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Chromosomal plasticity: mitigating the impacts of herbivory

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Abstract. Endoreduplication, the replication of the genome without mitosis, leads to endopolyploidy, an increase in cellular chromosome number. Although endoreduplication is widespread among angiosperms and other groups of eukaryotes, the degree to which this process is plastic under varying environmental conditions and its potential adaptive significance are not known. Here, using flow cytometry, we measured plasticity in chromosome number following the removal of apical dominance (simulating natural herbivory) in two ecotypes of *Arabidopsis thaliana*: Columbia and Landsberg *erecta*. We report that endopolyploidy of clipped Columbia plants was significantly different than unclipped controls following the removal of apical dominance and regrowth, and that cellular ploidy is positively associated with attributes of fitness (biomass, flower, fruit, and seed production). In contrast, clipped Landsberg *erecta* showed no significant differences in endopolyploidy and a decrease in seed production compared to unclipped controls; representing a significant genotype × environment interaction between ecotypes. Altering ploidy via endoreduplication adds a previously unknown way in which plants may be able to cope with environmental stress: enhancing regrowth rates and fitness following plant damage.

Key words: Arabidopsis; *chromosomal plasticity; compensation; DNA content; endoreduplication; fitness.*

Introduction

Plant tissue loss to herbivores is an important selective agent shaping plant phenotypes. To date, most studies of plant adaptation have focused on the evolution of defensive traits that reduce or prevent tissue damage by herbivores (Berenbaum et al. 1986, Mauricio and Rausher 1997, Agrawal 1998). However, herbivores may also select for traits that allow plants to maintain fitness in the face of tissue loss (Stowe et al. 2000). Plant genotypes that can compensate for tissues lost with little or no decrement in fitness relative to those that are undamaged represent such an example and are termed tolerant (see Stowe et al. 2000 for a review). Interest in tolerance was stimulated by empirical studies demonstrating that herbivore damage can, under certain circumstances, increase, rather than decrease, plant reproductive success (a specialized case of tolerance, termed overcompensation, i.e., increased flower, fruit, and seed production following herbivory). Specifically, studies by Paige and Whitham (1987) showed that when ungulate herbivores removed 95% or more of the aboveground biomass of the monocarpic biennial scarlet gilia, *Ipomopsis aggregata*, the product of lifetime seed production, seed germination, and seedling survival averaged 3.0 times that of uneaten controls (see also Paige 1992, 1994, 1999). The increase in relative fitness was largely due to architectural changes in the plant; removal of scarlet gilia's single inflorescence resulted in the production of multiple flowering stalks due to the release of apical dominance and an overall increase in both above- and belowground biomass.

With an increasing number of investigators seeking evidence for overcompensation, more supportive evidence is being uncovered. For example, evidence for increased flower, fruit, and seed production following herbivory has been found for a number of plant species including two species of *Ipomopsis*, *I. aggregata* and *I. arizonica* (Paige and Whitham 1987, Maschinski and Whitham 1989), and several unrelated to *Ipomopsis* including *Gentianella campestris*, *G. amarella* (Nilsson et al. 1996, Lennartsson et al. 1997), *Sanicula arctopoides* (Lowenberg 1994), *Bouteloua gracilis* and *Bouteloua hirsute* (Alward and Joern 1993), *Ipomoea purpurea* (Hougen-Eitzman and Rausher 1994), *Arabidopsis*

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thaliana (Mauricio et al. 1997, Weinig et al. 2003), and *Erysimum strictum* (Rautio et al. 2005).

There is also evidence that genetic variation for tolerance exists. Specifically, some families exhibit overcompensating tolerance, whereas others express incomplete tolerance (Mauricio et al. 1997, Tiffin and Rausher 1999, Juenger and Bergelson 2000, Weinig et al. 2003). Heritability of traits associated with tolerance has been demonstrated in one population of scarlet gilia as well (Juenger and Bergelson 2000). In addition, recent studies comparing historically grazed and ungrazed populations of the plant *Gentianella campestris* indicate that repeatedly grazed populations can evolve overcompensating tolerance, while ungrazed populations remain completely intolerant (Lennartsson et al. 1997). Although these observations provide evidence that genetic variation for tolerance exists, little is known about the genetic mechanisms that may lead to enhanced growth and reproduction in plant species exhibiting growth compensation.

