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Dynamics of chromosome number and genome size variation in a cytogenetically variable sedge (*Carex scoparia* var. *scoparia*, Cyperaceae)[1]

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- *Premise of the study*: High intraspecific cytogenetic variation in the sedge genus *Carex* (Cyperaceae) is hypothesized to be due to the "diffuse" or non-localized centromeres, which facilitate chromosome fission and fusion. If chromosome number changes are dominated by fission and fusion, then chromosome evolution will result primarily in changes in the potential for recombination among populations. Chromosome duplications, on the other hand, entail consequent opportunities for divergent evolution of paralogs. In this study, we evaluate whether genome size and chromosome number covary within species.
- *Methods*: We used flow cytometry to estimate genome sizes in *Carex scoparia* var. *scoparia*, sampling 99 plants (23 populations) in the Chicago region, and we used meiotic chromosome observations to document chromosome numbers and chromosome pairing relations.
- *Key results*: Chromosome numbers range from $2n = 62$ to $2n = 68$, and nuclear DNA 1C content from 0.342 to 0.361 pg DNA. Regressions of DNA content on chromosome number are nonsignificant for data analyzed by individual or population, and a regression model that excludes slope is favored over a model in which chromosome number predicts genome size.
- *Conclusions*: Chromosome rearrangements within cytogenetically variable *Carex* species are more likely a consequence of fission and fusion than of duplication and deletion. Moreover, neither genome size nor chromosome number is spatially autocorrelated, which suggests the potential for rapid chromosome evolution by fission and fusion at a relatively fine geographic scale (<350 km). These findings have important implications for ecological restoration and speciation within the largest angiosperm genus of the temperate zone.

Key words: agmatoploidy; aneuploidy; chromosome evolution; flow cytometry; genome size.

Chromosome numbers in the sedge genus *Carex* L. (Cyperaceae) exhibit high variance among and within species. In the more than 500 species in the genus with reported chromosome counts, chromosome numbers vary from $n = 6$ to $n = 66$ (Tanaka, 1949; Hipp et al., 2009), including every haploid number between $n = 6$ and $n = 48$ (Davies, 1956; Roalson et al., 2007; Roalson, 2008). This remarkable chromosome number variation has been proposed to be primarily a consequence of chromosome fission and fusion rather than duplication and deletion. Knowing whether chromosome duplication is involved in chromosome number change is key to understanding the role of chromosome evolution in this highly diverse genus (about 2000 species worldwide; Reznicek, 1990). If chromosome duplication is involved, then gene subfunctionalization or neofunctionalization may play an important role in species evolution (Osborn et al., 2003). If not, then chromosome evolution is likely to play a role in species evolution primarily through reduction of recombination or hybridization among populations (Rieseberg, 2001; Ayala and Coluzzi, 2005) or through selection on recombination rates (Bell, 1982, pp. 426ff.).

Sedges possess holocentric chromosomes, which are defined as chromosomes with "diffuse" or nonlocalized centromeres (Wahl, 1940; Davies, 1956; Hoshino, 1981; reviewed in Hipp et al., 2009, and citations therein). Holocentric chromosomes are found in every species studied in the angiosperm families Cyperaceae and Juncaceae, as well as in numerous unrelated species of flowering plants, algae, and arthropods (Darlington, 1973, p. 101; Dernburg, 2001; Mola and Papeschi, 2006; Roalson et al., 2007). No flowering plant clade exhibits greater chromosome number variation than *Carex*, and the genus has consequently long been of interest to evolutionary biologists (e.g., Heilborn, 1924; Stebbins, 1971; Bell, 1982; Briggs and Walters, 1997, p. 356). Because holocentric chromosomes readily produce viable chromosome fragments by fission and fusion (agmatoploidy and symploidy, respectively; Malheiros-Gardé and Gardé, 1950; Davies, 1956; Darlington, 1973, p. 15; Luceño and Guerra, 1996), the potential exists for high chromosome number variation and rapid chromosome evolution in taxa such as *Carex* without significant changes in DNA content. Although chromosome duplications and deletions (aneuploidy sensu stricto [s.s.]) have been proposed as explanations of the wide variation in chromosome number found in *Carex* (Heilborn, 1924; Tanaka, 1949), most investigations have suggested agmatoploidy and symploidy to be the dominant mode of evolution on the basis of meiotic chromosome pairing relations

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(Luceño and Castrovejo, 1991; Roalson et al., 2007; Hipp et al., 2009).

In contrast to the large amount of chromosome counting in the genus, there has been little study to date of genome size variation in *Carex*. Nishikawa et al. (1984) reported a negative correlation between chromosome number and genome size in 26 *Carex* species, suggesting that chromosome number increase is associated with loss in DNA content. Macroevolutionary studies such as this one are important for investigating the effects of genome size variation on ecology and diversification (Grime et al., 1985; Bennett and Smith, 1991; Bennett and Leitch, 1995; Cox et al., 1998; Grime, 1998; Leitch et al., 1998; Bennett et al., 2000; Soltis et al., 2003; Achigan-Dako et al., 2008; Chrtek et al., 2009; Leitch et al., 2009). They are not, however, ideally suited to investigating the question of how chromosome rearrangements affect genome size, as they risk conflating among-species differences in genome size with differences due to chromosome number changes alone.

