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THE LEVEL OF AGAMOSPERMY IN A NEBRASKA POPULATION OF *SPIRANTHES CERNUA* (ORCHIDACEAE)¹

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This study investigated reproduction in a prairie population of *Spiranthes cernua* in eastern Nebraska. *Spiranthes cernua* reproduces both sexually and asexually, through adventitious embryony. Agamospermous seeds are monoembryonic, polyembryonic, have extruded or free embryos, or lack embryos. These kinds of seeds are extremely rare in *Spiranthes vernalis*, a close relative that relies exclusively on sexual reproduction. Sexually derived seeds are monoembryonic or lack embryos. One objective was to quantify the amount of sexual reproduction in a natural population. In 1985 and 1986, maximum estimates of sexual seed production per capsule were 19.2% and 33.6%, respectively. Thus, every capsule examined contained a high proportion of agamospermous seeds. In addition, attempts were made to manipulate the reproductive system with experimental crosses performed in the field. The treatments examined agamospermy, autogamy, and outcrossing. In all treatments the majority of seeds in a capsule were agamospermous.

Plants are notorious for their variety of reproductive strategies. Many reproduce both sexually and asexually (Lloyd, 1984). Asexual reproduction can occur by vegetative propagation and/or agamospermy (Gustafsson, 1946; Richards, 1986). Agamospermy is the formation of seeds without fertilization and can be obligate, if there is a complete loss of sexual function (Grant, 1981). However, most if not all agamospermous plants are facultative, capable of some sexual reproduction (Clausen, 1954; de Wet and Stalker, 1974; Asker, 1979). Even "obligate" apomicts may continue to produce viable pollen and rudimentary megagametophytes (Mogie, 1988; Mogie and Ford, 1988), indicating the retention of some sexual function.

The degree of sexuality exhibited by an agamospermous species may be a function of genetic and environmental factors. Asexual populations tend to occupy ranges of greater area, higher altitude, higher latitude, and are often peripheral to their sexual counterparts (Glesner and Tilman, 1978; Bierzychudek, 1985, 1989). The significance of these ecological differences between predominantly sexual and asexual taxa is subject to debate (Bierzychudek, 1985, 1989; Michaels and Bazzaz, 1986, 1989; Mogie and Ford, 1988). One explanation often advanced is that pollinator activity is limited at the periphery of a species range. Agamospermy assures reproductive success in the absence of pollinators (Manning, 1981; Lloyd, 1988). The purpose of this study was to quantify the expression of sexual reproduction in a population at the edge of its range where apomixis would be expected to be high.

The experimental organism was the terrestrial orchid, *Spiranthes cernua* (L.) L. C. Richard, which produces seed both sexually and asexually. In this species the mechanism

of agamospermy is adventitious embryony (Leavitt, 1900; Ames, 1921; Swamy, 1948; Catling, 1980, 1982; Lakshmanan and Ambegaokar, 1984). This type of embryonic development often results in the production of polyembryonic seeds, which can be easily distinguished from monoembryonic seeds (Nogler, 1984). Unlike other types of agamospermy, adventitious embryony does not directly prevent the development of the megagametophyte. Sexual and asexual ovules can coexist in the same ovary (Swamy, 1948; Catling, 1982). Since the work of Ames (1921), the production of monoembryonic seeds has been associated with sexual reproduction and polyembryonic seeds with agamospermy in *S. cernua* (Sheviak, 1976; Sheviak and Catling, 1980; Catling, 1982). Swamy (1948) distinguished three races of *S. cernua* based on this seed character: sexual, agamospermous, and intermediate. In the agamospermous race, Swamy noted that "the occurrence of a single embryo per seed is exceptional." The "intermediate" plants had both kinds of seeds, which vary in proportion. In a herbarium survey of the eastern half of the United States, Swamy reported 81.4% of the plants were agamospermous, 10.4% were sexual, and 8.2% were intermediate. Attempts to quantify agamospermy in other species have involved microscopic examination of embryo sacs during megasporogenesis (Marshall and Brown, 1974). In order to examine a natural population, we used differences in seed morphology to distinguish between asexual and sexual reproduction.

Sporophytic cells from the micropylar region of the two-layered inner integument differentiate into proembryos in *S. cernua*. The megagametophyte usually degenerates at the four nucleate stage (Swamy, 1948; Catling, 1982; Lakshmanan and Ambegaokar, 1984), although complete development of the embryo sac has been reported in the "intermediate race" (Swamy, 1948). When and what triggers the degeneration is unknown. In addition to polyembryony, agamospermy can also produce seeds with single embryos and can result in extruded embryos, free embryos, and variations in embryo shape and/or position (Sheviak, 1982). The testa, which consists of dead cells derived from the outer integument, can develop in the absence of an embryo. This results in an empty seed coat (Catling, 1982). This condition can arise in unfertilized sexual ovules or in asexual ovules that fail to produce

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TABLE 1. *Pollination manipulations conducted on Spiranthès cernua in 1985*

Treatment	Number of plants per treatment ^a	Date performed ^b
1 Pollinator exclusion (bagged)	15	8 September
2 Outcrossing (emasculated)	19	8 September
3 Agamospermy (bagged, emasculated)	8	16 September
4 Autogamy (bagged, self-pollinated within a flower)	16	9 September
5 Geitonogamy (bagged, self-pollinated with a different flower on the same plant)	16	15 September
6 Xenogamy (bagged, cross-pollinated with a different plant)	14	16 September
7 Unmanipulated	19	8 September

^a Five plants from each treatment were scored. Ten capsules from each plant were counted.

