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The consequences of monoecy and protogyny for mating in wind-pollinated *Carex*

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Summary

- Monoecy and protogyny are widespread in wind-pollinated plants and have been interpreted as outcrossing mechanisms, though few studies have investigated their function. *Carex*, a large genus of anemophilous herbs, is predominantly monoecious and many species are protogynous. We investigated whether monoecy and protogyny limit self-pollination in seven *Carex* species.
- We conducted field experiments comparing stigmatic pollen loads and seed set between intact and emasculated stems. We tested for self-compatibility and evaluated pollen limitation of seed set by supplemental pollination. Finally, we measured outcrossing rates in open-pollinated and emasculated stems using allozyme markers.
- Emasculated stems captured significantly less pollen than open-pollinated stems and set less seed. Pollen deposition during the female-only phase for intact stems was only 12% of the total captured. Outcrossing rates for three species indicated high selfing (range $t = 0.03$–0.39). Allozyme loci in the remaining species were monomorphic also suggesting high selfing. These results demonstrate that neither monoecy nor protogyny is particularly effective at limiting self-fertilization.
- Selection for the avoidance of selfing is unlikely to maintain monoecy in many *Carex* species although protogyny may provide limited opportunities for outcrossing. We propose that geitonogamy in self-compatible wind-pollinated species with unisexual flowers may be widespread and provides reproductive assurance.

Introduction

Flowers of wind-pollinated plants are frequently unisexual (dicliny), or if hermaphroditic separate their sex functions temporally, through well-developed dichogamy (Charlesworth, 1993; Renner & Ricklefs, 1995; Ackerman, 2000). Many monoecious species are wind-pollinated and, in general, abiotic pollination is strongly associated with this sexual system (Webb & Lloyd, 1986; Renner & Ricklefs, 1995; Friedman & Barrett, 2008). In some cases monoecy may have evolved as a mechanism to promote outcrossing (Lloyd, 1972; Charlesworth & Charlesworth, 1978). However, a survey of 588 angiosperms by Bertin (1993) found that the presence of self-compatibility versus self-incompatibility was independent of whether species were monoecious or possessed hermaphroditic flowers. This result is inconsistent with a major role for the avoidance of selfing in the evolution of monoecy. Because wind direction is often unpredictable, the spatial segregation of sex functions through monoecy may be ineffective at preventing geitonogamy in wind-pollinated plants (Harder *et al.*, 2000).

The common association between monoecy and protogyny in wind-pollinated plants (Lloyd & Webb, 1986; Bertin & Newman, 1993; Sargent & Otto, 2004) may largely function to reduce the incidence of self-fertilization. Abiotically pollinated species are more frequently protogynous than protandrous, whereas biotically pollinated species exhibit the reverse pattern (Sargent & Otto, 2004). Moreover, Bertin (1993) and Routley *et al.* (2004) demonstrated that protogyny was associated with self-compatibility, and protandry with self-incompatibility, more often than would be expected under a random model. This finding is consistent with the inbreeding avoidance hypothesis for the evolution of protogyny (Lloyd & Webb, 1986). The associations between protogyny and anemophily and protogyny and self-compatibility suggest that protogyny may be an effective outcrossing mechanism in many anemophilous species.

In animal-pollinated plants, geitonogamy involves mating costs as it reduces outcross siring success through pollen (Harder & Barrett, 1995; Harder & Wilson, 1998; Lau *et al.*, 2008) and seed discounting (Lloyd, 1992), and can also result in inbreeding depression (Eckert & Barrett, 1994a; Eckert, 2000). However, neither the frequency nor the reproductive consequences of geitonogamy in wind-pollinated species have been investigated (but see Pannell, 1997; de Jong *et al.*, 1999). Wind-pollinated plants have a bimodal distribution of outcrossing rates (Schemske & Lande, 1985; Aide, 1986; Barrett & Eckert, 1990; Goodwillie *et al.*, 2005), with species more frequently either predominantly selfing or outcrossing. It has been argued that the apparent low incidence of mixed mating, compared with animal-pollinated plants, might reflect different selective pressures in wind-pollinated species, including infrequent geitonogamy (Aide, 1986; Vogler & Kalisz, 2001; Goodwillie *et al.*, 2005).

The occurrence of separate female and male flowers in monoecious species requires that any selfing that occurs through geitonogamy must involve the same pollination processes as would occur with outcrossing. Because in animal-pollinated species geitonogamy requires the action of a pollinator, it offers no reproductive assurance in the absence of pollinators (Lloyd, 1992). Reproductive assurance in animal-pollinated plants is achieved through autonomous selfing, and is particularly beneficial when pollinators are scarce or mates are lacking (Herlihy & Eckert, 2002; Kalisz *et al.*, 2004). Wind-pollinated plants do not suffer from unpredictable pollinators, so it is unclear whether the capacity for self-fertilization provides reproductive assurance. Eckert *et al.* (2006) surveyed the literature for experimental tests of reproductive assurance and found results for 29 species, all animal-pollinated (but see Jacquemyn & Brys, 2008 for an ambophilous example). Reproductive assurance can be tested by comparing seed set of intact control flowers with those of emasculated flowers, where anthers are removed before they dehisce (Cruden & Lyon, 1989; Lloyd & Schoen, 1992; Eckert *et al.*, 2006). In wind-pollinated plants the capacity for pollen to fall from anthers onto stigmas below may provide a simple mechanism for self-pollination (Lloyd & Webb, 1986). However, the degree to which monoecy and protogyny may limit geitonogamy is currently unknown and therefore forms the basis of this study.

