ORIGINAL ARTICLE

Mendelian segregation for two-factor apomixis in *Erigeron annuus* (Asteraceae)

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The inheritance of asexual seed development (apomixis) in *Erigeron annuus* (Asteraceae) was evaluated in a triploid ($2n=3x=27$) population resulting from a cross between an apomictic tetraploid ($2n=4x=36$) pollen parent and a sexual diploid ($2n=2x=18$) seed parent. Diplospory (unreduced female gametophyte formation) and autonomous development (embryo and endosperm together) segregated independently in the population yielding four distinct phenotype classes: (1) apomictic plants combining diplospory and autonomous development, (2) diplosporous plants lacking autonomous development, (3) meiotic plants with autonomous (though abortive) development and (4) meiotic plants lacking autonomous development. Each class was

represented by approximately one-quarter of the population ($n=117$), thus corresponding to a two-factor genetic model with no linkage ($\chi^2=2.59$, $P=0.11$). Observations demonstrate that autonomous embryo and endosperm development (jointly) may occur in either reduced or unreduced egg cells. The cosegregation of the traits is attributed to tight linkage or pleiotropy. The data are consistent with the hypothesis that autonomous development in *E. annuus* is regulated by a single fertilization factor, *F*, which initiates development of both the embryo and the endosperm in the absence of fertilization.

Heredity (2007) **98**, 92–98. doi:10.1038/sj.hdy.6800907; published online 4 October 2006

Keywords: apomixis; diplospory; parthenogenesis; polyploidy

Introduction

Great strides have been made in the last decade to elucidate the genetics of asexual seed development, that is, apomixis, in flowering plants (Bicknell and Koltunow, 2004). The majority of research has focused on gametophytic apomicts; those apomicts that include in their development a fully unreduced female gametophyte bearing an unreduced egg cell. Gametophytic apomicts are traditionally classified as aposporous, the unreduced gametophyte arising by mitotic divisions of nucellar cells surrounding the megaspore mother cell (MMC), or diplosporous, the unreduced gametophyte arising directly via mitotic or mitotic-like divisions of the MMC (Gustafsson, 1946–1947; Asker and Jerling, 1992).

There is a conspicuous lack of consensus regarding the number of independent genetic factors that control asexual seed development in gametophytic apomicts. A few earlier studies of aposporous apomicts indicated that all of apomictic development was controlled by a single dominant genetic locus (Grimanelli *et al.*, 2001; Grossniklaus *et al.*, 2001). However, more recent studies, of both aposporous and diplosporous taxa, indicate that different elements of apomixis (i.e., unreduced female gametophyte formation, autonomous embryo development, autonomous endosperm development) may be controlled by separate genetic loci. Examples include

Taraxacum officinale (van Dijk *et al.*, 1999, 2003), *Hieracium* (Bicknell and Koltunow, 2004), *Poa pratensis* (Albertini *et al.*, 2001) and *Ranunculus megacarpus* (Nogler, 1984). In each case, segregation indicated either close genetic linkage between separate apomixis factors, or an imprecise genetic relationship.

The focus of this work is on apomixis in the daisy fleabane, *Erigeron annuus* (Asteraceae). This taxon, an annual to short-lived perennial herb, is predominantly triploid ($2n=3x=27$), and is diplosporous with autonomous endosperm and embryo development. *E. annuus* and most other apomicts in the Asteraceae contrast with the majority of apomictic plants that require fertilization of the central cell for endosperm formation (pseudogamy). Apomictic development in *E. annuus* involves a mitotic or mitotic-like division of the MMC that produces two nuclei, each of which undergoes two subsequent mitotic divisions to yield a mature, 8-nucleate female gametophyte (*Antennaria* type; Gustafsson, 1946–1947). Sexual relatives, in *E. strigosus*, are diploid ($2n=2x=18$), outcrossing and have tetrasporic female gametophyte development in which all four products of meiosis participate in the formation of the haploid female gametophyte (Noyes and Allison, 2005).