^b Date when the majority of individuals in the treatment were manipulated.

adventitious embryos. Clearly, the sexual or asexual origin of seeds cannot be determined unambiguously without developmental or genetic information (Catling, 1982; Sheviak, 1982). Estimates based on seed morphology represent minimum values for agamospermy and maximum for sexual reproduction. Despite their "crudeness," such estimates are valuable because more individuals can be sampled and the variation within a population determined. Further, the level of agamospermy can be quantified rather than observing simply presence or absence of agamospermy.

Terrestrial orchids are frequently reported to be pollinator limited (Mosquin, 1970; Thien and Marcks, 1972; Ackerman, 1975, 1981; Boyden, 1982; Mehrhoff, 1983; Cole and Firmage, 1984; Davis, 1986; Snow and Whigham, 1989; Robertson and Wyatt, 1990). Therefore, manipulations were conducted in the field to test the effects of various pollination regimes on the occurrence of polyembryony. Pollination experiments have been performed on the sexual species of *Spiranthès* (Catling, 1982) but not the agamosperous species. The field experiment examined the effect of pollinator exclusion, agamospermy, autogamy, and outcrossing on the level of asexual seed production. We predicted that pollen limitation would favor agamospermy, while pollination would induce a sexual response.

MATERIALS AND METHODS

Description of the study site—The study site was located at Nine-Mile Prairie approximately 14.5 km northeast of Lincoln, Nebraska, Lancaster County (lat. 40°52' long. 96°46'). This unplowed, tallgrass prairie supports a diverse flora of 392 species dominated by *Andropogon gerardii* Vitm. and *A. scoparius* Michx. (Steiger, 1930; Kaul and Rolfsmeier, 1987). The study population was distributed over an area of approximately 200 m².

Description of the experimental organisms—*S. cernua* is a widely distributed perennial herb in North America. It flowers in September–October, after most other sym-

patric species. Following anthesis, new overwintering shoots form from tuberous roots. New shoots develop in early spring and persist into late summer. Morphological variation has led to the description of several ecotypes (Sheviak, 1982). The leaves of the plants in this study were fugacious. *S. vernalis* has a similar phenology to *S. cernua* with the exception that it flowers somewhat earlier. *S. cernua* has a polyploid chromosome number of 60, while in *S. vernalis* the number is 30. The inflorescence of *S. vernalis* is taller and single-ranked, which easily distinguishes it from its close relative. Its breeding system is exclusively sexual.

Flowering individuals of *S. cernua* were marked and numbered in September 1985. Voucher specimens of *S. cernua* and *S. vernalis* were collected in 1986 and are available at the University of Nebraska at Omaha Herbarium. The identification of these specimens was confirmed by C. J. Sheviak, New York State Museum. The *S. cernua* were members of the Boone Platte race with reduced floral structures (Sheviak, 1982). Eastern Nebraska is at the western edge of its range (Luer, 1975; Sheviak, 1982). Note that neither *S. cernua* nor *S. vernalis* was listed at Nine-Mile Prairie by Steiger in 1930.

Pollination treatments—Seven treatments were used to test the effects of pollination on apomixis (Table 1). The first treatment tested the effects of pollinator exclusion by bagging the plants before anthesis. In treatment 2, all flowers on a spike were emasculated, preventing self-fertilization. Plants that were bagged and emasculated before anthesis could produce seeds only by agamospermy (treatment 3). In treatment 4, plants were bagged before anthesis. When the flowers opened, they were self-pollinated. Sexual seed production in these flowers would thus simulate autogamy. Treatment 5 was similar to treatment 4, except flowers were pollinated with pollen from different flowers on the same plant. In this case, sexual reproduction resulted from geitonogamy. In treatment 6, plants were bagged before anthesis, emasculated, and then outcrossed with pollen from at least two individuals. The natural condition of the plants was represented by treatment 7 (unmanipulated). Reproduction in these individuals could theoretically occur by autogamy, geitonogamy, xenogamy, and/or agamospermy.

