Two Independent Loci Control Agamospermy (Apomixis) in the Triploid Flowering Plant *Erigeron annuus*

Richard D. Noyes and Loren H. Rieseberg

Department of Biology, Indiana University, Bloomington, Indiana 47405

Manuscript received November 8, 1999
Accepted for publication January 31, 2000

ABSTRACT

Asexual seed production (agamospermy) via gametophytic apomixis in flowering plants typically involves the formation of an unreduced megagametophyte (via apospory or diplospory) and the parthenogenetic development of the unreduced egg cell into an embryo. Agamospermy is almost exclusively restricted to polyploids. In this study, the genetic basis of agamospermy was investigated in a segregating population of 130 F_1's from a cross between triploid ($2n = 27$) agamospermous *Erigeron annuus* and sexual diploid ($2n = 18$) *E. strigosus*. Correlations between markers and phenotypes and linkage analysis were performed on 387 segregating amplified fragment length polymorphisms (AFLPs). Results show that four closely linked markers with polysomic inheritance are significantly associated with parthenogenesis and that 11 cosegregating markers with univalent inheritance are completely associated with diplospory. This indicates that diplospory and parthenogenesis are unlinked and inherited independently. Further, the absence of agamospermy in diploid F_1's appears to be best explained by a combination of recessive-lethal gametophytic selection against the parthenogenetic locus and univalent inheritance of the region bearing diplospory. These results may have major implications for attempts to manipulate agamospermy for agricultural purposes and for interpreting the evolution of the trait.

ASEXUAL seed production, agamospermy, occurs in >40 flowering plant families but is most prevalent in the grasses (Poaceae), Asteraceae, and Rosaceae (Grant 1981; Asker and Jerling 1992). The ability of plants to produce asexual seed has been known since the middle of the 19th century (Smith 1841) and the principal alternative developmental pathways by which agamospermous seed is produced have been well characterized (Gustafsson 1946–1947; Nogler 1984a). The two main types of agamospermy, gametophytic apomixis and adventitious embryony, differ in that the former includes the formation of unreduced but functional megagametophytes via apospory or diplospory. In diplospory, the megagametophyte arises from mitotic or mitotic-like divisions of the megasporocyte, while in apospory, the unreduced megagametophyte arises from divisions of cells in the nucellus or chalazal region. The embryo typically develops from the unreduced egg cell of the aposporous or diplosporous megagametophyte via parthenogenesis. In adventitious embryony, the embryo develops directly from nucellar or chalazal tissue without an intervening gametophyte stage. Agamospermous plants may also be pseudogamous, *i.e.*, requiring fertilization of polar nuclei for endosperm formation, or nonpseudogamous. In the latter, endosperm develops autonomously and fertilization of polar nuclei is not required for agamospermous seed formation.

In agriculture, it would be desirable to use agamospermy to perpetuate hybrid cultivars via seed (Koltunow *et al.* 1995; Vielle-Calzada *et al.* 1996). However, most of the world's economic crop species reproduce sexually and efforts to introgress gametophytic apomixis from related species have met only highly qualified success. Proper manipulation appears to require knowledge of the genetics of at least four different aspects of the trait: (1) unreduced megagametophyte formation, (2) parthenogenesis, (3) endosperm development, and (4) mechanisms prohibiting expression or inheritance of agamospermy in diploids. Although complete characterization has been elusive, recent investigations for several grass species show that the genetics of unreduced megagametophyte formation is remarkably simple. Analyses of experimental populations segregating for diplospory in Tripsacum (Leblanc *et al.* 1995; Grimanelli *et al.* 1998a), and apospory in Pennisetum (Ozias-Akins *et al.* 1993, 1998; Gustine *et al.* 1997) and Brachiaria (Pessino *et al.* 1997), for instance, indicate that these traits are principally controlled by a single genetic locus, though of as yet undetermined size or structure (see review, Pessino *et al.* 1999). In these studies, discovery of randomly amplified polymorphic DNA (RAPD), cDNA, or restriction fragment length polymorphism (RFLP) markers closely linked to apospory or diplospory was achieved through bulked segregant analysis (Michelmore *et al.* 1991) and facilitated, in some instances, by comparative mapping approaches.

Corresponding author: Richard D. Noyes, Plant Genome Mapping Laboratory, University of Georgia, Riverbend Research Ctr., Rm. 162, 110 Riverbend Rd., Athens, GA 30602.
E-mail: rnoyes@arches.uga.edu

In contrast, the genetic basis of parthenogenesis is poorly understood. In part, the reason is historical as most classical studies failed to consider parthenogenesis to be a distinct and tractable trait (Asker and Jerling 1992). Data from the few studies that have been conducted are equivocal. Parthenogenesis is interpreted to be closely linked to apospory in Ranunculus (Nogler 1984b) and completely linked to apospory in Panicum (Savidan 1980), for instance. These and similar observations provided the rationale for models for agamospermy (Nogler 1984b; Mogie 1988, 1992) in which the expression of parthenogenesis is presumed to be merely a pleiotropic manifestation of a properly integrated gene for apospory or diplospory rather than a trait under distinct genetic control. On the other hand, older (Sörenson 1958; Richards 1973) and recent (Van Baarlen *et al.* 1999) studies suggest that parthenogenesis and diplospory are inherited independently in Taraxacum. Complex inheritance patterns in other taxa have prompted some authors (*e.g.*, Asker 1979) to speculate that parthenogenesis may be quantitative in nature and genetically complex. Genetic map-based investigations on the inheritance of parthenogenesis are wanting, however, and thus the question remains largely open.

The basis for the absence of gametophytic apomixis in diploids (except in rare cases of diplohaploidy; *e.g.*, De Wet and Harlan 1970) is also a contentious issue. Two divergent opinions currently prevail. According to Mogie (1988, 1992) expression of agamospermy is dosage dependent and although diploids may carry agamospermy genes, they occur below a prescribed dosage ratio and are therefore not expressed. In contrast, according to the gametophytic selection model of Nogler (1984b), the genes for agamospermy are dominant but have recessive lethal activity that is exhibited when they occur in haploid pollen grains (as well as aneuploid and higher euploid grains) in the absence of compensatory wild-type alleles. Diploid plants, according to this hypothesis, thus lack agamospermy genes because they cannot be effectively transmitted in haploid gametes. Direct tests of these hypotheses are lacking in part because most molecular marker-based investigations of agamospermy have been conducted using tetraploids. Claims that a complex *trans*-acting haploid segregation distortion system is linked to agamospermy in Tripsacum (Grimanelli *et al.* 1998b) are difficult to evaluate because of the complexity of the crossing design employed and differences in basic chromosome number of the parental materials (maize, $2n = 20$; Tripsacum, $4x = 2n = 72$).

In this work we explore the genetic basis of agamospermy in the flowering plant genus Erigeron (Asteraceae). Erigeron (>400 spp.) is a genus of perennial herbs, biennials, and annuals that is widespread in temperate North America, Europe, and South America (Cronquist 1947; Solbrig 1962; Nesom 1989). Agamospermy has been well documented for six Erigeron species and circumstantial evidence indicates that it occurs in several other species as well (Noyes 2000a). Agamospermy in Erigeron is diplosporous, parthenogenetic, and nonpseudogamous (Gustafsson 1946–1947; Harling 1951). A recent genetic study of agamospermy in the genus (Noyes 2000b) was based on the analysis of reproductive phenotypes in an experimental population of 130 individuals resulting from a cross between agamospermous triploid ($2n = 27$) *Erigeron annuus* and sexual diploid ($2n = 18$) *E. strigosus*. *E. annuus* is considered to be an obligate agamosperm but produces pollen that can function in crosses. The population is cytologically complex, exhibiting a bimodal chromosome number distribution centering near diploid and triploid levels (Figure 1). Diplospory and parthenogenesis were scored quantitatively for each F_1 but the resulting bimodal distribution of phenotypes, with a substantial proportion of individuals with phenotype scores of zero, suggests that the genetic basis of each trait is fundamentally qualitative. Furthermore, the overall pattern of variation indicates independent segregation and inheritance of the two traits (Figure 1). Finally, both traits occur predominantly in hybrids with $2n = 24$ and above and are rare in hybrids with $2n = 18$–23 (Figure 1).