The cytogenetically variable species *Carex scoparia* Schkuhr ex Willdenow var. *scoparia* ($2n = 56–70$; Hipp et al., 2010) provides an opportunity to test whether genome size and chromosome number variance are correlated, as expected if chromosome number changes are a consequence of genetic duplications or deletions (aneuploidy s.s.), or are uncorrelated, as expected if chromosome number changes entail rearrangements without duplication or deletion (agmatoploidy/symploidy). The species is particularly interesting because it was the subject of recent work demonstrating a positive correlation between genetic and karyotypic differentiation (Hipp et al., 2010). In this study, we use flow cytometry (FCM) and chromosome counts to evaluate the alternative hypotheses of aneuploidy and agmatoploidy/symploidy in the evolution of chromosome numbers in *Carex scoparia* var. *scoparia* (Cyperaceae).

MATERIALS AND METHODS

Sampling—Live plants of *Carex scoparia* var. *scoparia* were collected from 23 populations in the greater Chicago region during the summer of 2007 (Fig. 1, Table 1) and cultivated in a greenhouse at The Morton Arboretum. Ninety-nine individuals were sampled for FCM, and chromosome numbers were counted from at least one individual of each of 20 populations and at least two individuals per each of 15 populations.

Flow cytometry—Flow cytometry is an efficient method of estimating DNA content (Doležel, 1998; Lysák et al., 2000; Doležel and Bartoš, 2005; Doležel et al., 2007; Greilhuber et al., 2007). Samples were prepared following Doležel and Bartoš (2005) and Doležel et al. (2007), with modifications as described here. Young, fresh leaf tissues (15–20 mg) were chopped using a razor blade in 1 mL De Laat buffer (de Laat and Blaas, 1984, as modified in Kron and Husband, 2009), then filtered through nylon mesh (40-μm pore size). Chopped samples were kept on ice for about 30 min and then stored in

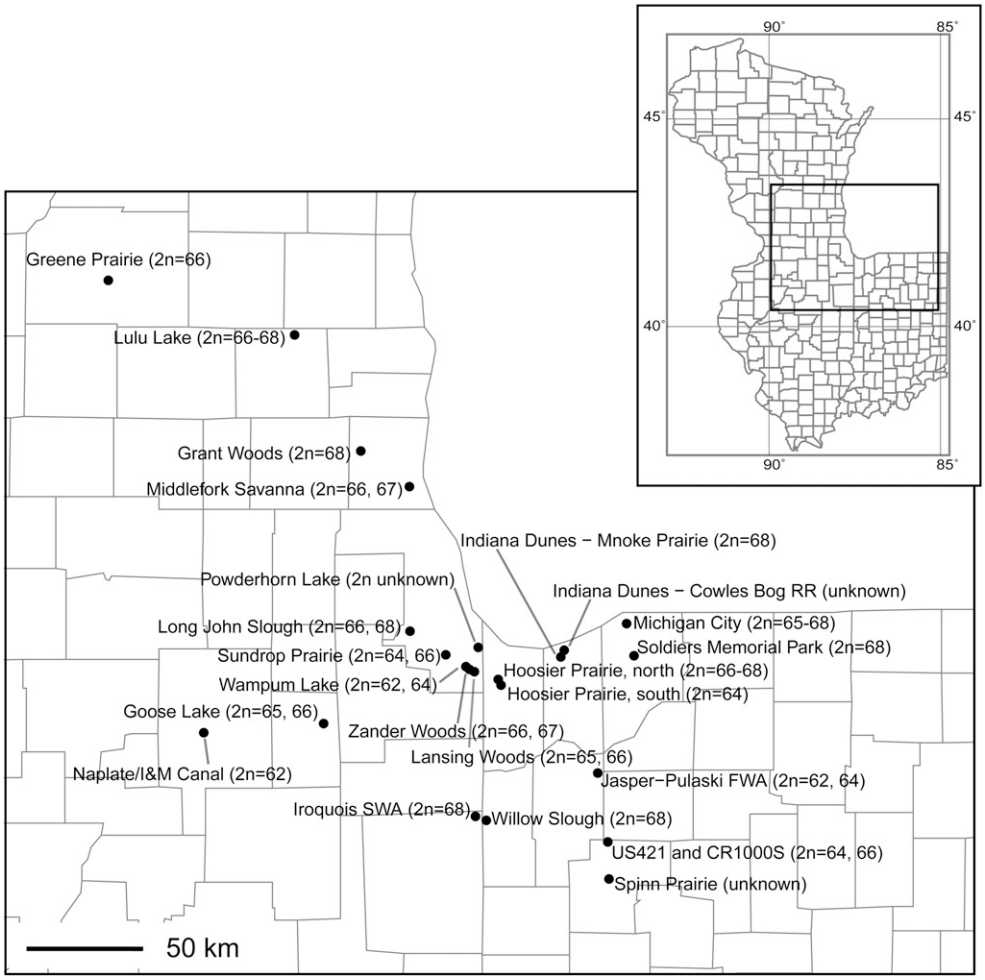

Fig. 1. Map of sites sampled. Diploid ($2n$) chromosome counts are inferred from meiotic counts (cf. Table 1).

TABLE 1. Nuclear DNA content and diploid number in examined populations of *Carex scoparia* var. *scoparia.* All vouchers are archived at The Morton Arboretum Herbarium (MOR).

