Chromosomes tell half of the story: the correlation between karyotype rearrangements and genetic diversity in sedges, a group with holocentric chromosomes

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Abstract

Chromosome rearrangements may affect the rate and patterns of gene flow within species, through reduced fitness of structural heterozygotes or by reducing recombination rates in rearranged areas of the genome. While the effects of chromosome rearrangements on gene flow have been studied in a wide range of organisms with monocentric chromosomes, the effects of rearrangements in holocentric chromosomes—chromosomes in which centromeric activity is distributed along the length of the chromosome—have not. We collected chromosome number and molecular genetic data in Carex scoparia, an eastern North American plant species with holocentric chromosomes and highly variable karyotype (2n = 56–70). There are no deep genetic breaks within C. scoparia that would suggest cryptic species differentiation. However, genetic distance between individuals is positively correlated with chromosome number difference and geographic distance. A positive correlation is also found between chromosome number and genetic distance in the western North American C. pachystachya (2n = 74–81). These findings suggest that geographic distance and the number of karyotype rearrangements separating populations affect the rate of gene flow between those populations. This is the first study to quantify the effects of holocentric chromosome rearrangements on the partitioning of intraspecific genetic variance.

Keywords: amplified fragment length polymorphisms, Carex, chromosome rearrangements, Cyperaceae, holocentric chromosomes, karyotype evolution

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Introduction

Chromosome rearrangements play an important role in partitioning genetic variance, both within and among species (White 1969; Rieseberg 2001; 2007; Noor et al. 2002; Ayala & Coluzzi 2005; Feuk et al. 2005; Noor et al. 2007). Although many studies have focused on the role of inversions in protecting species-specific or population-specific regions of the genome from recombination and thus preserving adaptive gene combinations, chromosome fission and fusion also have the potential to limit gene flow and drive speciation (Baker & Bickham 1986; Basset et al. 2006). Most work on the effects of chromosome fission and fusion has been undertaken in organisms that exhibit Robertsonian rearrangements (Bantock & Cockayne 1975; Searle 1986; Davisson & Akeson 1993; Nachman & Searle 1995; Hauffe & Searle 1998; Bidau et al. 2001; Pardo-Manuel de Villena & Sapienza 2001; Rowell et al. 2002; Dumas & Britton-Davidian 2002; Panithanarak et al. 2004), i.e. non-reciprocal translocations involving fission and fusion at or near a centromere. These studies demonstrate the potential for Robertsonian fusions to decrease recombination in rearranged areas of the genome among populations that are connected by gene flow.

The effects of fission and fusion on gene flow in organisms whose chromosomes lack localized
centromeres—holocentric chromosomes—is not as well understood. In holocentric chromosomes, spindle fibers (microtubules) attach along the entire length of the chromosome arm, dragging the chromosome broadside toward the poles at anaphase (Dernburg 2001; Nagaki et al. 2005). Chromosome fragments that would be acentric and consequently lost in an organism with localized centromeres may be inherited in Mendelian fashion in organisms with holocentric chromosomes (Faulkner 1972; Luceño 1993), and gametes involving chromosome fragments are consequently expected to be viable. Holocentric chromosomes are known in plants primarily from the angiosperm sedge family Cyperaceae (c. 5000 species) and its sister family, the rushes (Juncaceae, c. 430 species), but they are also known in at least four other angiosperm genera, a few algae, several arthropod orders, and nematodes, including the model system Caenorhabditis elegans (Godward 1954; King 1960; Flach 1966; Tanaka & Tanaka 1977; Sheikh et al. 1995; Pazy 1997; Perez et al. 1997; Buchwitz et al. 1999; Nokkala et al. 2002; Guerría & García 2004; Wang & Porter 2004). Although strong selection against structural heterozygotes appears to maintain karyotypic stability in C. elegans (Dernburg 2001), holocentricity is accompanied by extensive and rapid karyotypic variability within and among species in the sedge genus Carex and in some arthropod genera (e.g., Heilborn 1924; Hoshino 1981; Normark 1999; Cook 2001; Kandul et al. 2007). In Carex, extensive studies of chromosome pairing relationships in meiosis show an abundance of univalent and heteromorphic trivalent associations, with quadrivalents less common. The abundance of univalents and trivalents suggests that chromosome number changes are due to breakages, fusions, or translocations (Wahl 1940; Faulkner 1972; Hoshino 1981, 1992; Hoshino & Okamura 1994; Hoshino & Onimatsu 1994; Hoshino et al. 1994). Moreover, these findings suggest that pairing relationships are often not adversely affected by these rearrangements. It is consequently unclear what effects we should expect holocentric chromosome rearrangements to have on gene flow within species.

