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Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae). II. Artificial populations

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Polyploidization has long been recognized as an important force in the diversification of plants. Theoretical models predict that polyploids may be expected to exhibit higher rates of self-fertilization than do closely related diploid species. Wild populations of the neopolyploid *Tragopogon mirus* (4*n*) exhibited slightly higher rates of outcrossing than did populations of one of its progenitors, *T. dubius* (2*n*). In the current study, outcrossing rates in populations of *T. dubius* and *T. mirus* were estimated using artificial arrays constructed to maximize the chances of detecting outcrossing events. The artificial diploid population is more highly outcrossing ($t = 0.727$; family-level estimates range from 0.00 to 1.32) than the tetraploid population ($t = 0.591$; family-level estimates range from 0.00 to 1.14), although the difference between them is not statistically significant. The results of this study, combined with those of the previous work on wild populations, suggest that mating systems in these species vary more among populations than between ploidal levels. This could be because of the relatively recent origins of the tetraploid species; there may have been insufficient time since the formations of the tetraploids for shifts in mating systems to occur.

Keywords: allozymes, mating system, polyploidy, *Tragopogon*.

Introduction

Although polyploidy has long been recognized as an important evolutionary force in the plant kingdom (reviewed in Cook & Soltis, 1999), many aspects of evolution via polyploidy remain poorly understood. Among the remaining unresolved issues surrounding polyploidy is the question of whether polyploids exhibit elevated levels of self-fertilization relative to their diploid progenitors. Several authors have predicted that one evolutionary consequence of polyploidization in plants is a shift in the mating system of the polyploid towards selfing (e.g. Busbice & Wilsie, 1966; Dewey, 1966; Townsend & Remmenga, 1968; Stebbins, 1971; Bennett, 1976; Grant, 1981; Lande & Schemske, 1985; Schemske & Lande, 1985; Richards, 1986; Hedrick, 1987; Barrett & Shore, 1989), and at least one neopolyploid is known to reproduce mainly by selfing (Ingram & Noltie, 1995). Such a shift might be precipitated by the necessity for the newly formed polyploid to be self-fertilizing to ensure its initial establishment and survival (e.g. Stebbins, 1971; Grant, 1981); later, it might be reinforced because of reduced genetic load in the polyploid (Hedrick, 1987) and the corresponding lower inbreeding depression (e.g. Lande & Schemske, 1985).

Estimates of plant mating systems can be obtained from either artificial or natural populations. Estimates from natural populations might be assumed to portray more accurately the patterns of mating in nature. However, most methods for estimating mating systems rely on genetic variation within a population to provide markers for detecting outcrossing events and calculating outcrossing rates. If there is low genetic variability in a natural plant population, outcrossing events between genetically identical individuals will go undetected, and outcrossing rates will be underestimated.

Artificial populations can be constructed such that the genetic variability required to detect outcrossing or gene flow is built in. Such a design can help to ensure that most, if not all, outcrossing events will be detected, resulting in much more accurate estimates of outcrossing

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or gene flow. This approach has been used successfully to measure gene flow and neighbourhood size in *Carduus nutans* (Smyth & Hamrick, 1987) and density-dependent outcrossing rates in *Mimulus ringens* (Karron *et al*., 1995), and to compare outcrossing rates among various floral morphs (Epperson & Clegg, 1987; Barrett & Eckert, 1990; Kohn & Barrett, 1992). The use of artificial arrays can eliminate certain confounding factors present in natural populations, such as population shape, size or density (Karron *et al*., 1995). However, artificial populations will not mimic exactly the conditions of their wild counterparts, and outcrossing estimates from artificial populations may also deviate from estimates from natural populations. A field site that is not visited by pollinators at the same rate as an established wild population, for example, might lead to lower pollinator activity and hence lower outcrossing rates in the artificial plot. By arranging the genetic variation in a constructed population, the experimenter might unwittingly also introduce bias towards or against outcrossing, if a particular genotype happened to be linked to an allele that promoted or inhibited outcrossing.

Combining the two approaches and comparing results from both artificial and natural populations can help overcome some of the difficulties inherent in each individual approach. To our knowledge, there are no examples in the literature that have compared outcrossing rates in artificial and natural populations, particularly with respect to comparing polyploids and their diploid progenitors. In the current study, results obtained from artificial populations are compared with those from an earlier study of natural populations (Cook & Soltis, 1999) of the same two plant species, a tetraploid and one of its diploid progenitors.

