

## REPRODUCTIVE BIOLOGY OF TWO DOMINANT PRAIRIE GRASSES (*ANDROPOGON GERARDII* AND *SORGHASTRUM NUTANS*, POACEAE): MALE-BIASED SEX ALLOCATION IN WIND-POLLINATED PLANTS?<sup>1</sup>

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It has been proposed that some wind-pollinated plants have the necessary conditions for an optimal sex allocation that is male biased, though there are few data that address this prediction. We determined that two prairie grass species (*Andropogon gerardii* and *Sorghastrum nutans*) had reproductive characteristics that theoretically would result in a male-biased allocation: both species were self-incompatible and neither species had increased seed set after supplemental hand pollination. The relative allocation to pollen and seed production was measured in terms of biomass, energy, nitrogen, phosphorus, potassium, magnesium, and calcium. Sex allocation in *A. gerardii* was significantly male biased (from 60 to 89% male) when measured in currencies of biomass, energy, potassium, and calcium; there was no significant bias in the sex allocation (from 49 to 57% male) when measured in currencies of nitrogen, phosphorus, and magnesium. Sex allocation in *S. nutans* was significantly male biased (from 69 to 81% male) for all currencies except phosphorus (61% male). This is the first evidence for male-biased sex allocation in any plant or animal hermaphrodite. Though the necessary conditions may be uncommon, male-biased allocation may be found in other species with similar reproductive biology.

**Key words:** *Andropogon gerardii*; pollen limitation; Poaceae; self-incompatibility; sex allocation; *Sorghastrum nutans*; tallgrass prairie; wind pollination.

Cosexual plants allocate resources to both female reproduction (i.e., seed production) and male reproduction (i.e., pollen production), and the optimal relative allocation of resources to the sexes can be predicted by sex-allocation models (e.g., Charlesworth and Charlesworth, 1981; Charnov, 1982; Lloyd, 1984; Charlesworth and Morgan, 1991). The allocation prediction depends on the shapes of both the female and male fitness–gain curves (the relationship between relative allocation of resources to a sex and the realized fitness via that sex). If both fitness curves are linear, then simple models of sex allocation predict that cosexual species should allocate equal resources to the two sexes (Maynard Smith, 1971; Charnov, 1979; Lloyd, 1984). Nonlinearity in one or both curves can cause the optimal allocation to be unequal. For instance, the possibility of saturating animal pollinators with pollen may cause the male fitness–gain curve to level off as male allocation increases (Charnov, 1979; Lloyd, 1984). If this concave male curve occurs in a species with a linear female curve, the resulting optimal allocation is female biased; this has been proposed as the cause for female-biased sex allocation in some animal-pollinated species (Lloyd, 1984)

Outcrossed wind-pollinated species constitute a special situation in which male-biased sex allocation might be

expected. Unlike animal pollen vectors, wind cannot be saturated with pollen; thus it is frequently suggested that the male fitness–gain curve is linear in outcrossed wind-pollinated plants (Charlesworth and Charlesworth, 1981; Charnov, 1982; Lloyd, 1984; Charlesworth and Morgan, 1991; Ganeshaiah and Uma Shaanker, 1991) as long as the pollen dispersal distance is relatively large (Burd and Allen, 1988; de Jong and Klinkhamer, 1994). The shape of the female fitness–gain curve will depend on the nature of seed dispersal. If seed-dispersal distance is short, then there will be local resource competition for safe sites among the seeds of a particular plant as female investment by that plant increases. This would produce a concave female fitness–gain curve, since increasing seed production does not lead to a proportional increase in female fitness at high levels of investment (Charnov, 1979; Lloyd, 1984; Ganeshaiah and Uma Shaanker, 1991; de Jong and Klinkhamer, 1994). Together, a linear male curve and a concave female curve would produce a male-biased optimal sex allocation.

Two additional conditions must be satisfied for the prediction of male-biased allocation to be realized. First, selfing has a large impact on sex allocation; greater selfing rates increasingly skew the predicted sex allocation toward female investment (Charlesworth and Charlesworth, 1981; Charnov, 1987), a prediction of sex-allocation theory that is particularly well supported empirically (reviewed in Brunet, 1992). Second, pollen limitation of seed set can affect the predicted sex allocation (Olivieri, Couvet, and Slatkin, 1994) since the realized allocation to female resources by an individual will be dependent on the stochastic events of pollen arrival during pollination. Consistent pollen limitation has an impact on the optimal sex allocation, but the exact response is dependent on the specific assumptions about pollen dispersal

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(Olivieri, Couvet, and Slatkin, 1994). Thus both self-pollination and pollen limitation could change the prediction of male-biased allocation in wind-pollinated species.

We measured sex allocation in two wind-pollinated grasses that seem to satisfy the theoretical conditions for male-biased allocation. *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash (Poaceae: Andropogoneae) are wind-pollinated (and hence likely to have linear male fitness-gain curves) and have dry fruits with no obvious adaptations for animal dispersal (and hence likely to have saturating female fitness-gain curves due to local competition among seedlings).

