

Plasticity in ploidy underlies plant fitness compensation to herbivore damage

DANIEL R. SCHOLES and KEN N. PAIGE

School of Integrative Biology, University of Illinois at Urbana-Champaign, 515 Morrill Hall, 505 S. Goodwin Ave., Urbana, IL 61801, USA

Abstract

How plants mitigate damage by animal herbivores is a fundamental ecological and evolutionary question of plant–animal interactions. Some plants can increase their fitness when damaged in a phenomenon termed ‘overcompensation’. Despite overcompensation being observed in a variety of plant species, its mechanistic basis remains elusive. Recent research has shown that the *Arabidopsis thaliana* genotype Columbia-4 employs endoreduplication, the replication of the genome without mitosis, following damage and that it overcompensates for seed yield. The related genotype Landsberg *erecta*, in contrast, does not increase its endoreduplication following damage and suffers reduced seed yield. While these results suggest that a plant’s ability to plastically increase its ploidy during regrowth may promote its mitigation of damage, no studies have explicitly linked the endoreduplication genetic pathway to the regrowth and fitness of damaged plants. By comparing fitness and ploidy between undamaged and damaged plants of Columbia-4, Landsberg *erecta* and their offspring, we provide evidence that endoreduplication is directly involved in compensatory performance. We then overexpressed an endoreduplication regulator and compared this mutant’s endoreduplication and compensation with its background genotype Columbia-0, an undercompensator. Enhancing Columbia-0’s ability to endoreduplicate during regrowth led to the complete mitigation of the otherwise detrimental effects of damage on its fitness. These results suggest that the ability of these plants to increase their ploidy via endoreduplication directly impacts their abilities to compensate for damage, providing a novel mechanism by which some plants can mitigate or even benefit from apical damage with potential across the wide range of plant taxa that endoreduplicate.

Keywords: *Arabidopsis*, endopolyploidy, endoreduplication, herbivory, overcompensation, plant–animal, tolerance

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Introduction

A broad, consequential issue in the ecology and evolution of plant–animal interactions regards the mechanisms by which plants defend against and compensate for herbivore-induced damage. One potential outcome of the interaction, however, is overcompensation, where a plant displays increased reproductive output after sustaining damage. The first clear evidence of overcompensation was demonstrated in *Ipomopsis aggregata*,

where a 2.4-fold increase in seed yield was observed following the removal of 95% of the aboveground biomass of this monocarpic biennial (Paige & Whitham 1987). In addition, the product of lifetime seed production, seed germination and seedling survival averaged 3.0 times that of uneaten controls (Paige 1992). While initially controversial, similar results supporting this apparent plant–herbivore mutualism have been reported in numerous other studies and systems, providing substantial evidence that overcompensation does indeed occur in certain situations (e.g. Paige & Whitham 1987; Maschinski & Whitham 1989; Hougen-Eitzman & Rausher 1994; Lowenberg 1994; Nilsson *et al.*

Correspondence: Daniel R. Scholes, Fax: (217) 244 1224;
E-mail: scholes2@illinois.edu

1996; Lennartsson *et al.* 1997; Mauricio *et al.* 1997; Weining *et al.* 2003). Of course, it is known that extremes in environmental conditions (such as severe drought) can influence the degree of compensation within a normally overcompensating population (Levine & Paige 2004).

While the ecology of overcompensation has been studied in considerable depth, its genetic basis has only begun to be elucidated. Increases in fitness following damage are likely due to the release of apical dominance; the removal of the apical meristem by herbivores eliminates apical production of the plant hormone auxin, leading to a rapid break in dormancy of axillary buds and subsequent stem elongation, which changes plant architecture, increases photosynthetic capacity and in some cases enhances fitness (e.g. Paige & Whitham 1987). Given that the release of apical dominance probably alters both physiological and genetic processes to maximize rapid regrowth and fitness compensation, recent studies have targeted integrative mechanisms for facilitating rapid regrowth in overcompensating genotypes. Studies by Siddappaji *et al.* (2013) used transcriptomic, quantitative trait loci mapping, and candidate gene knockout and complementation methodologies with *Arabidopsis thaliana* to uncover one key metabolic pathway – the oxidative pentose phosphate pathway (OPPP) – with strong evidence supporting its role in influencing compensation via the action of glucose-6-phosphate dehydrogenase 1 (G6PD1; Scholes *et al.* 2013). The OPPP is a component of the generalized stress response that synthesizes intermediates from glucose for biosynthesis in the Calvin cycle, the production of secondary defensive compounds and the production of ribo- and deoxyribonucleotides (Kruger & von Schaewen 2003).

