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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 (2*n* = 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* (2*n* = 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

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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; Guerra & García 2004; Wang & Porter 2004). Although strong selection against structural heterozygotes appears to maintain karyotypic stability in *C. elegans* (Dernburg 2001), holocentry 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 hetermorphic 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 karytotypic 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 postmating, 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

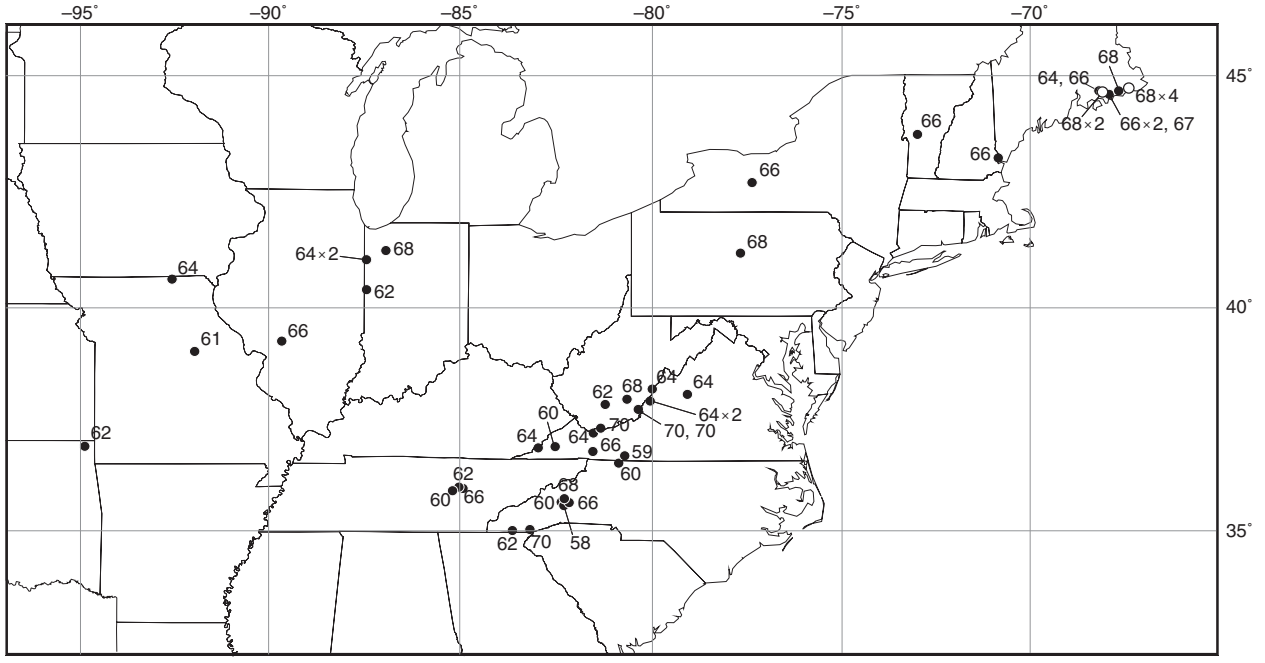

Fig. 1 Map of sites sampled, with inferred diploid chromosome counts. Multiplication sign ('×') indicates that multiple individuals of the same count were found at a site. The two sites where *C. scoparia* var. *tessellata* was collected are indicated with open circles; all other sites were collections of *C. scoparia* var. *scoparia* exclusively.