Attempts to test sex-allocation theory have been hampered by the conceptual and methodological difficulties of measuring absolute allocation to pollen and seed production (Brunet, 1992). A particular ongoing problem has been choice of the resources in which to measure allocation (Goldman and Willson, 1986; Reekie and Bazzaz, 1987a, b; Chapin, 1989; Ashman, 1994). The correct resource for measuring sex allocation would be the one (or more) that ultimately limits reproductive success, but this is known for few species. Since we do not know the limiting resources for our study species, we measured allocation of biomass, energy, nitrogen, phosphorus, potassium, magnesium, and calcium.

In addition to measuring the sex allocation in the study species, we tested two of the conditions for male-biased allocation. We determined (1) whether the species were obligately outcrossing by testing for self-incompatibility and (2) whether availability of pollen limited seed production by using supplemental hand pollination.

## MATERIALS AND METHODS

*Andropogon gerardii* and *Sorghastrum nutans* are tall (flowering shoots often >2 m), warm-season, sod-forming grasses that are among the most common species throughout North American tallgrass prairie (Curtis, 1959; Weaver, 1968). The two species occur in "almost identical" habitats (Weaver, 1968), though *S. nutans* is typically less common within sites (Curtis, 1959; Weaver, 1968).

The study populations were located on the Hillside Prairie restoration in the Carleton College Arboretum in Northfield, Minnesota (44°28'15"N and 93°08'50"W). Restoration of the prairie began in 1978, and the study populations were located in an area planted in 1981. Seed in the prairie came from source populations within 300 km of the site. By 1989 the prairie was dominated by native species, with *S. nutans* and *A. gerardii* among the dominant plants. Individual genets of both *S. nutans* and *A. gerardii* could be easily distinguished in the field, since they were derived from seed and had not yet grown together in most cases.

All measurements were made in 1989, except for measurements of pollen and seed composition, which were made in 1993. Before flowering had begun in 1989, two parallel transects were established through an area where both study species were common. Study plants of each species were chosen at 5-m intervals along the transects. When flowering shoots appeared, eight experimental shoots were chosen at random from each study plant and were assigned randomly to the following treatments: three for male measurements, three for female measurements, and two for supplemental hand-pollination.

**Sex allocation**—We estimated relative allocation to male and female reproduction for 19 *A. gerardii* plants and for 15 *S. nutans* plants. For most of these individuals, independent estimates of allocation were made on three separate flowering shoots; these shoots were then used as replicates for among-plant statistical comparisons. For relative allo-

cation, plant-wide male and female reproductive effort were calculated on a per-floret basis as follows:

- 1) Male allocation per floret = proportion of functionally male florets × number of anthers per floret (always three in these species) × anther length × dry mass per unit anther length × amount of resource per unit mass.
- 2) Female allocation per floret = proportion of functionally female florets × seed set × seed dry mass × amount of resource per unit mass.

Of the parameters needed to estimate allocation, proportions of functionally male and female florets, anther length, seed set, and seed mass were all measured for multiple florets per plant (see below). Other parameters were calculated from bulk samples. Dry mass of anthers per floret was based on the mass of oven-dried samples of anthers with known total length. Air-dried seed masses were converted to dry mass based on mass loss of oven-dried samples.

The determination of energy and nutrient composition required large bulk samples of >10 000 anthers and 3000–4000 seeds from each species. Mature anthers and seeds were dissected from individual florets from large samples of inflorescences collected haphazardly from the study site in 1993. Energy content of samples was determined by bomb calorimetry by Hazleton Laboratories of Madison, Wisconsin. Elemental composition was determined by the Research Analytical Laboratory of the Department of Soil Science, University of Minnesota, St. Paul. Nitrogen was measured as total Kjeldahl nitrogen; phosphorus, potassium, magnesium, and calcium were measured by inductively coupled plasma (ICP) atomic emission spectroscopy.

**Floral ratios**—Like many species in the tribe Andropogoneae (Connor, 1981), *A. gerardii* and *S. nutans* bear spikelets in pairs, one sessile and one pedicelled (Fig. 1). Each spikelet contains one functional floret. The floret of the sessile spikelet (hereafter referred to as "sessile floret") is hermaphroditic in both species. In *A. gerardii* the floret of the pedicelled spikelet (hereafter referred to as "pedicelled floret") is often described as male (Gleason and Cronquist, 1991), though it is sometimes hermaphroditic (Boe, Ross, and Wynia, 1983) or nonfunctional. In contrast, the pedicelled florets of *S. nutans* are vestigial (Fig. 1).

To determine frequency of different floral types (male, hermaphrodite, and neuter), we used observations made during the measurement of seed set. Neuter florets were much smaller than normal, and had neither functional stamens nor functional pistils; these were recognized by size. All florets of normal size had stamens (see Results). The pedicelled florets of *A. gerardii* were the only ones of normal size that sometimes lacked pistils. If a pedicelled floret contained an ovary (either expanded into a seed or the same size as at anthesis), it was tallied as a hermaphrodite. If a pedicelled floret was empty, it was tallied as a male.

**Male characteristics**—Almost all *A. gerardii* shoots had several inflorescences (Fig. 1); to control for possible effects of inflorescence position, an inflorescence was collected from the top, middle, and bottom of the shoot. Each *S. nutans* shoot produced a single terminal inflorescence.