Here we propose taking the first steps toward testing a novel idea: that endoreduplication leads to enhanced growth and reproduction following herbivory, explaining the phenomenon of tolerance/overcompensation in plants. Endoreduplication is the replication of the genome without mitotic cell division, leading to endopolyploidy, where individual cells within an organism produce higher nuclear DNA content from consecutive doublings (Nagl 1976, Brodsky and Uryvaeva 1977, Melaragno et al. 1993). This process is common in many groups of eukaryotes and nearly the rule in angiosperms (Nagl 1976, Sugimoto-Shirasu and Roberts 2003). Endoreduplication may have genetic and/or nucleotypic effects (an effect based on DNA content alone) that could lead to rapid regrowth and enhanced fitness (see *Discussion*). Interestingly, removal of plant apical dominance, which causes a reduction in the level of auxin and leads to axillary bud break and stem regeneration, is known to trigger an exit from mitotic cycles and an entry into endocycles (Ishida et al. 2010). In contrast, high levels of auxin repress the endocycle (Ishida et al. 2010). Thus, there appears to be a direct link between endoreduplication and the removal of apical dominance. However, the degree to which endoreduplication might be plastic following herbivory, or its adaptive significance, is unknown. In this study we assess (1) whether there is plasticity in endoreduplication following the removal of apical dominance and (2) whether there is a positive relationship between endoreduplication and fitness compensation.

Here we use the model system *Arabidopsis thaliana* to address these issues. Our previous studies established that ecotypes of *A. thaliana* differ in their ability to compensate for tissue loss due to herbivory, ranging from lowered fitness after damage (undercompensation; e.g., Landsberg *erecta*, Ler-0) to increased fitness after damage (overcompensation; e.g., Columbia, Col-4). Landsberg *erecta* (hereafter Ler.) shares a common genetic background with Columbia (hereafter Col.), given that Col. was derived from the nonirradiated Laibach Landsberg population (from the Nottingham Arabidopsis Stock Center; description *available online*).[2] *A. thaliana*, a diploid with five chromosomes, has been shown to endoreduplicate extensively in nearly all of its tissues (Sugimoto-Shirasu and Roberts 2003), producing nuclei with DNA content as high as 64C (Melaragno et al. 1993), which represents five endoreduplication cycles. In addition, endopolyploidy in *Arabidopsis* likely represents a form of polyteny (i.e., the quantity of DNA doubles with each endocycle but the number of chromosomes remain the same due to sister chromatid cohesion; Melaragno et al. 1993, Sugimoto-Shirasu and Roberts 2003).

Methods

To assess the relationship between plant compensation and the degree of plasticity in endoreduplication following the removal of apical dominance, we planted 170 individuals each of Ler. and Col. and grew them under greenhouse conditions. At 2.5 weeks, prior to bolting, 10 rosettes of each ecotype were harvested and analyzed for DNA content to establish baseline ploidy. When inflorescences reached 6 cm (~3.5 weeks), 80 plants of each ecotype were clipped, leaving approximately 1 cm of inflorescence (comparable to mammalian herbivory). At 4.5 weeks, 20 plants of each ecotype (10 clipped, 10 unclipped) were analyzed for DNA content, independently analyzing rosettes and inflorescences (stems, flower buds, leaves). At approximately 6.5 weeks (nearing senescence), 30 additional inflorescences (stems, leaves, flowers, flower buds, and valves of siliques) of each ecotype (15 clipped, 15 unclipped) were analyzed for DNA content.

Tissue for flow cytometric analysis was prepared via standard protocols (see Galbraith et al. 1983). In brief, plant tissue was chopped with a razor blade, matched for tissue type (main stems, lateral stems, leaves, etc.) and biomass, sheared in a nuclear isolation buffer (sodium citrate, MOPS, magnesium chloride, Triton X-100; Galbraith et al. 1983), filtered for debris removal, and stained with propidium iodide. Suspended nuclei were analyzed via a BD Biosciences FACScanto flow cytometer (San Jose, California, USA) for measurement of nuclear DNA content. Background correction and nuclei population gating were performed using De Novo Software FCS Express (v.3; Los Angeles, California, USA) to measure the proportion of nuclei at each ploidy level (2C, 4C, 8C, 16C) per plant sample. Mean nuclear DNA content per cell per plant was estimated via calculating a weighted average fluorescence value per plant based on the number of nuclei and average fluorescence of each ploidy level. The 2C ploidy level of each plant was used as the internal standards