Carex (Cyperaceae) is near-exclusively wind pollinated and is one of the largest genera of angiosperms with over 2000 species (Kükenthal, 1909; Reznicek, 1990). Flowers are exclusively unisexual and species are predominantly monoecious and rarely dioecious (Standley, 1985a; Ball & Reznicek, 2002). Sedge flowers are arranged on a condensed spike, which may contain flowers of only one sex or both sexes (Smith & Faulkner, 1976). Most species exhibit interfloral protogyny where stigmas on a given stem are receptive before male flowers on the same stem release their pollen (Bertin & Newman, 1993). Despite the ubiquitous occurrence of *Carex* species in diverse plant communities in Northern temperate regions (Ball, 1990), there have been virtually no experimental field studies on the floral biology of populations (but see Handel, 1976; Stenström, 1999). Recently, Bertin (2007) investigated whether monoecy in six *Carex* species permitted flexibility in the allocation of resources to female and male function under changing environmental conditions. He found only modest effects of altered nutrients, water and light on sex allocation and suggested that the avoidance of self-pollination may be particularly important in the evolution and maintenance of monoecy in sedges.

Here, we investigate seven sympatric *Carex* species to evaluate the functional roles of monoecy and protogyny in pollination and mating. Three main questions were addressed: through the use of pollen enclosures and supplemental pollen, we investigated whether plants are self-compatible and if they are pollen limited; using emasculation experiments and genetic markers, we investigated whether monoecy inhibits the capture of self-pollen and therefore limits rates of self-fertilization; by comparing pollen loads on stigmas of intact and emasculated stems throughout flowering, we investigated whether protogyny influences pollen capture and promotes outcrossing. We use results from these experiments to evaluate the efficacy of monoecy and protogyny in limiting selfing, and discuss the implications of geitonogamy for wind-pollinated plants. We also comment briefly on the rarity of dioecy in *Carex* and speculate on the puzzle of such a species-rich but potentially highly selfing group.

Materials and Methods

Species and location of study

We selected seven sympatric *Carex* species representing six sections of the genus: *C. arctata* Boott (sect. Sylvaticae), *C. hirtifolia* Mack. (sect. Hirtifoliae), *C. laxiflora* Lam. (sect. Laxiflorae), *C. pedunculata* Muhl. ex Willd. (sect. Digitatae), *C. pensylvanica* Lam. (sect. Montanae), *C. plantaginea* Lam. (sect. Laxiflorae), and *C. scabrata* Schwein. (sect. Anomalae). Experiments were conducted from April to July in 2005 and 2006 in a single population of each species growing at the Koffler Scientific Reserve (KSR) at Jokers Hill, in Southern Ontario, Canada (44 03′N, 79 29′W). The seven species were all monoecious and protogynous and flower in late spring–early summer in mixed deciduous-evergreen forests (Ball & Reznicek, 2002). *Carex plantaginea* and *C. scabrata* grow primarily along moist depressions or creek edges whereas the remaining species grow on well-drained soils.

All *Carex* species in our study consist of many flowering stems (or culms) on a plant. Each stem is characterized by a terminal staminate spike comprising numerous stamens, with several pistillate spikes arranged throughout the inflorescence below. Each pistillate spike produces many single-ovule flowers. The mean number of stems per plant for each species was: *C. arctata* (4.62, SE = 0.29); *C. hirtifolia* (5.75, SE = 0.36); *C. laxiflora* (6.25, SE = 0.37); *C. pedunculata* (16.10, SE = 0.93); *C. pensylvanica* (29.49, SE = 2.11); *C. plantaginea* (6.58, SE = 0.55); and *C. scabrata* (30.44, SE = 3.06). The differences among species in stem number reflect, in part, variation in overall plant size.

Pollination and seed set

For each species we used 30 pairs of plants separated by at least 5 m to ensure that they were distinct clones. We applied treatments to two labeled stems on each experimental plant and these were either left intact or emasculated before flowering. Emasculation involved removing the staminate spike from the stems. One stem was used for stigma collection and the second for measuring seed set. For each day during flowering we recorded the sexual phase of stems. All species are protogynous and, within a stem, stigmas mature from the top of the inflorescence to the bottom. After several days of stigma exposure, anthers emerge at the top of the stem, while female flowers continue to open below. Thus, initially only female flowers are functional, followed by a phase where both female and male flowers are in anthesis. For intact and emasculated stems we collected stigmas every other day during the flowering period and stored them in 70% ethanol. To assess pollen capture, we stained stigmas with basic fuchsin and counted all pollen on the three stigmas of a flower using a compound microscope (20×).

An additional 40 plants per species were tagged; 20 stems were bagged and received only self pollen to test for self-compatibility, and 20 received supplemental cross pollen using hand pollination to test for pollen limitation by comparing them to open-pollinated flowers. To determine whether plants are self-compatible, we secured pollen enclosures around the stems of plants before stigmas had emerged to exclude wind-borne pollen, and then we applied self pollen to stigmas. The enclosure consisted of three layers of pollen-proof spun-nylon material secured to the ground with thin metal spikes. When anthers were dehiscent, we opened the enclosure around the bagged stem and brushed the stigmas with anthers from the same plant. The bag was then re-secured. For each bagged stem, pollen was applied on at least two separate days. Supplemental pollen was applied to stigmas by brushing them with anthers from three donors, located at least 25 m away. Again, pollen was applied on at least two separate days.

Once seeds were fully mature (4–6 wk) we harvested all treatment infructescences from intact, emasculated, bagged and supplementally-pollinated stems, and counted the number of mature seeds and the number of aborted or unfertilized flowers to assess proportion seed set. We then used seeds from intact and emasculated infructescences to investigate mating patterns.

We analysed pollen receipt with repeated-measures, general linear models (Neter *et al.*, 1996; SAS PROC MIXED 9.1; SAS Institute 2002, Cary, NC, USA), with the dependent variable log-transformed to assure normally distributed residuals. We analyzed seed set using generalized linear models (Allison, 1999: SAS PROC GENMOD 9.1; SAS Institute 2002, Cary, NC, USA) with logit transformations to accommodate the binomial distribution of data. Likelihood-ratio tests were used to determine the significance of each effect in the model. Analyses considered species, treatment, and sex phase (where appropriate) as categorical independent variables, and their interactions. We excluded terms from the model by backward elimination ($\alpha = 0.05$) if they did not explain a significant proportion of the variation in the dependent variable. The interaction between species and treatment was always retained in the model to reflect the experimental design. Because we sampled multiple flowers per stem, we employed restricted maximum likelihood (Jennrich & Schluchter, 1986) to characterize the covariance between responses by individual plants. We calculated denominator degrees of freedom for *F*-tests of the general linear models by Kenward & Roger's (1997) approximation, which can result in fractional degrees of freedom. We adjusted all plotted values to account for the other components in the model.

