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How Far is Too Far? Genetic consequences of Seed Provenance Decisions in Sedges

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ABSTRACT

We investigated the regional patterns of karyotype (chromosome) variation and population genetic structure in 22 Chicago region populations of *Carex scoparia*, a sedge species that exhibits remarkable chromosomal variation. Karyotype rearrangements are common, with our counts in the species ranging from $2n = 62$ to $2n = 68$ and multiple counts fixed within at least some populations. As would be expected under these conditions, we found substantial genetic differentiation among populations and significant evidence of inbreeding (low heterozygosity) in most populations. One population, Powderhorn Lake Forest Preserve, showed an intriguing pattern: heterozygosity levels compatible with outbreeding, in combination with evidence of hybridization among two source populations and strong genetic linkage among most markers. This combination may suggest a lack of recombination between two source populations due to karyotype differences.

There is a management implication in this interpretation. If in fact non-local genotypes are unable to recombine with local genotypes, then introduction of non-local plants will most likely result in coexistence of the two populations or competitive exclusion of one. The risk is that in the absence of recombination between genotypes, locally adapted genotypes may be outcompeted by nonlocal genotypes, reducing the overall genetic diversity of the species. Whether this theoretical risk, suggested by our study, is a risk in fact is a subject of our ongoing work.

Pending further study, our recommendation based on this work is that restorationists should limit seed sources to a broad range of the local populations nearest the restoration site *when working with sedges*. Unfortunately, the data we have gathered does not present evidence on the relevant spatial scale; however, our interpretation of the data suggests that collecting as locally as possible from a range of sites will minimize the risk of genetic homogenization due to nonrecombination between local and nonlocal populations and maximize genetic variation at the site level.

INTRODUCTION

Sound ecological restoration is generally thought to depend on sampling appropriately at two scales: from the local flora and from the local gene pool. Yet the scale at which a gene pool may be considered "local" varies widely from organism to organism, with local adaptation evident on scales that range from less than a kilometer to hundreds of kilometers. Moreover, it is not clear what the implications of utilizing local genotypes are for ecological restoration, especially in our rapidly changing climate (see, for example, Pergams and Lacy 2008). No studies of local adaptation and few studies of gene flow between populations have been undertaken in the temperate zone's largest flowering plant genus, *Carex* (ca. 140 species in the Chicago region alone), despite the fact that the genus is ecologically important in fens, wet prairies, and woodlands.

In this project, we began an investigation of regional patterns of karyotype (chromosome) variation and population genetic structure in *Carex scoparia*, a sedge species that exhibits remarkable chromosomal variation. Understanding the pattern of genetic and chromosomal differentiation at regional scales is an essential first step to answering the question of whether populations across the region have the potential to be genetically differentiated from one another. If populations are strongly differentiated at neutral loci—regions of the genome that are presumably not under natural selection—then this provides evidence that there is restricted gene flow among populations; whether this restricted gene flow also limits the movement of broadly-selected alleles that affect species coherence (Morjan and Rieseberg 2004) or locally-selected alleles that are responsible for local adaptation requires additional work. The pattern of chromosome rearrangements is equally important and confounding in genera like *Carex*, in which chromosome rearrangements are rapid and have the potential to limit gene flow among populations. Recent work in the genus *Carex* has demonstrated high levels of gene flow among populations at regional scales, with deep divergences associated only with substantial geographic barriers to gene flow (Escudero et al. 2008, Schönswetter et al. 2008). However, our own work in *Carex scoparia* based on amplified fragment length polymorphisms (AFLP) and a microsatellite survey of exemplars from populations distributed across the species' range demonstrates continent-scale limitations to gene flow associated with both chromosome rearrangements and geographic distance (Hipp et al. 2008). Recent microsatellite investigations of a distantly related sedge species likewise suggest a high degree of inbreeding (King and Roalson 2008). Work conducted to date is insufficient to allow a general response to the question of the scale of gene flow in sedges.

Our work focuses on *Carex scoparia* both because the genus is our largest in number of species but among the most poorly studied from an ecological and management standpoint, and because *Carex scoparia* is a common species of wet prairies, which are among the most poorly represented of Illinois' prairie types. In this report, we will address two key recommendations in the Chicago Wilderness Biodiversity Recovery Plan, namely the need to "research, develop, and implement strategies to maintain genetic diversity" and to "study gene flow in plants."