Pollinators were excluded with nylon mesh bags (1 mm²), supported on a wire frame (Whigham and McWethy, 1980; Hogan, 1983; Cole and Firmage, 1984). The spike was firmly tied to the wire support before anthesis. The bags were large enough for the flowers to grow and mature. The effectiveness of the bags was tested on the closely related, sympatric, sexual species, *S. vernalis*. Bagged *S. vernalis* failed to set seed. A ×2.5 binocular magnifying lens (Optivisor, Donnegan Optical Co., Lenexa, KS) was used for pollination manipulations. Flowers were emasculated by depressing the labellum with forceps exposing the rostellum. Intact pollinaria were removed in an upward motion away from the rostellum. This prevented contact with the stigmatic surface. In crosses, an entire pollinarium was deposited on the sticky surface of the stigma and held there by repositioning the labellum under the rostellum. At least 20 flowers were manipulated on each inflorescence. The pollen adhered readily to the stigma even though many inflorescences exhibited varying

levels of protandry. If a treatment was unsuccessful, the flower was removed.

The pollination treatments were performed over a 9-day period of peak flowering, 8–18 September 1985 (Table 1). Pollen from 15 different individuals stained positive with lacto-phenol (Radford et al., 1974), suggesting the pollen was viable. Pollinations in treatment 6 (xenogamy) were performed after most of the flowers on an inflorescence had opened. The flowers develop sequentially from the bottom up; thus the flowers within a given inflorescence differ in their date of anthesis, depending on their position. The pollen collected from treatment 2 (emasculated) was transferred to plants in treatment 6 on the same day. A flower was considered receptive if pollinia readily stuck to the stigma. Geitonogamous crosses were performed by systematically emasculating one rank and immediately pollinating the adjacent rank of an individual. Some plants were too mature to manipulate, especially the bagged, emasculated flowers (treatment 3) which were the last to be treated. When this happened, new individuals were located and randomly assigned to the treatment. Inflorescences were collected before dehiscence from 1 to 11 October 1985. The capsules were separated from the inflorescence and stored at 4 C.

Seeds were removed from the capsule and placed in concentrated phloxine B which stained the embryos red. After staining for 48 hours, the seeds were rinsed twice in 50% ethanol. The alcohol was decanted and the seeds were allowed to air dry on microscope slides. Melted glycerol jelly was placed on the dry seeds and the seeds stirred into the mounting medium until an even distribution was achieved. Similar techniques have been described by Owczarzak (1952) and Catling (1982). The slides were read using Hoffman optics ($\times 100$).

The design of the pollination experiment sampled variation among treatments, among plants within a treatment, and among capsules within a plant. Five plants from each of the seven pollination treatments were selected at random, for a total of 35 plants. The ten undehisced capsules in best condition were selected for study. To reduce the experimental error, one observer scored the seeds (JMS). Observer bias was further reduced by randomizing the capsules so that the treatment was unknown at the time of scoring. The entire slide was scanned and an effort made to classify each seed. An average of 150 seeds from each capsule were classified. A total of 349 slides were read (one capsule lacked seeds). Capsules of *S. cernua* were collected from ten unmanipulated plants (five capsules each) in 1986. These plants were used to test for yearly differences in the natural level of agamospermy and seed viability. The sexual species, *S. vernalis*, was sampled from the same location in 1986 for comparison with *S. cernua*. Inflorescences from ten unmanipulated individuals of *S. vernalis* were collected, and five capsules from each plant were scored ($N = 50$).

Initial observations of seeds resulted in six seed types (Table 2). Polyembryonic, extruded, free, and unknown seed categories represent seeds of asexual origin (Swamy, 1948; Catling, 1982; Sheviak, 1982). This group of seeds will be referred to as "polyembryonic." Seeds with single extruded embryos or seeds with single embryos not centrally located were classified as unknown. Catling (1982) similarly considered aberrations in embryo shape and

TABLE 2. Description of seed categories observed in *Spiranthes cernua*

Seed type	Origin	Description
Monoembryonic	Sexual/ Asexual	Seeds with a single, well-defined oval or circular embryo
Empty	Sexual/ Asexual	Seed coats with no apparent embryo (infertile)
Polyembryonic	Asexual	Seeds with two or more distinct embryos
Extruded	Asexual	Seeds with one or more embryos protruding from the seed coat
Free	Asexual	Free embryos without a seed coat
Unknown	Asexual	Seeds with embryos of indefinite shape and/or number

location to be a direct result of asexual development. Inviolate empty seed coats were present in all treatments but were most frequent in sexual crosses and in *S. vernalis*, suggesting a sexual origin. Monoembryonic seeds can be produced sexually or asexually, i.e., normal monoembryonic seeds were observed in treatment 3. We will refer to monoembryonic and empty seeds as the "nonpolyembryonic" seed group and they may represent either reproductive mode. Fluctuations in the proportion of nonpolyembryonic seeds/capsule are believed to represent shifts between sexual and asexual reproduction.

