continuing contemporary chromosome work in *Carex* (reviewed in Roalson (2008)). This distinction is crucial to understanding the role of chromosome evolution in species diversification. That genetic divergence between populations is not a prerequisite to cytogenetic divergence is evidenced by the facts that different meiotic chromosome pairings (Escudero et al., 2008b; Luceño, 1994) and chromosome numbers (Luceño, 1992a; Schmid, 1982) have been detected within single individuals. Moreover, our study shows that almost 90% of cytogenetic variance is apportioned among populations within species. The question of whether chromosome rearrangement drives genetic divergence, however, remains.

Cytogenetic variation in plants has long attracted biologists' interest (Bell, 1982; Stebbins, 1950). The current study provides an important step forward in our understanding of the effects of phylogeny and species-specific properties on the evolution of chromosome cytogenetic diversity in the most species-rich angiosperm genus of the temperate zone. Specifically, our finding that intraspecific chromosome number variance is correlated with mean coalescent depth suggests that chromosome rearrangements accrue gradually with population age and substructure. Older species

Table 4
Cytogenetic sampling indicating taxon, chromosome number (2*n*), number of populations (NPOP), country (lineage in brackets), coordinates (Longitude/Latitude), and publication.

Taxon	2*n*	NPOP	Country	Longitude	Latitude	Publications
Subsect. *Elatae*						
C. binervis	72	1	(2) Spain	−3.868	40.746	Luceño and Castroviejo (1993)
	73	1	(2) Spain	−5.233	40.277	Published here
	74	1	(1) Great Britain	−1.975	54.550	Davies (1956a,b)
	74	1	(1) Belgium	6.077	50.545	Dietrich (1972)
	74	4	(1) Portugal			
				−8.577	40.148	Queirós (1980)
	74			−8.196	41.812	Luceño and Castroviejo (1993)
				−7.796	41.372	
				−7.854	41.140	
	74	19	(1) Spain	−4.644	43.343	Luceño and Castroviejo (1993)
				−5.240	43.423	
				−3.878	43.046	
				−4.109	43.389	
				−1.795	43.391	
				−1.809	43.348	
				−6.046	43.046	
				−8.873	41.903	
				−8.354	42.212	
			(2) Spain	−5.692	40.253	Luceño and Castroviejo (1993)
				−5.092	40.209	
				−5.232	40.275	
				−5.265	40.258	
				−4.476	40.281	
				−3.764	40.869	
				−3.868	40.746	
				−4.975	40.399	Published here
C. camposii	72	3	Spain	−2.549	37.235	Luceño (1992b)
				−3.401	37.091	
				−3.030	37.113	
C. diluta	70	1	Armenia	39.768	45.308	Escudero and Luceño (2009)
C. distans	68	2	(1) Morocco	−7.954	31.393	Luceño and Castroviejo (1993)
				−4.880	33.050	Escudero and Luceño (2009)
	68	1	(2) Turkey	33.588	40.538	Escudero and Luceño (2009)
	70	3	(1) Morocco	−6.567	34.250	Luceño and Castroviejo (1993)
				−5.367	34.083	Escudero et al. (2008a)
				−6.283	34.877	Escudero and Luceño (2009)
	70	7	(1) Spain	−5.274	36.916	Luceño and Castroviejo (1993)
				−4.188	43.018	
				−4.398	43.386	
				−6.479	37.101	
				−6.477	37.102	
				0.8001	42.505	
				−5.933	37.350	Escudero et al. (2008a)
				−6.444	36.989	Escudero and Luceño (2009)
	70	4	(1) Greece	22.483	40.167	Escudero et al. (2008a)
				22.633	39.500	
				21.050	40.053	Escudero and Luceño (2009)
	70	1	(1) France	−1.404	43.363	Escudero and Luceño (2009)
	70	2	(2) Turkey	32.896	39.688	Escudero and Luceño (2009)
				31.596	40.740	
	71	1	(1) Spain	−4.398	43.386	Luceño and Castroviejo (1993)
	71	1	(1) Greece	20.909	40.041	Escudero and Luceño (2009)
	72	1	(1) Italy	15.910	41.627	Dietrich (1972)
	72	1	(1) Spain	−4.398	43.386	Luceño and Castroviejo (1993)
	72	3	(1) Greece	22.483	40.167	Escudero et al. (2008a)
				21.165	39.802	Escudero and Luceño (2009)
				20.770	40.051	
	72	2	(2) Turkey	27.178	39.123	Escudero and Luceño (2009)
				26.578	40.249	
	74	2	(1) Germany	9.696	54.221	Wulff (1937)
				No locality	No locality	Tischler (1935)
	74	10	(1) Spain	−3.008	41.791	Kjellqvist and Löve (1963)
				−5.060	43.462	Luceño and Castroviejo (1993)
				−3.444	37.111	
				−2.029	40.643	
				−6.477	37.102	
				−3.566	37.667	
				−3.350	40.017	
				−3.160	40.118	
				−2.800	42.167	
				−1.549	40.959	