[2] ⟨http://arabidopsis.info/CollectionInfo?id=94⟩

TABLE 1. (A) Percentage of rosette nuclei at each ploidy level for Columbia and Landsberg *erecta* of *Arabidopsis thaliana* prior to bolting at 2.5 weeks ($n = 10$ rosettes per ecotype). (B) Percentage of rosette nuclei at each ploidy level for Columbia and Landsberg *erecta* one week after clipping, i.e., at 4.5 weeks of age. (C) Percentage of inflorescence (stems, leaves, flowers, flower buds, and valves of siliques) nuclei at each ploidy level for clipped and unclipped Columbia and Landsberg *erecta* at 4.5 weeks ($n = 10$ plants per treatment per ecotype) and 6.5 weeks after germination ($n = 15$ plants per treatment per ecotype).

		Columbia			Landsberg *erecta*		
Time	Ploidy	Clipped	Unclipped	*P*	Clipped	Unclipped	*P*
A) Rosettes							
2.5 weeks	2C		63.23 ± 3.47			61.29 ± 2.40	0.652†
Baseline	4C		36.77 ± 3.47			38.71 ± 2.40	0.652†
B) Rosettes							
4.5 weeks	2C	48.03 ± 1.73	46.37 ± 1.79	0.514	41.27 ± 2.39	41.20 ± 2.93	0.986
	4C	29.24 ± 0.83	32.27 ± 0.91	0.024	32.14 ± 1.88	33.92 ± 1.43	0.460
	8C	14.50 ± 0.94	14.78 ± 1.16	0.855	19.85 ± 1.04	17.56 ± 1.13	0.155
	16C	8.24 ± 0.81	6.59 ± 0.63	0.126	6.75 ± 0.59	7.32 ± 1.06	0.645
C) Inflorescences							
4.5 weeks	2C	51.88 ± 0.79	46.35 ± 0.75	0.0001*	51.12 ± 1.04	46.97 ± 1.95	0.085
	4C	31.93 ± 0.35	34.69 ± 0.51	0.0001*	31.64 ± 0.84	33.88 ± 0.66	0.054
	8C	12.15 ± 0.45	14.55 ± 0.54	0.003*	13.14 ± 0.43	14.67 ± 1.31	0.294
	16C	4.04 ± 0.27	4.42 ± 0.24	0.319	4.10 ± 0.42	4.48 ± 0.43	0.540
6.5 weeks	2C	50.02 ± 1.69	60.48 ± 1.74	0.0001*	55.47 ± 1.63	54.98 ± 1.78	0.839
	4C	31.75 ± 1.04	26.67 ± 0.78	0.001*	29.03 ± 1.22	30.46 ± 1.46	0.461
	8C	14.52 ± 0.78	10.72 ± 1.00	0.006*	13.01 ± 0.57	11.78 ± 1.06	0.316
	16C	3.71 ± 0.48	2.13 ± 0.58	0.046*	2.49 ± 0.28	2.79 ± 0.51	0.609

Notes: Values are means ± SE. Data were analyzed using independent *t* tests followed by a sequential Bonferroni adjustment to correct for multiple comparisons. Asterisks (*) indicate a significant ($P < 0.05$) difference following adjustment. Rosettes at 2.5 weeks, rosettes and inflorescences at 4.5 weeks, and inflorescences at 6.5 weeks for each ecotype were analyzed separately given that they represented independent sets of plants.

† *P* values are for comparison of the two ecotypes.

for DNA content calculation for the 4C, 8C, and 16C levels of each plant (*A. thaliana* genome size = 0.32 pg DNA/2C nucleus).

Upon senescence (~8 weeks), the remaining plants of each treatment were analyzed for fitness (48–54 plants per treatment). Fitness included number of flowers, siliques, average number of seeds per silique, seed mass/100 seeds, number of main stems, number of lateral stems, total stem length (total length of all lateral and main stems combined), plant height, and total above-ground dry biomass at senescence. Because flower production is highly correlated with silique production ($R^2 = 0.841$, $P < 0.0001$), only the more ultimate assessments of fitness are discussed. Statistical analyses were conducted in Systat (version 13; Systat, Chicago, Illinois, USA) using independent *t* tests followed by a sequential Bonferroni adjustment (Rice 1989) for number of variables measured within each ecotype and experiment. As noted above, fitness was measured from a separate set of plants for each ecotype due to the destructive sampling necessary for obtaining nuclear DNA content. Thus, separate analyses were applied to DNA content (including separate analyses for basal ploidy [rosettes] and time points of 4.5 weeks [rosettes and inflorescences from same plants] and 6.5 weeks of age [inflorescences only]) and fitness measures for each ecotype. A genetic × environment (G × E) interaction was estimated using a multifactorial ANOVA for DNA content (combining 4C, 8C, and 16C) per cell at 6.5 weeks of age.