Data analysis—The raw data were counts of each seed type per capsule in each pollination treatment. Two types of analyses will be presented. The first is a nested ANOVA with the following sources of variation: among treatments (= pollination manipulation or year or species) and among plants within treatment. The error term represents variation among capsules within plants. The proportions were transformed with an arcsin transformation. The treatment MS was tested against the plant (treatment) MS. The components of variance were estimated for each level in the hierarchy using the SAS procedure NESTED (VAX version, SAS Institute, 1985). This analysis depends on how well the transformation meets the normality assumption of ANOVA. We also used a chi-square contingency analysis to detect the association between pollination treatment and seed type. The SAS procedure FREQ was used for chi-square analyses. The advantage of contingency analysis is that the raw data can be used and the table can be partitioned. The disadvantage is that the hierarchical nature of the design resulted in pooling of data and loss of some information.

RESULTS

Natural level of agamospermy—The proportion of polyembryonic seeds/capsule was determined in 1985 and 1986 from five and ten unmanipulated plants, respectively. In 1985, the average value was 80.2%, but there was considerable variation among capsules within a plant (overall range: 69.5%–88.1%). The majority of seeds produced were polyembryonic in all capsules. In 1986, the average was 66.2% with a range of 32.8% (43.9%–76.7%). The greater variability in 1986 may be due to sampling more individuals ($N = 10$, five capsules each). Only two capsules contained a higher proportion of nonpolyem-

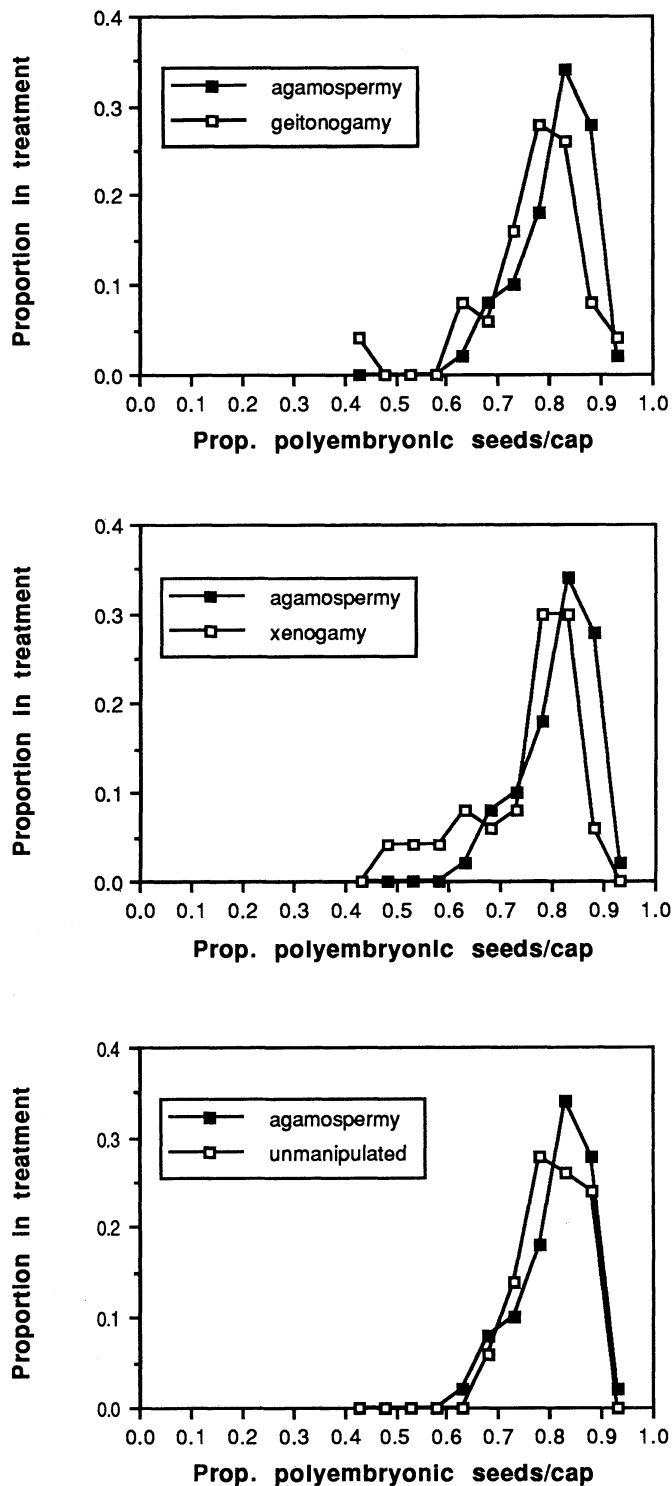


Fig. 1. Relative frequency polygons of the proportion of polyembryonic seeds/capsule within pollination treatments. Symbols occur at the class midpoint. Pairwise comparisons are shown between treatment 3 (agamospermy) and treatments 5 (geitonogamy), 6 (xenogamy), and 7 (unmanipulated).

bryonic than polyembryonic seeds. A nested ANOVA showed that the difference between years was statistically significant ($F = 70.02$; $P = 0.0001$). The plant (year) effect was also significant ($F = 2.82$; $P = 0.0003$). Most of the

TABLE 3. Contingency analysis of pollination treatment differences. The observed counts and percentages are given. The direction of deviations from expected values are shown by (+) or (-)