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. vexans*) 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 $N = 17.4 \pm 3.5$ [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

Table 1 Individuals sampled for this study

Taxon	State	County	Voucher	1st Meiotic interphase	Inferred 2*n*	Latitude	Longitude
Carex scoparia	Illinois	Macoupin	PER 3548	33	66	39.175594	−89.704181
var. *scoparia*	Indiana	Jasper	PER 3355	34	68	41.157473	−86.970325
	Indiana	Newton	PER 3633A	32	64	40.958330	−87.480000
	Indiana	Newton	PER 3633B	32	64	40.958330	−87.480000
	Indiana	Warren	PER 3631	31	62	40.301993	−87.476548
	Maine	Hancock	PER 3489A	33	66	44.627500	−68.152025
	Maine	Hancock	PER 3489B	33	66	44.627500	−68.152025
	Maine	Hancock	PER 3489C	32	64	44.627500	−68.152025
	Maine	Washington	PER 3485A	33	66	44.575068	−67.977294
	Maine	Washington	PER 3485B	32 + III	67	44.575068	−67.977294
	Maine	Washington	PER 3485C	33	66	44.575068	−67.977294
	Missouri	Schuyler	PER 3556	32	64	40.531767	−92.585667
	Missouri	Tucker	PER 3555	29 + III	61	38.950573	−91.989983
	New Hampshire	Strafford	PER 3473	33	66	43.184198	−70.889683
	New York	Yates	PER 3466	33	66	42.645267	−77.352949
	North Carolina	Buncombe	PER 3321	30	60	35.620755	−82.328900
	North Carolina	Buncombe	PER 3322	29	58	35.608803	−82.335208
	North Carolina	Clay	PER 3316	31	62	35.030700	−83.648460
	North Carolina	Macon	PER 3319	35	70	35.052046	−83.187687
	North Carolina	McDowell	PER 3325	33	66	35.641472	−82.157455
	North Carolina	Surry	PER 3327	30	60	36.506640	−80.856401
	North Carolina	Yancy	PER 3326	31 + 2III	68	35.727759	−82.283272
	Oklahoma	Ottawa	AAR 9760	31	62	36.874547	−94.877425
	Pennsylvania	Centre	PER 3493	34	68	41.101511	−77.659223
	Tennessee	Cumberland	PER 3639	31	62	35.957013	−84.987427
	Tennessee	Cumberland	PER 3641	30	60	35.979903	−85.053682
	Tennessee	White	PER 3644	33	66	35.902156	−85.220550
	Vermont	Rutland	PER 3468	33	66	43.701199	−73.011241
	Virginia	Alleghany	PER 3335	32	64	37.863087	−80.030607
	Virginia	Alleghany	PER 3336	32	64	37.863087	−80.030607
	Virginia	Augusta	PER 3332	30 + IV	64	38.009750	−79.052011
	Virginia	Carroll	PER 3328	28 + III	59	36.665502	−80.697779
	Virginia	Lee	PER 3648	32	64	36.839953	−82.969855
	Virginia	Scott	PER 3649	30	60	36.866691	−82.525050
	Virginia	Smythe	PER 3650	33	66	36.759396	−81.535413
	Virginia	Tazewell	PER 3652	32	64	37.164350	−81.520833
	Virginia	Tazewell	PER 3653	35	70	37.269922	−81.328458
	West Virginia	Greenbrier	PER 3342	34	68	37.903288	−80.637762
	West Virginia	Monroe	PER 3656A	35	70	37.682135	−80.340423
	West Virginia	Monroe	PER 3656B	35	70	37.682135	−80.340423
	West Virginia	Pocahontas	PER 3661	32	64	38.127510	−79.974120
	West Virginia	Raleigh	PER 3654	31	62	37.790254	−81.210573
Carex scoparia	Maine	Hancock	PER 3488B	34	68	44.627500	−68.152025
var. *tessellata*	Maine	Washington	AAR 10319	34	68	44.715267	−67.461222
	Maine	Washington	PER 3484A	34	68	44.575068	−67.977294
	Maine	Washington	PER 3484B	34	68	44.575068	−67.977294
	Maine	Washington	PER 3484C	34	68	44.575068	−67.977294
	Maine	Washington	PER 3733B	34	68	44.653164	−67.726929
Carex longii	Mexico	Michoacan	Zamudio *et al.* 11237	–	–	–	–
Carex vexans	Florida	Paseo	PER 2379	–	–	–	–