RESULTS

At 2.5 weeks of age, prior to elongation of the inflorescence and thus before clipping, ploidy did not differ between rosettes of the two ecotypes, Col. and Ler. ($P = 0.659$ at each of the two ploidy levels, 2C and 4C), each representing a basal diploid (2C) level with 4C cells likely representing cells with replicated nuclear DNA prior to mitotic cell division (Table 1A). At approximately 4.5 weeks of age, 1 week after clipping, clipped inflorescences of Col. differed from unclipped inflorescences of Col. at 2C, 4C, and 8C (Table 1C), whereas clipped individuals of Ler. showed no significant differences from unclipped individuals at any ploidy level (Table 1C). In Col. the percentage of nuclei at the 2C level was significantly higher for clipped plants than unclipped plants. As a result, clipped inflorescences had lower proportions of cells at higher ploidy levels than unclipped plants (Table 1C), representing a delay in the degree of endopolyploidy achieved via endoreduplication during the regeneration period. Ploidy of clipped rosettes did not differ from unclipped rosettes for either Col. or Ler. at any level of ploidy ($P > 0.05$; at this point in time plants were beginning to endoreduplicate with ploidy levels of 2C, 4C, 8C, and 16C present in rosette tissues of both ecotypes; Table 1B). Because

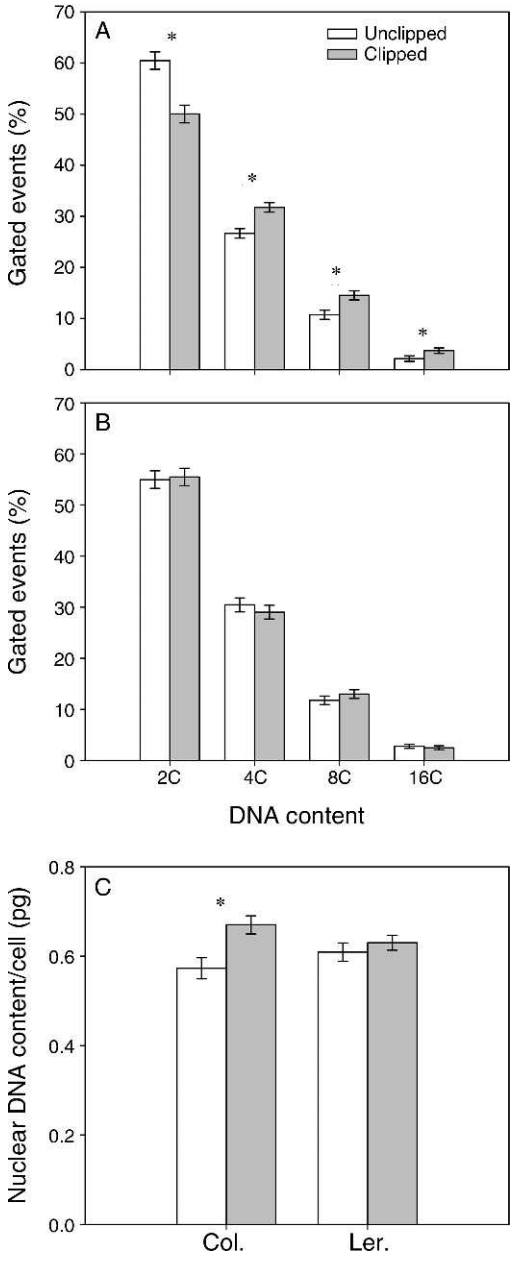

FIG. 1. Measures of DNA content. The percentage of cells at each of four nuclear DNA content levels in (A) Columbia (Col.) and (B) Landsberg *erecta* (Ler.) ecotypes of *Arabidopsis thaliana* clipped and unclipped plants at 6.5 weeks after germination. "Gated events" refers to fluorescence events measured by flow cytometry of propidium-iodide-stained DNA within suspended nuclei. Shown are means ± SE (n = 15 plants per treatment). Asterisks (*) indicate a significant difference at the $P < 0.05$ level following a sequential Bonferroni adjustment. (C) Nuclear DNA content (pg) per cell of Columbia and Landsberg *erecta* clipped and unclipped plants. Shown are means ± SE ($n = 15$ plants per treatment). Clipped Columbia plants have higher nuclear DNA content per cell than unclipped plants ($P = 0.004$).