Upon the release of apical dominance by apical meristem damage, nucleotides produced via the OPPP in the regenerating tissue may be used to increase cellular ploidy by endoreduplication (Kruger & von Schaewen 2003). Because auxin maintains a cell's undifferentiated state, removing the site of apical auxin production triggers axillary cell expansion and differentiation via an exit from mitotic cycles and an entry into endocycles (Ishida *et al.* 2010). The endocycle is characterized by successive genome replications without cell division, a process termed endoreduplication, such that cellular ploidy increases (Nagl 1976; Brodsky & Uryvaeva 1977). Endopolyploidy, the condition of having increased ploidy via endoreduplication, is common in eukaryotes and particularly in plants with approximately 90% of herbaceous angiosperms being endopolyploid (Nagl 1976; Sugimoto-Shirasu & Roberts 2003). Due to its commonality, endoreduplication is suspected to play critical roles in generalized cellular development, expansion and differentiation. It is hypothesized that endoreduplication achieves this by increases in cell size, metabolism

and gene expression owing to the exponential increase in genome copy number with each replication (Nagl 1976, 1978; Barow 2006). Thus, endoreduplication following the release of apical dominance may theoretically promote compensation, or even overcompensation, to damage by mediating lateral growth whereby stem number, biomass, fruit number and seed yield may increase relative to undamaged plants.

Recent studies have uncovered correlative evidence implicating endoreduplication in this role – Scholes & Paige (2011) demonstrated for the first time that the induction of endoreduplication following experimental clipping is positively related to fitness compensation for apical damage, where clipped plants of the *A. thaliana* genotype Columbia-4 experienced a 10% increase in whole-organism ploidy relative to unclipped controls during their regrowth and ultimately produced 40% more seed when damaged than when undamaged (i.e. they overcompensated). Clipped plants of the closely related genotype Landsberg *erecta*, in contrast, elicited no such increase in ploidy and suffered lower seed yield relative to undamaged plants. The correlation between ploidy and seed yield was also reported in recombinant inbred lines produced by crossing Columbia-4 and Landsberg *erecta* (Scholes *et al.* 2013), suggesting the interrelatedness of these traits. The ability to increase cellular ploidy in response to damage is thus ecologically important but genetically constrained – those genotypes that are able to enhance endoreduplication following damage may benefit from increased regrowth and fitness via the increased cell volume, metabolism and transcriptional output that comes with increased ploidy (Nagl 1976, 1978; Barow 2006).

Here, we extend recent studies on the basis of overcompensation and address an issue unresolved since the inception of the compensation debates: by what mechanism does plant compensation to damage occur? Specifically, we provide a direct test of the hypothesis that endoreduplication underlies the compensatory response of *A. thaliana* to apical damage. Because Columbia-4, Landsberg *erecta*, and their offspring genotypes were used to provide the initial relationship between ploidy and seed yield (Scholes & Paige 2011; Scholes *et al.* 2013), we first assess whether the compensation of more comprehensive measures of fitness (seed yield \times seed weight) and biomass production are related to endoreduplication in this family despite genetic recombination of these parental genotypes. Further, and more importantly, we test whether the experimental manipulation of the endoreduplication genetic pathway causes predictable changes in fitness compensation upon apical damage. Together, our findings indicate that endoreduplication is employed generally and acts with direct causality in the compensatory

response, demonstrating that it is an important, although as yet overlooked, mechanism by which plants can mitigate or even benefit from apical damage.