In this study, we use a combination of molecular genetic data (amplified fragment length polymorphisms, AFLPs) and chromosome counts in the widespread and karyotypically diverse sedge Carex scoparia Schkuhr ex Willdenow var. scoparia (2n = 56–70) to test two interrelated hypotheses: (i) that the various chromosome numbers within C. scoparia var. scoparia represent chromosome ‘races’ that are genetically differentiated from one another to a degree comparable to species or infraspecific taxa and (ii) that chromosome rearrangements have an effect on molecular genetic structure within species. If chromosome ‘races’ represent cryptic species, we expect to see deep genetic breaks that correspond to chromosome rearrangements. Similarly, if chromosome rearrangements restrict gene flow, we expect to see a correlation between chromosome number differences and genetic distance at least at small geographic scales. We compare our results with a reanalysis of isozyme and chromosome number data gathered in the western North American species Carex pachystachya (Whitkus 1988b, 1991, 1992), which is found in a different Carex clade, to investigate whether our findings are unique to C. scoparia or applicable to a broader phylogenetic range of the genus.

Materials and methods

Study organism

The eastern North American C. scoparia var. scoparia exhibits substantial karyotypic diversity, ranging from 2n = 56 to 2n = 70 (Fig. 1; Table 1; note that the 2n = 56 count has not been confirmed through our own work, and no populations of this count are consequently represented in the current study). A sister variety, C. scoparia var. tessellata Fernald & Wiegand, is a regional endemic apparently limited to two counties in Maine with only one known chromosome number (2n = 68; Table 1). The western North American C. pachystachya Chamisso ex Steudel is widely distributed, ranging from northern California and Colorado to Alaska. These species are in different major clades of a species-rich and karyotypically diverse group, Carex section Ovales, which has been the focus of substantial phylogenetic and cytological research (Whitkus 1988a,b, 1991; Rothrock & Reznicek 1996, 1998, 2001; Reznicek & Rothrock 1997; Hipp 2007; Hipp et al. 2006, 2007). Detailed investigations in the genus have demonstrated that hybrids tend to be either rare and sterile (Cayouette & Catling 1992; Waterway 1994; Ball & Reznicek 2002; Smith & Waterway 2008), or frequent in a limited number of taxa (Cayouette & Catling 1992). In section Ovales, there is no demonstration of well established, naturally occurring hybrids. All available information on reproductive isolation between species indicates that chromosomal differences do not play a role, but instead, genetically determined barriers, either pre- or post-mating, are the norm (Whitkus 1988a,b, 1991, 1992). Previous study of C. pachystachya demonstrated that populations are typically invariant in chromosome number, and that when there is variation (in 3 of 20 populations studied), individuals differ by at most one chromosome pair (Whitkus 1991). Variation within sibling families has also been observed; variants all possessed the same inferred diploid count, differing only in the possession of tetravalents at meiosis I, which is evidence of rearrangements (e.g. translocations or
inversions) that affect pairing relationships but not chromosome number (Whitkus 1991). Polyploidy is unknown in the section (Whitkus 1988a, 1991; Rothrock & Reznicek 1998), with the possible exception of a putative allotetraploid not closely related to *C. scoparia* (Hipp et al. 2006). All observations of chromosome pairing relationships in the genus support the stance that chromosome evolution in *Carex* is predominantly by fission and fusion (reviewed in Hipp et al. 2009).

**Sampling and chromosome data**

Examplars were collected from 35 populations of *C. scoparia* var. *scoparia* (*N* = 42 individuals) and two populations of *C. scoparia* var. *tessellata* (*N* = 6), a total of 48 individuals (Table 1; Fig. 1). This sampling was designed to maximize population sampling, based on previous studies finding that genetic and karyotypic variance are extremely limited within populations of related *Carex* species in the section *Ovales* (Whitkus 1991, 1992). Additional genetic sampling within populations would very likely reduce sampling error in estimating genetic distances among populations, but was not conducted because the initial study of chromosome variance from which our samples were drawn was not built around a population-genetic study. Two species (*C. longii* and *C. texans*) were included as outgroups based on previous phylogenetic studies (Hipp et al. 2006, 2007). Because analyses were conducted at the level of the individual, on the assumption that individuals are an appropriate proxy for populations, *C. scoparia* var. *scoparia* specimens were subsampled to a single individual per population for most analyses (referred to as unique-by-population samples; *N* = 35 individuals, 24 permutations based on removal of one individual per permutation from each of populations 3485, 3633, and 3656 and removal of two individuals from 3489). Analyses were conducted over all unique-by-population permutations and statistics are presented as mean ± standard error of the mean (SEM) calculated over permutations. *Carex pachystachya* samples were analysed at the population level (average population size *N* = 17.4 ± 3.5 [SEM]), and consequently no subsampling was necessary. Latitude and longitude were estimated for each population to < 1 km precision by mapping populations in Google Earth (Google, Inc., Mountain View, CA, USA). These coordinates were used to generate a pairwise geographic distance matrix of great-circle distances using the Haversine formula (Sinnott 1984).