only inflorescence tissue was removed during clipping, rosettes were not expected to differ between treatments within an ecotype, and may serve as verification that changes in ploidy in the inflorescences were due to the clipping treatment.

At 6.5 weeks of age, 3 weeks after clipping, Col. clipped plants differed from unclipped plants at the 2C, 4C, 8C, and 16C levels (Table 1C, Fig. 1A). Clipped plants of Col. had a lower proportion of cells at the 2C level than unclipped plants, indicating higher endopolyploidy in clipped plants. This is confirmed at the 4C, 8C, and 16C levels, where clipped plants had a significantly greater proportion of cells at these higher ploidy levels than did unclipped plants (Table 1C, Fig. 1A) with an overall higher DNA content (Fig. 1C, $P = 0.004$). Although the degree of endoreduplication was initially set back in Col. clipped plants at 4.5 weeks, DNA content not only recovered three weeks after clipping but was significantly higher than in unclipped control plants. In contrast, at 6.5 weeks after clipping, clipped plants of Ler. showed no significant differences in ploidy than unclipped plants (Table 1C, Fig. 1B). There was also a significant genotype X environment interaction between Ler. and Col. for higher level ploidy ($P = 0.008$; see Fig. 2).

As expected, clipped plants of Col. produced significantly greater numbers of siliques and seeds (1.51- and 1.4-fold greater, respectively, Fig. 3A and B, $P < 0.0001$ and $P < 0.0001$, Table 2) than plants that were not clipped while clipped plants of Ler. produced an equal number of siliques and fewer seeds when compared to those that were not clipped (Fig. 3A and B, for siliques $P = 0.453$ and number of seeds $P = 0.002$, Table 2). No significant differences were found for seed masses between clipped and unclipped plants of Col. (1.162

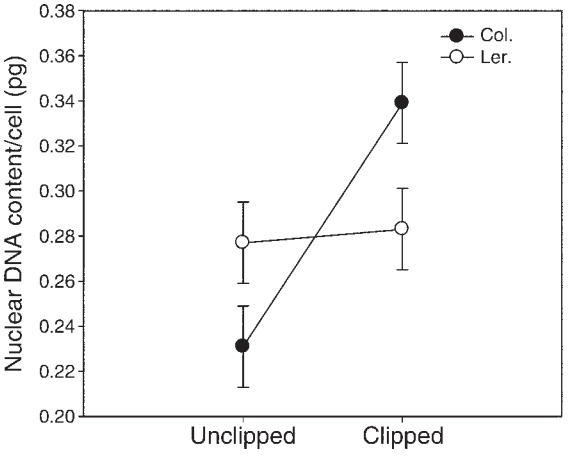

FIG. 2. Norm of reaction. Combined (4C, 8C, 16C) DNA content of Columbia and Landsberg *erecta* clipped and unclipped plants at 6.5 weeks after germination. Shown are means ± SE ($n = 15$ plants per treatment). There is a significant genotype × environment interaction ($P = 0.008$).