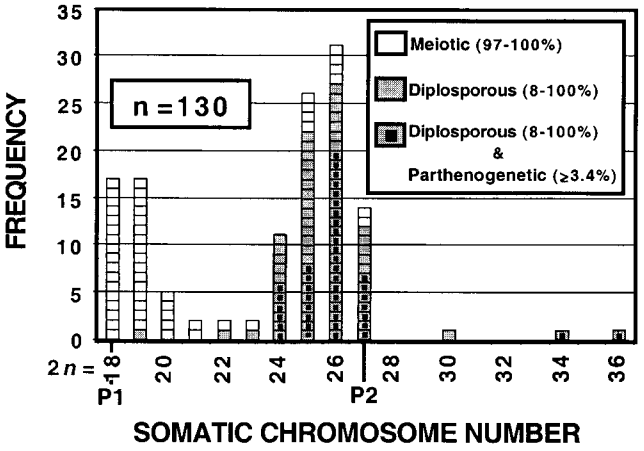

Figure 1.—Chromosome numbers, diplospory, and parthenogenesis for F_1's from a cross between diploid ($2n = 18$) sexual *E. strigosus* (P1) and triploid ($2n = 27$) agamospermous *E. annuus* (P2) [modified from Noyes (2000b)]. Fifty-two F_1's are predominantly meiotic (97–100% meiotic ovules) and 78 F_1's are diplosporous (0–92% meiotic ovules). Scores based on observations of ≥100 cleared ovules per F_1. Parthenogenetic seed production estimated from average seed counts for three capitula per F_1 and verified with progeny tests.

Because the Noyes (2000b) population appears to segregate for both diplospory and parthenogenesis, and includes a mixture of diploid, aneuploid, and higher polyploid hybrids, it provides an opportunity, exploited here, to investigate multiple aspects of the genetics of gametophytic agamospermy simultaneously. Our strategy was first to identify molecular markers [amplified fragment lenth polymorphisms (AFLPs)] correlated

with reproductive phenotypes in the Noyes experimental population and then to examine the relationship of these markers on genetic maps constructed for the triploid agamospermous parent. Mapping strategies for polyploids, even those at the mere triploid level, are complicated by the diversity of chromosome pairing possibilities and the distribution of markers into multiple dosage classes (Da Silva and Sorrells 1996). Furthermore, correct interpretation of patterns resulting from selection, like those encountered in this study, presents a considerable challenge. Despite impediments such as these, genetic maps based on segregating molecular markers have been generated for a diversity of polyploids including tetraploid cotton (Reinisch *et al.* 1994), hexaploid Festuca (Xu *et al.* 1995), hexaploid wheat (Gill *et al.* 1991), and octoploid sugar cane (Da Silva *et al.* 1993; Ming *et al.* 1998). Here, marker-based mapping methodology was used in Erigeron to evaluate (1) the genetic basis of diplospory, (2) the genetic basis of parthenogenesis, (3) genomic structure in a triploid agamospermous species, and (4) factors responsible for the absence of agamospermy in diploids.

MATERIALS AND METHODS

Plant materials: The experimental F_1 population was generated from a cross using sexual diploid ($2n = 18$) *E. strigosus* (P1) as the female parent and agamospermous triploid ($2n = 27$) *E. annuus* (P2) as the pollen parent (Noyes 2000b). In the previous study, a total of 129 progeny were identified as hybrid on the basis of patterns of inheritance of 13 pollen parent-specific RAPD markers. Subsequent analysis with AFLP markers of a few progeny of ambiguous paternity led to the identity of one additional F_1 hybrid (130 total hybrid progeny). The cytological data (Figure 1), in combination with molecular marker tests of progeny indicating that sexual P1 produces predominantly haploid ($n = 9$) gametes, were interpreted (Noyes 2000b) to indicate that microsporogenesis in agamospermous P2 yields functional gametes that are approximately haploid ($n = 9$) and diploid ($n = 18$). Complete details on the origin of the parental plants, the cross, paternity analysis, and chromosome counts are provided elsewhere (Noyes 2000b).

Diplospory and parthenogenesis estimates: All 130 hybrids flowered over a 3-year period. Analysis of megagametophyte formation and seed production for the F_1's indicated that both diplospory and parthenogenesis segregate in the experimental population (Noyes 2000b; Figure 1). Diplospory was measured quantitatively for each hybrid by scoring as either meiotic or diplosporous $\geq$100 cleared ovules per plant. Meiotic megagametophytes are tetrasporic, developing from the four haploid nuclei resulting from meiosis of the megasporocyte. Diplosporous megagametophytes, on the other hand, arise from two unreduced nuclei that result from a mitotic or mitotic-like division of the megasporocyte.

Many of the F_1's produced at least some seed, though often at a very low level (Noyes 2000b). However, high levels of phenotypic variation in F_2 cohorts indicated that predominantly meiotic hybrids likely produced seed via selfing. In contrast, F_2 cohorts of a sample of 28 F_1's characterized by moderate to high levels of diplospory (percentage of meiotic ovules ranging from 1 to 30%) were with rare exception phenotypically uniform (Noyes 2000b). This is evidence that diplosporous F_1 hybrids produce seed predominantly via diplospory and parthenogenesis even when the proportion of meiotic ovules is substantial. Parthenogenesis was estimated quantitatively as the average percentages of achenes with embryos (determined microscopically) for three capitula per F_1 (see Noyes 2000b). Parthenogenesis scores range from 0 to 68%, and the trait appears to segregate among experimental hybrids with moderate to high diplospory scores (Noyes 2000b; Figure 1).

DNA isolations and genotyping: Total genomic DNA was isolated from 100 mg of fresh leaf tissue using DNeasy plant mini kits (QIAGEN, Chatsworth, CA) for P1, P2, and all F_1 progeny. All individuals were genotyped with AFLPs (Vos *et al.* 1995). Digestion of genomic DNA, ligation of adapters, preamplification, and selective amplification followed standard protocols (Vos *et al.* 1995). All enzymes and buffers were purchased from New England Biolabs (Beverly, MA). HPLC-purified *Eco*RI + 3 primers, 5′ labeled with either Texas Red or Oregon Green fluorescent dyes, were obtained from GIBCO-BRL-Life Technologies (Rockville, MD).

Reaction products were denatured at 90° with an equal volume (10 μl) of formamide dye (98% formamide, 10 mm EDTA pH 8.0, bromphenol blue) and separated on 5% denaturing polyacrylamide gels run at constant power (50 W) for 1 hr 30 min. Bands were visualized using an FMBIO II scanner and image analysis software (Hitachi Software Engineering, Tokyo, Japan) with wavelengths of 625 nm for Texas Red-labeled and 505 nm for Oregon Green-labeled reactions, respectively. Fragment sizes of the Texas Red- and Oregon Green-labeled fragments were estimated from the comigration of markers constituting Prism Genescan-500 ROX (PE Applied Biosystems, Warrington, Great Britain) and MapMarker LOW 70-400BP fluorescein (Bioventures, Murfreesboro, TN) ladders, respectively. Each AFLP marker is identified with a unique label that consists of the last two selective nucleotides of the *Eco*RI and *Mse*I primers followed by the estimated fragment size (for example, GCCC.209 refers to a 209-bp fragment produced by the primer combination *Eco*RI + AGC and *Mse*I + ACC).