Mating patterns

We used allozyme markers and starch gel electrophoresis to determine the outcrossing rate of a single population of each species. Seed families of a minimum of 10 seeds from each maternal family (all harvested intact and emasculated stems) were ground in three drops of 0.1 M Tris-HCl extraction buffer (Soltis *et al.*, 1983) and the extract absorbed onto 3-mm chromatography paper wicks and placed directly onto 11–12% starch gels. We used a histidine–citrate (pH 6.5) and lithium–borate (pH 8.3) buffer system, and stained gels for enzyme activity following recipes in Wendel & Weeden (1991). Four species (*C. hirtifolia*, *C. arctata*, *C. pedunculata* and *C. pensylvanica*) exhibited no allozyme polymorphism among a minimum of 11 loci. For the other three species, we resolved two variable loci each (*C. scabrata*: triosephosphate isomerase (*Tpi*: 2 alleles), phosphoglucomutase (*Pgm*: 2 alleles); *C. laxiflora*: alcohol dehydrogenase (*Adh*: 2 alleles), triosephosphate isomerase (*Tpi*: 2 alleles); *C. plantaginea*: phosphoglucomutase (*Pgm*: 3 alleles), phosphoglucose isomerase (*Pgi*: 2 alleles)).

Genotypes were inferred based on segregation patterns characteristic of either dimeric or monomeric codominant enzymes. For each species we estimated the outcrossing rate (*t*) using the program MLTR (version 3.0, Ritland, 1990). This program uses maximum-likelihood procedures to infer the genotypes of maternal parents, allele frequencies in the pollen pool and the proportion of progeny that are the result of outcrossing. We derived the standard errors of the outcrossing rate estimates from the standard deviation of 1000 bootstrap values, using the seed family as the unit of resampling.

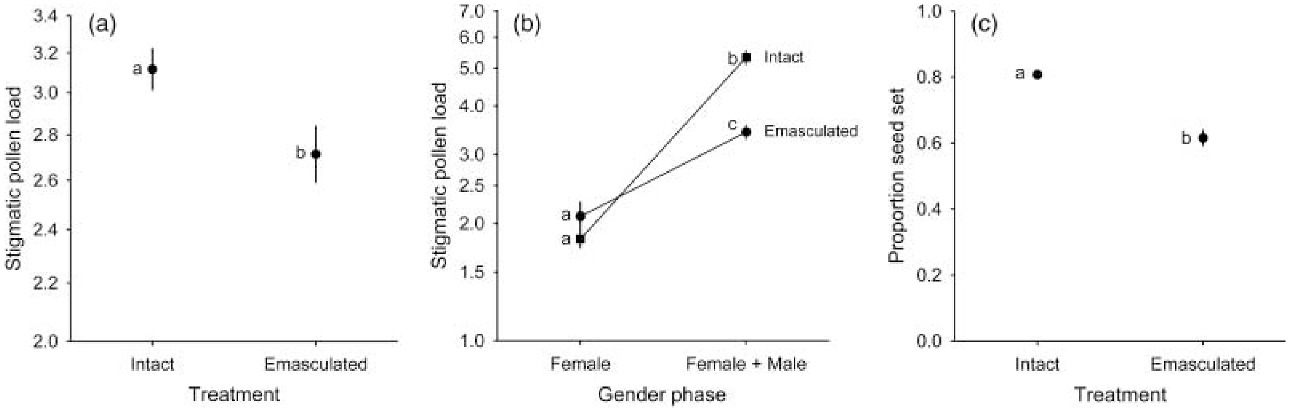

Fig. 1 Results from emasculation experiments on seven monoecious *Carex* species at Koffler Scientific Reserve, Ontario, Canada. (a) Influence of emasculation treatment on mean (SE) stigmatic pollen load; (b) interaction between emasculation treatment and gender phase during flowering on mean (SE) stigmatic pollen load; and (c) influence of emasculation treatment on mean (SE) proportion seed set. Letters indicate significant differences. See the Supporting Information, Table S1, for species means and Table 2 for statistical details.

To determine whether estimates of outcrossing rates were significantly different between emasculated and intact stems, we examined the pairwise comparisons of 1000 bootstrap estimates following methods outlined in Eckert & Barrett (1994b). Using this method, treatments were considered to differ significantly if $100[1 - (\alpha_{PC}/2]$ percentage of the differences between randomly paired bootstrap values were all greater than zero or all less than zero (where α_{PC} represents the Type 1 error rate per contrast).

Results

Pollen receipt

Overall, stigmas of emasculated stems captured significantly less pollen than those of open-pollinated (intact) stems (Table 2, Fig. 1a, Supporting information Table S1; mean decrease in pollen capture: 33.83%, range: 0–83%). Average stigmatic pollen loads of intact stems ranged from 4.13 to 13.19 among the seven species (Table 1). There was no significant species by treatment interaction indicating that the *Carex* species do not generally differ in their response to emasculation (see Supporting Information, Table S1). However, emasculated stems of *C. plantaginea* received higher pollen loads than intact stems (emasculated adjusted mean = 3.11, SE = 0.36; intact adjusted mean = 2.67, SE = 0.22), although this difference was not significant ($t_{922} = 1.07$, $P > 0.25$).

In each *Carex* species, stigmas received significantly more pollen during the combined sex phase than during the female-only phase (Table 2, Fig. 1b; percentage of pollen capture during female-only phase, 12.44%, range 6.46–30.87%). There was a significant treatment by gender phase interaction (Table 2). This interaction arises because stigmas of intact stems received significantly more pollen than those of emasculated stems during the combined sex phase (intact mean = 11.33, range = 6.82–21.91; emasculated mean = 5.15, range = 1.60–11.30; $t_{681} = 6.88$, $P < 0.0001$; Fig. 1b). However, there was no significant difference between intact and emasculated stems during the female phase when self-pollen was unavailable (intact mean = 1.41, range = 0.69–2.17; emasculated mean = 1.65, range = 0.89–2.51; $t_{915} = 1.38$, $P > 0.15$; Fig. 1b).