mg/100 seeds for clipped plants and 1.159 mg/100 seeds for unclipped plants; $P = 0.382$) or Ler. (1.161 mg/100 seeds for clipped plants and 1.159 mg/100 seeds for unclipped plants; $P = 0.94$) (Table 2). Lateral stem production was significantly higher for clipped individuals of both Col. and Ler. ($P < 0.0001$) but the magnitude was slightly higher in Col. than Ler. (1.75-fold higher for clipped plants of Col. and 1.62-fold higher for clipped plants of Ler. over unclipped controls; Table 2). Main stem production was also significantly higher in clipped plants of both Col. and Ler. ($P < 0.0001$) but the magnitude was almost twice as high in Ler. as in Col. (2.60-fold higher for clipped plants of Ler. and only 1.43-fold higher for clipped plants of Col. over unclipped controls; Table 2). Total stem lengths combined was also significantly higher in clipped plants of both Col. and Ler. compared to their unclipped controls ($P < 0.0001$) but the magnitude was slightly higher in Ler. (1.46-fold higher for clipped Ler. and 1.2-fold higher for clipped Col. over unclipped controls; Table 2). Plant height was significantly reduced in clipped plants of both Ler. and Col. compared to their unclipped controls (each by approximately 7%; Table 2). However, Col. is approximately half the height of Ler. (Table 2). Furthermore, aboveground biomass was significantly greater for both clipped plants of Col. ($P < 0.0001$) and Ler. ($P < 0.0001$) when compared to their unclipped controls, although the magnitude of increase was greater for Col. (1.49- vs. 1.24-fold greater, respectively, Fig. 3C). In sum, clipped Col. can be described as short plants with thicker heavier stems whereas clipped Ler. can be described as tall plants with thinner lighter stems.

Discussion

The fitness consequences of endoreduplication have not been previously assessed. Here we show that there is a correlation between fitness and endoreduplication—higher levels of endoreduplication, following the removal of apical dominance, are positively associated with greater biomass and higher levels of silique and seed production (relative to their uneaten controls). As an aside, total DNA content in Col. is likely greater than that reported here given that tissues were matched for biomass and biomass is significantly greater in clipped Col. than in clipped Ler., thus, our estimates are at best conservative. Based on these results, we suggest that increasing ploidy within an individual during its lifetime may add a previously unknown way in which some plants may be able to cope with herbivory and may help to explain ways in which some plant species are able to overcome and take advantage of being eaten, i.e., increasing fitness following damage (see Paige and Whitham 1987 and Lennartsson et al. 1997 for examples). In other words, we suggest that there may be a direct tie between plant compensation and

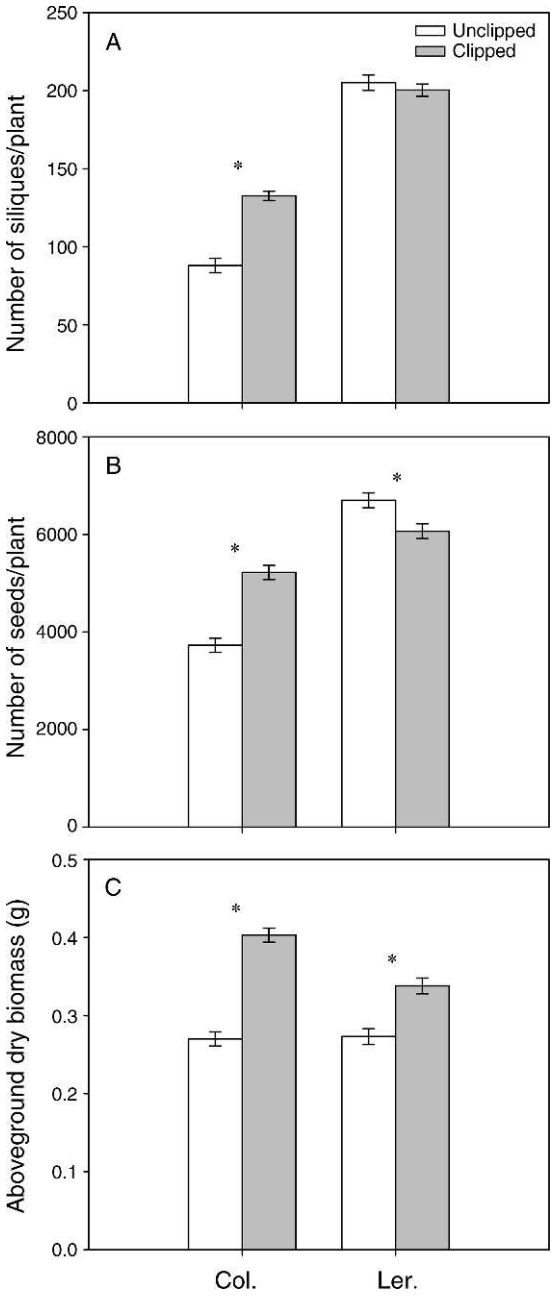

Fig. 3. Measures of fitness. Number of (A) siliques and (B) seeds per plant for Columbia and Landsberg *erecta* clipped and unclipped treatments at senescence. Shown are means ± SE ($n =$ 48–54). Asterisks (*) indicate significance ($P < 0.05$) following a sequential Bonferroni adjustment. (C) Aboveground plant biomass (g) for Columbia and Landsberg *erecta* clipped and unclipped treatments at senescence. Shown are means ± SE ($n =$ 48–54). Clipped plants of Columbia and Landsberg *erecta* have significantly higher biomass than unclipped plants.