Marker classification: All segregating markers were tested for fit to six single-dose (1–6) and four double-dose (7–10) inheritance models (Table 1). Model parameters differ in allele dosage [single dose (SD) *vs.* double dose (DD)], selection (neutral *vs.* complete), and segregation type (chromosome *vs.* chromatid). Selection models (2, 4, 6, 8, and 10) assume complete elimination of gametes bearing either a single (A) or double dose (AA) of an allele in the absence of a wild-type (a) allele. Consequently, the expected frequency of markers in diploid $2n = 18$ progeny for these models is zero. Chromatid segregation (models 3, 4, 9, and 10) is unique to autopolyploids and occurs when a marker lies >50 cM from the centromere of a chromosome (Stansfield 1983). Under this condition, random assortment of chromatids rather than chromosomes is expected. All models assume trisomic inheritance except for disomic models 5 and 6, which assume preferential pairing of homologues (with and without complete gametophytic selection, respectively), to the complete exclusion of the third nonrecombinant homologue. A nonrecombinant homologue, effectively a univalent in meiosis, is here assumed to segregate with one or the other of the homologous synaptic chromosomes. Under this assumption, the inheritance of a marker lying on a univalent corresponds exactly to that of a DD marker under complete gametophytic selection (model 8). However, note that the exclusion from diploid progeny of markers located on a univalent occurs due to meiotic behavior rather than gametophytic selection. Expected ratios for dominant markers at aneuploid chromosome levels for all models were calculated assuming stepwise increase in likelihood from haploid to diploid gametic levels.

TABLE 1

Ten models for inheritance of dominant markers in triploid Erigeron

Model	Probabilities for dominant markers	
	n	2*n*
Single dose		
1. (Aaa) trisomic, chromosome, neutral	1/3	2/3
2. (Aaa) trisomic, chromosome, selection[a]	0	2/3
3. (Aaa) trisomic, chromatid, neutral	1/3	9/15
4. (Aaa) trisomic, chromatid, selection[a]	0	8/14
5. (Aa) disomic[b], chromosome, neutral	1/2	1/2
6. (Aa) disomic[b], chromosome, selection[a]	0	1/2
Double dose		
7. (AAa) trisomic, chromosome, neutral	2/3	1
8. (AAa) trisomic, chromosome, selection[a]; or univalent[c]	0	1
9. (AAa) trisomic, chromatid, neutral	2/3	14/15
10. (AAa) trisomic, chromatid, selection[a]	0	8/9

Probabilities calculated for haploid and diploid gametes produced by a triploid pollen donor.

[a] Models with selection assume complete elimination of gametes bearing either a single (A) or double dose (AA) of an allele in the absence of a wild-type (a) allele.

[b] Disomic models assume complete preferential pairing of two homologs to the exclusion of a third univalent.

[c] Our assumption is that a nonrecombining univalent will segregate with one or the other preferentially pairing homolog. The inheritance of markers on a univalent is coincidentally equivalent to that of double-dose markers under complete recessive lethal-gametophytic selection.

To classify markers according to inheritance model, we explored diverse approaches for obtaining a statistical fit between observed and expected marker frequencies. Simple comparisons of observed *vs.* expected sums of markers across the population proved to be inadequate for marker classification as, for instance, a DD marker under complete homozygote gametophytic selection yields a net expected value nearly equal to a neutral SD marker. Logistic goodness-of-fit methods (*e.g.*, Hosmer and Lemeshow 1989) were determined to be unsatisfactory because of the small size of intermediate chromosome number classes. Ultimately, we achieved satisfactory classification by comparing the expected number of marker observations (Table 2) for the 43 F_1's with low ($2n$ = 18–22) and the 85 F_1's with high ($2n$ = 23–27) chromosome numbers, with observed values for each marker using a 4×2 goodness-of-fit (G) test with d.f. = 3. This method is effective because each inheritance model provides a unique combination of predictions for these two sets of progeny. To simplify marker classification, the three F_1's with $2n > 27$ were ignored. All genetic models providing a fit with $P > 0.01$ were considered for each marker. For markers with multiple candidate models, a single model was selected if its G score was $\geq$2.2 units less than other competing models. This corresponds to a minimum threefold difference in likelihood. Otherwise, a marker was considered to correspond with equal likelihood to multiple models.

Marker-phenotype correlations: For both diplospory and parthenogenesis, the population could be readily divided into roughly equal-sized groups on the basis of trait absence (approximately) and presence (Figure 1). This segregation pattern suggests that the underlying genetics of each trait are qualitative rather than quantitative, and t-tests, which are recommended for normally distributed quantitative traits (Doerge *et al.* 1997), were therefore deemed inappropriate. Instead, we estimated associations between markers and reproductive phenotypes with 2×2 G-tests (d.f. = 1) conducted separately for the two traits.

To discover markers associated with diplospory, 52 F_1's with a high proportion of meiotic ovules (97–100%) were compared with 78 individuals with a low proportion of meiotic ovules (0–92%, corresponding to proportion of diplosporous ovules ranging from 8 to 100%; Figure 1). G-tests were performed for all markers. However, the two groups are distinct cytologically (nondiplosporous plants average $2n$ = 20.27; diplosporous plants average $2n$ = 25.69) and preliminary comparisons showed that most of the markers determined to be under gametophytic selection (models 2, 4, 6, 8, and 10) showed significant associations with diplospory. Thus, to avoid spurious association, G-tests were performed with expected values for each marker derived from probabilities of the single model (1–10, Table 1) providing the greatest likelihood of fit. This approach effectively factors out the influence of marker class, selection, and chromosome number on marker-phenotype association.

To test for markers correlated to parthenogenesis, only diplosporous plants were considered. This was done to eliminate the possibility that individuals with parthenogenesis genes, but lacking those for diplospory, might not produce seed and thus be misclassified. In addition, to achieve better chromosome number balance between the two groups, the three individuals with $2n > 27$ were removed from analysis. For the G-tests, 71 F_1's with diplospory markers were divided into those 30 individuals (average $2n$ = 25.10) with parthenogenesis scores of 0–1.6% and 41 (average $2n$ = 25.68) with scores ranging from 3.4 to 67.7%. Because the seed production estimates are evaluated qualitatively rather than quantitatively, our classification of individuals as nonparthenogenetic or parthenogenetic is likely robust to low levels of error in the raw quantitative scores. Although the parthenogenetic and nonparthenogenetic groups are similar in chromosome number, G-tests for parthenogenesis were performed with expected values taking into account chromosome number as well as marker class.

Linkage analysis: MAPMAKER 3.0 (Lander *et al.* 1987; Lincoln *et al.* 1992) for UNIX was used to infer linkage relationships among all single-dose markers plus those for which univa-

TABLE 2

Expected values for dominant markers in F_1 population

Inheritance model	$2n$ = / N =	Probabilities and expected values per F_1 chromosome number										Pooled expected values ($\Sigma N \cdot P$)	
		18	19	20	21	22	23	24	25	26	27	$2n$ = 18–22	$2n$ = 23–27
		17	17	5	2	2	2	11	26	31	14		
1	P	0.333	0.370	0.407	0.444	0.481	0.519	0.556	0.593	0.630	0.667	15.9	51.4
	$N \cdot P$	5.7	6.3	2.0	0.9	1.0	1.0	6.1	15.4	19.5	9.3		
2	P	0.000	0.074	0.148	0.222	0.296	0.370	0.444	0.519	0.593	0.667	3.0	46.8
	$N \cdot P$	0.0	1.3	0.7	0.4	0.6	0.7	4.9	13.5	18.4	9.3		
3	P	0.333	0.363	0.393	0.422	0.452	0.481	0.511	0.541	0.570	0.600	15.5	46.7
	$N \cdot P$	5.7	6.2	2.0	0.8	0.9	1.0	5.6	14.1	17.7	8.4		
4	P	0.000	0.063	0.127	0.190	0.254	0.317	0.381	0.444	0.508	0.571	2.6	40.1
	$N \cdot P$	0.0	1.1	0.3	0.4	0.5	0.6	4.2	11.6	15.7	8		
5	P	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	21.5	42.0
	$N \cdot P$	8.5	8.5	2.5	1.0	1.0	1.0	5.5	13.0	15.5	7		
6	P	0.000	0.056	0.111	0.167	0.222	0.278	0.333	0.389	0.444	0.500	2.3	35.1
	$N \cdot P$	0.0	0.9	0.6	0.3	0.4	0.6	3.7	10.1	13.8	7		
7	P	0.667	0.704	0.741	0.778	0.815	0.852	0.889	0.926	0.963	1.000	30.2	79.4
	$N \cdot P$	11.3	12.0	3.7	1.6	1.6	1.7	9.8	24.1	30.0	14.0		
8	P	0.000	0.111	0.222	0.333	0.444	0.556	0.667	0.778	0.889	1.000	4.6	70.2
	$N \cdot P$	0.0	1.9	1.1	0.7	0.9	1.1	7.3	20.2	27.6	14.0		
9	P	0.667	0.696	0.726	0.756	0.785	0.815	0.844	0.874	0.904	0.933	29.9	74.7
	$N \cdot P$	11.3	11.8	3.6	1.5	1.6	1.6	9.3	22.7	28.0	13.1		
10	P	0.000	0.099	0.198	0.296	0.395	0.494	0.593	0.691	0.790	0.889	4.0	62.4
	$N \cdot P$	0.0	1.7	1.0	0.6	0.8	1.0	6.5	18.0	24.5	12.4		