Chromosome analyses for *C. scoparia* followed the technique of Cooperrider & Morrison (1967), as described by Rothrock & Reznicek (1996). For each individual studied, an average of five pollen mother cells were inspected at first meiotic metaphase. Drawings, photographs (Fig. 2), and voucher specimens have been
Chromosome numbers (Table 1) are reported as the number of bivalents observed at first meiotic metaphase, followed by the number of univalents ('I'), trivalents ('III'), or tetravalents ('IV'). Inferred diploid chromosome numbers were inferred using the following formula: 

\[ 2n = \text{univalents} + 2 \times \text{bivalents} + \cdots + x \times \text{valents}, \]

where \( x \) indicates the number of chromosomes.

### Table 1: Individuals sampled for this study

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deposited at the University of Michigan Herbarium. Chromosome numbers (Table 1) are reported as the number of bivalents observed at first meiotic metaphase, followed by the number of univalents ('I'), trivalents ('III'), or tetravalents ('IV'). Inferred diploid chromosome numbers were inferred using following the formula: \( (1 \times \text{univalents}) + (2 \times \text{bivalents}) + \cdots + (x \times \text{x-valents}), \) where \( x \) indicates the number of chromosomes.

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associated with one another at first meiotic metaphase. Some individuals counted have odd inferred diploid counts as a consequence of hybridization between individuals with differing chromosome numbers (Whitkus 1988a). Chromosome counting methods and results for *C. pachystachya* were reported in previous publications (Whitkus 1988a,b, 1991, 1992).

**Molecular data**

AFLP methods for *C. scoparia* follow Vos et al. (1995), as adapted in a previous study in the genus (Hipp et al. 2007). Briefly, DNA was extracted from oven-dried leaf tissue from individuals grown in the greenhouse for cytological study, using the DNeasy filter method (Qiagen, Valencia, CA, USA). Total genomic DNA was digested using *Mse*I and *Eco*RI, then amplified using seven primer pairs selected based on an earlier screening (Hipp et al. 2007). Primer pairs utilized were E-ACT / M-CTT, E-ACT / M-CCG, E-ATG / M-CAG, E-AGC / M-CAG, E-ATG / M-CGA, E-ATG / M-CTC, and E-ATT / M-CGT (see Hipp et al. 2007 for full primer sequence). Each primer pair differs by at least two base pairs from the other primer pairs used. Final PCR amplifications were performed with a 6-FAM labelled forward primer, cleaned using the CleanSEQ kit (Agencourt, Beverly, MA, USA), and run out on an ABI 3730 (Applied Biosystems, Foster City, CA, USA) capillary sequencer using a ROX-labelled internal lane standard (GeneFlo 625; CHIMERx, Milwaukee, WI, USA) that runs from 50 to 625 base-pairs (bp) in length at 25 bp intervals. Final amplifications were performed twice and scored as present or absent in GeneMapper vers. 3.7 (ABI). Bands were scored only if they could be unambiguously scored in all individuals, resulting in a total of 652 markers, 499 of which are present in *C. scoparia* var. *scoparia*. Pairwise genetic distances between individuals were calculated using the simple matching distance (for Mantel tests) or Nei & Li’s (1979) restriction site distance (for minimum-evolution tree). Analyses performed using Jaccard’s distance in lieu of simple matching distance did not affect results and are not reported. Genetic data for *C. pachystachya* were based on 21 allozyme loci analysed for 24 populations described in Whitkus (1992). Three populations which were part of the original study but did not have chromosome counts have been included in this study as their numbers were determined at a later date (2440, *n* = 39; 2276 and 2502, *n* = 41). Pairwise genetic distances between populations were calculated using Nei’s unbiased genetic distance (Nei 1978, eqn 6).
Analyses

AFLP data were analysed using minimum evolution (ME; Rzhetsky & Nei 1992) on a pairwise distance matrix calculated using Nei & Li’s (1979) restriction site distance to assess whether there are there deep phylogenographic breaks within C. scoparia comparable to the break between species. Branch support was estimated using 10,000 non-parametric bootstrapping replicates. Analyses were performed in PAUP* 4.0b10 (Swofford 2002).