Materials and methods

A. thaliana genotypes

Recombinant inbred lines (RILs) were produced through eight generations of single-seed descent from the crossing of the Columbia-4 (Col-4; TAIR stock number: CS933; The Arabidopsis Information Resource, <http://www.arabidopsis.org>) and Landsberg *erecta* (Ler-0; TAIR stock number: CS20) genotypes (Lister & Dean 1993). Landsberg *erecta* and Columbia-4 both originate from the Laibach Landsberg population near Gorzów Wielkopolsk, Poland (Nottingham Arabidopsis Stock Center, <http://www.arabidopsis.info>), and thus are related by descent. Because endoreduplication and fitness compensation are both polygenic traits, crossing the Columbia-4 and Landsberg *erecta* genotypes should break up any chance association between endoreduplication genes and genes associated with fitness through recombination of the parental haplotypes. The production of the RILs should thus result in the loss of the previously observed correlation by Scholes & Paige (2011) if there is no mechanistic link (i.e. direct interaction) between the two phenomena. Eight RILs were selected for analysis from the Col-4 × Ler-0 cross (TAIR stock numbers: CS1906, CS1913, CS1941, CS1942, CS1948, CS1968, CS1985 and CS1999), in addition to the Col-4 and Ler-0 parental genotypes. These specific RILs were selected due to their range in compensatory abilities, as measured in a preliminary analysis of 93 Col-4 × Ler-0 RILs (Siddappaji *et al.* 2013). Specifically, because approximately 78 of the total 93 RILs equally compensated at least one of the 2 years they were assessed (Siddappaji *et al.* 2013), we selected three that undercompensated both years (two RILs and Landsberg *erecta*), four that equally compensated both years and three that overcompensated both years (two RILs and Columbia-4). This selection reduced the number of equally compensating, and thus probably uninformative, genotypes to achieve a balanced sampling of the variation that exists for compensatory ability in this family. Initial assessment of these genotypes revealed a positive relationship between endoreduplication and compensation for seed yield following clipping (Scholes *et al.* 2013).

We additionally sought to experimentally manipulate the endoreduplication pathway and measure the effect of the manipulation on compensation. *INCREASED LEVEL OF POLYPLOIDY1* (*ILP1*; TAIR locus: At5G08550; The Arabidopsis Information Resource, <http://www.arabidopsis.org>) encodes a transcriptional

repressor of the 'A2' variety of cyclins (CYCA2; Yoshizumi *et al.* 2006). The CYCA2 family, and in particular CYCA2;3 (Imai *et al.* 2006), are key activators of cyclin-dependent kinase B (CDKB), which is a component of the protein complex that induces mitosis in *A. thaliana* (Boudolf *et al.* 2004). Overexpressing *ILP1* thus causes enhanced repression of both CYCA2 and mitotic cell division. S-phase DNA replication is allowed to continue occurring successively, however, which results in an increase in the cell's nuclear ploidy (generating endopolyploidy; Yoshizumi *et al.* 2006). An *ILP1* overexpression genetic line (*ILP1-ox*), obtained from the laboratory of Minami Matsui at the RIKEN Yokohama Institute (Plant Functional Genomics Research Group, Plant Science Center; Yokohama, Japan), was created from the addition of a CaMV 35S promoter to *ILP1* on a Columbia-0 (Col-0) genetic background as described by Yoshizumi *et al.* (2006). Yoshizumi *et al.* (2006) report that *ILP1-ox* has approximately 29x greater *ILP1* expression than the Col-0 wild type. Col-0 was also selected for analysis, and we note that it is related to, yet genetically distinct from, the Columbia-4 genotype used here and in our previous studies (Scholes & Paige 2011). Given the action of *ILP1*, we specifically sought to test whether the compensatory ability of an undercompensator, in this case the Col-0 wild type, could be enhanced by an enhanced ability to endoreduplicate. We believe that our selection of an undercompensating background genotype provides the greatest power for detecting the positive effects of endoreduplication on fitness compensation, rather than using the already overcompensating Col-4 genotype, which may experience marginal benefit by experimentally increasing its endoreduplication even more.

