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Comparisons of Genetic Variation and Outcrossing Potential Between the Sensitive Species *Rudbeckia fulgida* var. *sullivantii* (Asteraceae) and Its Cultivar

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ABSTRACT

Rudbeckia fulgida var. *sullivantii* (Sullivant's Coneflower, Asteraceae) is a target species for conservation and restoration at the Midewin National Tallgrass Prairie in Will County, Illinois because it is classified as a sensitive species by the USDA Forest Service. This species also has an extensively used cultivar named 'Goldsturm.' To aid in the restoration efforts of this species, the genetic diversity and cross pollination potential of *R. fulgida* var. *sullivantii* populations in Illinois were investigated. Random amplified polymorphic DNA results show high levels of genetic diversity in most of the populations surveyed. Little difference in genetic variation was observed between wild and cultivar populations. Cross pollination potential was confirmed as reciprocal crosses between wild and cultivar plants set seeds. Until additional research is conducted to understand better the reproductive and ecological consequences of cross pollination between wild and cultivar populations, these populations should be kept separate to prevent gene flow and maintain the genetic integrity of the wild populations.

Key words: cross pollination, cultivar, genetic diversity, Goldsturm, Midewin National Tallgrass Prairie, RAPD, *Rudbeckia fulgida* var. *sullivantii*

INTRODUCTION

A major problem for conservation managers in the preservation, (re)introduction, and management of sensitive, threatened, or endangered species is the lack of species-specific information. The successful implementation of an effective management plan for these

species depends on understanding the basic biology of the species as well as the overall genetic diversity found in populations (Timmerman-Erskine and Boyd, 1999). Knowledge of this information helps managers decide how to alleviate stresses brought through demographic, environmental, and genetic stochasticity. Specifically, loss of genetic variation is thought to reduce the ability of populations to adapt to changing environments, and to increase their susceptibility to pest and disease pressures (Barrett and Kohn, 1991).

The genetic variation of a species can be impacted by its reproductive biology (Wong and Sun, 1999), population size (Goodell et al., 1997), and proximity of populations to one another that affects gene flow (Foré and Guttman, 1999; Morris, 1993). In small, isolated populations, genetic drift combined with the potential increase in inbreeding can reduce genetic variation (Ellstrand and Elam, 1993; Foré and Guttman, 1999). This reduction is especially of concern in outcrossing species, which can be more susceptible to inbreeding depression than species with a long history of inbreeding (Treuren et al., 1993). Historically inbreeding populations may show a decrease in deleterious recessive alleles as these alleles become homozygous and are purged by selection against them. Outbreeding populations, on the other hand, are expected to show a higher frequency of deleterious recessive alleles that are not purged during many generations of inbreeding because they can be hidden from selection in heterozygous individuals (Treuren et al., 1993). This increased susceptibility to inbreeding depression is why sufficient gene flow in outbreeding populations is especially important.

On the other hand, gene flow between populations also can lead to outbreeding depression; the reduction of fitness following intraspecific hybridization between individuals from spatially separated genetic sources (Barrett and Kohn, 1991). This outbreeding depression can occur when populations are locally adapted to environmental conditions and hybrids between the populations are less fit in either location. A decrease in fitness could eventually decrease population size and therefore reduce genetic variability. Both inbreeding and outbreeding depressions reduce genetic variation, and affect the flexibility with which a population can respond successfully to changing abiotic and biotic environmental conditions by limiting the expression of beneficial alleles (Ellstrand and Elam, 1993; Hueneke, 1991; Luijten et al., 2000). Therefore, it is important to determine the distribution and levels (i.e., within and among) of a species' genetic diversity in order to more effectively apply conservation and restoration programs (Holsinger and Gottlieb, 1991).