Treatment	Seed categories	
	Nonpolyembryonic	Polyembryonic
1 Autogamy	1,625 (19.8%) 1,647 ^a (-)	6,580 (80.2%) 6,558 (+)
2 Outcrossing	1,492 (18.8%) 1,593 (-)	6,443 (81.2%) 6,342 (+)
3 Agamospermy	1,422 (18.5%) 1,543 (-)	6,265 (81.5%) 6,144 (+)
4 Self-compatibility	1,311 (17.6%) 1,495 (-)	6,137 (82.4%) 5,953 (+)
5 Geitonogamy	1,730 (22.9%) 1,517 (+)	5,824 (77.1%) 6,037 (-)
6 Xenogamy	1,637 (24.2%) 1,358 (+)	5,126 (75.8%) 5,405 (-)
7 Unmanipulated	1,381 (19.2%) 1,444 (-)	5,812 (80.8%) 5,749 (+)

^a Expected values.

Null hypothesis: seed category is independent of treatment. $\chi^2 = 161.17$; $P < 0.001$.

variance was due to the year component (58%). The remaining variance was equally divided among plants and among capsules (21% each).

Pollination treatments—A significant association was found between pollination treatment and seed type (Table 3). In all treatments the difference between the two seed type groups was great and all capsules had large proportions of polyembryonic seeds. The average proportion of nonpolyembryonic seeds/capsule ranged from 17.6% in treatment 4 (a test of self-compatibility) to 24.2% in treatment 6 (outcrossed by hand). As predicted, xenogamy and geitonogamy produced fewer polyembryonic seeds than the other treatments. However, the large proportion of polyembryonic seeds in treatment 4 was unexpected. The 7×2 table was then partitioned to examine the contributions of the various treatments (Table 4). All 2×2 contingency tables were corrected for continuity (Zar, 1984). Compared to obligate agamospermy, treatments 1, 5, and 6 (tests for autogamy, geitonogamy, and xenogamy) were statistically different. The other comparisons were not significant. Of the pairwise comparisons among sexual crosses (4, 5, and 6), two were significant. Autogamous selfing produced a smaller proportion of nonpolyembryonic seeds/capsule than geitonogamy (0.1764 vs. 0.2289). The statistical detection of such small percentage differences was due to the large sample size (i.e., 52,785 seeds were counted). In the nested ANOVA, the treatment effect was not significant ($F = 1.11$; $P = 0.3796$), but the plant (treatment) effect was very significant ($F = 7.70$; $P = 0.0001$). Estimation of the components of variance showed that 18% of the variation was accounted for by the treatment groups, 51% by plants within treatment, and 31% by capsules within plants. The distribution of proportions of polyembryonic seeds/capsule is illustrated in Fig. 1. Obligate agamospermy (3) is compared to geitonogamy (5), xenogamy (6), and unmanipulated (7) treatments. The distribution of unmanipulated capsules was very similar to agamospermy. The distribution of geito-

nogamous and xenogamous capsules are shifted to the left relative to agamospermy. While differences in the average values were not detected by ANOVA, at least 20% of the xenogamous capsules produced fewer polyembryonic seeds than agamospermy (as low as 40%).

The percentage of seeds with embryos has been used to estimate seed fertility in terrestrial orchids (Kallunki, 1981). The proportion of fertile seed was lowest in the outcrossed plants (87%) and the geitonogamous crosses (88%). All other treatments were greater than 91%. This may be the result of a higher incidence of embryo abortion in sexually derived seeds (Wiens, 1984; Wiens et al., 1987). There was no significant difference among treatments in fertility ($F = 1.07$; $P = 0.4049$), but there were significant differences among plants ($F = 4.90$; $P = 0.0001$).

Differences in the relative frequency of seed types readily distinguished *S. cernua* from *S. vernalis* ($F = 441.60$; $P = 0.0001$). There were also among-plant differences ($F = 3.84$; $P = 0.0001$). The species component of variance was large (92.2%), while among-plant (6.4%) and among-capsules (1.4%) components were small. Most of the seeds of *S. vernalis* were nonpolyembryonic (90.6%). Of the 6,909 seeds viewed, only five were clearly polyembryonic. The remaining agamospermous seeds were classified into the extruded, free, or unknown categories.

DISCUSSION

Reproduction in the Nine-Mile Prairie population of *S. cernua* was predominantly agamospermous in both 1985 and 1986. If some of the nonpolyembryonic seeds were produced sexually, then the agamospermous plants were facultative even at the edge of their geographic range. A small amount of sexual reproduction each year could supply new genetic variants (Marshall and Weir, 1979). This would account for the allozyme variation observed in the Nine-Mile population (A. Antlfinger, unpublished data) and in other "obligately" apomictic populations (Lyman and Ellstrand, 1984; Mogie, 1985).