A previous genetic map for *E. annuus* was based on an experimental population formed by crossing a triploid apomict with a sexual diploid seed parent (Noyes, 2000; Noyes and Rieseberg, 2000). Results from that study indicated that apomixis was regulated by two independently segregating loci. One locus was associated with parthenogenetic seed formation, whereas the other was associated with diplospory. That analysis, however, suffered a few key shortcomings. First, there was nearly

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Received 14 January 2006; revised 22 August 2006; accepted 31 August 2006; published online 4 October 2006

continuous variation in the chromosome numbers of F1s from diploid to triploid levels, which complicated phenotypic characterization and molecular marker analyses. Second, the capacity for autonomous development in the mapping population was inferred from seed production rather than from direct observation of development. This method failed to consider the likelihood that autonomous endosperm and embryo development in meiotic plants, particularly those with low chromosome number, could be abortive. Third, using seed formation as a proxy for autonomous development assumed that autonomous embryo and endosperm formation were genetically regulated by a single pleiotropic locus. However, the two traits were not evaluated independently.

The objective of this study was to explore the inheritance of apomixis in an experimental population generated by crossing a recombinant apomictic tetraploid pollen parent with a sexual diploid seed parent. This experiment was used to (1) investigate the segregation of apomixis in a chromosomally uniform (triploid) population, (2) evaluate, quantitatively, the expression of autonomous embryo and endosperm development in hybrids and (3) test the hypothesis that autonomous embryo and endosperm formation are pleiotropically linked in *E. annuus*.

Materials and methods

The pollen parent used in this experiment was a tetraploid ($2n = 4x = 36$) apomict ($D \times P1.005$) of hybrid origin formed by a recombination between diplosporous and parthenogenetic parents (Figure 1; Noyes, 2006). $D \times P1.005$ thus contained a mixture of the genome of wild-type apomictic triploid ($2n = 3x = 27$) *E. annuus* and sexual diploid ($2n = 2x = 18$) *Erigeron strigosus* grandparents. Ovule development for $D \times P1.005$ included 50.4% unreduced ovules by diplospory and 49.6% reduced ovules by meiotic tetraspory. In addition, $D \times P1.005$ capitula yielded an average of 24.3% seed, and produced high-quality pollen of uniform size as estimated with Cotton Blue in lactophenol staining

(estimated viability = 98%). The seed parent used in this experiment was sexual diploid ($2n = 2x = 18$) *E. strigosus* var. *strigosus* (RDN #1618) obtained from Jenkins Co., GA, USA. This plant was 100% meiotic via tetraspory. To evaluate developmental attributes of mature ovules of both parents, capitula at full anthesis (4–5 days post-initiation of anthesis in the centripetally flowering capitulum) were fixed in formalin:acetic acid:ethanol (FAA), cleared in methyl salicylate and >100 ovules were viewed with differential interference contrast optics (DIC) as described previously (Noyes, 2000, 2006).

Pollinations ($D \times P1.005 \times \#1618$) were performed over a 1 week period in a greenhouse at the University of Colorado. Crosses yielded abundant seed, of which 121 progeny were grown to maturity under standard greenhouse conditions. Plants bolted after approximately 6 months. Chromosome counts were performed for all hybrids using the acetocarmine squash technique and visualized with bright field microscopy as previously described (Noyes, 2000). Five to ten spreads with well-separated chromosomes were evaluated for each plant.

All progenies were scored quantitatively for mode of development. Capitula (comprising on average ca. 90 ray and 250 disc florets) were fixed in FAA, cleared in methyl salicylate and whole ovaries (each bearing a single ovule) were visualized using DIC. Two different stages of development were scored for each plant: (1) young ovules to characterize the first division of the MMC as either meiotic or mitotic (diplosporous) and (2) mature ovules at full anthesis to examine the condition of mature megagametophytes. At both stages for all plants, >50 each of ray and disc ovules from a single capitulum were evaluated. Mature stages for parental plants were also characterized. Scoring of early megagametophyte development in *Erigeron* is simple because diplospory (two large nuclei) versus meiosis (four relatively smaller nuclei) is readily determined (Noyes, 2000, 2006). Mature resting megagametophytes in *E. annuus* (consisting principally of a large domed egg cell, fusion nucleus and micropylar synergids), as well as embryos and endosperm, are also easily viewed in cleared, mature ovules. Seed production for hybrids, as applicable, was estimated by simple counts of total number of filled achenes over the total number of florets within a single capitulum. Statistical analyses were performed with the Analyse-It plug-in program (version 1.60.0.1, Analyse-it Software, Ltd, Leeds, UK) for Excel (version 10.0.4, Microsoft Corp., Redmond, WA, USA).