Pooled expected values are provided for the 10 inheritance models presented in Table 1. N, number of F_1's at each chromosome number. P for haploid (n = 9; $2n$ = 18 in the F_1) and diploid (n = 18; $2n$ = 27 in the F_1) gametic levels are from Table 1. P for aneuploids assumes stepwise increase in the likelihood of the inheritance of a marker from haploid to diploid gametic levels. Pooled expected values were compared with pooled observed values with G-tests (see text).

lent inheritance was suspected. Because triploid parent P2 is likely to be heterozygous for many loci, the cross in terms of marker expectations is identical to a BC1, and thus the options "F_2 backcross" with "$a = 0$" and "$h = 1$" were selected in MAPMAKER. Only those markers scored for P2 were considered in the analysis. Further, as only single-dose, dominant AFLP markers were considered, linkage maps potentially correspond to each of P2's $2n = 27$ chromosomes, rather than to the $n = 9$ sets of homologues (Wu *et al.* 1992).

Analyses were first carried out on all SD markers that were inferred to be neutral (corresponding to models 1, 3, 5, above). This was done to avoid artifactual linkage of independent markers under parallel selection. Markers were first divided into linkage groups using the "group" command (parameters LOD > 6.0; $\theta < 0.20$). This recombination fraction corresponds to a maximal two-point map distance of 25 cM. These conservative levels seemed warranted given the complexity of the study population. Furthermore, P2 is slightly biased toward the production of diploid *vs.* haploid gametes (Figure 1), resulting in an expected neutral frequency for a SD marker of 0.54 rather than 0.50 as assumed by linkage algorithms, potentially resulting in a modest underestimation of θ.

Putative univalent markers and those inferred to be under selection were then added sequentially to the genetic map using the "links" command. Linkage groups containing markers significantly associated with diplospory and parthenogenesis were subjected to exhaustive analysis using "compare" and the ordered marker sequences were confirmed using "ripple." Haldane's (1919) mapping function was used to transform the recombination frequency between linked loci into centimorgan distances. Linkage groups were also analyzed using the "error detection on" option to aid in the identification of misscored data. Linkage groups were designated "LG-*X*," where *X* is an arbitrarily assigned consecutive number, except those associated with parthenogenesis and diplospory, which were designated "LG-P" and "LG-D," respectively.

RESULTS

Marker segregation and classification: Over 100 AFLP primer combinations were surveyed and 26 primer pairs, providing the sharpest bands and highest level of polymorphism, were used to genotype the experimental population. A total of 387 polymorphic loci occurring in the triploid agamospermous parent P2 were scored ($\sim$15 loci/primer combination). The presence or absence of fragments was interpreted conservatively; nonetheless, the 136 instances of questionable bands scored as "missing data" form a relatively modest proportion of the data set (0.27%).

The fit of observed marker frequencies to models indicates that inheritance for P2 is complex (Figure 2). A total of 380 of 387 loci were readily classified into SD or DD categories. Although the majority of markers (294; 76%) are interpreted as SD and neutral, pairing behavior for this subset is highly variable with evidence of three different types of SD segregation (trisomic chromosome, trisomic chromatid, preferential-bivalent; Figure 2). Furthermore, for many SD markers, it was not possible to select one best inheritance model with our methods. This is not surprising, however, given that the expected probabilities for models 1 and 3, in particular,

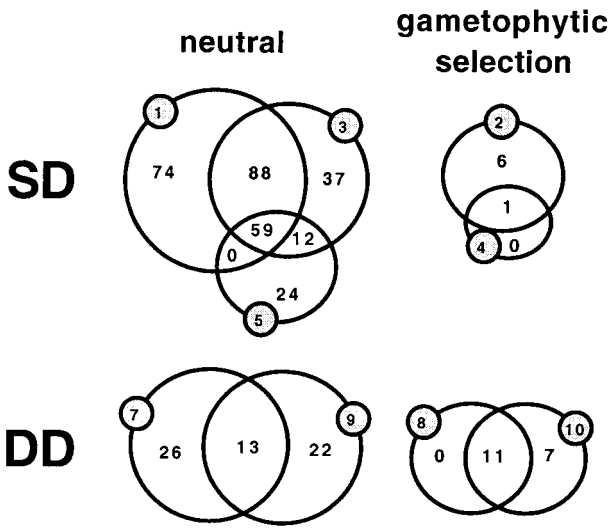

Figure 2.—Classification of AFLP markers into four principal inheritance groups: single dose (SD) and double dose (DD) under neutral conditions or recessive lethal-gametophytic selection. Shaded number in ring refers to inheritance models as given in Table 1. Markers classified to model based on 4×2 *G*-test with $P > 0.01$ (see text). Markers corresponding to multiple inheritance models with $G < 2.2$ are cross classified and appear within the intersecting region between model areas. Note: DD complete selection and univalent inheritance are equivalent (see text).

are similar (Table 1). Relatively few (61; 16%) of the total markers are DD neutral. These conform in nearly equal numbers to models 7 and 9 with chromosome or chromatid segregation, respectively.

Only 7 SD markers appear to be subject to complete homozygote selection as their distribution matches that expected under inheritance models 2 and 4 (Figure 2). In addition, 18 markers are inferred to be DD with complete homozygote selection with chromosome or chromatid inheritance. Alternatively, 11 of these latter markers, corresponding to model 8, may be interpreted as representing strict SD univalent inheritance.

A total of seven markers could not be readily classified into one of the four principal categories (Figure 2). Three of these did not provide a fit ($P \geq 0.01$) to any of the 10 models. All are similar, however, in featuring marker frequencies intermediate to expected values for neutral SD and DD models. This pattern suggests that these markers are either SD and under uniform positive selection, or, equally likely, DD and under uniform negative selection. Four other markers are equally likely in more than one of the four model groups. The frequency distribution of these in the F_1 population, however, is consistent with the hypothesis that each is a SD marker under intermediate lethal-recessive gametophytic selection.