Table 1 Summary of average stigmatic pollen load and average proportion seed set for seven open-pollinated *Carex* species at Koffler Scientific Reserve, Ontario, Canada

Species	Stigmatic pollen load (SE)	Proportion seed set (SE)
C. arctata	8.87 (2.67)	0.81 (0.01)
C. hirtifolia	5.49 (0.53)	0.80 (0.05)
C. laxiflora	4.33 (0.83)	0.73 (0.03)
C. pedunculata	13.19 (2.63)	0.90 (0.01)
C. pensylvanica	10.41 (3.61)	0.65 (0.03)
C. plantaginea	4.13 (0.58)	0.82 (0.03)
C. scabrata	4.73 (0.70)	0.82 (0.02)

Table 2 Summary of analyses on pollen receipt and seed set during emasculation experiments on seven *Carex* species at Koffler Scientific Reserve, Ontario, Canada

Source of variation	Stigmatic pollen load	Proportion seed set
Species	$F_{6,896} = 12.34$***	$F_{6,336} = 6.70$***
Treatment	$F_{1,1017} = 5.51$*	$F_{1,366} = 19.41$***
Gender	$F_{1,1069} = 182.38$***	NA
Treatment × Species	$F_{6,749} = 2.01$	$F_{6,366} = 1.50$
Gender × Species	$F_{6,887} = 2.98$**	NA
Gender × Treatment	$F_{1,678} = 25.01$***	NA

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$. NA, not applicable.

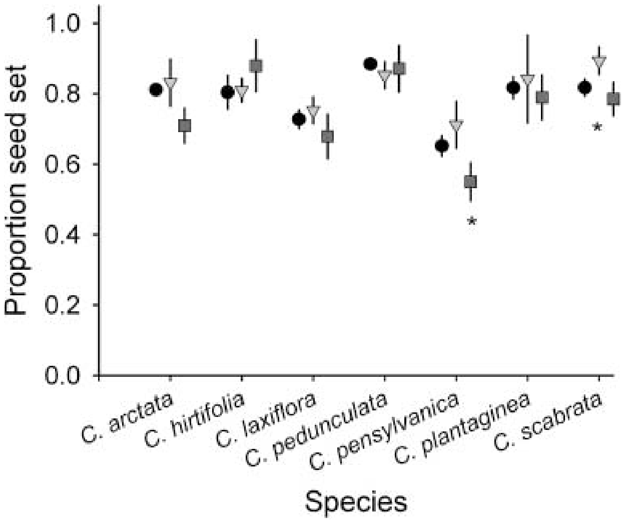

Fig. 2 Comparison of three treatments (open-pollinated (circles), supplemental pollen (triangles), bagged plus self pollen (squares)) on mean (SE) proportion seed set for seven *Carex* species. Asterisks indicate treatments that differed significantly from the open-pollinated treatment, see text for statistical details.

Patterns of seed set

Open-pollinated stems set significantly more seed than emasculated stems (Tables 2, S1, Fig. 1c; mean decrease in seed set: 24.01%, range: 10–47%). The absence of a significant species by treatment interaction (Table 2) indicates that all species responded similarly to emasculation. Maximum seed set ranged from 65% to 88% among intact stems of the seven species (Table 1).

In six of the seven species investigated, stems that received supplemental pollen set equivalent amounts of seed as open-pollinated stems (Fig. 2; statistics not shown). *Carex scabrata* was the only species that set significantly more seed with the addition of supplemental pollen (intact mean = 0.82, SE = 0.02; supplemental mean = 0.89, SE = 0.04; $F_{1,33} = 11.33$, $P < 0.001$; Fig. 2) indicating some pollen limitation of seed set.

Bagged stems that had self-pollen applied to stigmas set the same amount of seed as intact stems in six of the seven species investigated (Fig. 2; statistics not shown). This demonstrates that the species investigated are fully self-compatible. Stems of *C. pensylvanica* that were bagged and received self-pollen set significantly less seed than open-pollinated stems (intact mean = 0.65, SE = 0.03; bagged mean = 0.55, SE = 0.05; $F_{1,57} = 22.34$, $P < 0.0001$; Fig. 2).

Outcrossing rates

Open-pollinated stems of *Carex* species with polymorphism at allozyme loci exhibited high levels of self-fertilization with two species predominantly selfing (intact: *C. laxiflora* $t = 0.09$, SE = 0.03; *C. scabrata* $t = 0.03$, SE = 0.02) and the remaining species showing mixed mating (intact: *C. plantaginea* $t = 0.39$, SE = 0.06). Comparisons of outcrossing rate between intact and emasculated stems in each species failed to detect significant differences. However, in all three species emasculated stems had slightly higher outcrossing rates than intact stems (emasculated: *C. laxiflora* $t = 0.16$, SE = 0.07; *C. plantaginea* $t = 0.45$, SE = 0.08; and *C. scabrata* $t = 0.09$, SE = 0.03) consistent with the occurrence of within-stem geitonogamy. The high degree of selfing in emasculated stems also indicates substantial between-stem geitonogamy.

Discussion

Pollination and mating in monoecious wind-pollinated species has seldom been investigated experimentally and we lack a functional explanation for the widespread association between monoecy and wind pollination. Here we used *Carex*, a species-rich and predominantly monoecious genus, to assess whether monoecy limits geitonogamous selfing. Field experiments with seven species demonstrated that monoecy is not effective at limiting this mode of self-fertilization. Self-pollen contributes significantly to stigmatic pollen loads and seed set. Emasculated stems captured significantly less pollen than intact stems (Fig. 1a) and set significantly fewer seeds (Fig. 1c). During the stage when most plants were in both female and male phases, intact stems received more pollen than emasculated stems (Fig. 1b), indicating that they are probably capturing a substantial portion of self pollen from the same inflorescence. However, the co-occurrence of monoecy and protogyny in *Carex* provides a brief window of opportunity for outcross pollen to be captured, although we found that only 12% of the total pollen is captured during the female phase (Fig. 1b). Our results demonstrate that geitonogamy is a pervasive feature of the floral biology of *Carex* species and that it occurs both within and between stems.