ENNUMERATION OF PROJECT GOALS AND ACCOMPLISHMENTS

Our project proposal laid out three primary goals. In this section of the paper, we describe our findings in light of these goals.

Goal 1. *Survey 20 populations of Carex scoparia for neutral genetic variation using microsatellite markers already developed in our lab, and chromosome diversity using standard methods also in regular use in our lab, using 10 to 20 individuals per population and 10 microsatellite markers.*

Accomplishments. We surveyed genetic variation in 22 Chicago region populations (Table 1, Figure 1), one southern Wisconsin population, and two New York state populations for comparison, including over 500 individuals surveyed for 11 polymorphic microsatellite markers, which is almost double our expected sample of 300 individuals. We found substantial genetic differentiation among populations ($P < 0.00001$ for all pairwise comparisons based on the exact G test as implemented in GENEPOP, as well as

high differentiation based on Bayesian analysis of population genetic structure in STRUCTURE; Figure 2), and significant evidence of inbreeding (low heterozygosity) in most populations. Two populations show heterozygosity levels compatible with outbreeding: Greene Prairie (in the UW Madison Arboretum) and Powderhorn Lake Forest Preserve. Powderhorn Lake Forest Preserve also shows very strong linkage disequilibrium involving most markers. This combination could be explained by seed introductions from a population with differing karyotype, in which high heterozygosity in F_1 hybrids would be associated with a lack of recombination between the two populations. In fact, analysis of the data using STRUCTURE suggests a high degree of genetic admixture (hybridization) in this population (Figure 2), a pattern not seen in any other population surveyed. There is no record of planting *C. scoparia* at Powderhorn Lake FP (W. Vanderploeg, pers.comm.), but we will be investigating chromosome pairing relationships as we continue our karyotype investigations.

Goal 2. *Correlate the pattern of genetic variation among populations with both geographic distance and chromosome number difference, to evaluate whether chromosomal variation plays a role in gene flow among chromosomally variable sedge populations in the Chicago region.*

Accomplishments. We analyzed genetic and chromosomal variation across 35 populations sampled from throughout the range of *Carex scoparia* and found a strong correlation between genetic distance and both chromosome number difference and geographic distance at this scale (Figure 3). Within the Chicago region alone, however, there is no evidence of isolation-by-distance ($P = 0.4037$, standard Mantel correlation, $N = 22$ populations). Within the Chicago region, we have sampled chromosome variation from examplars of ten populations (Table 1; Figure 4) and find that chromosome number varies widely among populations even within the Chicago region. (We are continuing our chromosome investigations and expect to have three individuals per each of 22 populations counted by the end of winter; however, timing greenhouse material for anther squashes proved to be more problematic this spring than we had expected, slowing our work.) For two populations from which we have counted chromosomes in at least two individuals, we find variance in chromosome numbers without evidence of irregular pairing relationships at meiosis, suggesting that multiple chromosome numbers may be fixed within those populations. With additional sampling, we will be investigating whether there is a correlation between chromosome karyotype variance and genotype variance within populations.

The geographic pattern of chromosome numbers (Figure 1) suggests that karyotype rearrangements are occurring rapidly enough that chromosome numbers alone do not adequately represent karyotype rearrangements. The number $2n = 62$, for example, is the lowest number we have found in the Chicago region so far, and it is found at two sites that are 115 miles apart from one another (Mnoke Prairie, Indiana Dunes; and Naplate, IL) as well as a third site, Jasper-Pulaski Fish and Wildlife Area, where it is found in a population with individuals of $2n = 64$. Further analysis of the relationship among genetic divergence and karyotype rearrangement within the Chicago region will be conducted as we gather more karyotype data.

Goal 3. *Test newly developed molecular markers for utility in studies of conservation genetics and gene flow across a range of species in Carex section Ovales, the largest group of sedges in the Midwest.*
Accomplishments. We tested the 11 polymorphic microsatellite markers for amplification in four species within *Carex* section *Ovales* and an additional *Carex* species (*C. stipata*) also common in wetlands but not closely related. All primer pairs but one amplified in *Carex stipata* and all but two amplified in the other *Ovales* species (Table 2), suggesting that these markers will be of use for understanding gene flow and conservation genetics in a wide range of *Carex* species. We have sent samples of these primers to colleagues working in other *Carex* labs and they are finding the primers useful in a broader range of species.