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 × univalents) + (2 × bivalents) + ··· + (x × x-valents), where x indicates the number of chromosomes

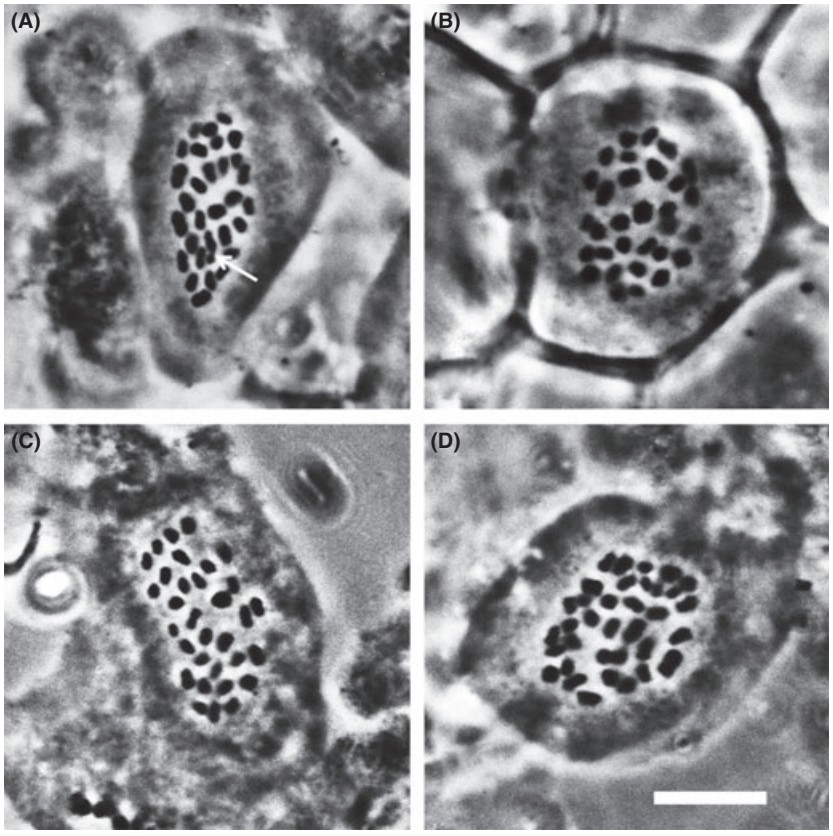

Fig. 2 Meiotic metaphase I from four collections of *C. scoparia*. (A) $n = 29 + III$ (Tucker Co., MO, USA, PER 3555). (B) $n = 31$ (Ottawa Co., OK, USA, AAR 9760). (C) $n = 32$ (Alleghany Co., VA, USA, PER 3335). (D) $n = 34$ (Centre Co., PA, USA, PER 3493). Arrow indicates trivalent. Scale bar = 10 μm.

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 phylogeographic 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