The significant between-year difference in agamospermy may reflect environmental influences on agamospermy, genetic differences among plants within the unmanipulated condition, and/or variation in pollinator availability. Climate may directly influence embryo development. For example, changes in photoperiod with latitude are known to affect rates of agamospermy in grasses (Knox, 1967). Light has been shown to be an important factor in the determination of sex in dioecious orchids (Gregg, 1975). Climate might also act indirectly through its effect on the pollinator population.

Pollinator limitation was probably contributing to the high level of agamospermy. There was evidence that pollinators were not visiting *S. cernua* in 1985. Flowers were carefully examined at the time of manipulation, and few pollinaria had been removed. This has been observed every year since as pollen could be collected routinely after capsule formation. Further, the unmanipulated plants had seed type proportions similar to bagged and emasculated plants (obligate agamospermy). Sheviak (1982) has reported that pollination of *S. cernua* is occasional or rare. Low rates of pollination are common in terrestrial and epiphytic orchids (Mosquin, 1970; Thien and Marcks, 1972; Ackerman, 1975, 1981, 1989; Boyden, 1982; Mehr-

TABLE 4. 2×2 contingency analyses comparing pairs of treatments and seed categories

Treatment pairs	Chi-square	P
Agamospermy (3) vs. Outcrossed (2)	0.22	NS ^a
vs. Selfed (4)	0.48	NS
vs. Unmanipulated (7)	1.15	NS
vs. Autogamy (1)	4.29	0.05
vs. Geitonogamy (5)	44.76	0.001
vs. Xenogamy (6)	69.86	0.001
Selfed (4) vs. Geitonogamy (5)	64.85	0.001
Xenogamy (6) vs. Selfed (4)	98.12	0.0001
vs. Geitonogamy (5)	3.79	NS
vs. Outcrossed (2)	72.04	0.001

^a NS = not significant.

hoff, 1983; Cole and Firmage, 1984; Davis, 1986). One explanation for lack of pollination is that *S. cernua* may not compete well with other fall-flowering species for pollinators (Anderson and Schelfhout, 1980; Kaul and Rolfsmeier, 1987). Agamospermy allows *S. cernua* to be independent of pollinators. This mating system contributes to its ability to colonize habitat that has been reduced to small, isolated remnants, such as prairie, and to persist in small numbers.

Two pollination treatments slightly increased the average proportion of nonpolyembryonic seeds: hand outcrosses and geitonogamy. We had expected pollination to produce a large sexual response. This did not occur in the majority of capsules, although some did produce as much as 60% nonpolyembryonic seed. Several factors could have contributed to the lack of response to pollination. First, because the pollen donor can influence rates of fertilization (Nogler, 1984), perhaps the male parents used in this study were uniformly poor in their ability to fertilize the female recipients. This might occur if the population was clonal (Catling, 1987). A preliminary study of allozyme variation showed a minimum of four genetically distinct groups based on four polymorphic loci (A. Antlfinger, unpublished data). At least, the population does not consist of a single clone.

A second factor was that the autogamous and geitonogamous crosses were hampered by the protandrous flowering of *S. cernua*. The difference in time of maturity of pollen and ovules was likely overcome in the geitonogamous crosses because flowers on an adjacent rank were mature. In contrast, autogamous selfing may have been unsuccessful because the flower was unreceptive and not because of self-incompatibility. Differences between the two kinds of selfing have also been reported in the terrestrial orchid, *Calopogon tuberosus* (Firmage and Cole, 1988, p. 1373).

The data analyses suggested that plant-to-plant variation was a major factor and partially explains why the treatment differences were not detected by ANOVA. Individual variation was observed in unmanipulated plants, but not nearly as much as in manipulated plants. Given these results, future experiments should perform all treatments on each plant and more plants should be examined within each treatment. Since unmanipulated plants showed less individual variation, our methodology must have contributed to the variability. The pollination treatments were performed over a short period of time relative to

the life of the inflorescence. Not all flowers had opened. Some were on the verge of senescence. Although these extremes were avoided, it is obvious that the flowers varied in their response to the pollination treatments. Natural pollinations may be more effective, if they occur when the flowers are most receptive.

Finally, high levels of polyembryonic seeds/capsule would result if the adventitious embryos formed before the crosses were performed. Precocious capsule development before anthesis has been observed in some individuals of *S. cernua* (Sheviak, 1982). If adventitious embryos developed early they might limit the resources available to meiotic embryos developing later (Williamson, 1981; Nogler, 1984). "Timing" may play a key role in regulating the amount of sexual vs. asexual reproduction in *S. cernua* (Catling, 1982). More megagametophytes could mature if the development of adventitious embryos was suppressed or delayed. There was no visible sign of early capsule formation in the treated flowers. Examination of ovules at different stages of flower development would help clarify this issue and has been suggested previously (Catling, 1982).