(*continued on next page*)

Table 4 (*continued*)

Taxon	2*n*	NPOP	Country	Longitude	Latitude	Publications
	74	1	(1) Greece	22.500	40.102	Strid and Franzen (1981)
	74	1	(1) Sweden	18.296	57.641	Heilborn (1924)
	74	1	(1) France	2.993	45.589	Davies, (1956a,b)
	75	1	(1) Crete, Greece	24.917	35.117	Escudero et al. (2008a)
C. extensa	60	1	Great Britain	−4.350	53.283	Davies (1956a,b)
	60	1	Italy	15.910	41.627	Dietrich (1972)
	60	1	Germany	9.696	54.221	Wulff (1937)
	60	3	Portugal	−8.864	40.148	Rodrigues (1953)
				−8.852	40.151	Queiros (1980)
				−9.220	39.396	Luceño and Castroviejo (1993)
	60	1	France	3.597	43.353	Labadie (1976)
	60	3	Spain	−7.051	43.465	Luceño and Castroviejo (1993)
				−0.333	39.383	
	60	1	Greece	20.456	39.279	Escudero et al. (2008a)
C. fischeri	68	1	Kenya	34.691	1.022	Escudero and Luceño (2009)
	70	1	Kenya	36.634	−0.804	Escudero and Luceño (2009)
	72	1	Kenya	37.212	−0.171	Escudero and Luceño (2009)
C. fissirostris	72	2	Morocco	−7.850	31.200	Luceño and Castroviejo (1993)
				−7.800	31.233	Escudero et al. (2008a)
C. fuscula	64	2	Chile	−73.227	−37.978	Escudero et al. (2008a)
				−73.054	−36.827	Escudero and Luceño (2009)
	66	1	Chile	−73.028	−36.862	Escudero et al. (2008a)
	68	1	Argentina	−64.787	−31.882	Escudero and Luceño (2009)
C. helodes	70	2	Spain	−6.250	37.580	Published here
				−6.585	37.463	
	71	1	Portugal	−8.720	37.780	Escudero et al. (2008b)
	72	2	Spain	−6.250	37.580	Published here
				−6.585	37.463	
	72	5	Portugal	−7.950	37.230	Luceño (1992b) and Escudero et al. (2008b)
				−7.940	37.220	
				−8.750	37.180	
				−8.520	37.050	
				−8.720	37.780	
	74	3	Morocco	−5.370	35.080	Escudero et al. (2008b)
				−5.330	35.100	
				−5.350	35.120	
	75	1	Portugal	−8.520	37.050	Escudero et al. (2008b)
C. hochstetteriana	76	1	Azores Islands, Portugal	−27.226	38.644	Luceño (1992a)
C. idaea	74	2	Crete, Greece	24.820	35.154	Escudero et al. (2008a), Escudero and Luceño (2009)
				24.833	35.200	Escudero and Luceño (2009)
C. laevigata	69	1	(1) Spain	−1.890	43.247	Luceño and Castroviejo (1993)
	70	1	(1) Portugal	−8.279	41.730	Luceño and Castroviejo (1993)
	71	2	(1) Spain	−1.890	43.247	Luceño and Castroviejo (1991)
				−8.880	41.822	
	72	1	(1) Great Britain	−2.613	54.461	Davies (1956a,b)
	72	1	(1) Germany	6.083	50.776	Dietrich (1972)
	72	5	(1) Spain	−1.890	43.247	Luceño and Castroviejo (1991)
				−4.234	43.346	
				−6.674	42.879	
				−8.516	42.181	
			(2) Spain	−5.476	36.606	Escudero et al. (2008a)
	72	4	(1) Portugal	−7.787	42.266	Luceño and Castroviejo (1991)
				−7.931	40.916	
				−7.930	41.006	
				−7.930	41.006	
	73	1	(1) Portugal	−7.930	41.006	Luceño and Castroviejo (1991)
	73	2	(1) Spain	−1.890	43.247	Luceño and Castroviejo (1991)
				−4.234	43.346	
	74	10	(1) Spain	−4.234	43.346	Luceño and Castroviejo (1991)
				−6.551	42.876	
				−8.467	42.233	
				−7.377	39.288	
			(2) Spain	−3.540	38.339	Luceño and Castroviejo (1993)
				−4.065	39.413	
			(2) Spain	−5.650	36.867	Escudero et al. (2008a)
				−4.033	38.417	
				−5.733	36.467	
				−5.476	36.606	
				−5.527	36.579	
				−5.583	36.517	
	74	6	(1) Portugal	−8.413	40.199	Luceño and Castroviejo (1991)
				−7.954	40.241	