Model	Total variance explained	Geographic distance	Chromosome number difference	Chromosome identity
Carex scoparia* var. *scoparia				
(1) $Y \sim \Delta km$	$R^2 = 0.0543 \pm 0.0024$	$r^2 = 0.0543 \pm 0.0024$, $P = 0.0019$	–	–
(2) $Y \sim \Delta 2n$	$R^2 = 0.0388 \pm 0.0014$	–	$r^2 = 0.0388 \pm 0.0014$, $P = 0.0020$	–
(3) $Y \sim$ (1 - identity)	$R^2 = 0.0188 \pm 0.0008$	–	–	$r^2 = 0.0188 \pm 0.0008$, $P = 0.0043$
(4) $Y \sim \Delta km + \Delta 2n$	$R^2 = 0.1047 \pm 0.0027$	$r^2 = 0.0686 \pm 0.0026$, $P =$ 5e-04	$r^2 = 0.0533 \pm 0.0016$, $P = 0.0004$	–
(5) $Y \sim \Delta km +$ (1 - identity)	$R^2 = 0.0739 \pm 0.0026$	$r^2 = 0.0562 \pm 0.0024$, $P = 0.0015$	–	$r^2 = 0.0207 \pm 0.0009$, $P = 0.0031$
(6) $Y \sim \Delta 2n +$ (1 - identity)	$R^2 = 0.0403 \pm 0.0014$	–	$r^2 = 0.0219 \pm 0.0011$, $P = 0.0105$	$r^2 = 0.0015 \pm$ 2e-04, $P = 0.1825$
Carex pachystachya				
(1) $Y \sim \Delta km$	$R^2 = 0.0117$	$r^2 = 0.0117$, $P = 0.0963$	–	–
(2) $Y \sim \Delta 2n$	$R^2 = 0.1844$	–	$r^2 = 0.1844$, $P =$ 2.00E-4	–
(3) $Y \sim$ (1 - identity)	$R^2 = 0.1644$	–	–	$r^2 = 0.1644$, $P <$ 5.00E-5
(4) $Y \sim \Delta km + \Delta 2n$	$R^2 = 0.2065$	$r^2 = 0.0271$, $P = 0.0358$	$r^2 = 0.1971$, $P =$ 1.00E-4	–
(5) $Y \sim \Delta km +$ (1 - identity)	$R^2 = 0.1906$	$r^2 = 0.0313$, $P = 0.0284$	–	$r^2 = 0.1810$, $P =$ 1.00E-4
(6) $Y \sim \Delta 2n +$ (1 - identity)	$R^2 = 0.1923$	–	$r^2 = 0.0333$, $P = 0.0244$	$r^2 = 0.0096$, $P = 0.1170$

Models are abbreviated as follows: Y, pairwise genetic distance; Δkm, 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).

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 ($R^2 = \boldsymbol{R_{XY}R_{XX}^{-1}R_{YX}}$) 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_1 as a measure of the proportion of variance in pairwise genetic distances explained by X_1 when all other predictors in the model are held constant with respect to X_1. Analyses were performed using the *morton* and *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 2*n* chromosome number and number of AFLP bands detected per individual. In the *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 *vegan*, *ape* (Paradis *et al.* 2004), *ncf* (Bjørnstad 2008), and *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), *C. scoparia* is separated from the outgroup (*Carex longii* and *C. vexans*) with bootstrap support of 1.00, and the two varieties of *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 (2*n* = 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 accessions 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 *C. scoparia* varieties is estimated at 0.487 million years ago (mya; 95% confidence interval = 0.050–1.61 mya), and the divergence time between the *C. scoparia* varieties and their sister clade (*C. albolutescens* and *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 *C. scoparia* dataset ($R^2 = 0.0543 \pm 0.0024$, mean $P = 0.0019$; Table 2; Fig. 4A). There is also a significant positive correlation between chromosome number difference and genetic distance ($R^2 = 0.0388 \pm 0.0014$, 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 $R^2 = 0.1047 \pm 0.0027$). The correlation between chromosome identity and genetic distance is significant but weaker than the correlation between chromosome number difference and genetic distance ($R^2 = 0.0188 \pm 0.0008$, mean $P = 0.0043$; Table 2). When data are stratified in 200-km intervals, the correlation between chromosome number difference and genetic distance is positive in all strata with more than five comparisons, and the 200–400 km stratum shows a stronger correlation than expected ($P = 0.0211 \pm 0.0013$, Bonferroni-corrected $P = 0.1899$; Fig. 5). Across the *C. scoparia* var. *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 $\rho = 0.2267$, $P = 0.1488$).