Results

Apomictic pollen parent $D \times P1.005$ produced mature megagametophytes of high quality, with only approximately 0.07 degenerate ovules, that is, with the gametophyte either completely absent, collapsed into a single narrow dense mass or lacking an egg apparatus. Of the remaining ovules, 0.73 showed evidence of endosperm (0.09), embryos (0.21) or both (0.44). The remaining 0.20 ovules exhibited resting mature gametophytes with no evidence of autonomous embryo or endosperm development. For the sexual diploid seed parent (#1618), 0.73 ovules exhibited the mature resting stage, and 0.26 were degenerate. One of 111 ovules (0.009) showed endosperm and embryo development, which was attributed to a rare self-fertilization event.

Figure 1 Crossing design. Experimental population of 119 triploid plants generated by crossing a tetraploid apomict of hybrid origin ($D \times P1.005$) with sexual diploid *E. strigosus* (#1618).

Figure 2 Proportion of diplospory for 119 triploid hybrids. Remaining ovules are meiotic in 117 of the hybrids except for a low proportion of ovules (mean=0.02) in each plant with degenerate morphology. 'D + F' = apomicts, that is, plants exhibiting both diplospory (D) and autonomous embryo and endosperm development (F); 'D + 0' = plants exhibiting diplospory, but lacking autonomous development; '0 + F' = meiotic plants with autonomous embryo and endosperm development (abortive); '0 + 0' = wild-type sexual plants, that is, meiotic and lacking autonomous development. Plants with exceptionally high levels of degenerate ovules (0.64 and 0.20) excluded from analyses.

Chromosome counts for 119 of 121 progeny (0.98) were approximately triploid with $2n=26$, 27 or 28. These plants were interpreted to have originated via the syngamy of a reduced egg cell ($n=x=9$) produced by the diploid seed parent with a reduced sperm cell ($n=2x-1$, $2x$, $2x+1=17$, 18, 19) produced by the tetraploid apomictic pollen parent. Eutriploid progeny ($2n=3x=27$) predominated (0.85), whereas aneuploid hybrids were relatively infrequent ($2n=3x-1=26$, 0.04; $2n=3x+1=28$, 0.11). Two hybrids were diploid ($2n=2x=18$) that likely arose via self-fertilization by the diploid seed parent; these were excluded from subsequent analyses.

Diplospory segregated in the population (Figure 2) with 60/117 plants exhibiting diplospory (D) levels ranging from 0.40 to 1.00 ovules per plant (mean = 0.85). The balance of young ovules was meiotic (mean = 0.14) or degenerate (mean = 0.02). Fifty-seven plants in the population exhibited either no or a very low proportion of diplosporous ovules (mean = 0.008). The majority of ovules in these plants exhibited normal appearing tetrads (mean = 0.97); degenerate ovules were infrequent (mean = 0.02). Two plants in the population (Figure 2) were exceptional in that they produced high levels of degenerate ovules. One of these (#93) produced 0.64 degenerate, 0.13 diplosporous and 0.23 meiotic ovules, whereas the other (#102) produced 0.20 degenerate, 0.09 diplosporous and 0.70 meiotic ovules. Although these plants are portrayed as diplosporous (Figure 2), their atypical development lent uncertainty as to their proper classification. Consequently, they were excluded from subsequent analyses.

Of 117 hybrids, 65 exhibited autonomous development of embryos and endosperm (F), whereas 52 showed no evidence of autonomous development. The plants with autonomous development included 29 plants that were also diplosporous, and 36 plants that were meiotic. The joint consideration of diplospory and autonomous development yielded four categories of hybrids (Figure 2): (1) apomictic plants (D + F), that is, combining diplospory and autonomous embryo and endosperm

Figure 3 Micrographs of cleared ovules viewed with DIC of development for each of four classes of progeny generated by the cross in Figure 1. (a) Embryo and endosperm development in apomict #27. (b) Unreduced megagametophyte produced by diplosporous plant #112 lacking autonomous development. (c) Embryo and endosperm in meiotic plant #1 with abortive autonomous development. (d) Reduced megagametophyte of meiotic origin in #106 lacking autonomous development. e = egg, fn = fusion nucleus, en = endosperm nucleus, em = embryo. Bar = 20 μ m.

development (Figure 3a), $n=29$; (2) diplosporous plants (D + 0) lacking autonomous development (Figure 3b), $n=31$; (3) meiotic plants (0 + F) with autonomous development (Figure 3c), $n=36$ and (4) wild-type sexual plants (0 + 0), that is, meiotic plants lacking autonomous development (Figure 3d); $n=21$. These data correspond to a two-locus genetic model with independent segregation of diplospory and autonomous development ($\chi^2=2.59$, $df=1$, $P=0.11$).