The *Tragopogon* polyploid complex in the Palouse region of eastern Washington and adjacent northern Idaho, U.S.A., is a model system within which to investigate differences in mating systems between polyploids and their diploid progenitors. This complex comprises three diploid species, *T. dubius*, *T. pratensis* and *T. porrifolius*, and their two tetraploid derivatives, *T. mirus* (the product of hybridization between *T. dubius* and *T. porrifolius*) and *T. miscellus* (the result of hybridization between *T. dubius* and *T. pratensis*) (Ownbey, 1950). The history of this complex is clearly documented (e.g. Ownbey, 1950; Ownbey & McCollum, 1953; 1954; Brehm & Ownbey, 1965; Roose & Gottlieb, 1976; Soltis & Soltis 1989; 1991; Soltis *et al*., 1995; Cook *et al*., 1998), and the group provides an excellent model for studying the evolutionary effects of polyploidy. Previous work used natural populations of *T. dubius* and *T. mirus* to test the prediction that polyploids will exhibit higher selfing rates than their diploid progenitors (Cook & Soltis, 1999); in the present study we use artificial population arrays to test further the hypothesis that populations of *T. mirus* will exhibit higher selfing rates than populations of its progenitor *T. dubius*.

Materials and methods

Wild populations as seed sources

Artificial populations were constructed of target plants, from a population known to be monomorphic for (or to have in very high frequency) one allele, and donor plants, from a population known to be monomorphic for (or to have in very high frequency) a different allele. Based on the results of an allozymic survey (Soltis *et al*., 1995), two wild populations of each species, *T. dubius* and *T. mirus*, were chosen as seed sources for the artificial arrays. Because these two species were evaluated in previous work (Cook & Soltis, 1999), they were again chosen for this study. Populations of *T. porrifolius*, the other diploid progenitor of *T. mirus*, had previously been shown to have very low levels of diversity (Soltis *et al*., 1995), and no diversity had been detected in the current seed sample (Cook & Soltis, 1999).

The population of *T. mirus* from Tekoa, WA, was fixed for *Lap-2a*/*a*, whereas the population of *T. mirus* from Pullman, WA, was fixed for *Lap-2a*/*b*. Thirty seeds collected from the Tekoa population were planted as targets, and 200 seeds from the Pullman population were planted as donors. The population of *T. dubius* from Juliaetta, ID, was reported to possess alleles *Est-3a* at a frequency of 0.818 and *Est-3b* at a frequency of 0.182, whereas the population of *T. dubius* from Troy, ID, was monomorphic for allele *Est-3b*. Thirty seeds from the population from Troy were planted as targets, and 200 seeds from the population in Juliaetta were planted as donors.

Growth

Seeds were planted in July 1996 in 30 cm tree pots in the WSU Botany Department Steffen Center Greenhouse. Seeds germinated in 7 to 10 days; none needed to be replanted. The seedlings were grown at ambient temperatures and watered daily until September 1996, when they were transported to a lathhouse at the WSU Botany Department Airport Gardens, where they overwintered. Seedlings were watered twice during the time it took them to enter dormancy; during the winter they were left alone. As a precaution against possible predation or losses during overwintering, 33 backup donor plants of each species were planted on 23 October 1996 and kept in the Steffen Center Greenhouse in a cold room to overwinter.

Artificial array design

One discrete array of target/donor blocks was used for *T. dubius*, and one for *T. mirus*. A target/donor block had the target plant at its centre, surrounded by eight donor plants. Arrays were laid out within a large ploughed area approximately 10×12 m. Individual plants were spaced at 45-cm intervals within each array, and arrays were spaced 12.75 m apart. The spacing within the arrays is comparable to that seen in natural populations (L. M. Cook, pers. obs.), and the spacing between arrays was designed to be great enough to discourage pollinator visits between arrays.

Overwintered seedlings from both the lathhouse and the greenhouse were planted in the arrays as follows (Fig. 1): the array of *T. dubius* comprised 10 target/donor blocks of nine plants each, as described above, and the array of *T. mirus* comprised nine target/donor blocks.