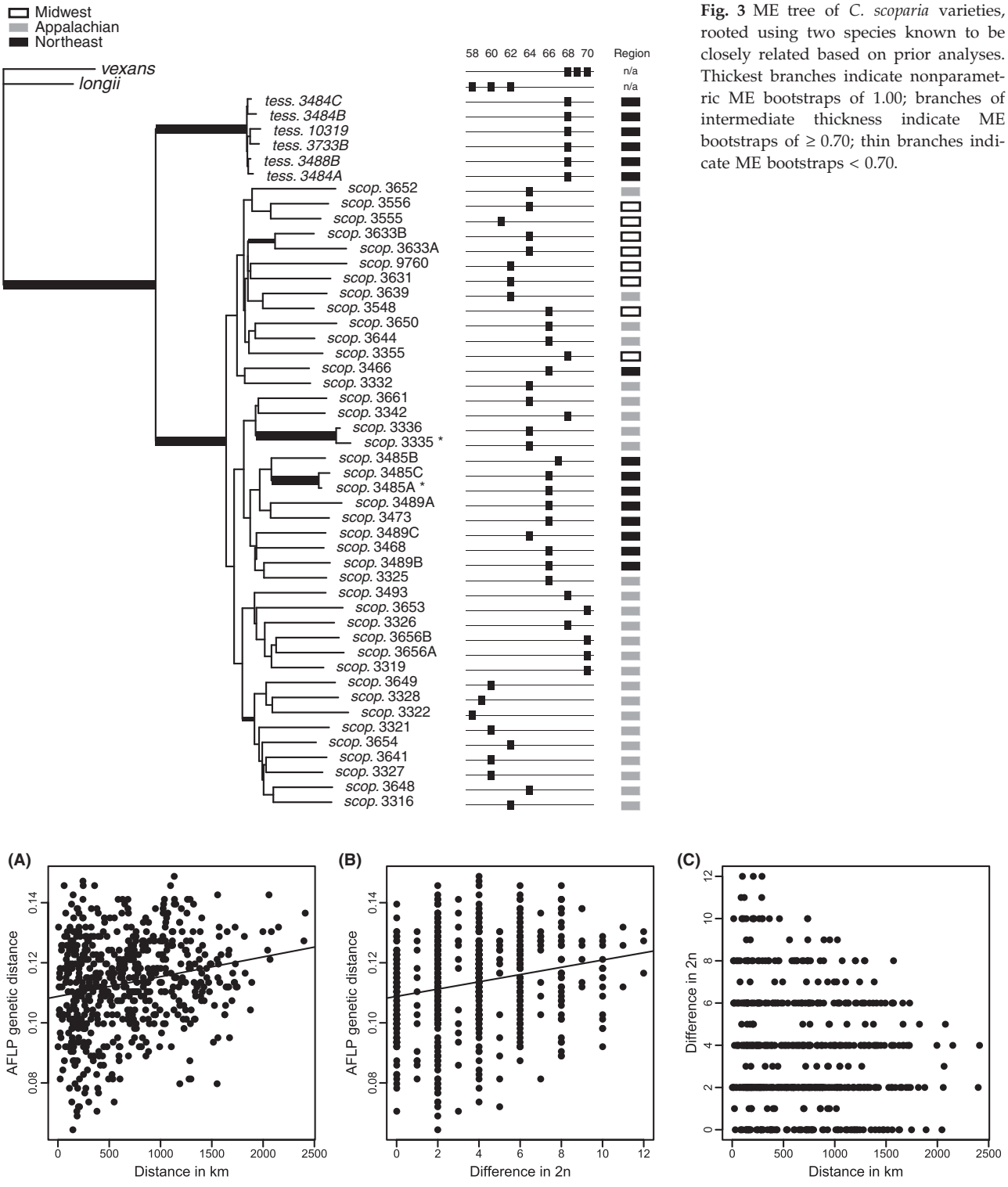

Fig. 3 ME tree of *C. scoparia* varieties, rooted using two species known to be closely related based on prior analyses. Thickest branches indicate nonparametric ME bootstraps of 1.00; branches of intermediate thickness indicate ME bootstraps of ≥ 0.70; thin branches indicate ME bootstraps < 0.70.