In the *Carex* species we investigated the combination of self-compatibility, modest clonal growth and multiple flowering stems per plant provides opportunities for between-stem geitonogamy. Protogyny provides some protection against within-stem geitonogamy, but asynchrony in sex phases among stems of a plant permits pollen transfer between stems. Although in our study it was not practical to quantify the between-stem component of geitonogamy by whole-plant emasculation, the high selfing rates in emasculated stems indicate that pollen dispersal between flowering stems on a plant likely contributes significantly to geitonogamous self-fertilization.

Monoecy and protogyny fail to limit self-pollination

Monoecious wind-pollinated plants may be unable to avoid self-pollination when female and male parts are presented simultaneously, which has been interpreted as an explanation for the high incidence of dichogamy in wind-pollinated species (Lloyd & Webb, 1986; Webb & Lloyd, 1986). Sargent & Otto (2004) found that transitions from biotic to abiotic pollination were more likely among protogynous species than among protandrous species. They suggested that this result occurs because protogynous species are less likely than protandrous species to suffer from increased self-fertilization upon a switch to wind pollination. An initial period of stigma presentation provides an opportunity for cross-pollination before selfing is possible. However, our results demonstrate that although the protogynous phase allows some pollen to be captured the amounts involved are significantly less than during the combined sex phase (Fig. 1b). Although theory suggests that protogyny provides better protection against selfing than protandry (Lloyd & Webb, 1986), our data indicate that protogyny is not particularly effective at preventing self-pollination, at least in the species we investigated. Authors of other studies of *Carex* (Vonk, 1979; Whitkus, 1992) have explained high selfing rates by suggesting that an overlap in anthesis of female and male flowers among stems could result in geitonogamy, although this was not demonstrated experimentally.

Despite wind pollination and unisexual flowers there is considerable evidence that many *Carex* species are highly selfing. Self-compatibility is widespread in *Carex* and in the Cyperaceae more generally (Faulkner, 1973; Handel, 1978; Vonk, 1979; Schmid, 1984; Standley, 1985b; Whitkus, 1988; Snyder & Richards, 2005). Although the possibility of self-incompatibility in a few species has been raised (Ford *et al.*, 1991; Stenström *et al.*, 2001) there is no convincing experimental evidence for its occurrence in *Carex*. Studies examining the partitioning of genetic diversity within and among populations using genetic markers have revealed low levels of intrapopulation variation, but high interpopulation genetic differentiation – a pattern consistent with high selfing (Bruederle & Fairbrothers, 1986; Waterway, 1990; Bruederle & Jensen, 1991; Schell & Waterway, 1992; Whitkus, 1992; Reinhammar, 1999; Tyler *et al.*, 2002; Kull & Oia, 2007). Other studies have provided direct evidence that some *Carex* species are predominantly selfing (Handel, 1978; Schmid, 1984; Whitkus, 1988). Handel (1985) and Ford *et al.* (1991) suggested that both inflorescence morphology and growth form facilitate selfing in many *Carex* species. Like most of the taxa in our study, selfing species are characterized by a caespitose growth habit and by having multiple pistillate and staminate spikes on each stem or spikes with both sexes. These traits increase opportunities for geitonogamous selfing, both within and between stems. By contrast, rhizomatous species with relatively few, widely-spaced, unisexual stems have a different population genetic structure with genetic diversity partitioned within rather than among populations (Ford *et al.*, 1991; McClintock & Waterway, 1993; Jonsson *et al.*, 1996; Stenström *et al.*, 2001), a pattern consistent with higher levels of outcrossing This suggests that clonal architecture plays an important role in governing mating patterns and population genetic structure in *Carex*.

In six of the seven species investigated there was no evidence that female fertility was pollen limited. The addition of supplemental pollen did not significantly increase seed set (Fig. 2). However, intact plants set significantly more seed than emasculated plants (Fig. 1c), demonstrating that self pollen enables plants to achieve maximum seed set. The overall prevalence of pollen limitation in wind-pollinated species is unclear, although it is often assumed that pollen availability does not limit reproduction. Recent empirical work suggests that pollen capture and seed set may decrease rapidly with increasing distance from pollen donors, and that pollen limitation in wind-pollinated plants may often occur in sparse populations (Handel, 1976; Knapp *et al.*, 2001; Koenig & Ashley, 2003; Davis *et al.*, 2004; Eppley & Pannell, 2007; Stehlik *et al.*, 2008). The *Carex* species in this study all grow in the understory of forests, which is a relatively unusual habitat for most wind-pollinated species. Forests are typically characterized by slow wind speeds and disrupted wind profiles. Although the seven species flower in early to late spring before the forest canopy has closed, wind speeds may still be low and unpredictable. Furthermore, the relatively small stature of the species investigated means that they may experience short pollen dispersal distances compared with wind-pollinated plants of open habitats. These factors, combined with the clonal growth patterns and presence of multiple spikes on a plant probably explain the low incidence of outcross pollination.

Geitonogamy and reproductive assurance in wind-pollinated plants

Virtually all work on geitonogamy deals with animal-pollinated plants where it is unlikely to be adaptive (Lloyd, 1992). This is, in part, because geitonogamy often results from pollinators successively visiting flowers of one plant (Lloyd, 1992). Thus, geitonogamous selfing limits opportunities for outcrossing because it removes pollen directly from the pollen pool that pollinators transport between flowers (Harder & Barrett, 1995; Harder *et al.*, 2008). However, in wind-pollinated species self-pollination between flowers should not directly affect opportunities for cross-pollination. Although a small portion of the total pollen produced will be used for selfing it is unlikely to diminish the success of the plant as a pollen donor. This arises because wind cannot be saturated with pollen and pollen removal is not limited by pollinator visitation. Thus geitonogamy can be increased without additional metabolic cost for the plant and without decreasing cross-pollination. De Jong *et al.* (1999) modeled a similar scenario involving the effects of geitonogamy on sex allocation in hermaphrodite plant populations. They found that the absence of pollen discounting resulted in increased relative male allocation and, in the absence of inbreeding depression, individuals benefited from the automatic advantage of selfing. Therefore, if pollen discounting is negligible the consequences of geitonogamy in wind-pollinated species may be quite different from animal-pollinated species, and may be beneficial.