Planting of seedlings and harvest of seeds

The potted seedlings were planted in this design on 11 May 1997. Seedlings were left in their tree pots, and each pot was buried in soil to a depth of 25 cm. The populations were watered daily, between 07.00 and 09.00 hours, for the remainder of the month of May and the entire month of June, except on days when it rained. A few volunteer plants of wild *T. dubius* were observed growing between or around the plots; these were destroyed. As the plants bolted and flowered, each capitulum was allowed to open-pollinate. When each closed up to set seed, a fine-mesh nylon bag was secured over it to prevent the loss of any seeds when the head ripened and opened. Heads opened inside the bags, indicating that the seeds were ripe, and then the heads were cut off, labelled, and laid out to dry thoroughly before being placed in labelled envelopes for later planting in progeny arrays. The number of heads collected varied from plant to plant; in some cases only a few heads set seed, whereas in others, the heads numbered more than 20. The last heads were collected on 5 August 1997.

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Fig. 1 Example of artificial array. This sample shows an array of six target/donor blocks. Target plants are represented by **T**, and donor plants by D.

Seeds collected from these open pollinations were planted in the WSU Botany Department Steffen Center Greenhouse on 13 September 1997. In each flat, 104 seeds from one maternal (target) plant were planted (a few seeds from each head collected; the number of seeds planted per head varied depending on how many heads there were for the family), so that each flat represented one family. Eight families of *T. mirus*, and seven families of *T. dubius* were represented. Seeds germinated in 4 to 10 days, and seedlings were allowed to grow for 4 to 6 weeks at ambient temperatures.

Enzyme electrophoresis

Leaf material from all of the seedlings was analysed for enzyme variation following the procedures of Soltis *et al.* (1983), with modifications as detailed in Cook & Soltis (1999). Leaf extracts were adsorbed onto paper wicks and frozen at −80°C. Frozen wicks were thawed for electrophoresis by adding a drop of a solution of 10 mL water and 0.5 mL 2-mercaptoethanol, and enzymes were electrophoresed through 12.5% starch gels. Esterase (EST) and leucine aminopeptidase (LAP) were resolved on gel and electrode buffer system 8 as modified by Haufler (1985); staining for enzymes followed Soltis *et al.* (1995) and was modified as described in Cook & Soltis (1999).

Data analysis

Gels were scored and genotypes were assigned to seedlings. Outcrossing estimates, t, were obtained for each family and artificial array, and standard errors for the arrays of *T. dubius* and *T. mirus* were derived with 1000 bootstrap replicates using the computer program MLT (Ritland, 1990). The standard errors for the family-level estimates were derived using 100 bootstrap replicates.

Results

Of the nine target plants of *T. mirus*, eight bolted and produced heads that set seed, and of the 10 *T. dubius* targets, seven bolted and set seed. Overall germination rates within progeny arrays differed: *T. dubius* had the higher rate, 74.6%, followed by *T. mirus*, 66.9%.

Outcrossing estimates (t) varied both within and between species (see Table 1 for standard errors). Estimates of outcrossing for individual families within *T. dubius* ranged from 0.00 to 1.32, with a mean of 0.696. Family-level outcrossing estimates within *T. mirus* ranged from 0.00 to 1.14, with a mean of 0.633. The array of *T. dubius* was the more highly outcrossing, with $t = 0.727$, compared to the estimate obtained for the array of *T. mirus*, with $t = 0.591$.

Discussion

The population of *T. dubius* from Troy, ID, from which the seeds to grow the target plants for this study were collected, had been inferred to be fixed for allele *b* at *Est-3* (Soltis *et al.*, 1995). However, the target plants that derived from this population had genotypes *ab*, *ab*, *aa*, *ab*, *ab*, *ab* and *aa* at this locus, indicating that the *a* allele was probably also present but undetected in the sample described in 1995. Heterozygous maternal genotypes are taken into consideration by the data analysis, but the presence of homozygous *a* individuals among the maternal plants in the study raises the probability that some outcrossing events in the array may have gone undetected. The majority of the donor plants should have been homozygous for allele *a*; seeds for these donor plants were selected from families that had been surveyed and exhibited only allele *a* at this locus, although the donor plants themselves were not genotyped. Because of *aa* × *aa* potential crosses, the estimate of outcrossing obtained for this array may actually represent an underestimate. Interestingly, one of the homozygous *a* targets (family C in Table 1) produced heterozygous progeny and gave the highest family-level estimate of t; the *b* allele in these progeny could only have come from another, heterozygous target plant. Removing the two homozygous individuals and their progeny (families C and G) from the analysis results in an essentially unchanged mean of 0.71.