Population (Number of individuals)	Mean DNA content (pg DNA/1C) ± s.d.	Mean CV (%) ± s.d.	2*n* (Voucher)	Latitude	Longitude
Goose Lake (4)	0.351 ± 0.0074	4.10 ± 0.48	65 (*n* = 31+III, 2830N), 66 (2830J, 2830W)	41.359583	–88.315900
Grant Woods (2)	0.360 ± 0.0072	3.94 ± 0.52	68 (2879A)	42.387900	–88.128067
Hoosier Prairie, North (5)	0.352 ± 0.0076	4.17 ± 0.55	66 (2863B, Q), 67 (*n* = 32+III, 2863M), 68 (2863A)	41.526361	–87.442680
Hoosier Prairie, South (3)	0.352 ± 0.0052	4.36 ± 1.76	66 (2864A)	41.504783	–87.437267
Indiana Dunes–Cowles Bog RR (1)	0.343 ± 0.0074	4.21 ± 0.49		41.636817	–87.100533
Indiana Dunes–Mnoke Prairie (5)	0.353 ± 0.0066	4.04 ± 0.47	68 (2865H, O)	41.617983	–87.108550
Iroquois SWA (4)	0.355 ± 0.0057	3.98 ± 0.36	68 (2894H)	41.008500	–87.548525
Jasper-Pulaski FWA (5)	0.352 ± 0.0067	4.05 ± 0.42	62 (2893O), 64 (2893A)	41.172383	–86.930767
Lansing Woods (6)	0.352 ± 0.0080	4.00 ± 0.59	66 (2880A, N, R), 65 (*n* = 31+III, 2880N)	41.555500	–87.554217
Long John Slough (5)	0.351 ± 0.0068	4.18 ± 0.37	66 (2878I), 68 (2787A)	41.708633	–87.879417
Lulu Lake (5)	0.357 ± 0.0069	4.19 ± 0.54	68 (2800), 67 (*n* = 32+III, 2807), 66 (2818)	42.826867	–88.462550
Michigan City/IN CR 900N (7)	0.350 ± 0.0068	3.99 ± 0.67	65 (*n* = 31+III, 2890J), 67 (*n* = 30+III+IV, 2890A), 68 (2890I)	41.737383	–86.784900
Middlefork Savanna (7)	0.352 ± 0.0088	4.18 ± 0.45	66 (*n* = 31+IV, 2895K), 67 (*n* = 32+III, 2895H)	42.253200	–87.882633
Naplate/I&M Canal (8)	0.353 ± 0.0088	4.30 ± 0.46	62 (2839B, O)	41.329700	–88.914233
Powderhorn Lake (1)	0.353 ± 0.0108	4.05 ± 0.19		41.647433	–87.534783
Soldiers Memorial Park (2)	0.347 ± 0.0063	4.06 ± 0.27	68 (2889A)	41.615900	–86.747283
Spinn Prairie (1)	0.351 ± 0.0082	4.55 ± 0.27		40.772083	–86.873900
Sundrop Prairie (5)	0.351 ± 0.0045	3.91 ± 0.49	64 (2891O), 66 (2891G)	41.619467	–87.698633
US421 and CR1000S (3)	0.352 ± 0.0101	4.18 ± 0.35	64 (2882D), 66 (2882A)	40.911967	–86.880083
Wampum Lake (4)	0.352 ± 0.0058	3.90 ± 0.47	62 (2875K), 64 (2875A)	41.576083	–87.589733
Willow Slough (2)	0.354 ± 0.0059	4.21 ± 0.48	68 (2892C)	40.993500	–87.494100
Zander Woods (5)	0.349 ± 0.0074	4.25 ± 0.45	66 (2881H, L), 67 (*n* = 33+I, 2881D)	41.569700	–87.585967
Greene Prairie (9)	0.357 ± 0.0095	4.01 ± 0.52	66 (2663, 2658_3/3)	43.026404	–89.437106
Mean	0.353 ± 0.0078	4.11 ± 0.56	65.88		

Note: pg = picogram; s.d. = standard deviation; CV = coefficient of variation; 2*n* = diploid chromosome number, calculated from meiotic observations (see text)

a refrigerator for approximately 16–20 h until analysis on a BD LSRII flow cytometer (BD Biosciences, San Jose, California, USA). RNase (50 µg/mL) and propidium iodide (50 µg/mL) were added for staining, and nuclei were stained on ice for 20–30 min or at room temperature for 15 min. Postchopping storage at 4°C was required because the flow cytometry facility we use is located in a collaborating laboratory. To determine whether this cold-storage period had an effect on the final FCM results, we replicated a subset of samples chopping directly in the collaborating laboratory, with no cold-storage period between chopping and staining. While peaks of samples without cold storage after chopping showed slightly lower coefficient of variation (CV) values, the peak mean values were not affected. *Raphanus sativus* 'Saxa' was used as an internal standard (Doležel et al., 1992), utilizing cultivar seed provided by J. Doležel. Measurements were performed at least three times per individual (except 2864A, 2865F, and 2880F, each of which was measured two times) on different days, and in each run 5000 nuclei were recorded within both the standard and sample peaks. Mean and CV values of each peak were calculated using FlowJo version 7.2.5 (Tree Star, Ashland, Oregon, USA; Fig. 2). Only samples with CV ≤ 5, relative standard error (RSE = CV/square root (events) × 100%) < 0.2, and nuclei counted for sample alone ≥ 1000 were included in analyses. Sample genome sizes were calculated following the Eq. 1C = (sample peak / standard peak) × standard genome size (*Raphanus sativus* 'Saxa', 1C = 0.555 picogram [pg] DNA; Doležel et al., 1992; Doležel and Bartoš, 2005). The Appendix presents means and standard deviations of CV, 1C, and RSE values of the samples.