IMPLICATIONS: CW BIODIVERSITY CONSERVATION AND RESTORATION

Our study contributes to an emerging picture of genetic differentiation in the largest genus in the temperate zone, demonstrating that population differentiation within sedges can be quite high, despite the fact that sedges are wind-pollinated plants. This finding stands at odds with two recent studies of other *Carex* species using AFLP data (Schönswetter et al. 2008, Escudero et al. 2008), which demonstrate limited interpopulation differentiation within regions; an exception is found in another recent AFLP study (Schönswetter et al. 2006), which found regional differentiation among populations in alpine conditions, where differentiation is expected to be higher. In the former two studies, chromosome variation is significantly less than we find in *C. scoparia*, which may account for this disparity.

Our work on the species across its range (Hipp et al. 2008) demonstrates that karyotype variance in *C. scoparia* does restrict gene flow among populations. Importantly, however, our work also demonstrates that *C. scoparia* is genetically coherent across the range of the species, and that gene flow among karyotypically divergent populations appears to proceed via karyotypically similar intermediates. At first glance, this might seem to suggest an important management implication: that the restorationist need not survey every population for karyotype prior to collecting seed for restoration, as gene flow is possible across a wide range of karyotypes. However, one of the populations we surveyed (the wet prairie at Powderhorn Lake Forest Preserve) suggests a different problem. At increasing geographic distance, nonlocal sedge populations are likely to be increasingly divergent from the local population genetically and chromosomally. (It is worth noting that the lack of evidence for isolation by distance in the Chicago region suggests that geographic distance may not be important in this regard, though workers in this genus—ourselves included—lack the genomic tools needed to directly test for the possibility that the karyotype rearrangements themselves exhibit isolation by distance.) Strong evidence of both hybridization and widespread disequilibrium in the Powderhorn Lake population suggests a lack of recombination between two populations that may be due to karyotype differences (this possibility will be tested as we continue our karyotype investigations). If in fact non-local genotypes are unable to recombine with local genotypes, then introduction of non-local plants will most likely result in coexistence of the two populations or competitive exclusion of one. The risk here is that in the absence of recombination between genotypes, locally adapted genotypes may be outcompeted by nonlocal genotypes, reducing the overall genetic

diversity of the species. Whether this theoretical risk, suggested by our study, is a risk in fact is a subject of our ongoing work.

As we continue our work, it is worth noting that we have not evaluated whether the risk of loss of local genotypes may be outweighed by the benefit of introducing new genotypes to an area as a way of providing new raw material for natural selection, especially in the light of rapid climate change. A mouse population studied in the Chicago region, for example, appears to have been replaced by another regional population in the period between 1976 and 2001 (Pergams and Lacy 2008). Such turnover may be a desirable response to climate change and other anthropogenic environmental shifts. However, the karyotype rearrangements that characterize *Carex scoparia* populations (and populations of many other sedges; see, for example, discussions and data in Hipp 2007 and Roalson 2008) may make sedges particularly susceptible to genetic homogenization if regional restoration efforts utilize genetically homogeneous seed sources.

Our recommendation based on this work is consequently that restorationists should limit seed sources to a broad range of the local populations nearest the restoration site when working with sedges. Unfortunately, the data we have gathered does not present evidence on the relevant spatial scale; however, our interpretation of the data suggests that collecting as locally as possible from a range of sites will minimize the risk of genetic homogenization due to nonrecombination between local and nonlocal populations and maximize the genetic variation at the site level. As was recently pointed out to me by a colleague (G. Morris pers.comm.), a similar recommendation may apply for species with variable ploidy, such as big bluestem (*Andropogon gerardii*), in which a robust, polyploid nonlocal population could outcompete the local population, without affording an opportunity for local alleles to introgress into the nonlocal population. Again, this is only a precautionary recommendation based on the possible implications of this work. Experimental field study will be needed to evaluate whether homogenization due to lack of recombination is a real concern for ecological restoration.