Table 1 Outcrossing estimates, t, obtained for artificial populations of *Tragopogon* and families within those populations, and the means of the family estimates. Standard errors are shown in parentheses

Species (Ploidy)	t (SE)	Family estimates of t (SE)	Mean
T. dubius (2*n*)	0.727 (0.082)	A: 0.06 (0.11) B: 1.15 (0.31) C: 1.32 (0.58) D: 1.15 (0.43) E: 0.58 (0.14) F: 0.61 (0.14) G: 0.00 (0.00)	0.696
T. mirus (4*n*)	0.591 (0.045)	A: 0.00 (0.00) B: 0.85 (0.20) C: 0.49 (0.19) D: 0.48 (0.17) E: 0.37 (0.16) F: 0.82 (0.25) G: 1.14 (0.26) H: 0.91 (0.22)	0.633

The estimate obtained for the array of *T. mirus* is expected to be more accurate; no unexpected alleles were detected in the target plants, so the appearance in the progeny of a *b* allele at *Lap-2* confirmed an outcrossing event.

The results obtained for these artificial population arrays of *T. dubius* and *T. mirus* seem to support the hypothesis that the polyploid species may show higher selfing rates than its diploid progenitor (however, see below for comparison with results from wild populations). The artificial array of *T. dubius* ($t = 0.727$) in this study was more highly outcrossing than that of *T. mirus* ($t = 0.591$). Although this difference was not statistically significant, it suggests that the tetraploid mating system might be in the process of evolving towards increased selfing, as population theory predicts.

These results are somewhat different from those obtained for wild populations of the same species (Cook & Soltis, 1999). Wild populations of the tetraploid, *T. mirus*, had higher outcrossing rates than populations of the diploid, *T. dubius* (Cook & Soltis, 1999). The outcrossing rate for the artificial array of *T. dubius* ($t = 0.727$) is significantly higher than that found for either of the wild populations ($t = 0.068$, $t = 0.242$). It is worth noting, however, that the population of *T. dubius* from which the target plants for this study were grown (Troy, ID) was the same one that exhibited the significantly higher outcrossing rate of the two wild populations of *T. dubius* ($t = 0.242$). If there exists in this population an allele or combination of alleles that promotes outcrossing, then the results from the artificial population could be easily explained. The population of *T. mirus* from which the target plants for this study were grown was not assessed in the previous study. In the present study, the artificial array exhibited a higher outcrossing rate ($t = 0.591$) than either tetraploid population assessed in the previous study ($t = 0.381$, $t = 0.456$). The difference between 0.591 and 0.381 is significant, but that between 0.591 and 0.456 is not.

The arrays in the present study represented artificial populations of these plants in order to allow comparisons with wild populations. The artificial arrays were designed specifically to allow detection of outcrossing events that might have gone unnoticed in the previous study, because of the low allozymic diversity in the wild populations surveyed. The higher estimates of outcrossing obtained

in these arrays may indicate that, to some extent, we succeeded in the latter objective, even though the values obtained for the array of *T. dubius* may be underestimates (see above).

The results here lend support to the hypothesis that tetraploid *T. mirus* exhibits lower outcrossing rates than at least one of its diploid progenitors, *T. dubius*. However, these results appear contradictory to those found in the study of wild populations, and taken together, the two studies suggest that mating system variation may be more dependent on individual- and population-level differences than on ploidal level in *Tragopogon*. There is wider variation in estimates of *t* among *T. dubius* populations (wild and artificial) than there is between *T. dubius* and *T. mirus* (current results and Cook & Soltis, 1999), and the ranges of estimates for individual families within each species in the present study overlap completely (Table 1). This variability mirrors that seen among individuals in natural populations (L. M. Cook & P. S. Soltis, unpubl. data) at both ploidal levels. Estimates of the mating system of *T. porrifolius*, the second diploid parent of *T. mirus*, are not available; we only know that there is evidence suggesting that outcrossing exists in natural populations of this species (Soltis *et al*., 1995; Cook & Soltis, 1999).

The mating systems of tetraploid and diploid species of *Tragopogon* may not have had time to diverge substantially. The tetraploid species are very young, having arisen sometime between 1900 and 1949 (and most likely post-1928, given that the first collection of *T. dubius* dates from 1928; Ownbey (1950)). A significant shift in outcrossing rates would presumably take time to become established throughout all populations of a species. Given the high level of variability in outcrossing rates within the diploid progenitor species, more time than has yet passed might be required for the tetraploid derivative to diverge significantly from its progenitor. Although recent work has shown that genomes in artificial polyploids can undergo very rapid divergence from their progenitors through chromosome rearrangements (e.g. Song *et al*., 1995), mating systems in *Tragopogon* may not undergo such rapid change.

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