An additional factor influencing the effectiveness of conservation and restoration programs is the threat of gene flow between wild species and their cultivated forms. Several studies have shown gene flow between wild types and cultivars resulting in the long-term establishment of cultivar alleles in wild populations (Barbour et al., 2002; Burke et al., 2002; Ellis et al., 2006; Ellstrand et al., 1999; Whitton et al., 1997). These studies also highlight additional concerns regarding the effects of this gene flow such as the facilitation and evolution of weedy and invasive species (Burke et al., 2002), pollen swapping resulting in extensive introgression and even extinction by hybridization (Barbour et al., 2002; Wolf et al., 2001), and the reduction of a population's growth rate by adversely affecting its reproductive effectiveness, its competitive status, and its interactions with pathogens and herbivores (Levin et al., 1996). While many studies have focused on

hybridization and gene flow within large-scale agriculture systems (Ellstrand, 1992; Ellstrand, 2003; Ellstrand et al., 1999; Linder et al., 1998; Lu and Snow, 2005; Raybould and Gray, 1993; Snow et al., 2005), the cultivation of one or more plants in a small scale backyard setting can also result in low levels of gene flow (Ellstrand et al., 1999).

At Midewin National Tallgrass Prairie (MNTP), a USDA Forest Service unit in Will County, IL, there are a number of sensitive, threatened, and endangered species. One of these species, *Rudbeckia fulgida* Ait. var. *sullivantii* (C.L. Boynt and Beadle) Cronq. (Sullivant's Coneflower, Asteraceae), a perennial prairie forb, is listed as sensitive by the USDA Forest Service (USDA Forest Service, 2001). The sensitive status indicates this species is susceptible or vulnerable to habitat alterations and/or management activities, resulting in viability concern for its long-term persistence (USDA Forest Service, 2001). As with many sensitive, threatened, and endangered species, no information is available about its population genetic diversity. In addition, this species has a very popular world-wide cultivar known as *R. fulgida* var. *sullivantii* 'Goldsturm.'

Because of limited information on the genetic diversity of this species and the popularity of its cultivar, the objectives of our study were to determine: 1) the genetic diversity of *R. fulgida* var. *sullivantii* populations found at MNTP and compare diversity levels with the cultivar 'Goldsturm' and, 2) whether cross pollination occurs between the wild type and cultivar forms. We believe that this information can aid in the proper development of a conservation management plan that addresses concerns of gene flow between wild type and the cultivar for this species.

MATERIALS AND METHODS

Species Description

Rudbeckia fulgida var. *sullivantii* - wild type. This species is found in nine states, mostly in the midwest to eastern United States (Urbatsch and Cox 2006; USDA, NRCS, 2004). Currently, only Illinois and Michigan consider the species rare, while states such as New York and Ohio consider it common (Molano-Flores, 2005). In Illinois, the species is documented in 12 of 102 counties. *Rudbeckia fulgida* var. *sullivantii* grows between 30-70 cm tall (USDA Forest Service, 1999). This species has alternate, lanceolate to ovate leaves with sharply dentate margins and primarily pinnate venation (Urbatsch and Cox 2006, USDA Forest Service, 1999). There are typically several terminal inflorescences on an individual, each with 2.5-4 cm orange-yellow rays (Gleason and Cronquist, 1991; USDA Forest Service, 1999). This species blooms from mid-July through late September and forms discrete colonies through asexual propagation via stolons (Gleason and Cronquist, 1991, Urbatsch and Cox 2006). Scott (2005) determined that *R. fulgida* var. *sullivantii* is mostly a self-incompatible species. The primary pollinators for *R. fulgida* var. *sullivantii* are considered generalists. The most common insect families visiting the inflorescences are: Apidae, Cantharidae, Halictidae, Hesperiidae, Nymphalidae, Pieridae, and Syrphidae (Scott, 2005). In Illinois, *R. fulgida* var. *sullivantii* is found in woodland edges, old pasture, mesic prairie, and along roadsides (Molano-Flores, 2005).

Rudbeckia fulgida var. *sullivantii* - 'Goldsturm' cultivar. This rhizomatous perennial cultivar can grow taller than the wild type (to heights of 75 cm). Like the wild type, leaves are dark green and range from lanceolate to ovate. 'Goldsturm's golden-yellow ray