Minimum number of karyotype rearrangements required to explain the observed chromosome distribution was estimated using ordered parsimony on a subsample of 1000 trees from the bootstrap analysis using Mesquite (Maddison & Maddison 2009). Clade ages for Carex section Ovales were estimated using a molecular clock calibration based on nuclear ribosomal DNA internal transcribed spacer (ITS) region (Kay et al. 2006) on published data (Hipp et al. 2006). Node age means and confidence intervals were estimated by drawing rates at random from absolute rates reported for herbaceous angiosperms (Kay et al. 2006) and node ages drawn at random from estimates based on the log-normal relaxed clock model as implemented in BEAST (Drummond et al. 2006; Drummond & Rambaut 2007) using the morton package (Hipp 2009) in R version 2.6.2 (R Development Core Team 2004).

To estimate the relative effects of geographic distance and chromosome number difference on genetic variance within species, we utilized the multiple-regression extension of the Mantel test (Mantel 1967; Smouse et al. 1986). We calculated the Pearson product-moment correlation coefficient for five models: (i) genetic distance affected by geographic distance only; (ii) genetic distance affected by chromosome number difference only; (iii) genetic distance affected by chromosome number identity only, where identity = 1 or 0 for pairwise comparisons that have or do not have the same chromosome number respectively; (iv) genetic distance affected by both chromosome number and geographic distance and (v) genetic distance affected by both chromosome number identity and geographic distance (Table 2). The two different chromosome number matrices (chromosome number difference and chromosome number identity) were analysed to assess whether genetic differentiation is better predicted by the presence of a chromosome number difference between populations or by the absolute difference in chromosome number. To determine whether chromosome rearrangements act in

Table 2 Standard and partial Mantel test significance levels for correlations among AFLP, geographic, and chromosome data

<table>
<thead>
<tr>
<th>Model</th>
<th>Total variance explained</th>
<th>Geographic distance</th>
<th>Chromosome number difference</th>
<th>Chromosome number identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carex scoparia var. scoparia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) $Y \sim \Delta km$</td>
<td>$R^2 = 0.0543 \pm 0.0024$</td>
<td>$r^2 = 0.0543 \pm 0.0024$, $P = 0.0019$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(2) $Y \sim \Delta 2n$</td>
<td>$R^2 = 0.0388 \pm 0.0014$</td>
<td>–</td>
<td>$r^2 = 0.0388 \pm 0.0014$, $P = 0.0020$</td>
<td>–</td>
</tr>
<tr>
<td>(3) $Y \sim (1 - \text{identity})$</td>
<td>$R^2 = 0.0188 \pm 0.0008$</td>
<td>–</td>
<td>–</td>
<td>$r^2 = 0.0188 \pm 0.0008$, $P = 0.0043$</td>
</tr>
<tr>
<td>(4) $Y \sim \Delta km + \Delta 2n$</td>
<td>$R^2 = 0.1047 \pm 0.0027$</td>
<td>$r^2 = 0.0686 \pm 0.0026$, $P = 5.0e-04$</td>
<td>$r^2 = 0.0533 \pm 0.0016$, $P = 0.0004$</td>
<td>–</td>
</tr>
<tr>
<td>(5) $Y \sim \Delta km + (1 - \text{identity})$</td>
<td>$R^2 = 0.0739 \pm 0.0026$</td>
<td>$r^2 = 0.0562 \pm 0.0024$, $P = 0.0015$</td>
<td>–</td>
<td>$r^2 = 0.0207 \pm 0.0009$, $P = 0.0031$</td>
</tr>
<tr>
<td>(6) $Y \sim \Delta 2n + (1 - \text{identity})$</td>
<td>$R^2 = 0.0403 \pm 0.0014$</td>
<td>–</td>
<td>$r^2 = 0.0219 \pm 0.0011$, $P = 0.0015$</td>
<td>$r^2 = 0.0015 \pm 2e-04$, $P = 0.1825$</td>
</tr>
<tr>
<td>Carex pachystachya</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) $Y \sim \Delta km$</td>
<td>$R^2 = 0.0117$</td>
<td>$r^2 = 0.0117$, $P = 0.0963$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(2) $Y \sim \Delta 2n$</td>
<td>$R^2 = 0.1844$</td>
<td>–</td>
<td>$r^2 = 0.1844$, $P = 2.00E-4$</td>
<td>–</td>
</tr>
<tr>
<td>(3) $Y \sim (1 - \text{identity})$</td>
<td>$R^2 = 0.1644$</td>
<td>–</td>
<td>–</td>
<td>$r^2 = 0.1644$, $P &lt; 5.00E-5$</td>
</tr>
<tr>
<td>(4) $Y \sim \Delta km + \Delta 2n$</td>
<td>$R^2 = 0.2565$</td>
<td>$r^2 = 0.2721$, $P = 0.0358$</td>
<td>$r^2 = 0.1971$, $P = 1.00E-4$</td>
<td>–</td>
</tr>
<tr>
<td>(5) $Y \sim \Delta km + (1 - \text{identity})$</td>
<td>$R^2 = 0.1906$</td>
<td>$r^2 = 0.0313$, $P = 0.0284$</td>
<td>–</td>
<td>$r^2 = 0.1810$, $P = 1.00E-4$</td>
</tr>
<tr>
<td>(6) $Y \sim \Delta 2n + (1 - \text{identity})$</td>
<td>$R^2 = 0.1923$</td>
<td>–</td>
<td>$r^2 = 0.0333$, $P = 0.0244$</td>
<td>$r^2 = 0.0096$, $P = 0.1170$</td>
</tr>
</tbody>
</table>