In animal-pollinated plants, selection for geitonogamy as a mechanism of reproductive assurance is unlikely because it requires the aid of a pollinator and so offers no reproductive assurance in the absence of pollinators. However, recent work by Pannell (2006) illustrates that in a metapopulation when mates are limiting, geitonogamy can be selected as a mechanism of reproductive assurance. Empirical work on *Mercurialis annua*, a wind-pollinated androdioecious annual, has shown that geitonogamous selfing gives hermaphrodites at low density a reproductive advantage over males during colonization (Pannell, 1997, 2001; Eppley & Pannell, 2007). Our data suggest that even in stable populations, geitonogamous selfing can be beneficial for wind-pollinated plants by alleviating pollen limitation in environments not conducive to extensive pollen dispersal.

Alternative functions for monoecy

There are several reasons why monoecy may be beneficial in wind-pollinated plants that are independent of any influences on selfing. Unisexual flowers in wind-pollinated plants may facilitate specialization for efficient pollen removal versus receipt. Unlike hermaphrodite animal-pollinated plants, where pollinators deliver pollen to stigmas and pick-up pollen from anthers in a single visit, in wind-pollinated plants the removal and capture of pollen are largely independent events. Interference between female and male structures within a flower may be directly disadvantageous in hermaphroditic wind-pollinated plants because the optimal conditions for pollen dispersal and pollen capture are usually different (Lloyd, 1982; Niklas, 1985; Young & Schmitt, 1995; Friedman & Harder, 2004). In accord with aerodynamic predictions, male flowers are most successful when presented at elevated heights, while female flowers capture more pollen when they are below the source of pollen release.

All *Carex* species in our study are characterized by several pistillate spikes throughout the inflorescence arranged adjacent to or below a staminate spike comprised of numerous stamens. This morphology suggests very different optima for female and male function in encouraging cross-pollination. However, this arrangement may also facilitate self-pollination within a stem as pollen can easily fall from anthers onto the stigmas below. *Carex plantaginea* differed from the other six species investigated in being the only one in which intact and emasculated plants captured equivalent amounts of pollen. Furthermore, it exhibited higher levels of outcrossing than the other species ($t = 0.39 \pm 0.06$). *Carex plantaginea* was shown by Handel (1976) to have less restricted pollen flow than the other *Carex* species in his study. He suggested that this difference resulted from the elevated height of the staminate spikes. Spatial segregation of female and male function may enhance outcrossing by allowing differential positioning and morphology of flowers for optimal pollen dispersal versus capture, but in the species investigated in this study it clearly did not preclude opportunities for selfing.

Rarity of dioecy and the puzzle of diversification in *Carex*

There is a strong association between anemophily and dioecy in flowering plants (Renner & Ricklefs, 1995; Linder, 1998; Vamosi *et al.*, 2003). A common evolutionary pathway to dioecy is from monoecy and the two sexual systems are strongly associated within genera (Bawa, 1980; Renner & Ricklefs, 1995). In *Carex* most species are monoecious and dioecy is relatively rare in the genus (*c.* 10 species) apparently having originated several times (Standley, 1985a). Given the very large size of the genus this raises the fascinating question as to why the evolutionary transition from monoecy to dioecy is so infrequent and why dioecious lineages are not more successful, particularly since monoecy appears to be ineffective as an outbreeding mechanism.

Stebbins (1957) first proposed that self-fertilization may be an evolutionary dead-end, since selfing lineages may have limited potential for adaptive diversification and are more likely than outcrossing lineages to become extinct (Takebayashi & Morrell, 2001; Igic *et al.*, 2008). Thus, the possibility that species-rich *Carex* may contain many selfing species is puzzling. Other features of the genetic system may be responsible for promoting diversification independent of mating patterns. For example, species richness in *Carex* may be a consequence of unusual and rapid chromosomal evolution (Hipp, 2007). Sedges have holocentric chromosomes, which evolve rapidly by fission and fusion, resulting in exceptional agmatoploid chromosome series ranging from $n = 6$ to $n = 66$ (Tanaka, 1949). Although unusual chromosomal evolution may, in part, account for the great diversity of sedges, the factors responsible for their remarkable radiation remain largely unresolved. Determining whether high selfing is widespread among *Carex* species is needed before this problem can be addressed in more detail. Nevertheless, at least in the *Carex* species investigated here, monoecy and protogyny are largely ineffective at limiting high rates of self-fertilization.

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References

Ackerman JD. 2000. Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution* **222**: 167–185.

Aide TM. 1986. The influence of wind and animal pollination on variation in outcrossing rates. *Evolution* **40**: 434–435.

Allison PD. 1999. *Logistic regression using SAS system: theory and application*. Cary, NC, USA: SAS Institute.

Ball PW. 1990. Some aspects of the phytogeography of *Carex*. *Canadian Journal of Botany* **68**: 1462–1472.

Ball PW, Reznicek AA. 2002. *Carex*. In: Flora of North America Editorial Committee, eds. *Flora of North America*, Vol. 23. New York, NY, USA: Oxford University Press, 254–572.

Barrett SCH, Eckert CG. 1990. Variation and evolution of mating systems in seed plants. In: Kawano S, ed. *Biological approaches and evolutionary trends in plants*. New York, NY, USA: Academic Press, 229–254.

Bawa KS. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* **11**: 15–39.

Bertin RI. 1993. Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. *American Journal of Botany* **80**: 557–560.

Bertin RI. 2007. Sex allocation in *Carex* (Cyperaceae): effects of light, water, and nutrients. *Canadian Journal of Botany* **85**: 377–384.

Bertin RI, Newman CM. 1993. Dichogamy in angiosperms. *Botanical Review* **59**: 112–152.

Bruederle LP, Fairbrothers DE. 1986. Allozyme variation in populations of the *Carex crinita* complex (Cyperaceae). *Systematic Botany* **11**: 583–594.