Fig. 4 Regression of pairwise distance matrices: (A) genetic distance on geographic (great-circle) distance, (B) genetic distance on chromosome number difference, and (C) chromosome number difference on geographic distance. Least-squared linear regression line is shown for illustrative purposes only on regressions that are significant by Mantel permutation.

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: $R^2 = 0.1844$, $P = 2.00\text{E-}4$; partial Mantel conditioned on geographic distance: $r^2 = 0.1971$, $P = 1.00\text{E-}4$; Table 2). The correlation between

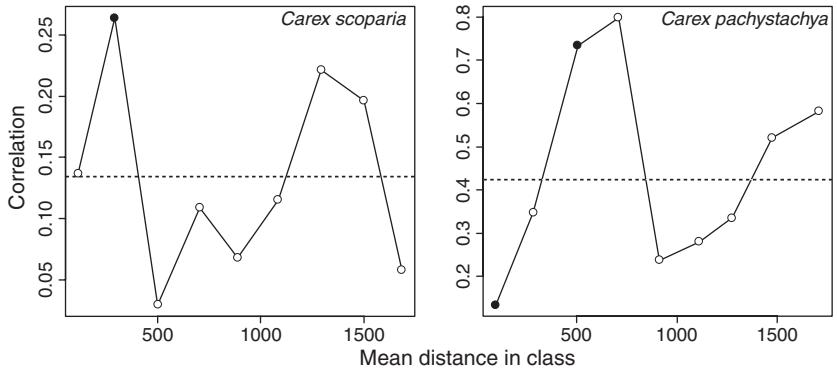

Fig. 5 Cross-correlogram of chromosome number difference on genetic distance, with correlations partitioned into 200-km strata. The top four strata for each species contain fewer than 15 data points (pairwise comparisons) and are consequently not shown. Dashed horizontal lines indicate mean correlation over all pairwise comparisons. Closed circles indicate data points that are significant at the 0.05 level; no points are significant after Bonferroni correction.

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 nonsignificant 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 ± 0.0010, P = 0.0796 ± 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 inter-population 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 ρ = 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

genetic and karyotypic similarity? One possible explanation is that relatively few rearrangement hotspots account for the chromosome number changes we observe, and that the changes differentiating two populations quickly saturate the hotspots. In this case, chromosome number would not be a better surrogate for karyotype in any but the most local scales, as karyotype rearrangements could be recomposed repeatedly at common rearrangement hotspots.

An alternative explanation is that long-distance gene flow in sedges is not uncommon. Geographic distance explains 5.4–6.9% of the total genetic variance in *C. scoparia* ($P < 0.005$ in all models incorporating geographic distance; Table 2), which is over half of the total variance explained in our models. This suggests that isolation-by-distance plays as great a role as karyotype rearrangement in the formation of genetic structure. However, in *C. pachystachya*, only 1.2–3.1% of genetic variance is explained by geographic distance, and the geographic correlation is not strongly significant ($P > 0.025$ in all models; Table 2). This fact, in combination with the geographic structure of karyotype rearrangement, suggests a potential for long-range gene flow within sedges, as has been demonstrated in other studies (Escudero *et al.* 2008; Schönswetter *et al.* 2008). Testing for the effects of long range gene flow and the possible effects of rearrangement hotspots in the observed pattern will require additional fine-scale geographic data.

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—derived 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 *Ovales* 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 52 095 years (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; Whitkus 1988a; Cayouette & Morisset 1992; Luceño 1994), and two of these demonstrate an inverse relationship between pollen viability and meiotic irregularities in first generation hybrids (Cayouette & Morisset 1985; Whitkus 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 intraspecific gene flow implicates karyotype evolution as

a potential player in speciation in one of the largest angiosperm genera and lays the ground work for teasing apart the relative effects of chromosome rearrangement, ecological divergence, and other factors in explaining the remarkable diversity of sedges.

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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.