Marker-phenotype associations: A total of 12 markers are significantly associated with diplospory (Table 3; Figure 3). Because of their fit, each was tested against

TABLE 3

Associations between markers and reproductive phenotypes

Model	No. markers tested	α'	Diplospory	*P*	Parthenogenesis	*P*
1	133	3.8E-4	—	—	—	—
2	9	0.0056	—	—	cccg.138	1.6E-14
					cccc.085	4.8E-07
3	115	4.4E-4	—	—	ccac.067	6.3E-09
					cccg.160	1.5E-07
4	1	0.05	—	—	—	—
5	49	0.0010	—	—	—	—
6	—	—	—	—	—	—
7	35	0.0014	—	—	—	—
8	—	—	—	—	—	—
9	27	0.0019	—	—	—	—
10	18	0.0028	tacg.130	3.5E-14	—	—
			tggc.400	1.1E-14		
			tagc.850	1.1E-14		
			ctcg.470	1.1E-14		
			cggc.340	1.1E-14		
			accg.230	1.1E-14		
			cggc.335	1.1E-14		
			tacg.095	1.1E-14		
			tgcg.115	1.1E-14		
			gccg.050	1.1E-14		
			ccgc.125	1.1E-14		
			cccg.170	1.1E-14		

All 387 AFLP markers were tested against the null hypothesis of no association with either diplospory or parthenogenesis with 2×2 *G*-tests (d.f. = 1). For diplospory, 52 F_1's with diplospory scores of 0–3% (meiotic scores 97–100%) were compared with 78 individuals with diplospory scores of 8–100% (meiotic scores 0–92%). For parthenogenesis, 30 individuals with seed production scores 0–1.6% were compared with 41 with scores ranging from 3.4 to 67.7%. Expected values were obtained from the inheritance model with the best fit for each marker. Model refers to those listed in Table 1. Four markers are significantly associated with parthenogenesis; 12 markers are significantly associated with diplospory with α' representing Bonferroni corrected α of 0.05.

expectations for inheritance model 10 (DD with complete selection). Eleven of the 12 markers always occur together in the same 74 F_1's. Because they never appear to segregate, we infer that these markers are completely linked. The 12th marker (tacg.130) is apparently closely linked as it occurs in all but two of the same group of individuals. The block of 11 markers occurs in all individuals with percentage of diplosporous ovules ranging from 28 to 100%. The four individuals with the lowest diplospory scores (8–18%) lack all 11 of the completely linked diplospory markers and we consider these plants to be sexual. The diplosporous ovules in these four plants (and in seven individuals classified as sexual with proportion of diplosporous ovules ranging from 1 to 3%) are interpreted to be artifactual and not homologous with those produced by plants with higher proportions of diplospory and exhibiting the linked diplospory markers. It is not known if rare production of aposporous or diplosporous ovules is characteristic of experimental sexual hybrids in other agamospermous taxa because, at least in all previous mapping studies (*e.g.*, Leblanc *et al.* 1995; Gustine *et al.* 1997; Pessino *et al.* 1997; Ozias-Akins *et al.* 1998), only a few (generally $\leq$20) or an undisclosed number of ovules were evaluated per F_1. Alternatively, the four individuals producing 8–18% diplosporous ovules may be truly diplosporous and the correlated markers may not be completely linked to the diplospory locus.

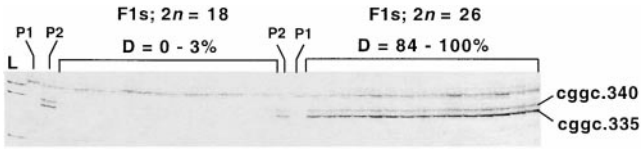

Figure 3.—AFLP markers cggc.340 and cggc.335 correlated with diplospory in Erigeron. P1, sexual diploid ($2n = 18$) seed parent; P2, agamospermous triploid ($2n = 27$) pollen parent. D, quantitative score (percentage) for production of diplosporous ovules. All $2n = 18$ F_1 hybrids (0–3% diplosporous ovules; 97–100% meiotic) fail to exhibit diplospory markers. All $2n = 26$ ($3x - 1$) hybrids shown, with high percentage diplosporous ovules, exhibit both bands. L, ladder.

Four SD markers are significantly associated with parthenogenesis (Table 3). Two of the markers conform to inheritance model 2 (SD with gametophytic selection), while the other two are SD and neutral. None of the

TABLE 4

Summary of AFLP linkage map of triploid ($2n = 27$) *Erigeron annuus*

Linkage	No. markers	Length (cM)	Average two-point distance	Linkage group	No. markers	Length (cM)	Average two-point distance
LG-1	3	13.5	4.5	LG-25	9	102.6	11.4
LG-2	6	68.6	11.4	LG-26	13	120.5	9.3
LG-3	3	24.4	8.1	LG-27	3	9.8	3.3
LG-4	6	56	9.3	LG-28	5	47.9	9.6
LG-5	15	112.7	7.5	LG-29	5	24.4	4.9
LG-6	12	82.4	6.9	LG-30	3	26.6	8.9
LG-7	11	102.8	9.3	LG-31	7	56	8
LG-8	10	55.2	5.5	LG-32	3	4.7	1.6
LG-P	10	78.7	7.9	LG-33	5	1.6	0.3
LG-10	3	5.8	1.9	LG-34	5	31.9	6.4
LG-11	14	84.1	6.0	LG-35	5	42.1	8.4
LG-12	4	32.8	8.2	LG-36	5	44.8	9.0
LG-13	4	8.1	2.0	LG-37	16	136.6	8.6
LG-14	2	12.1	6.1	LG-38	3	14.2	4.7
LG-15	14	100.6	7.2	LG-39	6	71.3	11.9
LG-16	5	40.6	8.1	LG-40	13	107.1	8.2
LG-17	7	50.8	7.3	LG-41	3	6.5	2.2
LG-18	6	25.6	4.3	LG-42	5	39.9	8.0
LG-19	7	47.5	6.8	LG-43	3	29.9	10.0
LG-20	8	77.4	9.7	LG-44	5	31.5	6.3
LG-21	2	23.3	11.7	LG-45	2	13.5	6.8
LG-22	2	5.8	2.9	LG-D	14	22.2	1.6
LG-23	6	44.5	7.4	LG-47	3	8.4	2.8
LG-24	5	23.6	4.7				
				Total	306	2171 ($3n$) 724 (n)	
				Average	6.5	46.2	7.1

Map constructed using the Haldane (1919) mapping function with linkage parameters $\theta < 0.20$, LOD > 6.0. LG-P and LG-D include markers statistically associated with parthenogenesis and diplospory, respectively. Homologous linkage groups were not determined.

four parthenogenesis markers is exclusively restricted to parthenogenetic individuals, although cccg.138 is closest, occurring in 36 of 41 F_1's categorized as parthenogenetic and in only 1 of 30 individuals classified as nonparthenogenetic.

Linkage maps: A total of 306 markers form a genetic map for triploid ($2n = 27$) Erigeron, comprising 47 linkage groups (Table 4). The mapped markers include 179 of 194 SD markers, 6 of 7 SD markers under complete homozygote selection, 3 of 4 SD markers hypothesized to be under intermediate selection, plus all 18 putative univalent markers. Linkage groups range in size from 2 to 16 markers (average = 6.5) with an average length of 46.2 cM. On average, linked markers are separated by 7.1 cM. The total length of the triploid map is 2171 cM, which corresponds to a haploid (n) map of 724 cM. Experimental analyses with $\theta > 0.20$ resulted in total linkage groups closer in count to the sporophytic chromosome number of P2. However, a number of the resulting larger linkages were suspect, consisting of loosely allied amalgams of tightly linked markers with high error rates at the junctions between groups. We estimate that a complete map, using conservative linkage parameters, would likely require the addition of 100–150 SD loci.

The distribution of SD markers under complete homozygote selection is nonrandom. The 6 mapped markers fall into groups of 3, 2, and 1 marker, respectively, that occur on three different linkage groups (LG-P, LG-11, and LG-16). This suggests that at least three different SD regions are under complete homozygote selection in the genome. The 22 mapped SD markers inferred to exhibit preferential pairing (model 5; Figure 2) fall into two classes. Ten of them occur singly or in pairs on seven different linkage groups. The other 12, however, occur in groups of 8 and 4 markers on only two different linkages (LG-7 and LG-42, respectively). LG-7 consists of 11 markers spanning 102.8 cM. This indicates that preferential pairing likely occurs for substantial contiguous regions of the genome. Because all markers were interpreted to be dominant, however, we were unable to establish that LG-7 and LG-42 correspond to preferentially pairing homologs.