florets can be slightly longer than the wild type ranging between 2.5-5 cm long. 'Goldsturm' tends to form tight clumps whereas the growth habit of the wild type is more spaced between ramets. This cultivar blooms from mid-July to October, and can survive in a wide range of temperatures from Florida to Alberta, Canada in hardiness zones 3-9 (Green Beam, 2004; PPA, 2002). 'Goldsturm' is said to grow best in well-drained, consistently moist soils, but will tolerate clay to sandy soils and mild droughts (PPA, 2002). This cultivar originated in Germany in 1937, but was not used extensively in Europe until 1949 (PPA, 2002). Between the 1960's and the 1980's, 'Goldsturm' was brought to the United States (Green Beam, 2004). Here its increase in popularity was aided by Kurt Bluemel, a perennial grower and Wolfgang Oehme, a landscape architect (Green Beam, 2004). This cultivar was originally selected for uniform height and bloom time; however, stringent selection for these traits has not been retained due to ease of seed propagation (R. Diblick, Northwind Perennial Farm Nursery Grower, pers. comm. 2003). In 1999, 'Goldsturm' was selected as the perennial plant of the year (PPA, 2002). One reason for this honored designation was this cultivar's ability to grow well in diverse climates (PPA, 2002). Finally, this cultivar is propagated by seed, division, or stem cuttings.

Study Sites

This study was conducted using eight populations in Will County northeastern Illinois from 2003-2004, primarily at MNTP (six populations), but also included one population at Grant Creek Prairie (GCP), and one population along Illinois Route 53 (Rt. 53). Habitat for these wild populations includes woodland edge, old pasture, mesic prairie, and roadside. At MNTP, the species is found from multiple dense populations to scattered individuals across the site.

Population Sampling Genetic Diversity

Wild Type. In 2003, leaf sampling for genetic analysis took place at MNTP, GCP, and along Illinois Rt. 53. The number of individuals sampled from each population ranged from 19-30 depending on population size. Two to four leaves per individual were collected, with particular attention paid to collecting leaf tissue from individuals separated by at least 91 cm (3 feet) to minimize collecting from genetically identical individuals. Leaf tissue was placed in a dry coin envelope and kept in a dry place until the tissue could be ground for DNA extraction.

Cultivar. In 2003, two nurseries were visited and at each nursery between two to four leaves were collected from 24 individuals to represent genetic diversity found in the cultivar. The first nursery, Green Glen Nursery (GGN), is located in Joliet, IL near MNTP. The second nursery, Country Arbors Nursery (CAC), is located in Urbana, IL. The leaves were placed in dry coin envelopes and kept in a dry place until they could be ground for DNA extraction.

Genetic Analysis

Random amplified polymorphic DNA (RAPD) procedures followed those of Koontz et al. (2001), with the exceptions listed below. DNA was extracted from approximately 20 mg of dried leaf tissue using the Wizard DNA extraction Kit (Promega, Madison, WI) following the manufacturer instructions. The ground leaf material was incubated in the nuclei lysis solution at 65° C for at least 45 minutes. DNAs were quantified with a SmartSpec 3000 spectrophotometer (Bio-Rad, Hercules, CA) and diluted to a standard 10

ng/μl with TE pH 8.0 buffer. The RAPD primers Operon (Qiagen; Valencia, CA) A1, A4, B3, B7, B8 and B12 were selected after screening a total of 36 primers. The primers selected showed the most robust, repeatable markers. To ensure repeatability, select samples were run multiple times. Loci that failed to repeat were dropped from the analysis. The RAPD reactions were separated on a 1.5% agarose (Amresco Type I, Solon, OH) gel using 0.5x TBE buffer. Gels were run until a bromophenol blue dye marker had migrated 8 cm. Fragments were sized with a 1 Kb DNA ladder (Promega). Gels were stained for 20 minutes using ethidium bromide, destained in distilled water for 30 minutes, and then visualized and photographed in UV light using the Kodak 1D analysis software (Kodak 1D Image Analysis Software, 2000). To help reduce scoring bias, this software was used to score and size the resulting RAPD loci. The band sensitivity was adjusted to -3 for the most conservative scoring.

Genetic diversity levels were determined using percent polymorphic loci. A locus was considered monomorphic if present in 95% or more of the individuals. To assess the similarity of wild and cultivar populations, Shannon's Information Index was calculated using POPGENE (Yeh and Boyle, 1997). DistAFLP (Mougel et al., 2002) was used to create a Jaccard similarity matrix from the raw RAPD data. The matrix then was used in ARLEQUIN (Schneider et al., 2000) to run two Analysis of Molecular Variance (AMOVA): one that separated the wild and cultivar populations, and one that combined all the populations together. A principal coordinates analysis (PCo) was used to analyze the raw binary presence absence data using the R-package (v. 4.0, Casgrain and Legendre, 2001). JMPin (v. 5.1, SAS Institute, 2003) was used to display graphically RAPD marker distributions by plotting the first four eigenvectors from the PCo.