Consideration of reproductive attributes (Figure 4) indicates the principal similarities and differences among the four phenotype classes. For instance, the four groups are similar in that each produced a low proportion of degenerate ovules at the time of division of the MMC (group means of 0.01, 0.02, 0.02, 0.02; analysis of variance (ANOVA), $P=0.36$). At anthesis, furthermore, although there is a high level of individual variation in the proportion of ovules that are degenerate (range = 0.0–0.73;

overall mean = 0.23), most of the variation resides within, rather than between groups (group means of 0.25, 0.27, 0.22, 0.20; ANOVA, $P=0.45$). These results indicate that production of degenerate ovules at anthesis occurred independently of either diplospory or autonomous development.

Differences among the four groups were observed in the condition of ovules at anthesis (Figure 4). For instance, an average of 0.60 ovules in apomictic hybrids (D + F) exhibited autonomous development, which was significantly higher than the corresponding mean of 0.26 for meiotic (0 + F) plants (Student's t -test, one-tailed, $P=9 \times 10^{-13}$). Note that for all 65 plants scored as possessing capacity for autonomous development, both endosperm and embryo formation were observed together, that is, a plant was never observed that produced only endosperm or only embryos. Correspondingly, neither endosperm nor embryo development, either separately or together, were ever observed in the other 52 hybrids (D + 0, 0 + 0).

The difference observed in autonomous development corresponded to a significant difference between diplosporous and meiotic plants in the proportion of mature, resting ovules (0.14 vs 0.52, respectively; Student's t -test, one-tailed, $P=7.9 \times 10^{-19}$). Because there was not a significant difference between the two groups in the proportion of degenerate ovules (0.25 vs 0.22; Student's t -test, two-tailed, $P=0.51$), the data are consistent with the hypothesis that autonomous development was simply initiated in a higher proportion of ovules in diplosporous than in meiotic plants. As might be expected, the mean proportions of resting ovules (0.73 and 0.80) in the two hybrid classes lacking autonomous development was considerably higher than in the two classes with autonomous development.

Most of the diplosporous plants with autonomous development (D + F; 27/29; 0.93) also produced seed

(Table 1). Two plants, #58 and #71, although producing 0.73 and 0.22 ovules, respectively, with evident capacity for autonomous development, yielded no filled seed at maturity. Among the 27 seed producers, production varied from 0.03 (#115) to 0.69 (#10). On average, the proportion of seed produced (0.31) was approximately half the estimated proportion of ovules exhibiting autonomous development (0.61). Differences among individuals were marked; in some (#8, #10, #17, #37, #79, #109, #113) the proportion of ovules exhibiting autonomous development and the proportion of seed produced were roughly equivalent. For many others, even though a substantial proportion of ovules exhibited autonomous development, a relatively small proportion of seed was observed. For instance, #27, which produced 0.99 unreduced ovules and 0.70 ovules with autonomous development, ultimately produced only 0.12 seed. Note that seed was never observed for the 36 meiotic plants with autonomous development, indicating that all embryos had aborted.

Linear regression was employed to explore the relationship between diplospory and embryo and seed formation for apomictic (D + F) hybrids (Figure 5). Prediction values factored-out degenerate ovules produced by subtracting the observed proportion of degenerate ovules at anthesis. This yielded an expected proportion of viable ovules at anthesis for each plant. For these viable ovules, the relative proportion of reduced and unreduced ovules was assumed to be the same as measured at the time of division of the MMC. Proportion of viable diplosporous ovules at anthesis was thus calculated as $(1.0 - P(\text{degenerate ovules})) \times P(\text{diplosporous ovules at division of MMC})$. The participation of meiotic ovules in embryo and seed production was considered by including relative proportions of 1.00, 0.50 or 0.00 in the prediction variable. Factors of 1.00 and 0.00 assumed, alternatively, that all or no meiotic ovules would exhibit autonomous development, whereas 0.50 assumed that the presence of a factor for autonomous development was required, but segregated among ovules.