All 12 of the markers significantly associated with

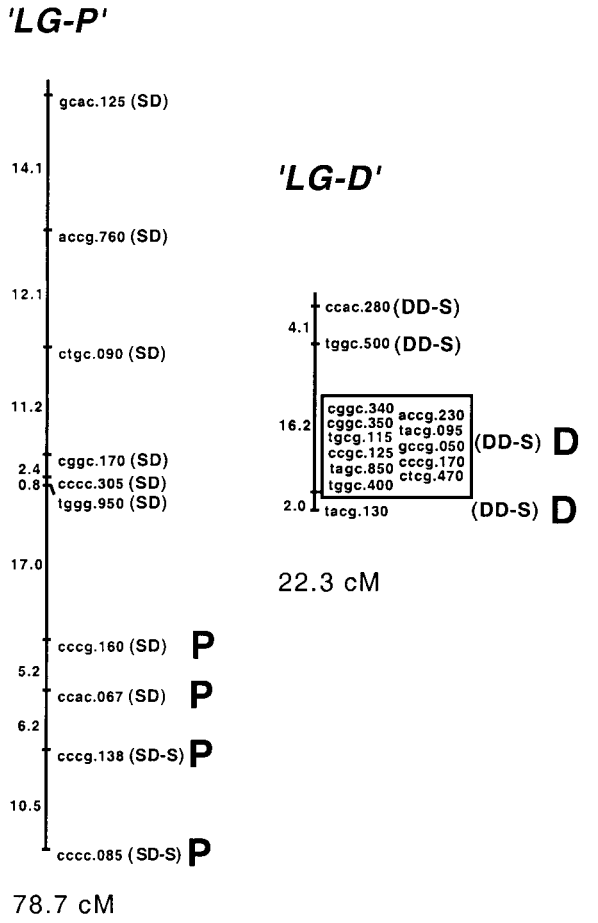

Figure 4.—Parthenogenesis (LG-P) and diplospory (LG-D) linkage groups in Erigeron. The relative positions of constituent AFLP markers are given in Haldane (1919) map distances. P and D denote markers statistically associated with parthenogenesis and diplospory, respectively (Table 3). SD, single-dose marker; SD-S, single-dose marker under recessive lethal-gametophytic selection; DD-S, double-dose marker under recessive lethal-gametophytic selection, or equivalently (favored here, see text), a single-dose marker with univalent inheritance.

diplospory occur on a single linkage group designated LG-D (Figure 4). The 11 nonsegregating correlated markers occur at a single point that is 2.0 cM from tacg.130. It is our interpretation that these markers represent a substantial genomic region and that they cosegregate due to lack of recombination between them. Although it is possible that the cosegregating markers are redundant, *i.e.*, they overrepresent by chance a limited genomic region, this seems unlikely because of the low incidence of other large cosegregating blocks among the remaining 376 markers studied. Although in one instance 6 markers cosegregate (LG-6), all other observations of cosegregation involve either 3 markers (five occurrences) or only 2 (18 occurrences). The average map distance among LG-D markers, when all 14 constituent markers are included, is 1.6 cM, which reflects this presumed decrease in recombination. Verification of our interpretation of recombination distances will require the development of codominant markers for this segment.

All 4 markers significantly associated with parthenogenesis are closely linked in LG-P (Figure 4). The 2 markers (cccg.138 and cccc.085) under complete gametophytic selection occur distal to the other 2 correlated markers (cccg.160 and ccac.067). All 10 markers constituting LG-P correspond to inheritance models 1, 2, or 3 (Table 1), indicating that inheritance for this entire linkage group is probably polysomic. In contrast to LG-D, LG-P appears to experience relatively high rates of recombination, as the total map distance for the group is 78.7 cM. To evaluate the possibility that parthenogenesis might be linked to diplospory, the associations of LG-P markers with those of LG-D were tested. No evidence for significant linkage was detected [LOD < 2.0 and $\theta > 0.33$ (>54 cM)].

Given the possibility that LG-D represents two homologous chromosomes, we evaluated the ability of MAPMAKER to detect a SD marker found on only one member of the homologous pair. Our hypothetical marker is present for exactly half of those individuals that carry diplospory markers and occurs in no other individuals. Analysis yields statistics [LOD = 5.41; $\theta = 0.28$ (42.1 cM)] indicating that MAPMAKER would be unlikely to detect linkage between a SD marker physically linked to a DD marker given the conservative values employed in this study.

DISCUSSION

Recombination in triploid Erigeron: Results show that the genome of Erigeron undergoes a high level of recombination resulting in a preliminary haploid genetic map (724 cM) that is comparable to those published for sexual species such as diploid Helianthus (1084–1983 cM; Knapp *et al.* 2000), Arabidopsis (501 cM; Chang *et al.* 1988), Gossypium [856 cM (A genome), 1486 cM (D genome); Brubaker *et al.* 1999], Eucalyptus (1462 cM; Byrne *et al.* 1995), and tetraploid Festuca [425 cM (*n*); Xu *et al.* 1995]. Furthermore, most of the AFLP markers analyzed conform to inheritance models 1, 2, or 3 (Table 1), indicating that the vast majority of the Erigeron genome, except for the region associated with diplospory, experiences polysomic inheritance, *i.e.*, random pairing involving all three homologs. Extensive disomic inheritance (models 5 and 6; Table 1) or univalent formation (model 8; Table 1) was not observed.

Genetic model for agamospermy in Erigeron: Upon consideration of marker frequencies alone, LG-D may be alternatively interpreted as either a single linkage group with univalent inheritance or two homologous linkages subject to complete gametophytic selection. The possibility that LG-D is represented twice in the genome is problematic as, shown above, it would be difficult to determine if there is genetic linkage between

SD parthenogenetic markers and one or the other duplicated LG-D blocks. However, we reject a two-allele interpretation for the following reasons. First, it is difficult to conceive of the mode of origin of a triploid plant with two homologous chromosomal segments (LG-D) because one or the other of the gametes (n or $2n$) contributing to the origin of the plant would have to be homozygous for the chromosomal segment, which appears to be unlikely because of recessive-lethal gametophytic selection. Although the plant could have arisen via the parthenogenetic development of a reduced 3X (AAa) egg produced by a tetraploid plant, tetraploids are rare in *E. annuus* and this phenomenon is unknown for the species. The origin of a triploid plant with only a single LG-D region is more easily achieved because it could occur via the union of a heterozygous diploid (Aa) gamete plus a haploid gamete (a) lacking the fragment.

Second, for a two-allele LG-D system to explain the occurrence of triploid sexual offspring and specific recombinant genotypes observed within the experimental population, we would have to invoke chromatid segregation (model 10, Table 1). However, the assumptions of chromatid inheritance require that a chromosome undergo sufficiently high rates of recombination such that genetic markers may occur >50 cM away from the centromere. The concentration of completely or closely linked markers in LG-D is suggestive of reduced recombination over an extensive chromosomal region and is thus at odds with the assumptions of chromatid inheritance. Observations instead are consistent with model 8 (Table 1) that assumes that LG-D is inherited principally as a univalent. Furthermore, occasional recombination between single-copy LG-D and its wild-type homologs would account for the recovery of sexual triploid offspring because each meiotic event would yield one in three viable gametes that would be diploid wild-type homozygous.

Third, evidence shows that two linkage groups (LG-7 and LG-42) are inherited as bivalents, whereas the markers in LG-D show little recombination. It is therefore possible that these three sets of markers represent homology, of which two (LG-7 and LG-42) exhibit strong preferential pairing, leaving LG-D as a univalent.

Last, our evaluation of diverse models revealed a fundamental incompatibility between the inheritance of LG-D (considered as single or double dose) and the polysomic inheritance of LG-P that precludes (1) complete linkage between LG-P and a univalent LG-D, (2) complete linkage between LG-P and one of two LG-D alleles, and (3) linkage between LG-P and LG-D in repulsion. In the light of these observations, our genetic model of agamospermy in Erigeron consists of (1) a diplospory locus located on a linkage group with (principally) univalent inheritance and (2) a parthenogenesis locus on a heterologous linkage group with polysomic inheritance. Our interpretation here of the inheritance of diplospory in Erigeron differs from that of Noyes (2000b), where, in the absence of molecular data, a one-gene, two-allele model was favored.