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Site Name	Voucher (MOR)	Latitude	Longitude	UTM-E	UTM-N	2*n*
Goose Lake	2830A-BB	41.35958	-87.68410	442780	4578901	66, 66
Grant Woods	2879A-E	42.38790	-87.87193	428229	4693214	68
Hoosier Prairie, north	2863A-X	41.52636	-87.44268	463068	4597285	66,68
Hoosier Prairie, south	2864A-O	41.50478	-86.56273	536493	4594888	
Indiana Dunes -- Cowles Bog	2857A-T	41.63682	-86.89947	508373	4609459	
Indiana Dunes -- Mnoke Prairie	2865A-X	41.61798	-86.89145	509043	4607369	62
Iroquois SWA	2894A-V	41.00850	-87.54853	453874	4539846	
Jasper-Pulaski FWA	2893A-U	41.17238	-85.06923	661961	4559690	62, 64
Lansing Woods	2880A-U	41.55550	-86.44578	546217	4600574	66 or 65
Long John Slough	2878A-U	41.70863	-86.12058	573163	4617801	
Lulu Lake	2800-2809, 2816-2819, 2821	42.82687	-87.53745	456070	4741729	
Michigan City / IN CR 900N	2703-2706, 2708, 2709, 2890A-Q	41.73738	-85.21510	648430	4622158	67
Middlefork Savanna	2895A-L	42.25320	-86.11737	572807	4678267	
Naplate / I&M Canal	2839A-R	41.32970	-87.08577	492823	4575362	62, 62
Powderhorn Lake	2866A-T	41.64743	-86.46522	544533	4610771	
Soldiers Memorial Park	2889A-V	41.61590	-85.25272	645576	4608606	65
Spinn Prairie	2884A-T	40.77208	-85.12610	658144	4515146	
Sundrop Prairie	2891A-T	41.61947	-86.30137	558203	4607763	
US421 and CR1000S	2882A-T	40.91197	-85.11992	658332	4530686	66
Wampum Lake	2875A-T	41.57608	-86.41027	549163	4602879	
Willow Slough	2678, 2681, 2683, 2684, 2688-2691, 2892A-T	40.99350	-86.50590	541559	4538153	
Zander Woods	2881A-U	41.56970	-86.41403	548854	4602168	

Table 1. Sites sampled for *Carex scoparia* work. 2*n* = diploid chromosome number, inferred from first meiotic prophase or pollen cell mitosis; counts separated by commas were made from separate individuals collected at the same population. Note that odd diploid counts are not uncommon in *Carex* due to irregular chromosome arrangements, presumed to be due to hybridization between two individuals of different chromosome numbers.

Section	Species	S047	S082	S087	S102	S119	S128	S175	S177	S180	S181	S245
Ovales	*C. scop.* var. *scoparia*	238-283	162-182	227-251	211-232	180-195	160-198	132-139	172-200	202-214	246-273	211-229
Ovales	*C. scop.* var. *tessellata*	249	163	230	205	201	176	138	191	201	250	214
Ovales	*Carex normalis*	237	167	220	211	204	197	138	180	204	247	211
Ovales	*Carex vexans*	--	167	214	213	189	179	135	186	--	262	211
Vulpinae	*Carex stipata*	268	164	214	221	177	142	129	200	--	235 / 238	217

Table 2 Cross-species amplification in *Carex scoparia* var. *tessellata* and three additional *Carex* species. A single specimen of each taxon other than *C. scoparia* var. *scoparia* was sampled. Sizes of alleles are reported in bp are reported. All sizes reflect amplification using M13-labeled primers, and sizes are consequently 18bp longer than sizes expected with standard labeled primers (see text and Schuelke 2000). **Source:** Hipp et al. in review, *Molecular Ecology Resources.*