The proportion of meiotic ovules included in the prediction variable affected the magnitude of regression coefficients. For instance, in predicting autonomous development, analyses including 1.0 and 0.5 meiotic ovules in the prediction variable ($R^2=0.75$ and 0.72, respectively; Figure 5a) were considerably higher than the value resulting when meiotic ovules were excluded ($R^2=0.55$). In contrast, for predicting seed production, analyses excluding meiotic ovules yielded a coefficient ($R^2=0.34$; Figure 5b) that was markedly higher than values when 0.5 or 1.0 meiotic ovules were included ($R^2=0.26, 0.15$, respectively).

Discussion

Before this study, evidence for multilocus control of apomixis has generally been weak. For instance, for *R. megacarpus*, initial inference that apospory and parthenogenesis were closely, rather than completely linked, was based on 1/41 experimental hybrids that exhibited apospory but not parthenogenesis (Nogler, 1984). Subsequent re-evaluation, based on further crosses and cytological study, led to the conclusion that capacity for parthenogenesis was likely present, although at a low

Figure 4 Developmental attributes of ovules for 117 hybrids. Four classes of progeny (D + F, D + 0, 0 + F, 0 + 0) as defined. 'Early' = stage of division of the MMC; 'Mature' = 4–5 days post-anthesis; 'Degen' = proportion of ovules with degenerate ovules (see the text); 'Emb ± End' = proportion of ovules exhibiting embryo and/or endosperm development; 'Undev' = proportion of mature gametophytes exhibiting the resting stage. ¹No significant differences among the four classes. ²Proportion of ovules exhibiting autonomous development significantly higher in D + F compared with 0 + F plants. ³Proportion of undeveloped ovules significantly higher in 0 + F compared with D + F plants.

Table 1 Reproductive attributes of 29 hybrid apomicts, by diplospory (P)

Plant ID	Chromosome number	Degenerate ovules (P) ^a	Diplospory (P) ^a	Degenerate ovules (P) ^b	Resting gametophytes (P) ^b	Ovules with embryos and/or endosperm (P) ^b	Seed (P) ^c
17	27	0.00	1.00	0.38	0.13	0.49	0.48
27	27	0.00	0.99	0.19	0.11	0.70	0.12
113	27	0.00	0.98	0.72	0.12	0.16	0.22
10	27	0.00	0.98	0.06	0.26	0.68	0.69
83	27	0.00	0.98	0.13	0.13	0.75	0.42
71	27	0.00	0.97	0.66	0.19	0.14	0.00
79	27	0.00	0.96	0.62	0.16	0.22	0.42
70	27	0.00	0.96	0.15	0.05	0.81	0.32
7	27	0.00	0.94	0.40	0.06	0.54	0.32
59	27	0.00	0.94	0.20	0.07	0.73	0.55
88	27	0.02	0.94	0.26	0.33	0.41	0.10
25	27	0.04	0.92	0.16	0.16	0.68	0.40
8	27	0.00	0.91	0.15	0.26	0.59	0.56
101	27	0.09	0.89	0.40	0.15	0.45	0.23
109	27	0.00	0.88	0.25	0.38	0.37	0.44
86	27	0.02	0.88	0.10	0.05	0.84	0.36
37	27	0.00	0.87	0.26	0.14	0.60	0.50
84	27	0.01	0.86	0.33	0.03	0.65	0.30
43	27	0.00	0.86	0.06	0.06	0.88	0.41
78	27	0.02	0.85	0.27	0.08	0.66	0.15
30	27	0.00	0.85	0.07	0.01	0.93	0.51
65	27	0.00	0.82	0.08	0.10	0.82	0.22
9	27	0.01	0.80	0.18	0.11	0.71	0.41
90	27	0.01	0.79	0.09	0.21	0.70	0.33
52	27	0.02	0.78	0.15	0.05	0.80	0.23
80	28	0.03	0.78	0.29	0.22	0.49	0.08
58	27	0.02	0.62	0.46	0.17	0.37	0.00
69	27	0.09	0.53	0.11	0.03	0.86	0.31
115	26	0.00	0.40	0.13	0.38	0.49	0.03
Avg.	27.0	0.01	0.86	0.25	0.14	0.60	0.31