Diplospory and parthenogenesis thus appear to be inherited independently in Erigeron as they may also be in Taraxacum (Richards 1973; Van Baarlen *et al.* 1999). Although relatively few taxa have been investigated, this observation suggests that there may be a fundamental difference between aposporous and diplosporous agamospermy with the former inherited as a single genetic factor and the latter requiring separate genes for megagametophyte and parthenogenetic development. In any case, our results indicate that there may be multiple genetic models for gametophytic apomixis in plants rather than a single one. This conclusion suggests that caution should be exercised by researchers aiming to introgress, clone, or otherwise manipulate agamospermy genes, because the trait may be fundamentally polygenic.

From an evolutionary perspective, the occurrence of independent factors for agamospermy and parthenogenesis is puzzling (Richards 1997). This is because it is theoretically difficult to explain how the two functions could simultaneously arise within a single organism. Furthermore, the bigenic nature of agamospermy in Erigeron would likely act to impede the spread of agamospermy within or between species. Out of the 57% of viable gametes observed to carry diplospory alleles, only ~60% of these also carry parthenogenesis alleles.

The diplospory and parthenogenetic loci in Erigeron: The apparent reduction in recombination rate associated with diplospory is not characteristic of large regions of the genome of Erigeron, but rather appears to be restricted to LG-D. Only one other region comprising 5 markers separated by an average of 0.3 cM (LG-33; Table 4) appears to experience similarly low recombination levels. Genetic analysis for *Pennisetum squamulatum* (Ozias-Akins *et al.* 1993, 1998) similarly reveals a large number of markers (12) completely associated with apospory, and results for Tripsacum (Grimanelli *et al.* 1998a) similarly indicate clustering of markers in the region related to diplospory.

The basis for the apparent reduction of recombination in regions associated with unreduced megagametophyte development is unknown. Ozias-Akins *et al.* (1998), on the basis of evidence that the apospory locus in Pennisetum is hemizygous, suggested that the fragment may be introgressed from distantly related species and is thus unable to pair at meiosis. The view expressed by Grimanelli *et al.* (1998a) is that this region could be a super-gene comprising many separate but functionally interdependent factors. Inversions or other structural features that would prohibit the breakup of the region due to recombination would then be evolutionarily favored. Low rates of recombination are unfortunate because it means that conventional genetic mapping techniques (based on recombination) will be of little use for dissecting the locus. The identification of individual

genes in the region may have to be accomplished through a combination of physical and comparative mapping techniques.

Evidence indicates that the inheritance of parthenogenesis in Erigeron is simple, a finding that is consistent with results obtained via mutation analysis in nonapomictic plant species. In Arabidopsis, for example, studies indicate that fertilization-independent seed development may be initiated by mutations in single genes (Chaudhury *et al.* 1997; Luo *et al.* 1999). The wild types of these genes (*e.g.*, *FIS1* and *FIS2*) possibly function to repress autonomous embryo and seed development. Mutant alleles are thought to interfere with repression and thus cause embryo development in the absence of fertilization. The high level of variation among quantitative scores observed in this study indicates that unlinked modifiers may also influence the expression of parthenogenesis.

An alternative interpretation is that the locus discovered here that is significantly associated with parthenogenesis actually governs some earlier stage in development or possibly autonomous endosperm formation, but not parthenogenesis, *per se*. The presence of this allele then conceivably permits development to proceed but is not directly responsible for parthenogenesis. This interpretation does not necessarily detract from the central importance of the locus, however, as it is nonetheless evidently required for parthenogenesis, and only when it is paired with the diplospory locus is full agamospermous reproduction achieved.

Agamospermy and gametophytic selection: This study provides direct molecular support for the recessive lethal-gametophytic selection hypothesis of Nogler (1984b) as a general explanation for absence of agamospermy in diploid plants. First, our ability to detect markers correlated with diplospory and parthenogenesis testifies that these factors are strictly dominant. There is no evidence to indicate that the dominance of agamospermy genes is dosage or ploidal level dependent as suggested by Mogie (1988, 1992). Second, diploid Erigeron hybrids apparently fail to exhibit agamospermy, not because agamospermy genes are recessive at the diploid level nor because there is something intrinsically different about gene expression in diploids *vs.* polyploids. Instead, diploids simply fail to inherit any of the genomic regions associated with parthenogenesis or diplospory.

Parthenogenetic capability is not inherited by diploid offspring because the terminal region of LG-P, bearing markers most closely linked to the putative parthenogenesis locus, is evidently subject to gametophytic selection and therefore precluded from viable haploid gametes. These results for the inheritance of parthenogenesis support the opinion (Nogler 1984b) that agamospermy genes are associated with recessive lethal effects.

Markers linked to diplospory, on the other hand, apparently fail to occur in diploid hybrids because of univalent inheritance rather than selection. According to our assumptions, a univalent will segregate at meiosis with one or the other of two preferentially pairing homologs and thus will always occur in diploid and never in haploid gametes. This makes it difficult to test whether gametophytic selection also occurs. These results indicate that in Erigeron, gametophytic selection (of parthenogenesis-linked markers) operates in tandem with univalent inheritance (of diplospory-linked markers) to exclude agamospermy genes from diploid hybrids.

In addition to LG-P, we detected two additional regions in the Erigeron genome subject to recessive lethal-gametophytic selection, LG-11 and LG-16. These regions potentially represent a considerable selective force as consideration of the joint segregation of these three fragments indicates that ~41% of the gametes produced by P2 may be inviable. These three regions may be primarily responsible for the low (50%) pollen fertility of P2 (Noyes 2000b). It is likely that with the accumulation of additional unlinked recessive lethals, as would be expected for a predominantly asexual species (Muller 1932; Williams 1974; Lynch *et al.* 1993), the probability that triploid Erigeron will produce viable haploid gametes will approach zero.

Although our knowledge of gametophytic agamospermy in flowering plants is accumulating at an accelerating pace due to the application of mapping methods such as those applied here, our understanding at the gene level is still primitive. Our discovery that different unlinked regions may have specific agamospermy functions and our overwhelming support for the hypothesis of haploid gametophytic selection are very basic, but potentially significant findings along the path of unraveling the genetic mechanisms behind this important mode of plant reproduction.

The authors thank Jeannette Whitton for stimulating discussion on the inheritance of agamospermy and two anonymous reviewers for useful criticism that greatly improved the manuscript.

LITERATURE CITED

Asker, S. E., 1979 Progress in apomixis research. Hereditas **91:** 231–240.

Asker, S. E., and L. Jerling, 1992 *Apomixis in Plants.* CRC Press, Boca Raton, FL.

Brubaker, C. L., A. H. Paterson and J. F. Wendel, 1999 Comparative genetic mapping of allotetraploid cotton and its diploid progenitors. Genome **42:** 184–203.

Byrne, M., J. C. Murrell, B. Allen and G. F. Moran, 1995 An integrated genetic linkage map for eucalypts using RFLP, RAPD and isozyme markers. Theor. Appl. Genet. **91:** 869–875.

Chang, C., J. L. Bowman, A. W. Dejohn, E. S. Lander and E. M. Meyerowitz, 1988 Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **85:** 6856–6860.

Chaudhury, A. M., L. Ming, C. Miller, S. Craig, E. S. Dennis *et al.*, 1997 Fertilization-independent seed development in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **94:** 4223–4228.

Cronquist, A., 1947 Revision of the North American species of Erigeron, north of Mexico. Brittonia **6:** 121–302.

Da Silva, J. A. G., and M. E. Sorrells, 1996 Linkage analysis in polyploids using molecular markers, pp. 211–228 in *Methods of*

Genome Analysis in Plants, edited by P. P. Jauhar. CRC Press, Boca Raton, FL.