Cytogenetics—From March to June of 2008, immature spikes were fixed in a mixture of methanol, chloroform, and propionic acid (6 : 3 : 2), then dissected anthers were squashed in 2% lactic acetic-orcein (Rothrock and Reznicek, 1996). Meiotic chromosome figures were observed in at least five pollen mother cells per sample using phase contrast at 1000× magnification and were documented using drawings and photographs (Fig. 3). All preserved slides, chromosome photographs and drawings, and vouchers are archived at The Morton Arboretum Herbarium (MOR). All counts including the number of univalents (I), trivalents (III), and tetravalents (IV) were converted to inferred diploid chromosome numbers following Luceño and Castroviejo (1991) (Table 1).

Data analysis—For statistical analyses, mean values of genome sizes and diploid chromosome numbers of individuals and populations were calculated, and analyses were performed both at the level of individuals and populations; only individuals with both chromosome counts and genome size estimates were included in these analyses. To test alternative models of chromosome evolution, we normalized data to unit variance and evaluated the confidence interval on the slope (beta or standardized regression coefficient) of a linear least-squares regression of genome size on chromosome number. Rescaling the data in this way has the effect of expressing chromosome number and genome size in common units but has no effect on r^2 or its significance. If chromosome number changes were due to unbiased deletion or duplication of chromosomes, the slope of this regression would approach 1.0 with increasing data. If chromosome number changes are due to fission and fusion with no change in DNA content, the regression slope will approach zero. This test assumes that any chromosome deletions or duplications are unbiased with respect to the DNA content of each chromosome duplicated or deleted, an assumption that is probably justified given the uniformity of chromosome sizes in *Carex scoparia* var. *scoparia*. Support for a model in which genome size is predicted by chromosome number (the quantitative aneuploidy model) was compared with support for a model in which genome size is not predicted by chromosome number (the agmatoploidy/symploidy model) using small-sample Akaike information criterion (AIC*c*) weights on models of the form Y = mX + b vs. Y ~ b. AIC*c* weights estimate the evidential support for a hypothesis or model, given the data and the pool of models considered, and are expected to be unbiased even with small sample sizes (Burnham and Anderson, 2002). Mantel tests of chromosome number and genome size differences relative to geographic distances between populations were used to evaluate whether spatial autocorrelation needed to be accounted for in estimating regression coefficients and their significance. As no spatial autocorrelation was found (see Results), no corrections were required.

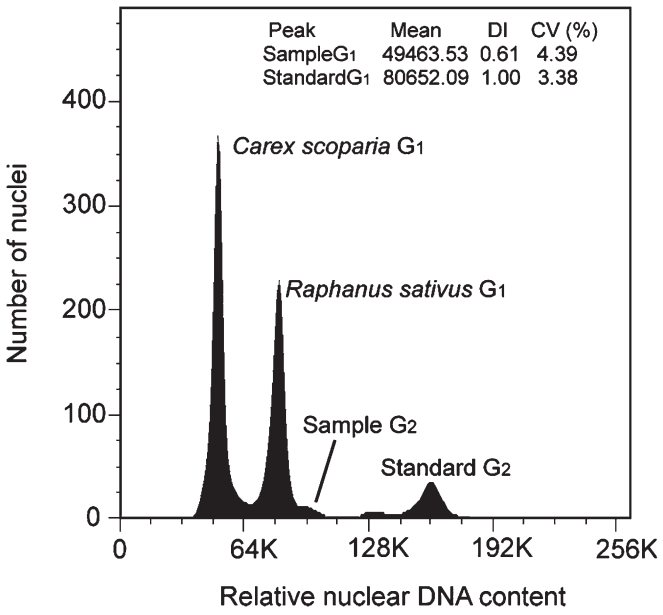

Fig. 2. Histogram of relative fluorescence intensity for nuclei in G_1 and G_2 phases of the cell cycle. *Raphanus sativus* 'Saxa' (1C = 0.555 pg DNA) was used as an internal reference standard. In this figure, 1C DNA content of *Carex scoparia* is equal to 49463.53 / 80652.09 × 0.555 = 0.340 pg. DNA index (DI) is calculated as mean channel number of sample / mean channel number of internal reference standard.

All analyses were conducted by using the base, stats, and vegan (Oksanen et al., 2007) packages of R v. 2.9.2 (R Development Core Team, 2009), and AIC*c* weights were calculated following the standard formula (Burnham and Anderson, 2002).