Bruederle LP, Jensen U. 1991. Genetic differentiation of *Carex flava* and *Carex viridula* in western Europe (Cyperaceae). *Systematic Botany* **16**: 41–49.

Charlesworth D. 1993. Why are unisexual flowers associated with wind pollination and unspecialized pollinators? *American Naturalist* **141**: 481–490.

Charlesworth D, Charlesworth B. 1978. Population genetics of partial male-sterility and the evolution of monoecy and dioecy. *Heredity* **41**: 137–153.

Cruden RW, Lyon DL. 1989. Facultative xenogamy: examination of a mixed mating system. In: Bock JH, Linhart YB, eds. *The evolutionary ecology of plants*. Boulder, CO, USA: Westview Press, 171–208.

Davis HG, Taylor CM, Lambrinos JG, Strong DR. 2004. Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). *Proceedings of the National Academy of Sciences, USA* **101**: 13804–13807.

Eckert CG. 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* **81**: 532–542.

Eckert CG, Barrett SCH. 1994a. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. *Evolution* **48**: 952–964.

Eckert CG, Barrett SCH. 1994b. Post-pollination mechanisms and the maintenance of outcrossing in self-compatible, tristylous, *Decodon verticillatus* (Lythraceae). *Heredity* **72**: 396–411.

Eckert CG, Samis KE, Dart S. 2006. Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In: Harder LD, Barrett SCH, eds. *Ecology and evolution of flowers*. Oxford, UK: Oxford University Press, 183–203.

Eppley SM, Pannell JR. 2007. Density-dependent self-fertilization and male versus hermaphrodite siring success in an androdioecious plant. *Evolution* **61**: 2349–2359.

Faulkner JS. 1973. Experimental hybridization of northwest European species in *Carex* section *Acutae* (Cyperaceae). *Botanical Journal of the Linnean Society* **67**: 233–253.

Ford BA, Ball PW, Ritland K. 1991. Allozyme diversity and genetic relationships among North American members of the short-beaked taxa of *Carex* sect. *Vesicariae* (Cyperaceae). *Systematic Botany* **16**: 116–131.

Friedman J, Barrett SCH. 2008. A phylogenetic analysis of the evolution of wind pollination in the angiosperms. *International Journal of Plant Sciences* **169**: 49–58.

Friedman J, Harder LD. 2004. Inflorescence architecture and wind pollination in six grass species. *Functional Ecology* **18**: 851–860.

Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology Evolution and Systematics* **36**: 47–79.

Handel SN. 1976. Restricted pollen flow of two woodland herbs determined by neutron-activation analysis. *Nature* **260**: 422–423.

Handel SN. 1978. Self-compatibility in *Carex plantaginea* and *C. platyphylla* (Cyperaceae). *Bulletin of the Torrey Botanical Club* **105**: 233–234.

Handel SN. 1985. The intrusion of clonal growth patterns on plant breeding systems. *The American Naturalist* **125**: 367–384.

Harder LD, Barrett SCH. 1995. Mating cost of large floral displays in hermaphrodite plants. *Nature* **373**: 512–515.

Harder LD, Barrett SCH, Cole WW. 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings of the Royal Society of London, Series B* **267**: 315–320.

Harder LD, Richards SA, Routley MB. 2008. Effects of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution* **62**: 157–172.

Harder LD, Wilson WG. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *American Naturalist* **152**: 684–695.

Herlihy CR, Eckert CG. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* **416**: 320–322.

Hipp AL. 2007. Nonuniform processes of chromosome evolution in sedges (*Carex* : Cyperaceae). *Evolution* **61**: 2175–2194.

Igic B, Lande R, Kohn JR. 2008. Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* **169**: 93–104.

Jacquemyn H, Brys R. 2008. Density-dependent mating and reproductive assurance in the temperate forest herb *Paris quadrifolia* (Trilliaceae). *American Journal of Botany* **95**: 294–298.

Jennrich RI, Schluchter MD. 1986. Unbalanced repeated-measures models with structured covariance matrices. *Biometrics* **42**: 805–820.

de Jong TJ, Klinkhamer PGL, Rademaker MCJ. 1999. How geitonogamous selfing affects sex allocation in hermaphrodite plants. *Journal of Evolutionary Biology* **12**: 166–176.

Jonsson BO, Jonsdottir IS, Cronberg N. 1996. Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii* (Cyperaceae). *Journal of Ecology* **84**: 449–459.

Kalisz S, Vogler DW, Hanley KM. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* **430**: 884–887.

Kenward MG, Roger JH. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **53**: 983–997.

Knapp EE, Goedde MA, Rice KJ. 2001. Pollen-limited reproduction in blue-oak: implications for wind pollination in fragmented populations. *Oecologia* **128**: 48–55.

Koenig WD, Ashley MV. 2003. Is pollen limited? The answer is blowin' in the wind. *Trends in Ecology & Evolution* **18**: 157–159.

Kükenthal G. 1909. Cyperaceae – Caricoideae. In: Engler A, ed. *Das pflanzenreich IV*. Leipzig, Germany: Englemann, 1–824.

Kull T, Oia T. 2007. Low allozyme variation in *Carex loliacea* (Cyperaceae), a declining woodland sedge. *Annales Botanici Fennici* **44**: 267–275.

Lau JA, Miller RE, Rausher MD. 2008. Selection through male function favors smaller floral display size in the common morning glory *Ipomoea purpurea* (Convolvulaceae). *American Naturalist* **172**: 63–74.

Linder HP. 1998. Morphology and the evolution of wind pollination. In: Owens SJ, Rudall PJ, eds. *Reproductive biology in systematics, conservation and economic botany*. Kew, UK: Royal Botanic Gardens, Kew, 123–135.

Lloyd DG. 1972. Breeding systems in *Cotula* L. (Compositae, Anthemidae). II. Monoecious populations. *New Phytologist* **71**: 1195–1202.

Lloyd DG. 1982. Selection of combined versus separate sexes in seed plants. *American Naturalist* **120**: 571–585.

Lloyd DG. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* **153**: 370–380.