Da Silva, J. A. G., M. E. Sorrells, W. L. Burnquist and S. D. Tanksley, 1993 RFLP linkage map of *Saccharum spontaneum*. Genome **36:** 782–791.

De Wet, J. M. J., and J. R. Harlan, 1970 Apomixis, polyploidy, and speciation in Dichanthium. Evolution **24:** 270–277.

Doerge, R. W., Z-B. Zeng and B. S. Weir, 1997 Statistical issues in the search for genes affecting quantitative traits in experimental populations. Stat. Sci. **12:** 195–219.

Gill, K. S., E. L. Lubbers, B. S. Gill, W. J. Raupp and T. S. Cox, 1991 A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome **34:** 362–374.

Grant, V., 1981 *Plant Speciation, Ed. 2*. Columbia University Press, New York.

Grimanelli, D., O. Leblanc, E. Espinosa, E. Perotti, D. G. de Leon *et al.*, 1998a Mapping diplosporous apomixis in tetraploid Tripsacum: one gene or several genes? Heredity **80:** 33–39.

Grimanelli, D., O. Leblanc, E. Espinosa, E. Perotti, D. G. de Leon *et al.*, 1998b Non-mendelian transmission of apomixis in maize-Tripsacum hybrids caused by a transmission ratio distortion. Heredity **80:** 40–47.

Gustafsson, A., 1946–1947 Apomixis in higher plants. Lunds Universitets Arsskrift **42–43:** 1–370.

Gustine, D. L., R. T. Sherwood and D. R. Huff, 1997 Apospory-linked molecular markers in buffelgrass. Crop Sci. **37:** 947–951.

Haldane, J. B. S., 1919 The combination of linkage values and the calculation of distances between the loci or linked factors. J. Genet. **8:** 299–309.

Harling, G., 1951 Embryological studies in the Compositae. III. Astereae. Acta Horti Bergiani **16:** 73–120.

Hosmer, D. W., and S. Lemeshow, 1989 *Applied Logistic Regression*. John Wiley & Sons, New York.

Knapp, S. J., S. T. Berry and L. H. Rieseberg, 2000 The genetic map of sunflower, in *DNA Markers in Plants*, edited by R. L. Phillipos and I. K. Vasil. Kluwer, Dordrecht (In press).

Koltunow, A. M., R. A. Bicknell and A. M. Chaudhury, 1995 Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiol. **108:** 1345–1352.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow and M. J. Daly, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

Leblanc, O., D. Grimanelli, D. G. de Leon and Y. Savidan, 1995 Detection of the apomictic mode of reproduction in maize-Tripsacum hybrids using maize RFLP markers. Theor. Appl. Genet. **90:** 1198–1203.

Lincoln, S., M. Daly and E. Lander, 1992 *Constructing Genetic Maps with MAPMAKER/EXP 3.0, Ed. 3*. Whitehead Institute Technical Report, Cambridge, MA.

Luo, M., P. Bilodeau, A. Koltunow, E. S. Dennis, W. J. Peacock *et al.*, 1999 Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **96:** 296–301.

Lynch, M., R. B. D. Butcher and W. Gabriel, 1993 The mutational meltdown in asexual populations. J. Hered. **84:** 339–344.

Michelmore, R. W., I. Paran and R. V. Kesseli, 1991 Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88:** 9828–9832.

Ming, R., S.-C. Liu, Y.-R. Lin, J. da Silva, W. Wilson *et al.*, 1998 Detailed alignment of Saccharum and Sorghum chromosomes: comparative organization of closely related diploid and polyploid genomes. Genetics **150:** 1663–1682.

Mogie, M., 1988 A model for the evolution and control of generative apomixis. Biol. J. Linnean Soc. **35:** 127–153.

Mogie, M., 1992 *The Evolution of Asexual Reproduction in Plants*. Chapman and Hall, New York.

Muller, H. J., 1932 Some genetic aspects of sex. Am. Nat. **66:** 118–138.

Nesom, G. L., 1989 Infrageneric taxonomy of New World Erigeron (Compositae: Astereae). Phytologia **67:** 67–93.

Nogler, G. A., 1984a Gametophytic apomixis, pp. 475–518 in *Embryology of Angiosperms*, edited by B. M. Johri. Springer-Verlag, Berlin.

Nogler, G. A., 1984b Genetics of apospory in *Ranunculus auricomus*. V. Conclusions. Bot. Helv. **94:** 411–422.

Noyes, R. D., 2000a Biogeographical and evolutionary insights on Erigeron and allies (Asteraceae) from ITS sequence data. Plant Syst. Evol. **220:** 93–114.

Noyes, R. D., 2000b Segregation for diplospory and seed production in sexual × agamospermous Erigeron hybrids. Int. J. Plant Sci. **161:** 1–12.

Ozias-Akins, P., E. L. Lubbers, W. W. Hanna and J. W. McNay, 1993 Transmission of the apomictic mode of reproduction in Pennisetum: co-inheritance of the trait and molecular markers. Theor. Appl. Genet. **85:** 632–638.

Ozias-Akins, P., D. Roche and W. W. Hanna, 1998 Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus that may have no allelic form in sexual genotypes. Proc. Natl. Acad. Sci. USA **95:** 5127–5132.

Pessino, S. C., J. P. A. Ortiz, O. Leblanc, C. B. do Valle, C. Evans *et al.*, 1997 Identification of a maize linkage group related to apomixis in Brachiaria. Theor. Appl. Genet. **94:** 439–444.

Pessino, S. C., J. P. A. Ortiz, M. D. Hayward and C. L. Quarin, 1999 The molecular genetics of gametophytic apomixis. Hereditas **130:** 1–11.

Reinisch, A. J., J. Dong, C. L. Brubaker, D. M. Stelly, J. F. Wendel *et al.*, 1994 A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: chromosome organization and evolution in a disomic polyploid genome. Genetics **138:** 829–847.

Richards, A. J., 1973 The origin of Taraxacum agamospecies. Bot. J. Linnean Soc. **66:** 189–211.

Richards, A. J., 1997 *Plant Breeding Systems, Ed. 2*. Chapman and Hall, New York.

Savidan, Y., 1980 Chromosomal and embryological analyses in sexual × apomictic hybrids of *Panicum maximum* Jacq. Theor. Appl. Genet. **57:** 153–156.

Smith, J., 1841 Notice of a plant which produces seeds without any apparent action of pollen. Trans. Linn. Soc. Lond. **18** (meeting of June 18, 1839, cited in Gustafsson 1946–1947).

Solbrig, O. T., 1962 The South American species of Erigeron. Contrib. Gray Herb. Harv. Univ. **191:** 3–79.

Sörenson, T., 1958 Sexual chromosome aberrants in triploid apomictic Taraxaca. Sven. Bot. Tidskr. **54:** 1–22.

Stansfield, W. D., 1983 *Schaum's Outline of Theory and Problems of Genetics, Ed. 2*. McGraw Hill, New York.

Van Baarlen, P., M. Verduijn and P. J. van Dijk, 1999 What can we learn from natural apomicts? Trends Plant Sci. **4:** 43–44.

Vielle-Calzada, J.-P., C. F. Crane and D. M. Stelly, 1996 Apomixis: the asexual revolution. Science **274:** 1322–1323.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee *et al.*, 1995 AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:** 4407–4414.

Williams, G. C., 1974 *Sex and Evolution*. Princeton University Press, Princeton, NJ.

Wu, K. K., W. Burnquist, M. E. Sorrells, T. L. Tew, P. H. Moore *et al.*, 1992 The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. **83:** 294–300.

Xu, W. W., D. A. Sleper and S. Chao, 1995 Genome mapping of polyploid tall fescue (*Festuca arundinacea* Schreb.) with RFLP markers. Theor. Appl. Genet. **91:** 947–955.

Communicating editor: J. A. Birchler