We measured length of mature anthers to determine allocation to pollen production, since anther length is highly correlated with pollen production in a number of grass species (McKone, 1989, and references therein), including those in the genus *Andropogon* (Campbell, 1982). Inflorescences for anther measurements were collected when they were actively flowering, and were stored in FAA (formalin-acetic acid-alcohol) for later dissection. Anther length was measured only in florets with mature anthers that generally appeared bright yellow. For each *S. nutans* plant we measured ~54 anther lengths: three anthers from each of six mature florets from three flowering shoots. For each *A. gerardii* plant we measured ~162 anther lengths: three anthers from each of six

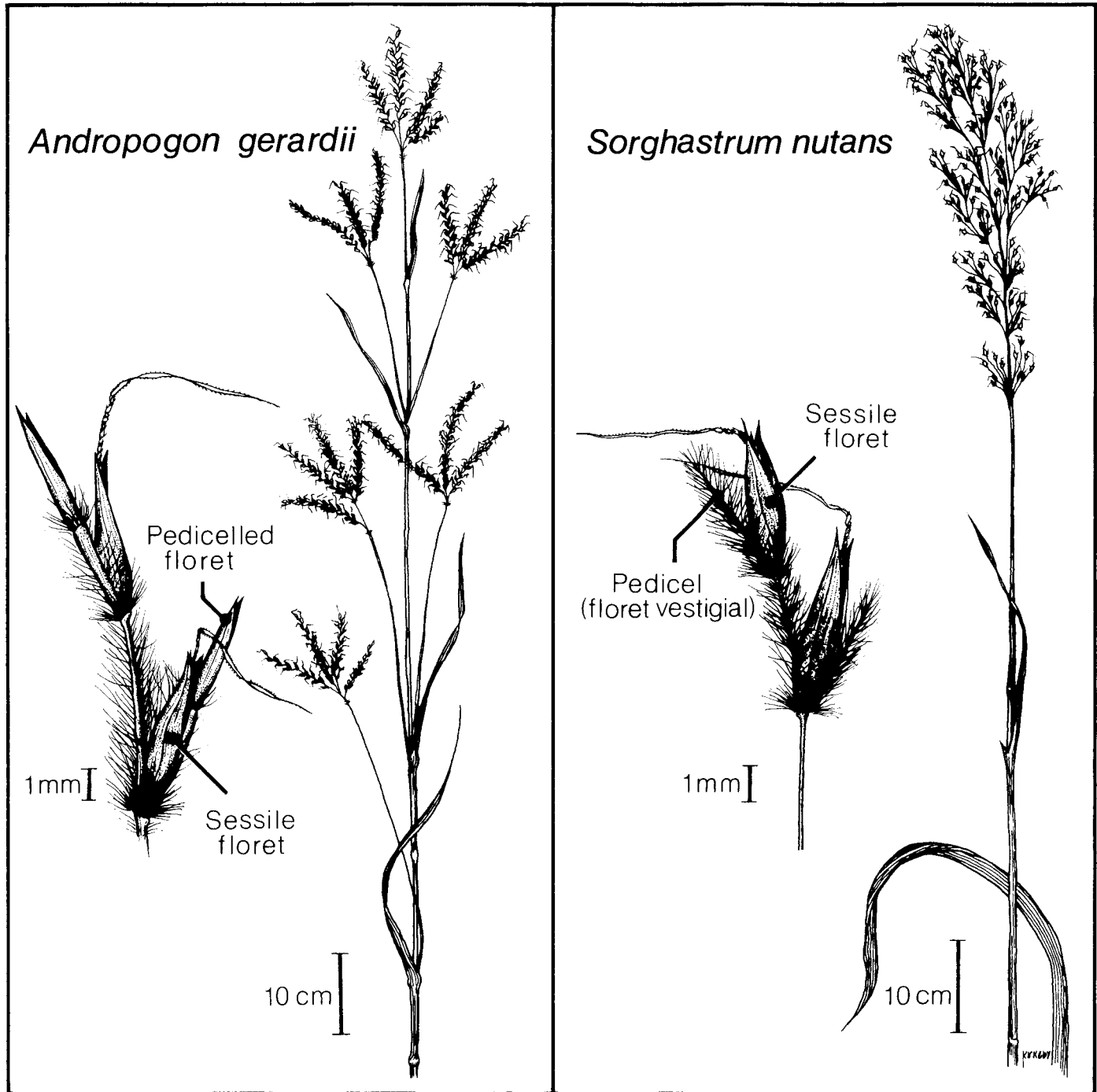


Fig. 1. Inflorescences of *Andropogon gerardii* and *Sorghastrum nutans*. The florets of *A. gerardii* occur in pairs; one is sessile and hermaphroditic, and the other is pedicelled and usually only male (but see Results). The pedicelled floret of *Sorghastrum nutans* is vestigial, and the sessile floret is hermaphroditic.

florets (three sessile and three pedicelled) from three inflorescences per shoot (from the top, middle, and bottom of the shoot) from three shoots; fewer anther lengths were measured when shoots had fewer than three inflorescences.

**Female characteristics**—Shoots designated for measurement of female characteristics were collected on 13 October, when seed was mature but before dispersal. Shoots were stored in paper envelopes in the

laboratory until they could be counted. For each plant, ~160 florets were dissected individually and classified as either (1) with a seed (technically a fruit, the caryopsis), including any expanded ovary (see below); (2) with an unexpanded ovary; (3) empty, with no visible ovary of any size; or (4) neuter, with the floret considerably smaller than average and never with a seed.