We have assumed that empty seeds represent aborted embryos caused by incompatible crosses. This assumption is supported by the observation that 29% of the seeds were empty in the sexual species, *S. vernalis*. Other orchid studies have used empty seed coats to estimate the success of hybrid crosses (Kallunki, 1981) and to determine self-compatibility (Ackerman, 1989). While both meiotic and mitotic embryos can abort, it appears to be more frequent in sexually derived seeds. The proportion of empty seeds was greatest in the xenogamous and geitonogamous crosses with maximum values of 0.306 and 0.316, respectively.

The use of seed morphology in the present study to quantify sexual vs. asexual reproduction in *S. cernua* has raised many questions. However, in comparisons with its sexual relative, *S. vernalis*, the species distinction was clear and individual variation was minimal. This method may be most valuable for comparing sexual species to agamosperous species. Complete understanding of the balance between agamosperous and sexual seed production in *S. cernua* awaits further developmental and genetic information.

LITERATURE CITED

- ACKERMAN, J. D. 1975. Reproductive biology of *Goodyera oblongifolia* (Orchidaceae). *Madrono* 23: 191-198.
- . 1981. Pollination biology of *Calypso bulbosa* var. *occidentalis* (Orchidaceae); a food deception system. *Madrono* 28: 101-110.
- . 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). *Systematic Botany* 14: 101-109.
- AMES, O. 1921. Notes on New England orchids. I. *Spiranthes*. *Rhodora* 23: 73-85.
- ANDERSON, R. C., AND S. SCHELFHOUT. 1980. Phenological patterns among tallgrass prairie plants and their implications for pollinator competition. *American Midland Naturalist* 104: 253-263.
- ASKER, S. 1979. Progress in apomixis research. *Heredity* 91: 231-240.
- BIERZYCHUDEK, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41: 1255-1264.
- . 1989. Environmental sensitivity of sexual and apomictic *Antennaria*: do apomicts have general-purpose genotypes? *Evolution* 43: 1456-1466.
- BOYDEN, T. C. 1982. The pollination biology of *Calypso bulbosa* var. *americana* (Orchidaceae). Initial deception of bumblebee visitors. *Oecologia* 55: 178-184.
- CATLING, P. M. 1980. Systematics of *Spiranthes* L. C. Richard in northeastern North America. Ph.D. dissertation, University of Toronto, Toronto.
- . 1982. Breeding systems of northeastern North American *Spiranthes* taxa (Orchidaceae). *Canadian Journal of Botany* 60: 3017-3039.
- . 1987. Notes on the breeding systems of *Sacoila lanceolata* (Aublet) Garay (Orchidaceae). *Annals of the Missouri Botanical Garden* 74: 58-68.
- CLAUSEN, J. 1954. Partial apomixis as an equilibrium system in evolution. *Caryologia* 6: 469-479 (Supplement).
- COLE, F. R., AND D. H. FIRMAGE. 1984. The floral ecology of *Platanthera blephariglottis*. *American Journal of Botany* 71: 700-710.
- DAVIS, R. W. 1986. The pollination biology of *Cypripedium acaule* (Orchidaceae). *Rhodora* 88: 445-450.
- DE WET, J. M. J., AND H. T. STALKER. 1974. Gametophyte apomixis and evolution in plants. *Taxon* 23: 689-697.
- FIRMAGE, D. H., AND F. R. COLE. 1988. Reproductive success and inflorescence size of *Calopogon tuberosus* (Orchidaceae). *American Journal of Botany* 75: 1371-1377.
- GLESNER, R. R., AND D. TILMAN. 1978. Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis in terrestrial animals. *American Naturalist* 112: 659-673.
- GRANT, V. 1981. Plant speciation. Columbia University Press, New York, NY.
- GREGG, K. B. 1975. The effect of light intensity on sex expression in species of *Cynoches* and *Catasetum*. *Selbyana* 1: 101-113.
- GUSTAFSSON, A. 1946. Apomixis in higher plants. *Lunds Universitets Asskrift* 42-43: 1-370.
- HOGAN, K. P. 1983. The pollination biology and breeding system of *Aplectrum hyemale* (Orchidaceae). *Canadian Journal of Botany* 61: 1906-1910.
- KALLUNKI, J. A. 1981. Reproductive biology of mixed-species populations of *Goodyera* (Orchidaceae) in northern Michigan. *Brittonia* 33: 137-155.
- KAUL, R. B., AND S. B. ROLFSMEIER. 1987. The characteristics and phylogeographic affinities of the flora of Nine-Mile Prairie, a western tall-grass prairie in Nebraska. *Transactions of the Nebraska Academy of Science* XV: 23-35.
- KNOX, R. B. 1967. Apomixis: seasonal and population differences in a grass. *Science* 157: 325-326.
- LAKSHMANAN, K. K., AND K. B. AMBEGAOKAR. 1984. Polyembryony. In B. M. Johri [ed.], *Embryology of angiosperms*, 445-474. Springer-Verlag, New York, NY.
- LEAVITT, R. G. 1900. Polyembryony in *Spiranthes cernua*. *Rhodora* 2: 227-228.
- LLOYD, D. G. 1984. Variation strategies of plants in heterogenous environments. *Biological Journal of the Linnean Society* 21: 357-385.
- . 1988. Benefits and costs of biparental and uniparental reproduction in plants. In R. E. Michod and B. R. Levin [eds.], *The evolution of sex*, 233-252. Sinauer, Sunderland, MA.
- LUER, C. A. 1975. The native orchids of the United States and Canada, excluding Florida. The New York Botanical Garden, New York, NY.
- LYMAN, J. C., AND N. C. ELLSTRAND. 1984. Clonal diversity in *Taraxacum officinale* (Compositae), an apomict. *Heredity* 53: 1-10.
- MANNING, J. T. 1981. The "survivor effect" and the evolution of parthenogenesis and self-fertilization. *Journal of Theoretical Biology* 93: 491-493.
- MARSHALL, D. R., AND A. H. D. BROWN. 1974. Estimation of the level of apomixis in plant populations. *Heredity* 32: 321-333.
- , AND B. S. WEIR. 1979. Maintenance of genetic variation in apomictic plant populations. *Heredity* 42: 159-172.
- MEHRHOFF, L. A. 1983. Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70: 1444-1453.
- MICHAELS, H. J., AND F. A. BAZZAZ. 1986. Resource allocation and demography of sexual and apomictic *Antennaria parlinii*. *Ecology* 67: 27-36.
- , AND ———. 1989. Individual and population responses of