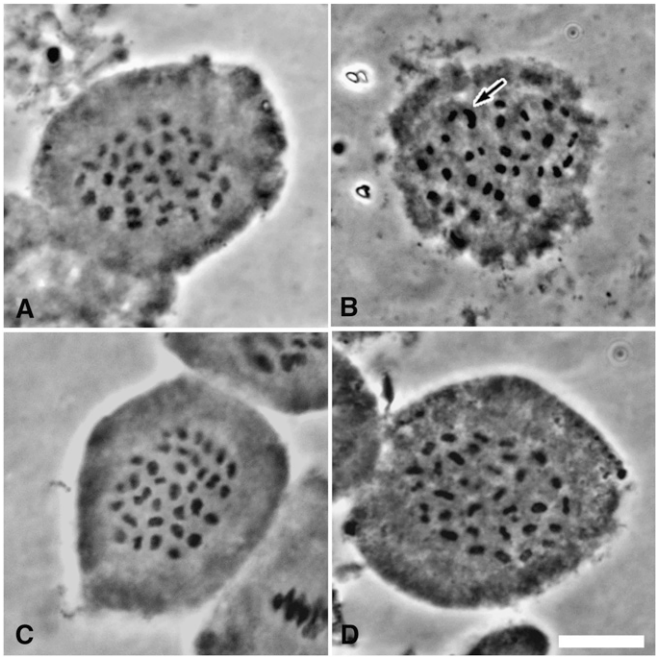

Fig. 3. Meiotic metaphase I photographs of *Carex scoparia* var. *scoparia*. (A) $n = 33$ (Goose Lake, 2030J). (B) $n = 33+III$ (Goose Lake, 2830N). Arrow indicates trivalent. (C) $n = 34$ (Hoosier Prairie, north, 2863A). (D) $n = 32$ (Hoosier Prairie, south, 2864M). Scale bar = 10 μm.

RESULTS

The DNA content estimates (1C-values) in this study varied more greatly among individual plants than among populations (Table 1). Among individuals, 1C-values ranged from 0.342 pg (2890D in Michigan City) to 0.369 pg (2656A in Greene Prairie), whereas the difference between maximum (Grant Woods) and minimum (Indiana Dunes–Cowles Bog RR) population-mean 1C values was 0.017 pg, a range of about 4.72% ((0.360–0.343)/0.360). The individual with minimum 1C-value (2882A) had $2n = 66$ chromosomes, close to the average chromosome number found in our study. Chromosome numbers ranged from $2n = 62$ to $2n = 68$ in the examined populations, a range of approximately 8.82%. Within-population differences in diploid chromosome number ranged from three (Michigan City/IN CR 900N and Soldiers Memorial Park populations, 65.88 ± 1.8102) to zero (Indiana Dunes–Mnoke Prairie, Naplate/I&M Canal, and Greene Prairie; Table 1). One individual (2880N from Lansing Woods) appeared to exhibit chromosome variation ($n = 33, 31+III$), which has been previously observed in the genus (Schmid, 1982; Luceño, 1994). Univalents, trivalents, and/or tetravalents were observed in seven populations (Table 1, Fig. 3). Variance in genome size within chromosome numbers exceeded the variance among chromosome numbers (Fig. 4).

The regression of DNA content (1C) on chromosome number ($2n$) was nonsignificant for data analyzed by individual ($r^2 =$ 3.74E-4, $P = 0.902$; 1C = –0.0193*$2n$ + –4.17E-15; 95% CI on standardized regression coefficient = –0.335, 0.296) or by population ($r^2 = 0.018$, $P = 0.570$; 1C = 0.135*$2n$ + 7.10E-15; 95% CI on standardized regression coefficient = –0.356, 0.626). In both cases, the 95% confidence interval on the standardized regression coefficient excluded slope = 1 and included slope = 0. As expected for a nonsignificant regression, the no-slope linear model was better supported than a linear model with chromosome number as a predictor (ΔAIC*c* = 2.43 for population-means data: log-likelihood of the no-slope model = –27.87, df = 2, AIC*c* = 60.4, AIC*c* weight = 0.77; log-likelihood of the model with chromosome number as a predictor = –27.68, df = 3, AIC*c* = 62.9, AIC*c* weight = 0.23; ΔAIC*c* and AIC*c* weight for individual data, no-slope model were 2.31 and 0.73, respectively). Neither chromosome number nor DNA content exhibited spatial autocorrelation on the basis of a Mantel correlation test between pairwise $2n$ or 1C differences and geographic distances ($2n$: $r = 1.55$E-3, two-tailed $P = 0.857$; 1C: $r = 0.309$, two-tailed $P = 0.108$; P values estimated by using 10 000 Mantel permutations).

DISCUSSION

Our analyses of the relation between DNA content and chromosome number support previous conclusions based on cytogenetic data alone that chromosome rearrangements within variable *Carex* species are more likely a consequence of fission and fusion than of chromosome duplication and deletion (aneuploidy s.s.). Among the *Carex scoparia* var. *scoparia* populations we sampled, the proportional range in chromosome number (8.82%) was nearly twice the genome size range (4.72%; Table 1), and genome size variance was not predicted by chromosome number variance (Fig. 4). Our data support an agmatoploid/symploid mechanism of chromosome evolution due to fission and fusion over a strict aneuploid mechanism, though the model in which chromosome number predicted genome