Cross Pollination Potential - Greenhouse Crosses

In June 2003, 30 wild plants were transplanted from population 4 at MNTP to the Illinois Natural History Survey (INHS) greenhouses in Champaign, IL. Transplanting took place early in the growing season before the development of stems to ensure individual plants would establish well in the greenhouse environment. Crosses were done in 2003 and 2004 at the INHS greenhouses between the transplanted wild individuals and cultivar individuals purchased from the Green Glen Nursery in Joliet, IL. It should be noted that the same wild individuals were used for the crosses in 2003 and 2004. However, in 2004 it became evident that the cultivar plants purchased from Green Glen Nursery in Joliet, IL were not going to bloom that year. Therefore, new cultivar plants that were expected to bloom were purchased from Country Arbors Nursery in Urbana, IL and used for the crosses. In both years, newly forming wild and cultivar inflorescences were bagged with bridal veil to reduce pollen contamination from stray pollinators in the greenhouse. Bridal veil bags have been extensively used to exclude the great majority of pollinators. Nonetheless small insects such as thrips, which have been shown to be effective pollinators may not have been excluded. This could account for the observed seeds that were found throughout the inflorescence where hand cross-pollination had not occurred in this study (see Results section). Reciprocal crosses between the wild type and cultivar were made as follows; florets used for crosses were marked with pink nail polish, and using fine tip forceps pollen was squeezed out from the anther tube of a donor plant and place on the receptive stigma of recipient plant. Twenty-six wild heads were bagged and pollinated with cultivar pollen. An average of 7 florets per head, were pollinated for a total of 165 wild type florets. Eighteen cultivar heads were bagged and pollinated with wild type pol-

len. On average, 6 florets per head were pollinated, for a total of 118 cultivar florets. A two-way ANOVA was used for the analysis followed by a post-hoc Fisher's LSD multiple comparison test (SigmaStat, 1997).

RESULTS

Genetic Diversity

RAPD results produced a total of 35 repeatable loci to score for each of the 10 populations. No loci were unique to individual populations; however, cultivar populations did not contain a locus that was observed in the wild populations (primer-fragment size: B7-1980; Table 1). In the eight wild and two cultivar populations tested, 25 loci were missing in individual populations (Table 1). Because of repeatability issues and the dominant nature of the RAPD data, population genetic inferences were restricted to percent polymorphic loci. The average percent polymorphic loci (*P*) for all 10 populations was 45%, ranging from 14.3% (population 6) to 68.6% (population 9; Table 2). Percent polymorphic loci for cultivar populations fell within the wild population range (CAC - 40.0% and GGN - 62.8%).

The Shannon's Information Index partitioned diversity between the wild and cultivar populations. Results from this test supported the percent polymorphic loci findings. The total population index was 0.61. Wild type populations had an index value of 0.59, while the cultivar populations had an index of 0.52.

When the wild and cultivar populations were combined, AMOVA results indicated significant structure both within and among populations, with most of the variation found within populations (64.24%; Table 3a). When the wild and cultivar populations were separated, significant structure existed at all hierarchy levels (among populations, among populations within groups, and within populations), again with most of the variation found within populations (63.06%; Table 3b).

The plot resulting from the PCo analysis of the RAPD data separates the wild populations Rt. 53 and population 5 from one another as well as the remaining wild and cultivar populations (Figure 1). All other populations (wild and cultivar) aggregate to form a relatively indistinguishable group of individuals (Figure 1).

Cross Pollination Potential

In 2003, 23 seeds developed from 165 wild florets crossed with cultivar pollen (Figure 2). The 118 cultivar florets crossed with wild pollen formed 26 seeds (Figure 2). In 2004, 50 seeds developed from 205 wild florets crossed with cultivar pollen. Of 135 cultivar florets crossed with wild pollen, 2 seeds were formed (Figure 2). A two-way ANOVA showed no significant differences between year or treatment ($F=1.705$, $df=1$, $P=0.195$ and $F=3.155$, $df=1$, $P=0.080$, respectively), but did show a significant interaction term ($F=14.359$, $df=1$, $P<0.001$). It should be noted that in all cases seeds were found throughout the inflorescence where hand cross-pollination had not occurred.