Table 4 (*continued*)

Taxon	2*n*	NPOP	Country	Longitude	Latitude	Publications
				−9.460	38.758	
				−7.583	38.733	Escudero et al. (2008a)
				−8.550	37.283	
				−8.517	37.300	
	75	1	(1) Portugal	−8.413	40.199	Luceño and Castroviejo (1991)
	76	3	(1) Spain	−6.530	36.930	Luceño and Castroviejo (1991)
			(2) Spain	−5.650	36.867	Escudero et al. (2008a)
				−5.450	36.683	
	76	3	(1) Portugal	−8.887	37.677	Luceño and Castroviejo (1991)
				−8.617	37.633	Escudero et al. (2008a)
				−8.517	37.300	
	76	1	(1) Morocco	−5.516	36.067	Escudero et al. (2008a)
	77	1	(1) Morocco	−5.516	36.067	Escudero et al. (2008a)
	78	3	(1) Spain	−6.530	36.930	Luceño and Castroviejo (1991)
				−5.553	36.027	
				−5.666	36.115	
	80	2	(1) Spain	−6.530	36.930	Luceño and Castroviejo (1991)
				−5.633	36.133	Escudero et al. (2008a)
	80	1	(1) Morocco	−5.517	36.067	Escudero et al. (2008a)
	82	1	(1) Spain	−5.567	36.183	Escudero et al. (2008a)
	84	1	(1) Spain	−5.687	36.142	Escudero et al. (2008a)
C. lainzii	68	1	Spain	−4.039	41.304	Luceño et al. (1988)
C. lowei	76	2	Madeira Islands	−16.967	32.733	Escudero et al. (2008a)
				−16.867	32.783	
C. mairii	68	7	Spain	−2.626	42.105	Davies (1956b)
				−3.008	41.791	Kjellqvist and Löve (1963)
				−2.495	38.672	Luceño and Castroviejo (1993)
				−0.335	38.674	
				−3.444	37.111	
				−2.029	40.643	
				−1.650	40.550	
	69	1	Spain	−2.029	40.643	Luceño and Castroviejo (1993)
	70	1	Italy	8.528	44.420	Dietrich (1972)
	70	5	Spain	−2.495	38.672	Luceño and Castroviejo (1993)
				−4.233	43.009	
				−1.979	40.224	
				−2.029	40.643	
				−3.565	37.677	
C. mannii	70	3	Kenya	36.634	−0.804	Escudero and Luceño (2009)
				37.212	−0.171	
				37.246	−0.059	
C. paulo-vargasii	74	5	Morocco	−5.333	35.100	Escudero et al., 2008a
				−4.517	34.867	
				−4.083	34.633	
				−4.533	34.917	
				−4.767	34.900	
C. perraudieriana	74	2	Canary Islands, Spain	−16.217	28.550	Luceño (1992a)
				−16.201	28.545	Published here
C. petitiana	70	1	Malawi	33.809	−10.575	Dietrich (1964)
	70	1	Kenya	34.691	1.022	Escudero and Luceño (2009)
	72	1	Kenya	37.246	−0.059	Escudero and Luceño (2009)
C. punctata	68	1	?	No locality	No locality	Heilborn (1924)
	68	1	Great Britain	−4.350	53.283	Davies (1956a,b)
	68	1	Italy	10.170	44.058	Dietrich (1972)
	68	3	Spain	−5.059	43.462	Luceño and Castroviejo (1993)
				−5.055	43.438	
				−1.985	43.321	
	68	1	Morocco	−5.600	35.783	Escudero et al. (2008a)
C. troodi	68	3	Cyprus	32.867	34.900	Escudero et al. (2008a), Escudero and Luceño (2009)
				32.993	34.933	
				32.683	34.983	
	69	1	Cyprus	32.833	34.950	Escudero et al. (2008a)

may be the engines of speciation in *Carex*. This effect may be enhanced if chromosome rearrangements strongly influence recombination rates (Bell, 1982) or produce novel linkage groups that have the potential to harbor locally adapted gene complexes (Heilborn, 1924; Faulkner, 1972). Moreover, the diminished ability of inter-populational hybrids to produce viable offspring when the parent populations differ by two or more chromosome rearrangements (Tanaka, 1949; Cayouette and Morisset, 1985; Whitkus, 1988) may enhance the potential for species with greater cytogenetic diversity to give rise to new species.

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Appendix A

See Table 4.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.07.009.

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