TABLE 2. Fitness traits of clipped and unclipped plants of Columbia and Landsberg *erecta* at senescence ($n = 48$–54 plants per treatment per ecotype).

Plant trait	Columbia			Landsberg *erecta*		
	Clipped	Unclipped	*P*	Clipped	Unclipped	*P*
Number of siliques	132.76 ± 4.06	88.00 ± 4.06	0.0001*	200.33 ± 4.26	205.13 ± 4.30	0.453
Number of seeds	5221 ± 145	3728 ± 145	0.0001*	6065 ± 152	6699 ± 154	0.002*
Seed mass (mg/100 seeds)	1.162 ± 0.003	1.159 ± 0.001	0.382	1.161 ± 0.003	1.159 ± 0.003	0.940
Number of lateral stems	5.09 ± 0.08	2.91 ± 0.14	0.0001*	5.33 ± 0.21	3.29 ± 0.08	0.0001*
Number of main stems	2.89 ± 0.09	2.02 ± 0.11	0.0001*	3.57 ± 0.10	1.38 ± 0.08	0.0001*
Total stem length (cm)	72.37 ± 1.98	60.39 ± 1.81	0.0001*	214.4 ± 5.69	146.8 ± 2.78	0.0001*
Aboveground biomass (g)	0.403 ± 0.011	0.270 ± 0.012	0.0001*	0.338 ± 0.006	0.272 ± 0.005	0.0001*
Plant height (cm)	19.57 ± 0.271	20.84 ± 0.260	0.001*	39.89 ± 0.582	42.80 ± 0.424	0.0001*

Notes: Means ± SE are shown. Data were analyzed using independent *t* tests followed by a sequential Bonferroni adjustment to correct for multiple comparisons. Asterisks (*) indicate significance ($P < 0.05$) following adjustment.

endoreduplication. The adaptive plasticity hypothesis (Dudley and Schmitt 1996) suggests that phenotypic plasticity has evolved to maximize fitness in variable environments. Our results suggest that this may be the case: higher ploidy is correlated with rapid regrowth rates and higher fitness following the removal of apical dominance. Increasing chromosome number, and thus gene copy number, may provide a means of increasing gene expression, likely by the up-regulation of selected genes or gene families. Over one hundred genes have been shown to be differentially expressed when comparing clipped and unclipped plants of the Col. ecotype, genes predominantly associated with metabolism (K. N. Paige, *unpublished data*) that may facilitate rapid regrowth. In addition, increasing chromosome number may also increase vast numbers of sequences that encode microRNAs, small interfering RNAs, and long noncoding RNAs; these factors have been shown to play major roles as post-transcriptional regulators (Taft et al. 2010) and their activities may be influenced by endoreduplication. Furthermore, increasing chromosome number increases the total DNA content and hence cell size, leading to extensive cell growth through endoreduplication, suggested by some to be the primary mechanism of endoreduplication (Barow 2006). The longstanding hypothesis is that a certain quantity of DNA is necessary for the maturation of a cell, and so endoreduplication may provide the cell volume and transcriptional output needed to sustain a fully differentiated cell's activities (Barow 2006). Growth by cell division along with growth by cell expansion through endoreduplication may be faster than growth by cell division alone (Barow 2006). Also, rapid growth and development following the removal of apical dominance may be further enhanced by an increase in cell size, maximizing nutrient transport, protein synthesis, and light and water absorption (Lee et al. 2009). Endoreduplication also occurs predominantly among plants adapted to habitats that require fast growth and development (Barow and Meister 2003, Barow 2006). This correlation between life history and endoreduplication suggests a possible functional relationship between enhanced chromosome production and rapid regrowth following the removal of apical dominance.