DISCUSSION

Conservation managers are continually challenged with the need to protect sensitive, threatened, and endangered species. In order to conserve these rare species effectively, adequate species-specific information is needed. In conservation, some of this information includes a thorough understanding of the species' genetic diversity (Holsinger and Gottlieb, 1991). The threat of gene flow between wild species and their associated cultivar also presents conservation managers with the need to understand the potential risk of hybridization (Ellstrand, 1992). This study on the sensitive species *Rudbeckia fulgida* var. *sullivantii* helps to provide information to conservation managers, enabling successful long-term management of this species. This information can also be applied to other species facing similar threats.

Genetic Diversity

Genetic diversity levels in *R. fulgida* var. *sullivantii* expressed as percent polymorphic loci (*P*), showed a wide range in diversity levels encompassing both the wild and cultivar populations (from 14.3% in population 6 to 68.6% in population 9). Of all the populations sampled, population 6 was the smallest in size (less than 50 ramets) and had the lowest percent polymorphic loci (14.3%). This result is similar to other studies that found smaller populations have lower genetic diversity than larger ones (Fischer and Matthies, 1998; Luijten et al., 2000). The lower levels of genetic diversity in this population may be the result of few genetically diverse founding individuals or loss of genetic diversity as the result of individual loss due to mowing, grazing, or herbicide application at the site.

Few studies on other *Rudbeckia* species have been done to compare levels of genetic diversity with *R. fulgida* var. *sullivantii*. However, one study that used Restriction Fragment Length Polymorphisms (RFLPs) to look at isolated *Rudbeckia missouriensis* populations in Missouri glades, found very low levels of overall diversity (5%; King and Schaal, 1989). While higher diversity levels are expected when using RAPDs, the discrepancy between 5% and an average of about 50% in *R. fulgida* var. *sullivantii* is substantially different. A study done on a related genus using allozyme markers showed varying degrees of genetic variation depending on whether the species was endemic or more widespread (widespread *Echinacea angustifolia* – 40.1% *P* and the endemic *Echinacea tennesseensis* – 23.0% *P*; Baskauf et al., 1994). Within Asteraceae, levels of percent polymorphic loci (using allozyme data) average 45.3% (Hamrick and Godt, 1996). While direct comparisons of these different studies are not possible given that they examined different genera and species as well as different molecular markers, the totality of data can at least be used to approximate expected levels of diversity for *R. fulgida* var. *sullivantii* and 'Goldsturm.'

Diversity levels of cultivar populations are a function of both the biological characteristics of the species and the cultural breeding practices (Godt and Hamrick, 1991). Within cultivated plant populations, genetic variation is either deliberately reduced or enhanced (Godt and Hamrick, 1991). In the case of *R. fulgida* var. *sullivantii*, the plant was cultivated initially for uniform height and bloom time; however, owing to the popularity of the plant and ease of seed and vegetative propagation, the rigorous standards for specific trait selection are no longer followed (R. Diblick, Northwind Perennial Farm Nursery Grower, pers. comm., 2003). This lack of trait selection may have contributed to the

similar diversity levels found in 'Goldsturm' when compared to the wild type (as seen here in percent polymorphic loci, Shannon's Information Index, and the Principal Coordinates Analysis). However, the unique wild locus (B7-1980) combined with loci that were common in wild populations, but only present in sporadic cultivars (B7-510 and B8-610), indicate the importance of maintaining the genetic integrity of the wild populations by reducing contact with the cultivar. Physical separation of the wild and cultivar is recommended since it could reduce possible gene flow which, as other studies have shown, can result in hybridization, loss of genetic diversity or even extinction of the wild type (Ellstrand et al., 1999; Ellstrand, 2003; Lu and Snow, 2005; Rhymer and Simberloff, 1996; Snow et al. 2005).

The analysis of molecular variance indicates most of the genetic differentiation is partitioned within as opposed to among both wild and cultivar populations of *R. fulgida* var. *sullivantii* (64.24%; Table 3b). This partitioning of variation is expected for a perennial, outcrossing species because of increased gene flow between individuals and populations (therefore populations are genetically more similar). It is also an important factor to keep in mind when considering management strategies that seek to monitor and maintain population-level genetic diversity (Hamrick et al., 1991).