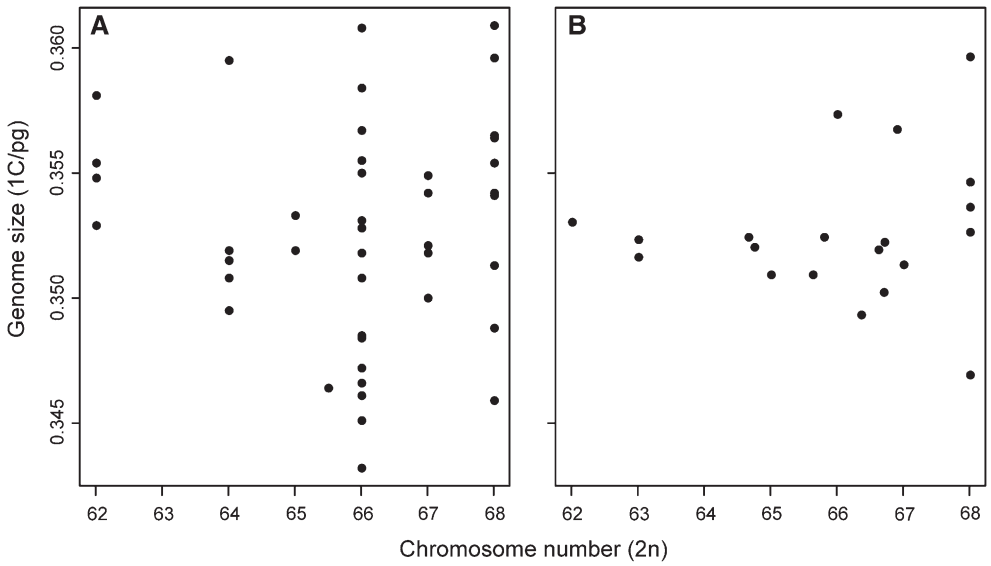

Fig. 4. Regression of genome size (1C) on chromosome number (2*n*). Both (A) individual-level regressions (r^2 = 3.74E-4, P = 0.902) and (B) population-level regressions (r^2 = 0.018, P = 0.570) are nonsignificant. Axes shown are raw data; regression formulae reported in the text are for data expressed in standard deviation units.

size was not strongly rejected (AIC*c* weight = 0.23 for analyses at the population level). In the model with chromosome number as a predictor of genome size, the simple aneuploid mechanism that would be implied by a standardized regression coefficient of 1 was soundly rejected (analyzed by individuals [N = 43], 95% CI on standardized regression coefficient = –0.335, 0.296; analyzed by population [N = 20], 95% CI = –0.356, 0.626). Admittedly, analyzing data by individual rather than by population risks introducing an obvious source of nonindependence into tests of alternative models of chromosome number change, but an aneuploid mechanism of chromosome number change within the species would nonetheless be expected to produce a positive correlation between chromosome number and DNA content in data analyzed both by individual and by population.

Whereas the model with chromosome number as a predictor (the aneuploidy s.s. model of form Y = mX + b) was weakly rejected, a slope of 1.0 was strongly rejected. Significant deviation from a slope of 1.0 could be a consequence of either of two factors. First, aneuploidy s.s. could be the actual mechanism at play if chromosome deletions and duplications draw from a biased subset of chromosomes or if reciprocal translocations of unequal fragment sizes effectively randomize genome size with respect to chromosome number. If, for example, all duplications and deletions were biased toward chromosomes with relatively low DNA content, then we would expect to find a positive relation between chromosome number and DNA content, with a standardized regression coefficient < 1. A strong enough bias to produce a standardized regression coefficient as low as 0.296 (the upper bound of the confidence interval for regressions on individual-level data) seems unlikely given the relatively even size of chromosomes within individuals sampled (Fig. 3). Moreover, if chromosome number increases were due to duplications of a subset of chromosomes, we would expect to regularly find tetravalents in meiosis (pairings of four rather than two chromosomes), due to homology between duplicated chromosome pairs. In the 42 individuals counted for this study, only two tetravalents were identified (Table 1), whereas 7 individuals exhibited trivalents, often with chain-like morphology, an expected consequence of agmatoploidy (Faulkner, 1972; Whitkus, 1988). Alternatively, deviation from a standardized regression coefficient of 1 in our study may be a consequence of fission and fusion as a mode of chromosome evolution, as has been suggested on the basis of previous cytogenetic work. For the reasons outlined above, we find this interpretation more plausible.

The finding that genome size and chromosome number variance do not correlate significantly with geography is particularly interesting given that our study covers a range of 328 km between the most distant populations. This finding supports previous work in *Carex* section *Ovales* demonstrating that chromosome evolution in the group is quite rapid (Hipp, 2007). In such a case, we expect to find a poor correlation between chromosome number and either geography or phylogeny. Our finding that chromosome fission and fusion explain the data better than chromosome deletions and duplications provides an explanation for this rapid chromosome evolution, as single chromosome fission and fusion events are not expected to be underdominant (Baker and Bickham, 1986).

Our findings have three more general implications. First, our finding of rapid chromosome evolution by fission and fusion at fine geographic scales in *Carex scoparia* supports previous arguments that chromosome evolution may play an important role in sedge diversification. The potential for chromosome rearrangements to limit gene flow among populations in the genus (Hipp et al., 2010), combined with the fine-scale patchwork of chromosome rearrangements observed on the landscape, increases the potential for local adaptation and differentiation of populations. Second, this patchwork distribution of chromosome rearrangements may have important consequences in ecological restoration efforts. Seed collection guidelines for ecological restoration frequently specify geographic distances from which seed should be collected (e.g., Meyer and Koenig, 2002, ch. 4.2.1.3). As chromosome rearrangements in sedges may occur on geographic scales much smaller than a county or watershed, the effects of these rearrangements on recombination between geographically proximate populations needs to be

investigated to determine whether geographic scale should be a primary concern in restoration of sedge-dominated habitats (e.g., fens, bogs, alpine meadows). Finally, this demonstration that quantitative aneuploidy is a poor explanation of chromosome evolution in *Carex scoparia* lays a framework for investigating whether chromosome rearrangements are occurring at a relatively small number of breakpoints and whether those breakpoints correlate with ecologically significant speciation genes (e.g., Larkin et al., 2009). If so, chromosome evolution in *Carex* may underlie the diversity of the temperate zone's most species-rich angiosperm genus.