Models are abbreviated as follows: $Y$, pairwise genetic distance; $\Delta km$, pairwise geographic distance; $\Delta 2n$, pairwise chromosome number difference; identity, pairwise chromosome number identity, where 1 denotes chromosome number is the same for individuals / populations, 0 denotes chromosome number differs between individuals / populations. Taxon sets are described in the text. All significance levels are based on 10,000 permutations and presented as the mean over taxon sets ± SEM for the C. scoparia tests (SEM calculated over $N = 24$ permutations).

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concert to effect genetic differentiation, a sixth model including both matrices—(6) genetic distance affected by chromosome number identity and chromosome number distance—was evaluated, and their partial correlation coefficients and significance calculated as a way of directly comparing the matrices. Because chromosome number identity is simply a rescaled chromosome number difference (where a difference > 0 is set to 1), the two are not taken to be independent predictors of genetic distance. Rather, the multiple and partial correlations are estimated as a way of evaluating whether chromosome number difference explains significantly more of the variance in genetic distance than chromosome number identity does. The multiple coefficient of determination \( R^2 \) was calculated as an estimate of the proportion of variance in the response variable (in this case, the pairwise genetic distances) explained by variance in the predictors. In the multiple models, the partial correlation coefficient \( r^2 \) was calculated for each predictor variable \( X_i \) as a measure of the proportion of variance in pairwise genetic distances explained by \( X_i \) when all other predictors in the model are held constant with respect to \( X_i \). Analyses were performed using the \textit{morton} and \textit{vegan} (Oksanen et al. 2007) packages in R.

Spatial cross-correlation among distance matrices was calculated to assess whether observed correlations between genetic distance and chromosome number difference differ significantly with varying geographic scale (Reich et al. 1994; Koenig 1999). To assess whether the correlation between chromosome number and genetic distance in the AFLP dataset might be biased by a positive correlation between chromosome number and the number of amplifying loci or alleles, Spearman rank-order was used to estimate correlation between \( 2n \) chromosome number and number of AFLP bands detected per individual. In the \textit{C. pachystachya} allozyme dataset, all populations exhibited allozyme patterns consistent with diploid expectations, so no analogous analysis was conducted. Analyses were conducted in R using the \textit{vegan}, \textit{ape} (Paradis et al. 2004), \textit{ncf} (Bjornstad 2008), and \textit{morton} packages.

Data availability

Data analysed in this study are archived in Dryad Digital Repository (hdl.handle.net/10255/dryad.1435).

Results

In the ME tree (Fig. 3), \textit{C. scoparia} is separated from the outgroup (\textit{Carex longii} and \textit{C. vexans}) with bootstrap support of 1.00, and the two varieties of \textit{C. scoparia} are each supported as monophyletic with bootstrap support of 1.00. Two additional nodes at the base of two pairs of individuals (3485A and 3485C; and 3335 and 3336) are supported at bootstrap support of 1.00. Both pairs represent individuals collected from the same site with the same chromosome number, and 3485A and 3335 were consequently excluded from subsequent analyses. One cluster of nine Appalachian accessions with relatively low chromosome numbers (2n = 58–64) is supported at bootstrap > 0.70. No other clusters of two or more individuals are strongly supported. There is some geographic signal in the data: all the Midwest accessions fall in a single cluster with three Appalachian accessions and one Northeastern accession, and all but one of the Northeast accession likewise fall in a cluster with one Appalachian accession.

Using a nuclear ribosomal DNA (ITS) clock on the previously published ITS dataset (Hipp et al. 2006), the divergence time between the \textit{C. scoparia} varieties is estimated at 0.487 million years ago (mya; 95% confidence interval = 0.50–1.61 mya), and the divergence time between the \textit{C. scoparia} varieties and their sister clade (\textit{C. alboluteascens} and \textit{C. suberecta}) is 1.66 mya (0.506–4.02 mya). The minimum number of karyotype rearrangements required to explain the observed data is 32 (range of parsimony reconstructions over 1000 trees subsampled from bootstrap set = 25–39).