Lloyd DG, Schoen DJ. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Sciences* **153**: 358–369.

Lloyd DG, Webb CJ. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms 1. Dichogamy. *New Zealand Journal of Botany* **24**: 135–162.

McClintock KA, Waterway MJ. 1993. Patterns of allozyme variation and clonal diversity in *Carex lasiocarpa* and *C. pellita* (Cyperaceae). *American Journal of Botany* **80**: 1251–1263.

Neter J, Kutner MH, Nachtsheim CJ, Wasserman W. 1996. *Applied linear statistical models*, 4th edn. Chicago, IL, USA: Irwin.

Niklas KJ. 1985. The aerodynamics of wind pollination. *Botanical Review* **51**: 328–386.

Pannell JR. 1997. The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution* **51**: 10–20.

Pannell JR. 2001. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. *Evolutionary Ecology* **14**: 195–211.

Pannell JR. 2006. Effects of colonization and metapopulation dynamics on the evolution of plant sexual systems. In: Harder LD, Barrett SCH, eds. *Ecology and evolution of flowers*. Oxford, UK: Oxford University Press, 223–238.

Reinhammar L-G. 1999. Allozyme differentiation between the lowland *Carex capitata* and the alpine *Carex arctogena* (Cyperaceae) in Scandinavia. *Biological Journal of the Linnean Society* **67**: 377–389.

Renner SS, Ricklefs RE. 1995. Dioecy and its correlates in the flowering plants. *American Journal of Botany* **82**: 596–606.

Reznicek AA. 1990. Evolution in sedges (*Carex*, Cyperaceae). *Canadian Journal of Botany* **68**: 1409–1432.

Ritland K. 1990. A series of Fortran computer programs for estimating plant mating systems. *Journal of Heredity* **81**: 235–237.

Routley MB, Bertin RI, Husband BC. 2004. Correlated evolution of dichogamy and self-incompatibility: a phylogenetic perspective. *International Journal of Plant Sciences* **165**: 983–993.

Sargent RD, Otto SP. 2004. A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. *Evolutionary Ecology Research* **6**: 1183–1199.

Schell CM, Waterway MJ. 1992. Allozyme variation and the genetic structure of populations of the rare sedge *Carex misera* (Cyperaceae). *Plant Species Biology* **7**: 141–150.

Schemske DW, Lande R. 1985. The evolution of self-fertilization and inbreeding depression in plants II. Empirical observations. *Evolution* **39**: 41–52.

Schmid B. 1984. Life histories in clonal plants of the *Carex flava* group. *Journal of Ecology* **72**: 93–114.

Smith DL, Faulkner JS. 1976. Inflorescence of *Carex* and related genera. *Botanical Review* **42**: 53–81.

Snyder JM, Richards JH. 2005. Floral phenology and compatibility of sawgrass, *Cladium jamaicense* (Cyperaceae). *American Journal of Botany* **92**: 736–743.

Soltis DE, Haufler CH, Darrow DC, Gastony GJ. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* **73**: 9–27.

Standley LA. 1985a. Paradioecy and gender ratios in *Carex macrocephala* (Cyperaceae). *American Midland Naturalist* **113**: 283–286.

Standley LA. 1985b. Systematics of the *Acutae* group of *Carex* (Cyperaceae) in the Pacific Northwest. *Systematic Botany Monographs* **7**: 1–106.

Stebbins GL. 1957. Self fertilization and population variability in the higher plants. *American Naturalist* **91**: 337–354.

Stehlik I, Friedman J, Barrett SCH. 2008. Environmental influence on primary sex ratio in a dioecious plant. *Proceedings of the National Academy of Science, USA* **105**: 10852–10857.

Stenström A. 1999. Sexual reproductive ecology of *Carex bigelowii*, an arctic-alpine sedge. *Ecography* **22**: 305–314.

Stenström A, Jonsson BO, Jonsdottir IS, Fagerstrom T, Augner M. 2001. Genetic variation and clonal diversity in four clonal sedges (*Carex*) along the Arctic coast of Eurasia. *Molecular Ecology* **10**: 497–513.

Takebayashi N, Morrell PL. 2001. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *American Journal of Botany* **88**: 1143–1150.

Tanaka N. 1949. Chromosome studies in the genus *Carex*, with special reference to aneuploidy and polyploidy. *Cytologia* **15**: 15–29.

Tyler T, Prentice HC, Widen B. 2002. Geographic variation and dispersal history in Fennoscandian populations of two forest herbs. *Plant Systematics and Evolution* **233**: 47–64.

Vamosi JC, Otto SP, Barrett SCH. 2003. Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *Journal of Evolutionary Biology* **16**: 1006–1018.

Vogler DW, Kalisz S. 2001. Sex among the flowers: the distribution of plant mating systems. *Evolution* **55**: 202–204.

Vonk DH. 1979. Biosystematic studies of the *Carex flava* complex 1. Flowering. *Acta Botanica Neerlandica* **28**: 1–20.

Waterway MJ. 1990. Genetic differentiation and hybridization between *Carex gynodynama* and *C. mendocinensis* (Cyperaceae) in California. *American Journal of Botany* **77**: 826–838.

Webb CJ, Lloyd DG. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. *New Zealand Journal of Botany* **24**: 163–178.

Wendel JF, Weeden NF. 1991. Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS, eds. *Isozymes in plant biology*. Portland, OR, USA: Dioscorides Press, 5–45.

Whitkus R. 1988. Experimental hybridizations among chromosome races of *Carex pachystachya* and the related species *Carex macloviana* and *Carex preslii* (Cyperaceae). *Systematic Botany* **13**: 146–153.

Whitkus R. 1992. Allozyme variation within the *Carex pachystachya* complex (Cyperaceae). *Systematic Botany* **17**: 16–24.

Young KA, Schmitt J. 1995. Genetic variation and phenotypic plasticity of pollen release and capture height in *Plantago lanceolata*. *Functional Ecology* **9**: 725–733.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Summary of average adjusted stigmatic pollen load and proportion seed set for intact and emasculated stems from seven *Carex* species at Koffler Scientific Reserve, Ontario, Canada