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APPENDIX. Nuclear DNA content (1C) and diploid number (2*n*) of individuals of *Carex scoparia* var. *scoparia* examined. All vouchers are archived at The Morton Arboretum Herbarium (MOR). *Abbreviations*: pg = picogram; s.d. = standard deviation; CV = coefficient of variation; RSE = relative standard error; FCM reps. = number of flow cytometry runs per individual; 2*n* = diploid chromosome number, calculated from meiotic observations (see text); NC = not counted.

Population (Number of individuals)	Individual (Voucher)	Mean DNA content (pg DNA / 1C) ± s.d.	Mean CV (%) ± s.d.	Mean RSE	FCM reps.	2*n*
Goose Lake (4)	2830D	0.353 ± 0.0107	4.46 ± 0.64	0.1295	3	NC
	2830J	0.347 ± 0.0073	3.93 ± 0.37	0.0813	4	66
	2830N	0.353 ± 0.0086	4.21 ± 0.42	0.0888	4	65
	2830W	0.352 ± 0.0023	3.82 ± 0.48	0.0802	3	66
Grant Woods (2)	2879A	0.361 ± 0.0055	3.82 ± 0.52	0.0856	6	68
	2879C	0.357 ± 0.0110	4.18 ± 0.53	0.0912	3	NC
Hoosier Prairie, North (5)	2863A	0.351 ± 0.0067	4.31 ± 0.48	0.1009	3	68
	2863B	0.358 ± 0.0051	4.13 ± 0.49	0.0978	4	66
	2863M	0.350 ± 0.0083	4.13 ± 0.56	0.0972	4	67
	2863P	0.347 ± 0.0121	4.31 ± 0.98	0.1058	3	NC
	2863Q	0.353 ± 0.0018	3.98 ± 0.52	0.0892	3	66
Hoosier Prairie, South (3)	2864A	0.355 ± 0.0012	3.80 ± 0.14	0.0865	2	66
	2864F	0.352 ± 0.0084	3.94 ± 0.52	0.0982	4	NC
	2864M	0.351 ± 0.0014	5.05 ± 2.81	0.1107	4	64
Indiana Dunes—Cowles Bog RR (1)	2857K	0.343 ± 0.0074	4.21 ± 0.49	0.0959	3	NC
Indiana Dunes—Mnoke Prairie (5)	2865B	0.354 ± 0.0093	4.54 ± 0.33	0.1120	3	NC
	2865F	0.356 ± 0.0067	3.81 ± 0.41	0.1054	2	NC
	2865H	0.355 ± 0.0023	4.03 ± 0.10	0.0895	4	68
	2865O	0.349 ± 0.0059	4.03 ± 0.69	0.0981	4	68
	2865S	0.351 ± 0.0088	3.80 ± 0.44	0.0838	4	NC
Iroquois SWA (4)	2894D	0.353 ± 0.0074	4.11 ± 0.29	0.1009	3	NC
	2894F	0.354 ± 0.0039	4.13 ± 0.44	0.0852	4	NC
	2894H	0.360 ± 0.0044	4.05 ± 0.23	0.0995	4	68
	2894U	0.350 ± 0.0044	3.58 ± 0.29	0.0819	3	NC
Jasper-Pulaski FWA (5)	2893A	0.351 ± 0.0040	3.90 ± 0.40	0.0872	4	64
	2893C	0.352 ± 0.0023	3.66 ± 0.25	0.0802	3	NC
	2893G	0.354 ± 0.0057	3.89 ± 0.24	0.0893	5	NC
	2893J	0.343 ± 0.0043	4.20 ± 0.46	0.0843	3	NC
	2893O	0.355 ± 0.0095	4.57 ± 0.26	0.0918	4	62
Lansing Woods (6)	2880A	0.357 ± 0.0055	3.90 ± 0.62	0.0964	4	66
	2880F	0.355 ± 0.0064	4.36 ± 0.60	0.0980	2	NC
	2880H	0.359 ± 0.0140	4.42 ± 0.24	0.0906	3	NC
	2880J	0.354 ± 0.0071	3.90 ± 0.90	0.0787	3	NC
	2880N	0.346 ± 0.0038	3.98 ± 0.27	0.0994	5	65.5
	2880R	0.348 ± 0.0066	3.71 ± 0.89	0.0801	4	66
Long John Slough (5)	2878A	0.354 ± 0.0073	3.93 ± 0.34	0.0989	3	68
	2878C	0.356 ± 0.0052	4.02 ± 0.34	0.0844	4	NC
	2878F	0.349 ± 0.0117	4.47 ± 0.26	0.0900	3	NC
	2878I	0.347 ± 0.0035	4.32 ± 0.31	0.1024	3	66
	2878T	0.348 ± 0.0030	4.23 ± 0.54	0.0865	3	NC
Lulu Lake (5)	2800	0.356 ± 0.0042	3.76 ± 0.52	0.0866	3	68
	2807	0.354 ± 0.0059	4.45 ± 0.40	0.0935	3	67