Mantel tests (Table 2) indicate a significant positive correlation between geographic distance and genetic distance in the \textit{C. scoparia} dataset \( R^2 = 0.0543 ± 0.0024 \), mean \( P = 0.0019 \); Table 2; Fig. 4A). There is also a significant positive correlation between chromosome number difference and genetic distance \( R^2 = 0.0388 ± 0.0014 \), mean \( P = 0.0020 \); Table 2; Fig. 4B). Both correlations are significant when the partial Mantel test is used to correct for correlation between the independent variables (Table 2), and the total variance explained by chromosome number difference and geographic distance combined is approximately additive (multiple \( R^2 = 0.1047 ± 0.0027 \)). The correlation between chromosome identity and genetic distance is significant but weaker than the correlation between chromosome number difference and geographic distance \( R^2 = 0.0188 ± 0.0008 \), mean \( P = 0.0043 \); Table 2). When data are stratified in 200-km intervals, the correlation between chromosome number difference and geographic distance is positive in all strata with more than five comparisons, and the 200–400 km stratum shows a stronger correlation than expected \( P = 0.0211 ± 0.0013 \), Bonferroni-corrected \( P = 0.1899 \); Fig. 5). Across the \textit{C. scoparia} var. \textit{scoparia} accessions (\( N = 42 \)), there is a positive but non-significant correlation between diploid chromosome number and number of AFLP bands (Spearman’s rank correlation \( \rho = 0.2267 \), \( P = 0.1488 \)).
There is a significant relationship between chromosome number difference and genetic distance in *Carex pachystachya* based on both the simple and partial Mantel tests (simple Mantel: \( R^2 = 0.1844, P = 2.00E-4 \); partial Mantel conditioned on geographic distance: \( r^2 = 0.1971, P = 1.00E-4 \); Table 2). The correlation between

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chromosome identity and genetic distance is significant and only slightly lower than the correlation between absolute chromosome number difference and genetic distance ($r^2 = 0.1644$, $P < 5.00E-5$; Table 2). However, in the model including both chromosome identity and chromosome number difference, the contribution of chromosome identity to explaining genetic distance is non-significant ($r^2 = 0.0096$, $P = 0.1170$), but the contribution of chromosome number difference is substantially stronger and significant ($r^2 = 0.0333$, $P = 0.0244$; Table 2). The correlation between genetic and geographic distances is positive and weakly significant in partial Mantel tests ($r^2 = 0.0271–0.0313$, $P = 0.0258–0.0358$; Table 2), but nonsignificant in the simple Mantel test ($r^2 = 0.0117$, $P = 0.0963$). This is to be expected given that the correlation between chromosome number and geographic distance is weakly though not significantly negative ($r = -0.0922$, $r^2 = 0.0085$, $P = 0.1387$).

When data are stratified at 200-km intervals, the correlation between chromosome number difference and genetic distance is positive in all strata. The 400–600 km stratum shows stronger-than-expected correlation ($P = 0.0490$), and the 0–200 km stratum shows weaker-than-expected correlation ($P = 0.0235$; Fig. 5), though neither of these is significant with Bonferroni correction.

Discussion

Karyotype rearrangements and gene flow

This study demonstrates that changes in chromosome number explain one-third to nearly one-half of the genetic variance that we were able to explain within C. scoparia and one-half to 90% of the genetic variance we were able to explain within C. pachystachya (Table 2). While the total variance explained by the models we tested is relatively low ($R^2 = 0.1047$ in C. scoparia var. scoparia, $R^2 = 0.2065$ in C. pachystachya), the contribution of karyotype variation to genetic variance is significant and strong in comparison to the contribution of geographic variation. The study also demonstrates that karyotype rearrangements alone are unlikely to drive speciation within sedges: despite the wide range of chromosome numbers found in C. scoparia var. scoparia, there is no evidence of deep genetic breaks within the variety comparable to the break between varieties (Fig. 3). Moreover, in both species, the correlation between chromosome number difference and genetic distance is much stronger than the correlation between chromosome number identity and genetic distance, and the effect of chromosome number identity on genetic distance is not significant in the model that includes both chromosome number difference and chromosome number identity (model 6, Table 2). If genetic distances were determined by the mere presence of a karyotype rearrangement rather than the number of karyotype rearrangements separating two individuals of populations, then chromosome number identity would be expected to explain genetic diversity as well as chromosome number difference does. The low and non-significant partial correlation coefficient of chromosome number identity in models in which chromosome number difference is included strongly suggests that genetic similarity is a decreasing function of the minimum number of karyotype rearrangements between populations. As these two species represent a broad ecological, geographic, and phylogenetic range of a diverse clade (Hipp et al. 2006), and as intraspecific karyotype variation is common in many other sedge species that also exhibit ecological and morphological coherence (Tanaka 1948; Hoshino 1981; Luceño & Castroviejo 1991; Escudero et al. 2008; Roalson 2008), our findings
likely reflect a general relationship between karyotype rearrangement and intraspecific genetic structure that is found throughout the genus *Carex*.