^aMeasured at division of MMC.^bMeasured at anthesis.^cMeasured as proportion of filled seed over total number of florets in a single mature capitulum. **Figure 5** Linear regression analysis for embryo and seed formation for 29 apomictic hybrids. (a) Proportion of ovules exhibiting autonomous development (embryos and/or endosperm) plotted against the proportion of unreduced ovules at maturity plus 1.00 meiotic ovules. (b) Proportion of seed produced against proportion of unreduced ovules at maturity plus 0.00 meiotic ovules.

level in the exceptional individual (Nogler, 1995). Similarly, for *P. pratensis*, inference for incomplete linkage of apospory and parthenogenesis was based on the occurrence of 2/18 aposporous hybrids that did not also exhibit parthenogenetic embryo formation (Albertini *et al.*, 2001). However, parthenogenesis in the 16 plants scored as possessing the trait ranged from 1.4 to 92.9%, which raises the possibility that the absence of embryos

could be explained by low penetrance of parthenogenesis in the two exceptional individuals. These re-interpretations would mean that apomixis in *R. megacarpus* and *P. pratensis* is most likely controlled by a single locus rather than multiple loci.

Evidence for multiple apomixis loci in the Asteraceae has been equivocal. Control of apomixis in aposporous *Hieracium*, for instance, was initially interpreted to be

monogenic (Bicknell *et al.*, 2000), but was subsequently considered to be uncertain, possibly involving three loci of undetermined linkage (Bicknell and Koltunow, 2004). In diplosporous *Taraxacum officinale*, experimental crosses (van Dijk *et al.*, 1999, 2003) indicated that apomixis was controlled by three separate loci, one each for diplospory, autonomous embryo formation and autonomous endosperm formation. This conclusion was based on analysis of hybrids from a cross in which 2/6 diplosporous individuals were inferred to possess capacity for autonomous endosperm but not embryo formation, and 4/6 individuals that possessed capacity for autonomous embryo but not endosperm formation. Although a genetic linkage map for diplospory has been produced for *Taraxacum* (Vijverberg *et al.*, 2004), the genetic relationship of factors for autonomous endosperm and embryo formation has not been determined.

For *E. annuus*, inferences regarding the precise nature of the two separate loci controlling apomixis (Noyes and Rieseberg, 2000) were weak owing to the complexity of the $3x$ (apomictic) $\times$ $2x$ (sexual) mapping population, and the incomplete characterization of development for F1s. Both of these shortcomings were alleviated in the present study. The quantitative analysis of triploid hybrids at two developmental stages permitted unequivocal categorization of hybrids into four groups that robustly support the hypothesis that apomixis in *Erigeron* is controlled by two completely independently acting loci; one locus regulates diplospory, and the other regulates, jointly, autonomous endosperm and embryo formation.

For all but two of 29 hybrids, autonomous development in combination with diplospory led to seed production. When compared with tetraploid apomict parent $D \times P1.005$, triploid hybrid apomicts showed, on average, markedly higher expression of diplospory (0.86 vs 0.50). Only one hybrid (#115) exhibited diplospory at less than the parental level. Mean seed production in hybrids, on the other hand, increased only modestly (0.31 vs 0.24). Variation was remarkable, however, with a few hybrids (#10, 0.69; #08, 0.56; #59, 0.55) approximating the value of 0.62 observed in a wild-type triploid apomict (Noyes, 2000).

Multiple factors apparently influenced the level of seed production in apomicts. First, developmental degeneration meant that at anthesis only 75% of ovules (on average) were viable (Table 1). The high level of variability (0.06 to 0.72; Table 1) is consistent with the hypothesis that deleterious factors segregated in the population. It is curious that sexual diploid parent (#1618) also exhibited a high level of degenerate ovules (0.26). Whether degenerate ovule production in hybrids was due to distinct deleterious factors being passed from parent to hybrids, or was the result of less tangible epistatic interactions is not known. Nonetheless, it is evident that natural levels of ovule degeneration should be considered in the selection of parents in future genetic studies.