Previous work also points to a tie between endoreduplication and seed development, as endoreduplication has been seen to occur widely in suspensor cells, which connect the embryo to the surrounding nourishing tissue and ensure nutrient transfer. Furthermore, plasmodesmata of the cell walls connecting the cytoplasm of adjacent cells slows down nutrient transport, thus, faster transport is facilitated by producing larger and fewer cells with fewer cell walls via endoreduplication (Barow 2006). Kowles and Phillips (1988) suggested that extra DNA produced by endoreduplication is important in maize endosperm development and kernel filling through increased gene expression and protein synthesis. Engelen-Eigles et al. (2001) and Lee et al. (2009) found that high temperatures or water deficits can cause the endosperm of developing seeds to remain primarily mitotic, reducing endoreduplication, leading to smaller endosperm that are ill suited in supporting the embryo. These results are consistent with our findings that lower levels of endoreduplication in clipped plants of Ler. are associated with lower seed production (in spite of equal silique production) whereas in clipped plants of Col. higher levels of endoreduplication are associated with higher seed filling and seed production. These results suggest a tie between at least one aspect of fitness and endoreduplication: seed development.

Of particular interest is the fact that allocation patterns/life histories are quite different between ecotypes following the removal of apical dominance. The allocation of the proportional mass of total seed produced to the total biomass produced following clipping in Col. is on average 6.0% lower than unclipped controls (15.1% vs. 16.0% biomass allocated to seed for clipped vs. unclipped plants), whereas the allocation of the proportional mass of total seed produced to the total biomass produced following clipping in Ler. is on the average 38% lower than unclipped controls (21.0% vs. 29.0% biomass allocated to seed for clipped vs. unclipped plants). Recall that the number of

seeds produced following clipping was significantly greater in Col. and significantly fewer in Ler., seed masses did not differ within or between Col. and Ler. for either treatment and biomass was significantly greater for both Col. and Ler. following clipping but the magnitude was about 20% greater biomass for Col. (see Table 2). Thus, we suggest that endoreduplication may contribute or altogether explain the differences we see in allocation patterns given the genetic and/or nucleotypic effects discussed above – the studies proposed below should help us in addressing this issue from an experimental standpoint.

We also observe that the magnitude and direction of the change in ploidy differs between ecotypes (i.e., there is a significant genotype × environment interaction). Phenotypic plasticity, the ability of an organism to express different phenotypes depending upon the biotic or abiotic environment, has been observed in the physiology, development, morphology, chemistry, and behavior of organisms in response to environmental cues (Agrawal 2001). Although plants are widely known to endoreduplicate, there are few examples of plasticity in the degree of endoreduplication or genotype × environment interactions in endopolyploidy. A noteworthy exception is the study by Ceccarelli et al. (2006) showing that roots of salt tolerant *Sorghum bicolor* endoreduplicate following exposure to NaCl, whereas roots of non-salt-tolerant genotypes of *Sorghum bicolor* do not endoreduplicate. Here we show that individuals of *Arabidopsis* can plastically alter ploidy during the course of their lifetimes in response to herbivore damage (in this case the removal of apical dominance). As noted above, removal of apical dominance in *Arabidopsis* is known to lower levels of auxin triggering an exit from mitotic cycles causing an entry into the endocycle (Ishida et al. 2010). Of course the variation that we see between these two genotypes in the degree of endoreduplication following the removal of apical dominance suggests that there are genetic differences in triggering this pathway that will require future investigation.

If endoreduplication leads to enhanced reproduction as these studies suggest, such phenotypic plasticity may be of an adaptive nature and widespread among plants in mitigating the detrimental effects of herbivory. To substantiate this claim, we are currently using a suite of knockout and overexpression mutants to make more direct connections between endoreduplication and fitness following the removal of apical dominance. In addition, we are conducting a wider screen of *Arabidopsis* ecotypes and several other plant species with similar compensatory responses to substantiate the broad-scale relationship between endoreduplication and fitness compensation in the face of herbivore impacts. Last, endoreduplication may have important effects beyond mitigating the impacts of herbivory as indicated by the studies of Ceccarelli et al. (2006) on *Sorghum* and salt tolerance noted above. Cookson et al. (2006) also recently showed experimentally, using a DEL1 mutant of *Arabidopsis*, that an increase in the extent of endoreduplication reduced the impact of water deficit on cell size, leaf expansion rate, final leaf size, and, by inference, fitness. In light of these results, an increase in endopolyploidy may provide an additional level of regulation of the genome, whether by transcriptional or nucleotypic effects (an effect based on DNA content alone), ultimately optimizing organismal fitness under ever-changing environmental stresses.

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