The seeds of both species were highly variable in size, ranging continuously from slightly enlarged ovaries to full seeds several times larg-

er. In all size categories, there was also a continuous range in shape from very shriveled to filled, plump seed. Since there was no discrete cutoff between seeds of different size or shape, we counted any ovary that had expanded since anthesis as a seed. At anthesis, ovaries measured from 0.5 to 0.7 mm in length; all ovaries longer than 1.0 mm were tallied as seed. This method probably included some aborted seeds, but it is reasonable to include these as part of female allocation. Air-dried seeds were weighed as a group for each inflorescence.

Shoots were subsampled for seed set and seed mass measurements. The single *S. nutans* inflorescence per shoot was broken into three roughly equal portions and ~15 florets were chosen at random from each portion for dissection; florets that had become detached from the inflorescence while in the bags were considered a fourth category and subsampled in the same way. In *A. gerardii* shoots with two inflorescences, both were counted; when there were more than two inflorescences per shoot, an inflorescence was counted from the top, middle, and bottom of the shoot. Each chosen *A. gerardii* inflorescence was subsampled by randomly counting half of a raceme from each selected inflorescence.

The different inflorescence positions that we defined for subsampling did not necessarily have the same number of florets. For instance, the top inflorescence of *A. gerardii* was typically considerably larger than lower inflorescences. As a result, a shoot-wide mean that gave equal weight to each subsample would be biased. This is particularly important in *A. gerardii*, since inflorescences in different shoot positions differed in seed set and seed mass (unpublished data). To compensate for the unequal number of florets at different shoot positions, shoot-wide averages for seed set and seed mass were calculated by weighing subsample values in proportion to the size of the floret pool being subsampled.

**Self-incompatibility**—To determine whether the species were self-incompatible, we used Lundqvist's (1961) method of hand pollinating pistils that have been removed from the plant and placed in a growth medium. Six intact pistils were dissected from flowering shoots from each of 12 *S. nutans* plants and 14 *A. gerardii* plants. Pistils were taken from florets that were near anthesis, as judged by the dark color and mature appearance of the anthers in the florets and by the orderly progression of flowering. Six pistils were chosen from each plant; three were self-fertilized and three were outcrossed. The pistils were "planted" into petri dishes containing the agar medium of Lundqvist (1961) and incubated overnight. Either self or nonself pollen was taken from recently collected inflorescences that flowered in the laboratory, and brushed onto the pistils in the petri dishes. Pollinations took place ~12 h after pistils were placed in the agar medium. Four hours after pollination, pistils were fixed in 3:1 ethanol:acetic acid for 3 h, cleared in 10 mol/L NaOH for 8 min, rinsed with water, stained with aniline blue, and mounted in 50% glycerine. Pollen germination and pollen tube development in the pistils were examined by fluorescence microscopy. To compare the selfed and outcrossed pistils, we counted the number of pollen tubes that had germinated in the stigmatic hairs, grown to the top of each of the two styles, and grown to the base of each style.

**Supplemental pollination**—For each experimental plant, two shoots were randomly assigned to receive supplemental hand pollination. These shoots were hand-pollinated daily throughout the flowering period. For a source of pollen, flowering shoots were chosen several meters away from the experimental transects. Shoots from pollen donors were collected from the study site, brought indoors, and placed in water; these cut shoots produced pollen for several days. Pollen was collected by tapping the shoots and collecting the pollen that fell onto paper beneath them. Pollen viability was verified by means of the FCR (fluorochromatic) test (Heslop-Harrison and Heslop-Harrison, 1970; Heslop-Harrison, Heslop-Harrison, and Shivanna, 1984). Grass pollen has a very short lifespan (Heslop-Harrison, 1979), so care was necessary to maintain viability long enough for hand pollination. Pollen was trans-

TABLE 1. Reproductive characteristics of *Andropogon gerardii* and *Sorghastrum nutans*, as population average  $\pm 1$  SE. Unless the population was uniform, minimum and maximum plant averages for each variable are given in parentheses. All calculations are based on plant-wide averages;  $N = 19$  plants for *A. gerardii*,  $N = 15$  plants for *S. nutans*. Approximately 162 anthers were measured for each *A. gerardii* plant and 54 for each *S. nutans* plant; these florets were taken from three randomly chosen flowering shoots per plant. In both species, ~160 florets from three randomly chosen flowering shoots were scored for female characteristics for each plant.