There are at least four possible explanations for the observed correlation between chromosome number difference and genetic distance. It could reflect an isolation-by-distance (or isolation with time since divergence) process acting on both the karyotype and population genetic structure. If this were the correct explanation, we would expect to see a positive correlation between geographic distance and chromosome number difference, but in fact this correlation is weakly negative (*Carex pachystachya*: $r^2 = -0.0085$, $P = 0.1387$; *C. scoparia*: $r^2 = -0.0133 \pm 0.0010$, $P = 0.0796 \pm 0.0087$).

If borne out with additional study at finer geographic scales, this negative correlation may suggest that sympatric populations differ in chromosome number more frequently than expected under the null hypothesis of no spatial structure, which would be expected if interpopulation hybrids tend to have reduced fitness. Alternatively, the correlation between genetic distance and chromosome number difference could be an artefact of a correlation between chromosome number and locus copy. This would be the expectation if chromosome number changes reflected chromosome deletion and duplication, but evidence across the genus *Carex* strongly suggests that chromosome number changes are due to fission and fusion rather than duplication (reviewed in Tanaka 1949; Hoshino 1981). Moreover, our data show no evidence for an increase in locus number with an increase in chromosome number: the correlation between number of AFLP bands and chromosome number in *C. scoparia* is positive but nonsignificant (Spearman’s rank correlation $\rho = 0.2267$, $P = 0.1488$), and the isozyme loci surveyed in *C. pachystachya* all suggest diploidy. A third possibility might be that genetic differentiation causes karyotype rearrangement. For this to be the case, the markers we sampled in estimating genetic differentiation would have to be linked to chromosome breakpoints or fusions. Given the large number of markers we sampled for this study (499 AFLP markers in *C. scoparia* alone), it seems unlikely that many of these would be linked with rearrangement breakpoints, especially given that chromosome breakpoints are relatively conserved in genome-level studies (Pevzner & Tesler 2003; Richard et al. 2003; Murphy et al. 2005; Ruiz-Herrera & Robinson 2007).

Without mapping our markers, it is impossible to rule out the possibility that the same mutations that drive chromosome rearrangement are dominating our estimates of genetic divergence. However, it seems unlikely that chromosome translocation hotspots would evolve rapidly enough due to base pair substitutions and that the mutations composing these hotspots would be a large enough component of total genetic variance to explain the significant correlations we find in this study.

The fourth possibility is that chromosome rearrangements limit gene flow among populations. Chromosome rearrangements have been shown in many cases to have an indirect effect on genetic differentiation among populations by reducing hybrid fertility or recombination in rearranged regions of the genome (Bidau 1991; Moulin et al. 1996; Dumas & Britton-Davidian 2002; Noor et al. 2002; Ortiz-Barrientos et al. 2002; Navarro & Barton 2003; Ayala & Coluzzi 2005; Lai et al. 2005; Basset et al. 2008; Skrede et al. 2008). Several studies have investigated patterns of gene flow among chromosome races in organisms that undergo Robertsonian translocations (Moulin et al. 1996; Britton-Davidian et al. 2002; Morgan-Richards & Wallis 2003; Chiappero et al. 2004; Panithanarak et al. 2004), but no studies we are aware of have found a correlation between genetic differentiation and chromosome number difference across a species’ range. Many studies have investigated the isolating role of fusions that are monobrachially homologous in two different populations (Baker & Bickham 1986). Simple structural heterozygotes between individuals that differ by fissions or a single fusion are less likely than reciprocal translocations, inversions, or duplications and deletions to suffer reduced fitness or recombination (e.g. Wallace et al. 1992). Our finding that genetic distance correlates more strongly with chromosome number difference than with chromosome number identity suggests that there is an interaction among karyotype rearrangements in their effect on genetic diversity within species: the greater the minimum number of karyotype rearrangements (i.e. the chromosome number difference) between two populations, the greater the barrier to gene flow between those populations.