The Principle Coordinates Analysis (PCo) showed all cultivar and wild populations (except populations 5 and Rt. 53) to have similar RAPD marker distributions (Figure 1). Of the 35 loci analyzed, no allele was observed for nine and six loci in populations 5 and Rt. 53, respectively. On average, the remaining populations sampled lacked the presence of an allele in only one of the 35 loci. For the Rt. 53 population, this difference could possibly be explained by its isolated location from other MNTP populations as well as by its small size (200 ramets or less). Both the degree of isolation and size of a population increase the risk of genetic drift, potentially decreasing genetic diversity in a population (Ellstrand and Elam, 1993). It is possible that a slight decrease in genetic diversity is already seen in Rt. 53 based on its lower percent polymorphic loci (34.3%) relative to other populations (Table 2). These results suggest within a small geographic range (5 miles) distinct genetic differences between populations can be found. Additional research should be done to see if differences exist between populations throughout Illinois as well as the entire range of *R. fulgida* var. *sullivantii*.

Unlike Rt. 53, population 5 is located within MNTP. A different feature of population 5 that could account for its separation in the PCo plot is the habitat where samples were collected. This dispersed population was small and located on either side of a railroad. One part of the population was adjacent to a forested area with taller vegetation surrounding individual plants, while the portion on the other side of the railroad was located in a more open old field. A majority of the other populations sampled were located in open prairie habitat. Another likely reason for the differences could be the reduced number of RAPD loci scored for this population (i.e., five loci were scored as missing).

Cross Pollination Potential

Studies have shown gene flow provides an avenue for the transfer of genes from cultivated to natural populations (Burke et al., 2002; Linder et al., 1998; Whitton et al., 1997). Through gene flow, these introduced genes have shown increased invasiveness, weediness, and even extinction (Barbour et al., 2002; Burke et al., 2002). In both 2003 and

2004, reciprocal greenhouse crosses of *R. fulgida* var. *sullivantii* and 'Goldsturm' resulted in seeds set, suggesting that gene flow is possible between the wild and cultivar form. The risk of the transfer of genes can be considered high because bloom time for both the cultivar and wild populations is the same. In addition, the growing popularity of 'Goldsturm' in home gardens (evidenced by its perennial plant of the year award in 1999) increases the likelihood of gene flow between the wild and cultivar forms.

Although the two-way analysis of variance did not show significant differences between years, the significant interaction draws attention to the low seed set found in 2004 when crossing the cultivar inflorescences with wild pollen. One possible reason for the large variation between cultivar seed set in 2003 and 2004 could be that the cultivar plants for each year were purchased from two separate nurseries. Cultivar varieties may differ between nurseries due to different seed sources, age, and/or size of plants resulting in varying levels of seed set. Also, issues of pollen contamination in 2003 due to the bridal veil touching the inflorescences may have inflated seed set and contributed to differences found between years. Finally, because the reciprocal crosses were conducted in a greenhouse, additional research is needed to determine if cross-pollination is actually occurring between populations within MNTP and cultivars planted at adjacent sites (e.g., home gardens). If this is the case, then, the conservation and ecological consequences for the wild type should be further investigated.

Conclusion

Overall, these results suggest that within MNTP unique genetic differences occur among the wild populations. While little genetic difference appears between *R. fulgida* var. *sullivantii* and 'Goldsturm' populations, loci differences combined with confirmed gene flow potential indicate the importance of keeping the wild and cultivar populations separate to preserve the wild populations' genetic integrity. Finally, this study adds to the increasing number of works (Van Gaal et al., 1998; Whelan et al., 2006) voicing concern about the potential of gene flow between cultivated forms of native plants in home gardens with their wild type relatives.

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Table 1. RAPD markers missing in individual wild and cultivar populations of *Rudbeckia fuligda* var. *sullivantii*. Asterisks indicate loci were present in two or fewer of the individuals sampled. CAC, Country Arbors Nursery; GGN, Green Glen Nursery; Rt. 53, Route 53; GCP, Grant Creek Prairie, and MNTP, Midewin National Tallgrass Prairie.