Reproductive trait	<i>Andropogon gerardii</i>	<i>Sorghastrum nutans</i>
Percentage of functional florets with anthers	100 $\pm$ 0	100 $\pm$ 0
Anther length (mm) (min-max)	3.54 $\pm$ 0.07 (3.06-4.22)	3.58 $\pm$ 0.10 (2.73-4.23)
Percentage of functional florets with ovaries (min-max)	61.8 $\pm$ 2.5 (50.0-85.5)	100 $\pm$ 0
Seed set (percentage of female florets) (min-max)	69.8 $\pm$ 3.5 (40.2-93.6)	62.5 $\pm$ 5.3 (25.1-90.0)
Dry seed mass (mg) (min-max)	0.61 $\pm$ 0.05 (0.14-0.93)	0.47 $\pm$ 0.10 (0.07-1.25)

ported to the field in a container with damp filter paper, so that high humidity would be maintained and pollen longevity increased (Heslop-Harrison, 1979). Since *A. gerardii* pollen is released usually from 0400 to 0700 (Jones and Newell, 1946; Norrmann, Quarín, and Keeler, 1997), it was pollinated starting at ~0800; *S. nutans* pollen is usually released from 0600 to 1000 (Jones and Newell, 1946), so it was pollinated starting at approximately noon. A small paintbrush was used to apply the pollen to all exerted stigmas of flowering inflorescences. To control for possible differences among inflorescences from different positions on a given shoot of *A. gerardii*, only the top inflorescence on the shoot was pollinated. After pollination, continued viability of the pollen was confirmed by use of the FCR test. Pollinated inflorescences were collected on 13 October. Seed set and seed size were determined as described above under "Female characteristics."

**Statistics**—Because of nonnormality in the data, we used nonparametric statistical tests. The difference between average male and female allocation per floret was tested by Wilcoxon paired-sample rank tests ("Wilcoxon test" hereafter). To control Type I error for multiple statistical tests, we used the sequential Bonferroni procedure within each species (Rice, 1989); all  $P$  values reported for allocation are adjusted accordingly.

## RESULTS

**Sex allocation**—Reproductive characteristics that were measured for each plant of the two species are summarized in Table 1. All examined *S. nutans* florets were hermaphroditic, but there was considerable variation in sex expression within *A. gerardii* (Fig. 2). Almost all sessile *A. gerardii* florets were hermaphroditic. Most pedicelled *A. gerardii* florets were male, but both hermaphroditic and neuter pedicelled florets were common. *A. gerardii* plants varied significantly in the proportion of pedicelled florets that were hermaphroditic (Kruskal-Wallis test,  $P < 0.05$ ; the three shoots counted per plant were used as replicates). The percentage of pedicelled florets that were hermaphroditic ranged among plants from 65.3% to zero. *A. gerardii* plants did not differ significantly in the proportion of pedicelled florets that were neuter (Kruskal-Wallis test,  $P > 0.10$ ).

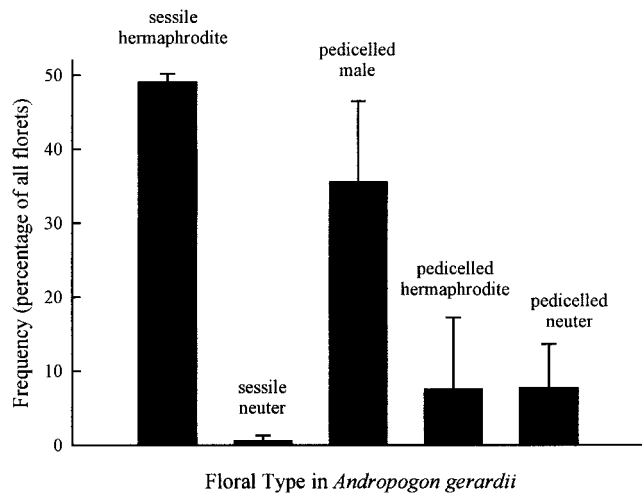


Fig. 2. Frequency of floral types in *Andropogon gerardii*. Error bars show standard deviation among the 19 sample plants; ~160 florets were scored per plant. See Fig. 1 for illustration of floral types. Neuter florets have neither functional stamens nor functional pistils.

Measurements on bulk samples yielded an average of 34.4  $\mu\text{g}$  dry mass/mm of anther in *A. gerardii* and 58.7  $\mu\text{g}$  dry mass/mm of anther in *S. nutans*. Oven-dried mass of seeds was 95.3% of air-dried mass for *A. gerardii* and 94.9% for *S. nutans*. Dry mass energy content and nutrient composition of anthers and seeds from both species are shown in Table 2.

Relative sex allocations were estimated from the equations in Methods and Materials. For each of 19 *A. gerardii* plants and 15 *S. nutans* plants, we calculated a single pooled value of male allocation per floret and a single pooled value of female allocation per floret.

Sex allocation per floret in *A. gerardii* ( $N = 19$ ; Figs. 3, 4) was significantly male biased when measured in terms of biomass (60% male, Wilcoxon test,  $P < 0.05$ ), energy (60% male, Wilcoxon test,  $P < 0.05$ ), potassium (74% male, Wilcoxon test,  $P < 0.001$ ), and calcium (89% male, Wilcoxon test,  $P < 0.001$ ). Allocation to anthers and seed per floret were not different (Wilcoxon tests,  $P > 0.10$ ) for nitrogen (53% male), phosphorus (49% male), or magnesium (57% male).