A second impediment to apomictic seed production appears to involve factors that terminate embryos or endosperm after their development has been initiated. These factors apparently influence the efficiency in which plants convert ovules exhibiting autonomous development into seed. Some plants appeared to lack these deleterious factors such that the proportion of ovules with autonomous development closely approxi-

mated seed production (e.g., #17, #113, #10; Table 1). However, for other plants (e.g., #27, #70), only a modest proportion of ovules with autonomous development developed into seed. The variation among hybrids is consistent with the hypothesis that these deleterious factors are unlinked to apomixis, and segregate. Further, they may correspond to parthenogenesis preventer genes hypothesized to be important in limiting expression of apospory in *P. pratensis* (Matzk *et al.*, 2005).

The data indicate that autonomous embryo and endosperm development can occur in diplosporous as well as meiotic individuals (Figures 2 and 4). This finding refutes the previous hypothesis for *E. annuus* that the expression of autonomous developmental is contingent upon a diplosporous background, and is consistent with the observation of abortive endosperm and embryo development in a single low chromosome number meiotic plant (Noyes, 2006). The expected ploidal level of 1.5 for parthenogenetic embryos produced by meiotic triploids in this study seems likely to have been the principal reason why young embryos were observed but never filled seed. Curiously, the genome region linked to autonomous development in *E. annuus*, in contrast to that linked to diplospory, appears to experience regular recombination (Noyes and Rieseberg, 2000). This indicates that the diplospory and autonomous development regions may evolve under different evolutionary constraints. Fortunately, recombination might make it possible to isolate candidate genes for autonomous development using positional cloning methods. This could potentially lead to the identification of important developmental genes that could then be manipulated in crop taxa.

The data as to whether expression of autonomous development requires a genetic factor to be present in each ovule, or is more a characteristic of an entire capitulum, are equivocal. The difference in levels of autonomous development in meiotic versus diplosporous plants (0.60 vs 0.26; Figure 4) is consistent with the hypothesis that the gene is expressed in only that fraction of ovules in which it occurs after segregation; in other words, the majority of ovules in diplosporous plants, but only half of the ovules in meiotic plants, would exhibit autonomous development. Nonetheless, the possibility that differences in expression are due, instead, to reduction of ploidal level in ovules of meiotic plants must also be considered. Regression analyses incorporating different proportions of reduced ovules, indicate that they are likely to contribute to the total population of ovules with autonomous development (Figure 5a), but are nonetheless inconclusive. This is because variables including 0.50 and 1.00 reduced ovules yield roughly equivalent coefficients. Further, autonomous development that may occur in reduced ovules appears to contribute little to seed production, most probably due to abortion (Figure 5b).

Because they always occurred in the same plant, autonomous development of embryos and endosperm in *E. annuus* appears to be a pleiotropic manifestation of a single genetic factor. For most apomicts that are not Asteraceae, pleiotropy of this type does not occur, and fertilization of the central cell is required for endosperm development. There is a rich literature on endosperm genome balance in sexual and apomictic plants (Haig and Westoby, 1991; Quarin, 1999; Spielman *et al.*, 2003).

It has been hypothesized that because seeds produced by Asteraceae are, independently of endosperm, resource rich, the genetic balance or even the requirement for endosperm altogether may be obviated. It appears the diminished importance of endosperm in Asteraceae has contributed to the evolution of a single factor that effectively links autonomous embryo and endosperm development. In addition, observations of endosperm but not embryos in some ovules in this study may indicate that the coordination of the development of these two structures, as observed in *Arabidopsis* (Nowack et al., 2006), may be disrupted in apomictic *Erigeron*.

This pleiotropic factor, hereafter identified as 'F', apparently indicates to the plant that fertilization of egg and central cell has occurred, thus initiating autonomous development of both structures in the absence of fertilization. This use of 'F' replaces the use of 'P' in *E. annuus*, which was used imprecisely to signify a gene for parthenogenetic seed formation (Noyes and Rieseberg, 2000). If 'F' is similar to FIE and FIS in *Arabidopsis* (Luo et al., 1999; Chaudhury et al., 2001), then it may act negatively by interfering with a regulatory block that normally prevents embryo and endosperm development until a signal is received upon fertilization. Alternatively, 'F' could positively initiate development by mimicking some aspect of fertilization. The precise nature of the gene must await further genetic characterization.

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