Populations	RAPD loci absent
CAC (cultivar)	B7-1980
GGN (cultivar)	B7-1980
GCP	A4-100
MNTP-9	A4-100, A1-920*
MNTP-7	A4-100
MNTP-6	A4-100
MNTP-5	A1-645*, A1-1800, A4-575*, A4-910*, B7-1500, B7-1980, B8-700*, B8-980*, B8-1150, B8-1500
MNTP-4	None
MNTP-3	None
Rt. 53	A1-730, A1-1800, A4-445, A4-630, A4-1450, B7-1500, B7-1710, B7-1980

Table 2. Percent polymorphic loci of eight *Rudbeckia fulgida* var. *sullivantii* populations and two cultivar "populations" based on RAPD markers. A population is considered monomorphic if the locus is present in 95% or more of the individuals. Asterisks indicate cultivar populations. Abbreviations: N, number of individuals sampled; *P*, percent polymorphic loci; CAC, Country Arbors Nursery; GGN, Green Glen Nursery; Rt. 53, Route 53; GCP, Grant Creek Prairie, and MNTP, Midewin National Tallgrass Prairie.

Population	N	P
*CAC	24	40.0
*GGN	24	62.8
GCP	30	37.1
MNTP-9	30	68.6
MNTP-7	24	57.1
MNTP-6	19	14.3
MNTP-5	30	65.5
MNTP-4	30	40.0
MNTP-3	30	51.4
Rt.53	23	34.3

Table 3. Results of analysis of molecular variance (AMOVA) for all tested *Rudbeckia fulgida* var. *sullivantii* populations (cultivar and wild) under two alternative groupings (a) combining wild and cultivar populations together and (b) separating wild and cultivar populations.

	Df	Sums of squares	Variance components	% of variance
(a) All populations (wild type and cultivar) combined				
Among populations	9	19.689	0.074	35.76*
Within populations	266	35.683	0.134	64.24*
(b) Wild type and cultivar populations separated				
Among populations	1	2.407	0.005	2.70*
Among populations within groups	8	17.283	0.072	34.24*
Within populations	266	35.683	0.134	63.06*

* P< 0.001; significance tests after 1023 permutations

Figure 1. Graphical representation of Principal Coordinate Analysis showing RAPD marker distributions of *Rudbeckia fulgida* var. *sullivantii* cultivar populations (●) in relation to wild populations (x). Two distinct wild populations are labeled; population 5 (■), and Rt. 53 (○).

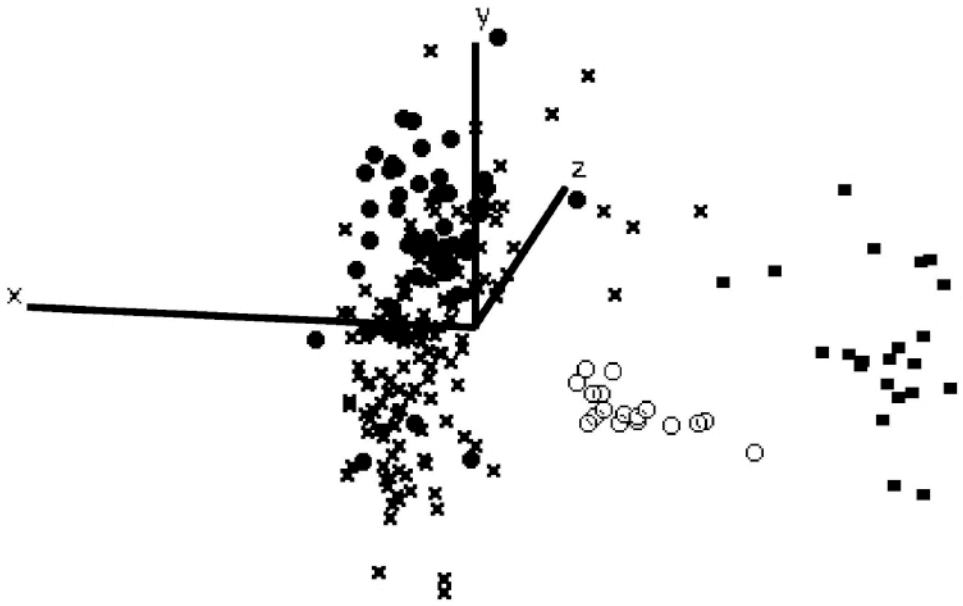

Figure 2. Percent seed set for 2003 and 2004 crosses between the wild type *Rudbeckia fulgida* var. *sullivantii* and cultivar 'Goldsturm.'