The sex allocation per floret in *S. nutans* ( $N = 15$ ; Figs. 3, 4) was significantly male biased when measured in terms of biomass (70% male, Wilcoxon test,  $P < 0.05$ ), energy (72% male, Wilcoxon test,  $P < 0.05$ ), nitrogen (70% male, Wilcoxon test,  $P < 0.05$ ), potassium (77%

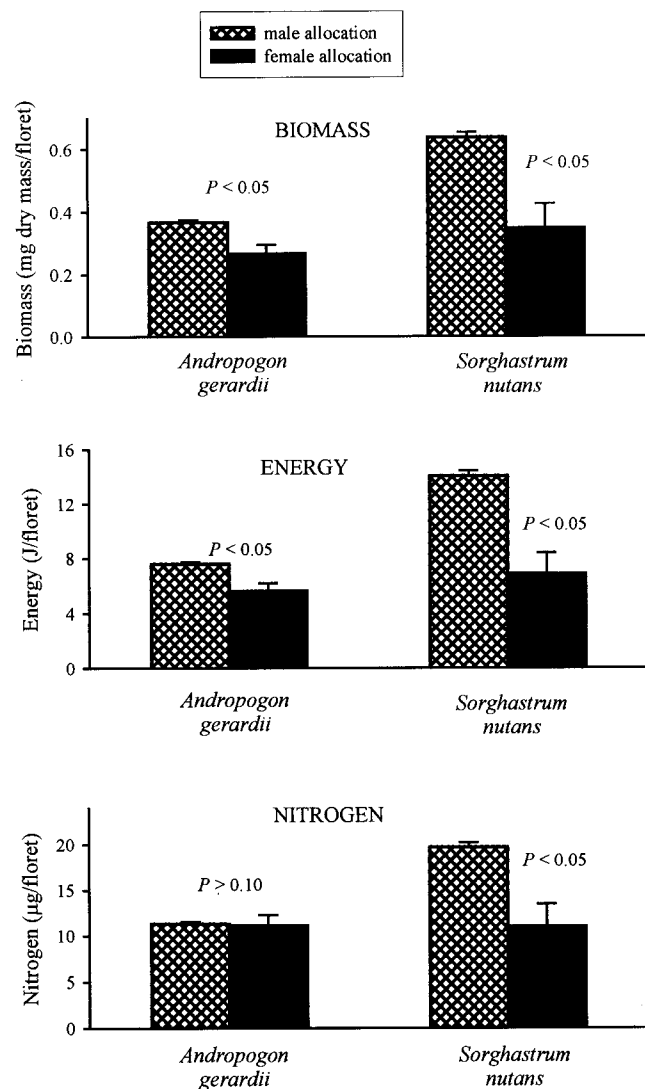


Fig. 3. Sex allocation in *Andropogon gerardii* and *Sorghastrum nutans* measured in currencies of biomass, energy, and nitrogen. Male allocation is the average investment in anthers per floret, as calculated from Eq. 1. Anther size and proportion of anther-containing florets were measured for each plant; resource concentration was based on bulk samples. Female allocation is the investment in seed per floret, as calculated by Eq. 2. Seed size, seed set, and proportion of ovule-containing florets were measured for each plant; resource concentration was based on bulk samples. Error bars show standard error among means of individual plants;  $N = 19$  for *A. gerardii* and  $N = 15$  for *S. nutans*. The  $P$  values are from Wilcoxon paired-sample rank tests; allocations are all significantly male biased except for nitrogen in *A. gerardii*.

TABLE 2. The energy and nutrient composition of anthers and seeds of *Andropogon gerardii* and *Sorghastrum nutans*, based on bulk samples. Energy is expressed as joules per milligram dry mass; elemental composition is percentage of dry mass.

Allocation currency	<i>A. gerardii</i> pollen	<i>A. gerardii</i> seed	<i>S. nutans</i> pollen	<i>S. nutans</i> seed
Energy (J/mg dry mass)	20.7	21.1	22.1	19.8
Nitrogen (%)	3.08	4.17	3.09	3.71
Phosphorus (%)	0.399	0.627	0.346	0.572
Potassium (%)	1.27	0.616	1.04	0.686
Magnesium (%)	0.207	0.236	0.221	0.230
Calcium (%)	0.223	0.0407	0.163	0.0801

male, Wilcoxon test,  $P < 0.01$ ), magnesium (69% male, Wilcoxon test,  $P < 0.05$ ), and calcium (81% male, Wilcoxon test,  $P < 0.01$ ). Only phosphorus allocation was not different (61% male, Wilcoxon test,  $P > 0.10$ ).

**Self-incompatibility**—Self-pollinated pistils of both *A. gerardii* and *S. nutans* clearly showed morphological signs of the incompatibility reaction (Heslop-Harrison, 1982). In all self-pollinated pistils, pollen tubes were often twisted and erratic in growth; many did not penetrate the stigmatic hairs and exhibited heavy callose deposi-