**Geographic structure**

It is striking that the correlation between chromosome number difference and genetic distance does not tail off with geographic distance. In both species, none of the 200-km strata analysed show significantly stronger or weaker correlation after Bonferroni correction, and both species show the most significant support for stronger correlation between 200 and 600 km (Fig. 5). In fact, the weakest correlation in *Carex pachystachya* is at the most local scale (0–200 km), though this correlation is not significantly lower than the global correlation after Bonferroni correction. Why should the effect not be strongest at local scales, where chromosome number differences are likely to more accurately reflect karyotype rearrangements, and where gene flow between populations would be expected to increase the correlation between
Implications for chromosomal speciation

The observed patterns of genetic variance, chromosome number diversity, and geography suggest that *C. scoparia* var. *tessellata*—a Maine endemic of sandy or gravelly, open sites—derives from a *C. scoparia* progenitor population during the Pleistocene (ITS-estimated divergence between *C. scoparia* varieties = 0.487 mya; 95% CI = 0.050–1.61 mya). *C. scoparia* var. *tessellata* is morphologically distinctive (Mastrogiuseppe et al. 2002) and, as the current study demonstrates, genetically divergent from the typical variety (Fig. 3). It is apparently limited in distribution to two counties, and its single observed karyotype (2n = 68) is accompanied by low genetic diversity (Fig. 3). The more widespread *C. scoparia* var. *scoparia* exhibits variance in chromosome number (2n = 56–70); ITS-estimated divergence between *C. scoparia* and sister group = 1.66 million years, 95% CI = 0.506–4.02 million years) that comprises much of the range found in the eastern North American clade that contains it (for *Carex* section *Ouales* of eastern North America: 2n = 48–80 in approximately 35 species; ITS-estimated age = 3.85 mya, 95% CI = 1.44–8.65 mya; chromosome counts and DNA data reported in Hipp 2007). Given the minimum number of karyotype rearrangements needed to explain the observed data in *C. scoparia* var. *scoparia* (32 rearrangements as estimated using maximum parsimony; 95% CI over bootstrap trees = 25–39), this age corresponds to an average interval of 52 (95% CI = 20 223–103 065 years) between the rearrangements observed in this study. Our sampling of 35 populations represents < 0.4% of an estimated minimal number of 9500 populations of the species across its range (based on a linear extrapolation from the population count in Wisconsin to the entire species range), and we know from recent work within the Chicago metropolitan region (Chung et al. in review) as well as sampling from geographically proximal populations in western North Carolina and east central Tennessee (Fig. 1) that chromosome number is highly variable even at local scales, suggesting that the observed pattern is not a consequence of chromosome diversification early in the species’ origin followed by a long period of stasis. Moreover, almost all northeastern North American populations of *C. scoparia* var. *scoparia* surveyed possess a single chromosome number, 2n = 66, which is compatible with an origin of *C. scoparia* var. *tessellata* from a progenitor population of the typical variety.

The pattern of variation within *C. scoparia* var. *scoparia* and *C. pachystachya* demonstrates that although rapid chromosome evolution plays an important role in patterning genetic variance within species, chromosome rearrangements alone are insufficient to cause speciation in at least some sedge lineages. Gene flow between karyotype races or species is typically expected to be a function of the number or complexity of chromosome rearrangements (Spirito et al. 1991; Basset et al. 2006), and the cumulative effect of numerous very weakly underdominant (nearly neutral) rearrangements is likelier to play role in reproductive isolation than the fixation of a small number of moderately to strongly underdominant rearrangements (Walsh 1982). Moreover, several studies in the genus *Carex* demonstrate that crosses involving larger differences in chromosome number exhibit greater meiotic irregularities (Faulkner 1973; Schmid 1982; Cayouette & Morisset 1985; Whittkus 1988a; Cayouette & Morisset 1992; Lučenčo 1994), and two of these demonstrate an inverse relationship between pollen viability and meiotic irregularities in first generation hybrids (Cayouette & Morisset 1985; Whittkus 1988a). One important outcome of the current study is that it demonstrates the inadequacy of thinking of different chromosome rearrangements as representing monophyletic ‘races’ or infraspecies (King 1993). Equally importantly, the finding that chromosome rearrangements in sedges have a cumulative effect on intra-specific gene flow implicates karyotype evolution as
Acknowledgements

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References


This study began as a survey of chromosome diversity in *C. scoparia* by Paul Rothrock and of genetic and chromosome diversity in *C. pachystachya* by Richard Whitkus. Andrew Hipp's research addresses the diversification of flowering plant lineages and traits, primarily in sedges and oaks. Paul Rothrock’s research focuses on the taxonomy of *Carex* section *Ovales*, a diverse New World clade with numerous regional endemics. Richard Whitkus researches the systematics and evolutionary genetics of plants and applications of molecular markers to mapping, diversity, and evolution. Jaime Weber works on quantitative and molecular genetics of cultivated and wild plants.