APPENDIX. Continued

Population (Number of individuals)	Individual (Voucher)	Mean DNA content (pg DNA / 1C) ± s.d.	Mean CV (%) ± s.d.	Mean RSE	FCM reps.	2*n*
	2816	0.358 ± 0.0104	4.73 ± 0.11	0.0989	3	NC
	2818	0.353 ± 0.0060	3.98 ± 0.70	0.0829	4	66
	2820	0.363 ± 0.0064	4.11 ± 0.32	0.1149	3	NC
Michigan City/IN CR 900N (7)	2703	0.351 ± 0.0105	4.38 ± 0.54	0.0953	3	NC
	2709	0.351 ± 0.0027	4.05 ± 0.74	0.0870	3	NC
	2890A	0.352 ± 0.0048	3.85 ± 0.76	0.0839	4	67
	2890D	0.342 ± 0.0040	4.32 ± 0.74	0.0933	4	NC
	2890H	0.352 ± 0.0038	3.73 ± 0.59	0.0957	3	NC
	2890I	0.354 ± 0.0038	3.80 ± 0.39	0.0913	3	68
	2890J	0.352 ± 0.0123	3.72 ± 1.06	0.0835	3	65
Middlefork Savanna (7)	2895C	0.345 ± 0.0035	4.19 ± 0.21	0.0836	3	NC
	2895E	0.353 ± 0.0081	4.27 ± 0.42	0.1058	4	NC
	2895F	0.352 ± 0.0115	4.07 ± 0.62	0.1007	7	NC
	2895G	0.355 ± 0.0056	4.50 ± 0.51	0.0841	3	NC
	2895H	0.352 ± 0.0060	4.14 ± 0.52	0.0904	5	67
	2895I	0.347 ± 0.0098	4.25 ± 0.39	0.0878	4	NC
	2895K	0.361 ± 0.0086	3.97 ± 0.30	0.0791	3	66
Naplate/I&M Canal (8)	2839B	0.353 ± 0.0031	3.80 ± 0.42	0.0861	3	62
	2839D	0.358 ± 0.0069	4.20 ± 0.64	0.0908	4	NC
	2839F	0.349 ± 0.0112	4.60 ± 0.37	0.0935	4	NC
	2839G	0.344 ± 0.0105	4.60 ± 0.11	0.1037	4	NC
	2839I	0.353 ± 0.0029	4.35 ± 0.58	0.0981	3	NC
	2839K	0.354 ± 0.0100	4.17 ± 0.50	0.0885	4	NC
	2839N	0.354 ± 0.0098	4.26 ± 0.39	0.0935	3	NC
	2839O	0.358 ± 0.0082	4.33 ± 0.46	0.0916	5	62
Soldiers Memorial Park (2)	2889A	0.346 ± 0.0082	4.19 ± 0.28	0.0911	4	68
	2889G	0.348 ± 0.0035	3.90 ± 0.17	0.0765	3	NC
Spinn Prairie (1)	2884A	0.351 ± 0.0082	4.55 ± 0.27	0.0937	4	NC
Sundrop Prairie (5)	2891D	0.356 ± 0.0012	3.52 ± 0.09	0.0762	3	NC
	2891G	0.351 ± 0.0008	4.00 ± 0.54	0.0894	3	66
	2891L	0.345 ± 0.0047	3.70 ± 0.51	0.0752	3	NC
	2891O	0.349 ± 0.0011	4.27 ± 0.42	0.1025	3	64
	2891S	0.353 ± 0.0044	4.07 ± 0.63	0.0906	3	NC
US421 and CR1000S (3)	2882A	0.343 ± 0.0068	4.28 ± 0.42	0.0873	3	66
	2882D	0.360 ± 0.0088	4.22 ± 0.37	0.0957	5	64
	2882G	0.349 ± 0.0078	4.04 ± 0.35	0.0843	4	NC
Wampum Lake (4)	2875A	0.352 ± 0.0025	3.71 ± 0.31	0.1000	3	64
	2875G	0.350 ± 0.0080	4.01 ± 0.65	0.0967	4	NC
	2875K	0.355 ± 0.0044	3.76 ± 0.16	0.0815	3	62
	2875R	0.353 ± 0.0073	4.09 ± 0.62	0.0880	3	NC
Willow Slough (2)	2892C	0.356 ± 0.0065	4.05 ± 0.54	0.1049	4	68
	2892N	0.350 ± 0.0004	4.41 ± 0.38	0.0961	3	NC
Zander Woods (5)	2881B	0.351 ± 0.0012	4.57 ± 0.30	0.1027	3	NC
	2881D	0.355 ± 0.0089	4.40 ± 0.43	0.0932	4	67
	2881H	0.349 ± 0.0060	3.95 ± 0.52	0.0913	4	66
	2881L	0.345 ± 0.0107	4.14 ± 0.56	0.0791	3	66
	2881O	0.345 ± 0.0062	4.26 ± 0.38	0.0991	3	NC
Greene Prairie (9)	2663	0.346 ± 0.0054	3.80 ± 0.60	0.0917	3	66
	2653C	0.362 ± 0.0088	4.06 ± 0.74	0.0823	3	NC
	2654A	0.358 ± 0.0087	4.53 ± 0.35	0.0948	3	NC
	2654B	0.362 ± 0.0132	3.97 ± 0.56	0.0897	3	NC
	2655B	0.348 ± 0.0051	3.58 ± 0.33	0.0755	3	NC
	2656A	0.369 ± 0.0016	4.06 ± 0.19	0.0894	3	NC
	2656B	0.364 ± 0.0024	4.34 ± 0.66	0.1095	3	NC
	2658_1/3	0.352 ± 0.0084	3.71 ± 0.32	0.0688	3	NC
	2658_3/3	0.355 ± 0.0049	4.10 ± 0.65	0.0858	3	66
Powderhorn Lake (1)	2668	0.353 ± 0.0108	4.05 ± 0.19	0.1013	3	NC