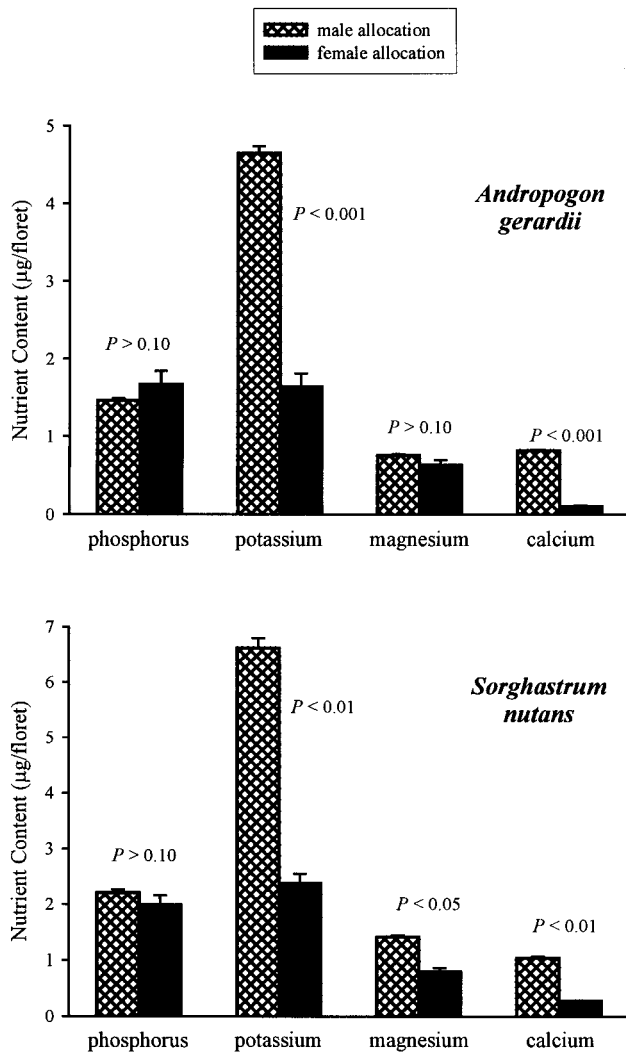


Fig. 4. Sex allocation in *Andropogon gerardii* and *Sorghastrum nutans* measured in currencies of phosphorus, potassium, magnesium, and calcium. Male allocation is the average investment in anthers per floret, as calculated from Eq. 1. Anther size and proportion of anther-containing florets were measured for each plant; nutrient concentration was based on bulk samples. Female allocation is the investment in seed per floret, as calculated by Eq. 2. Seed size, seed set, and proportion of ovule-containing florets were measured for each plant; nutrient concentration was based on bulk samples. Error bars show standard error among means of individual plants;  $N = 19$  for *A. gerardii* and  $N = 15$  for *S. nutans*. The  $P$  values are from Wilcoxon paired-sample rank tests; allocations were all significantly male biased except for phosphorus (both species) and magnesium in *A. gerardii*.

tion. These abnormal pollen tube features only occasionally occurred in the outcrossed pistils.

Selfed *A. gerardii* pistils had some pollen tubes in the stigmatic hairs, but rarely at the top of the style. There was a single pollen tube that had grown to the base of the style in only five of 37 pistils examined. Outcrossed *A. gerardii* pistils had ample pollen tube growth, and in 33 of 35 pistils there were at least two pollen tubes present at the base of the style.

Selfed *S. nutans* pistils also had some pollen tubes in the stigmatic hairs, but almost none of the tubes grew as far as the top of the style. There were pollen tubes at the

TABLE 3. The effect of supplemental hand pollination on seed set and seed mass in *Andropogon gerardii* and *Sorghastrum nutans*. In both species, hand pollination did not significantly affect either variable (Wilcoxon signed-rank test,  $P > 0.10$ ;  $N = 16$  plants for *A. gerardii*,  $N = 15$  plants for *S. nutans*).

Treatment	Seed set (%) (mean $\pm$ 1 SE)	Dry seed mass (mg) (mean $\pm$ 1 SE)
<i>Andropogon gerardii</i>		
Pollen addition	75 $\pm$ 3	0.66 $\pm$ 0.08
Control	69 $\pm$ 5	0.65 $\pm$ 0.06
<i>Sorghastrum nutans</i>		
Pollen addition	66 $\pm$ 5	0.56 $\pm$ 0.09
Control	63 $\pm$ 5	0.48 $\pm$ 0.09

base of the style in only two of 32 selfed pistils. All 34 outcrossed pistils had at least two pollen tubes at the base of the style.

**Supplemental pollination**—We pooled seed size and seed mass data for each treatment (supplemental pollination and control) within each plant. Significance of the treatment was determined by a paired comparison of the plant-wide averages across the population. Hand pollination did not significantly increase seed set or seed mass in either species (Wilcoxon signed-rank test,  $P > 0.10$ ; Table 3).

## DISCUSSION

There is evidence for male-biased sex allocation in both *Andropogon gerardii* and *Sorghastrum nutans* (Figs. 3, 4), though the currency used affects the estimate of sex allocation. These are the only male-biased sex allocation estimates of which we know in cosexual plants (or hermaphroditic animals; see Petersen, 1991; Petersen and Fischer, 1996). Though most previous estimates of plant sex allocation are in terms of biomass only, they are usually female biased (Cruden and Lyon, 1985; Goldman and Willson, 1986) as predicted for animal-pollinated or partially selfing species.

Even though *A. gerardii* is andromonoecious, with almost 40% of its florets producing pollen without functional ovules (Table 1), the evidence for male-biased allocation was stronger for *S. nutans*, the flowers of which are all hermaphrodite. This occurs at least partly because investment in pollen in the male florets of *A. gerardii* was offset by greater seed set and seed mass in hermaphrodite florets of *A. gerardii* relative to hermaphrodite florets of *S. nutans* (Table 1).