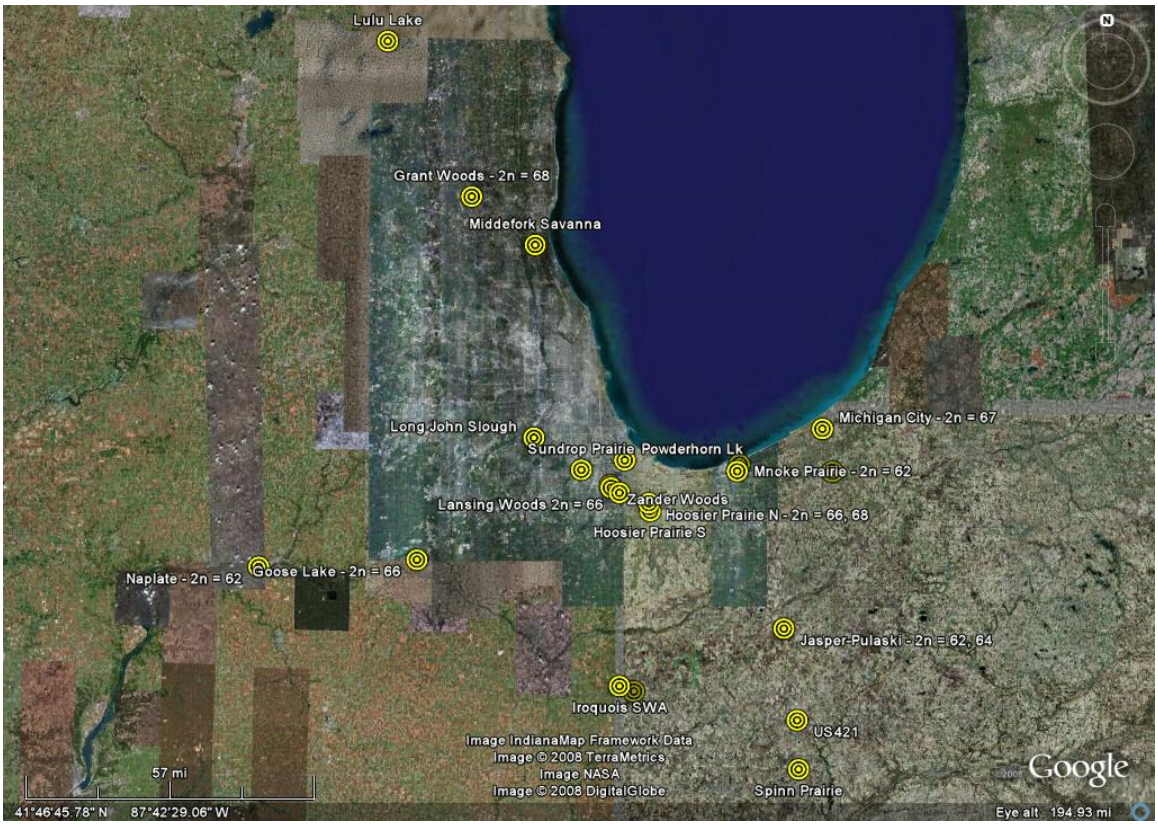

Figure 1 Locations of the 22 Chicago region *Carex scoparia* populations surveyed for this project, with chromosome numbers for populations from which we have made successful counts.

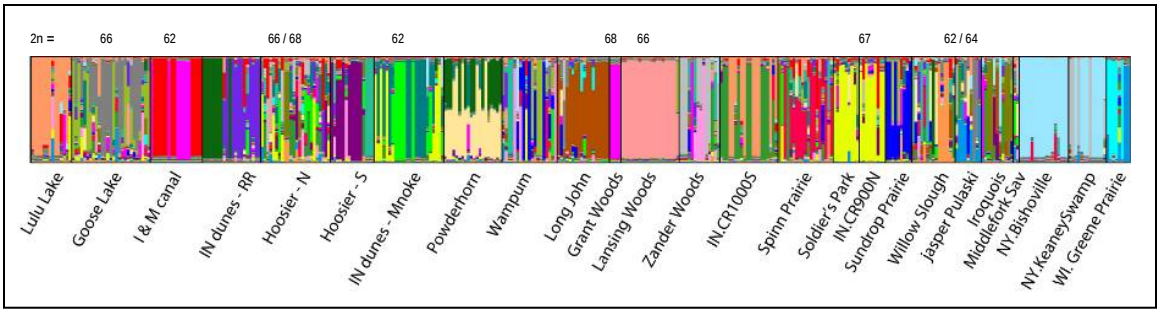

Figure 2 Bayesian clustering of *Carex scoparia* individuals sampled for this study, using STRUCTURE. The Bayesian method utilized is based on a likelihood model that assumes populations are at Hardy-Weinberg equilibrium with minimal linkage among loci. We set the number of populations (*K*) to 25 for this analysis, equal to our number of sampling localities (though population substructure may justify recognition of additional populations for further analysis), and allowed admixture (hybridization or introgression) among populations. Individuals sampled are represented by columns of color, estimating the percent of an individual's genome that derives from a given ancestral population. The things to note in this figure are that (1) most sites we sampled have one or two distinguishing, dominant colors, indicating that they are genetically distinct from other populations; and (2) the Powderhorn Lake population appears to be composed of hybrid individuals (see discussion in the text).