- sexual and apomictic plants to environmental gradients. *American Naturalist* 134: 190–207.
- MOGIE, M. 1985. Morphological, developmental and electrophoretic variation within and between obligately apomictic *Taraxacum* species. *Biological Journal of the Linnean Society* 24: 207–216.
- . 1988. A model for the evolution and control of generative apomixis. *Biological Journal of the Linnean Society* 35: 127–153.
- , AND H. FORD. 1988. Sexual and asexual *Taraxacum* species. *Biological Journal of the Linnean Society* 35: 155–168.
- MOSQUIN, T. 1970. The reproductive biology of *Calypso bulbosa* (Orchidaceae). *Canadian Field Naturalist* 84: 291–296.
- NOGLER, G. A. 1984. Gametophytic apomixis. In B. M. Johri [ed.], *Embryology of angiosperms*, 474–518. Springer-Verlag, New York, NY.
- OWCZARZAK, A. 1952. A rapid method for mounting pollen grains. *Stain Technology* 27: 249–251.
- RADFORD, A. E., W. C. DICKISON, J. R. MASSEY, AND C. R. BELL. 1974. *Vascular plant systematics*. Harper and Row, New York, NY.
- RICHARDS, A. J. 1986. *Plant breeding systems*. George Allen and Unwin, London.
- ROBERTSON, J. L., AND R. WYATT. 1990. Reproductive biology of the yellow-fringed orchid, *Platanthera ciliaris*. *American Journal of Botany* 77: 388–398.
- SAS INSTITUTE. 1985. *SAS user's guide: basics*, version 5 ed. SAS Institute, Inc., Cary, NC.
- SHEVIK, C. J. 1976. *Biosystematic study of the *Spiranthes cernua* complex with emphasis on the prairies*. Ph.D. dissertation, Harvard University, Cambridge, MA.
- . 1982. *Biosystematic study of *Spiranthes cernua* complex*. Bulletin No. 448. New York State Museum, Albany, NY.
- , AND P. M. CATLING. 1980. The identity and status of *Spiranthes ochroleuca* (Rydberg) Rydberg. *Journal of the New England Botanical Club* 82: 525–562.
- SNOW, A. A., AND D. F. WHIGHAM. 1989. Costs of flower and fruit production in *Tipularia discolor* (Orchidaceae). *Ecology* 70: 1286–1293.
- STEIGER, T. L. 1930. Structure of prairie vegetation. *Ecology* 11: 170–217.
- SWAMY, B. G. L. 1948. Agamospermy in *Spiranthes cernua*. *Lloydia* 1: 149–162.
- THIEN, L. B., AND B. G. MARCKS. 1972. The floral biology of *Arethusa bulbosa*, *Calopogon tuberosus*, and *Pogonia ophioglossoides* (Orchidaceae). *Canadian Journal of Botany* 50: 2319–2325.
- WIENS, D. 1984. Ovule survivorship, brood size, life history, breeding systems, and reproductive success in plants. *Oecologia* 64: 47–53.
- , C. L. CALVIN, C. A. WILSON, C. I. DAVERN, D. FRANK, AND S. R. SEAVEY. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 71: 501–509.
- WHIGHAM, D. F., AND M. McWETHY. 1980. Studies on the pollination ecology of *Tipularia discolor* (Orchidaceae). *American Journal of Botany* 67: 550–555.
- WILLIAMSON, C. J. 1981. The influence of light regimes during floral development on apomictic seed production and on variability in resulting seedling progenies of *Poa ampla* and *P. pratensis*. *New Phytologist* 87: 769–783.
- ZAR, J. H. 1984. *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, NJ.