The theoretical conditions that would lead to a male-biased optimal allocation (see Introduction) seem to be met in both *A. gerardii* and *S. nutans*: in addition to wind pollination and abiotic seed dispersal, our data show that these species are characterized by obligate outcrossing and by lack of pollen limitation. Outcrossing is enforced by self-incompatibility, in support of previous proposals that these species are generally outcrossing (Law and Anderson, 1940; Hanson and Carnahan, 1956). Normann, Quarín, and Keeler (1997) also observed pollen tube behavior after selfing in *A. gerardii* with results similar to ours, and found extremely low seed set after self-pollination. Our pollen addition experiment gave no evidence

of pollen limitation, as has been reported in some other wind-pollinated species (Allison, 1990; Bertness and Shumway, 1992). Pollen limitation would be unlikely in such ecologically dominant species as *A. gerardii* and *S. nutans*.

We do not know which resource or resources are limiting to growth and reproduction in *A. gerardii* and *S. nutans*. If fixed carbon (i.e., energy) is accepted provisionally as the best currency to measure reproductive costs (see Reekie and Bazzaz, 1987b; Chapin, 1989), both species have significantly male-biased allocations. Neither species had a biased sex allocation when phosphorus was used as the currency. Both *A. gerardii* and *S. nutans* are obligately dependent on mycorrhizal fungi (Hetrick, Kitt, and Wilson, 1988), and mycorrhizal plants do not respond to phosphorus fertilization in the greenhouse (Hetrick, Wilson, and Todd, 1990). This suggests that phosphorus is not usually directly limiting, though there is an energy cost to maintaining the mycorrhizal association.

The most male-biased previous estimate of sex allocation in a cosexual plant is for *Bromus inermis* (Poaceae), which has an allocation of nearly 50% male when measured in energy and various nutrients (McKone, 1987). *Bromus inermis* shares many of the characteristics of *A. gerardii* and *S. nutans* (wind pollinated, self-incompatible, no obvious adaptation for seed dispersal), but its flowering shoot is typically much shorter than in our study species. It is possible that the shorter inflorescence of *B. inermis* reduces pollen dispersal and allows for local mate competition among pollen grains on nearby stigmas (Burd and Allen, 1988). This would produce concave fitness-gain curves for both male and female reproduction, which could result in an equal optimal allocation (Lloyd, 1984). Another wind-pollinated grass, *Zizania aquatica*, has a female-biased allocation of biomass (Willson and Ruppel, 1984); however, the selfing rate of this species is apparently unknown.

In general, female characteristics (seed set and seed mass) were more variable than male characteristics (anther number and anther length) within both of our species (Table 1) and were therefore the more important determinants of sex allocation. Seed set and seed mass are both highly variable among years and among sites in *A. gerardii* and *S. nutans* (Knapp and Hulbert, 1986; Rabinowitz et al., 1989), so our results may not apply to different times and places. Some previous estimates of seed set are lower (Branson, 1941; Rabinowitz et al., 1989) and of average seed mass are higher (Kneebone and Cremer, 1955; Boe, Ross, and Wynia, 1983; Springer, 1991) than we found. This may be caused by differences among investigators in the operational definition of what is large enough to be considered a functional seed, or by the use of mechanical seed sorters, which would tend to miss small seeds. Our more inclusive criterion for what to count as a seed is conservative, since it would result in greater estimates of female allocation per floret.

Our measures of allocation were "static" (Ashman, 1994) and did not take into account the possibility of redeployment of resources into other structures later. The use of static measurements can introduce a serious bias in estimates of sex allocation, particularly for attractive structures such as petals and nectar (Ashman, 1994).

However, our measures were made when pollen and seed were mature and about to be dispersed from the parent plant. Thus, there was little possibility of resource recovery.

We did not measure some portions of reproductive effort. Neither the filaments of the stamens nor unfertilized ovules were collected, though these are only a small fraction of the size of the anthers and seeds that we did collect. Other parts of the inflorescence were not included in the sex allocation estimate, including the glumes and lemmas of the spikelets and the peduncle that holds the inflorescences above the surrounding vegetation (Fig. 1). These structures represent "shared costs" (Lloyd, 1984), which are not easily assigned to male or female allocation since they contribute to both. At least some of the resources used to make these structures could be recovered for other uses, reducing their ultimate cost to the plant (Ashman, 1994).

Our use of bulk samples to estimate nutrient and energy composition of pollen and seeds was based on the assumption that there was little interplant variation in these values. We were unable to test this explicitly, since such large samples were needed for the composition analysis. The relative consistency of the resource composition of seed and anthers between these two species (Table 2) and general similarity to other grasses (e.g., McKone, 1987) suggest that there would be relatively little intraspecific variation, but this remains untested.

In conclusion, we found that *S. nutans* had a male-biased sex allocation of all resources but phosphorus and that *A. gerardii* had a male-biased allocation for energy and some (but not all) of the nutrients measured. Only work on resource limitation in these species will give a clear indication of which resource ultimately limits their reproductive effort. Yet the relative consistency of the male bias, particularly in *S. nutans*, strongly supports the hypothesis of male bias. This is consistent with the prediction that wind-pollinated, outcrossed plants will have male-biased allocations if seed is not widely dispersed. Though these conditions may be relatively rare, we suspect that male-biased sex allocation will be found in other species with similar reproductive and ecological characteristics.

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