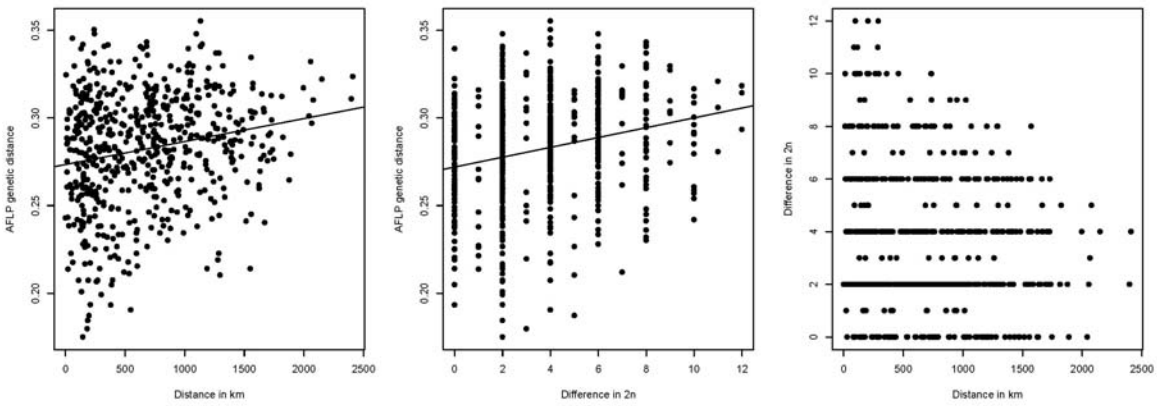

Figure 3 Relationship between geographic distance and genetic distance (left panel), chromosome number difference and genetic distance (middle panel), and chromosome number difference and geographic distance (right panel), based on AFLP survey of species from throughout its range in eastern North America (from Hipp et al. 2008). Both correlations involving genetic distance are positive and significant ($P < 0.001$ based on simple and partial Mantel tests). The relationship between chromosome number and geographic distance is not significant, perhaps reflecting the fact that chromosome number difference may significantly underestimate the number of karyotype rearrangements between two individuals.

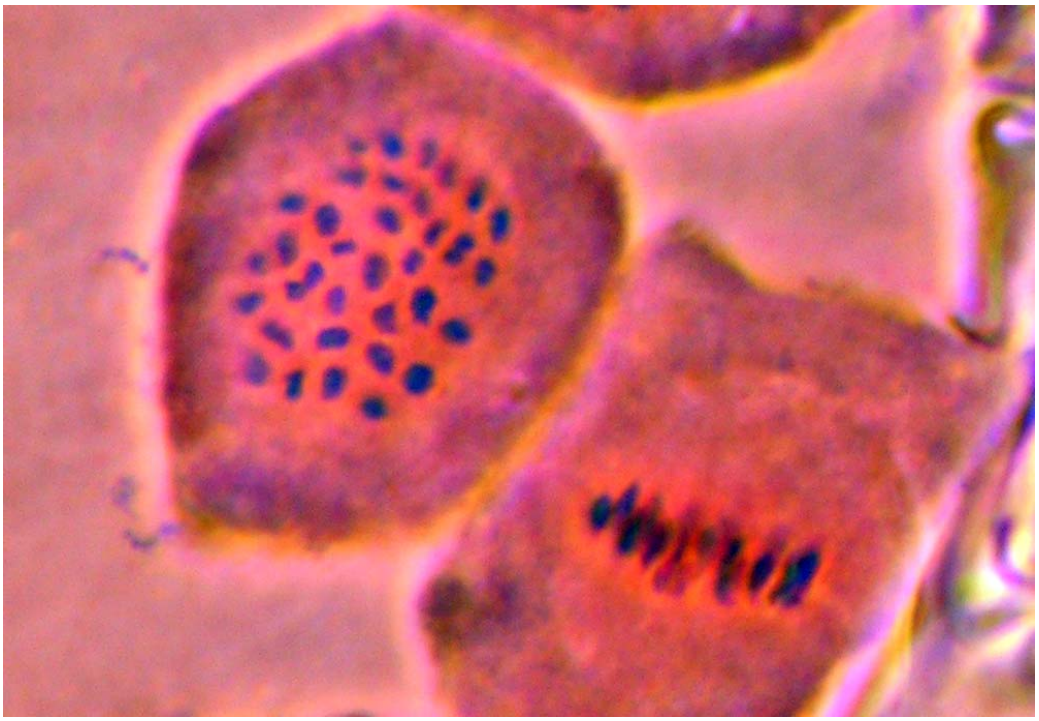

Figure 4 Meiotic cells from *Carex scoparia* 2863A (Hoosier Prairie, North; 2*n* = 68). The upper cell is at first meiotic prophase. The lower cell is at first meiotic metaphase.