Growth and experimental clipping

Seventy-five individuals of each genotype (Col-4, Ler-0, eight Col-4 × Ler-0 RILs, Col-0 and *ILP1-ox*) were grown in a greenhouse maintained at approximately 21 °C on a 12-hour light/dark cycle. At 4 weeks after planting, prior to bolting, rosette tissue of five plants of each genotype was analysed for baseline nuclear DNA content. When inflorescences reached 6 cm in height (approximately 5 weeks after planting), half (35) of the remaining plants were clipped, leaving 1 cm of inflorescence tissue (removing approximately 85% of the inflorescence, comparable to natural mammalian herbivory; Paige & Whitham 1987). The other half (35) remained unclipped.

Cytometric analysis

At the induction of senescence (ranging from approximately 9–12 weeks after planting), all inflorescence

tissues (stems, leaves, flowers, flower buds and valves of siliques) of 20 plants of each genotype (10 unclipped and 10 clipped) were analysed for nuclear DNA content. Nuclear DNA content of each sample was estimated by flow cytometry. Tissue for cytometric analysis was prepared by standard protocols (Galbraith *et al.* 1983). In brief, fresh tissue was matched for tissue type and mass, chopped with a razor blade, sheared in a nuclear isolation buffer (sodium citrate, 3-morpholinopropane-1-sulphonic acid, magnesium chloride, Triton X-100; Galbraith *et al.* 1983), filtered for debris removal and stained with propidium iodide. Suspended nuclei were analysed for DNA content via a BD Biosciences (San Jose, CA, USA) FACScanto flow cytometer. Background correction and nuclei population gating were performed using *De Novo* Software FCS Express (v.3; Los Angeles, CA, USA) to measure the number of nuclei at each ploidy level (2C, 4C, 8C, 16C) for each plant sample. The 'C' here reflects the number of copies of the basic genome in a cell, such that 2C denotes diploidy, 4C denotes tetraploidy, etc. Because estimations of absolute genome size are not necessary here, the 2C nuclei population was used as the internal standard to identify the nuclei populations at the higher ploidy levels, as is standard for assessments of endopolyploidy (Galbraith *et al.* 1991). The cycle value, calculated as the mean number of endoreduplication cycles per nucleus and thus an overall measure of endoreduplication (Barow & Meister 2003), was calculated from the number of nuclei at each ploidy level for each sample by the equation:

$$\text{Cycle value} = \frac{(0 \cdot n_{2C} + 1 \cdot n_{4C} + 2 \cdot n_{8C} + 3 \cdot n_{16C})}{(n_{2C} + n_{4C} + n_{8C} + n_{16C})},$$

where the cycle value is the sum of the number of nuclei at each ploidy level multiplied by the number of endocycles required to achieve each corresponding ploidy level, divided by the total number of nuclei measured. The resultant 'cycle values' are continuous numbers produced by averaging the number of endocycles that each nucleus in the sample had undergone to achieve its ploidy level. Cycle values therefore range from zero, which would indicate that all nuclei are diploid, to three, which would indicate that all nuclei are 16C. Here, we use the cycle value as a measure of endopolyploidy that is directly comparable across genotypes that may differ slightly in their genome size (pg DNA/1C).

Fitness analysis

After cytometric analysis, the 50 remaining plants of each genotype (25 unclipped and 25 clipped) were analysed

for fitness. Phenotypic measures included the total number of siliques per plant, the average number of seeds per siliques, average seed weight and the inflorescence dry biomass of each plant. The average number of seeds per siliques was determined by counting the number of seeds within each of ten siliques of representative length from each plant. For each plant, the average number of seeds per siliques for the respective genotype \times treatment group was multiplied by the total number of siliques for the plant, yielding an estimate of the total seed yield for each plant. Average seed weights were determined by measuring the weight of approximately 100 seeds of at least 10 plants of each genotype \times treatment group. Each weight measurement was divided by the number of seeds weighed to determine the average seed weight for each genotype \times treatment group. We then multiplied the seed yield of each plant by the average seed weight for the respective genotype \times treatment group to yield a measure of 'composite seed production', equalising the total weight of seeds produced by the plant. Because this measure reflects both seed number and quality (i.e. seed yield \times seed weight), we use composite seed production as our ultimate measure of fitness.

Statistical analyses

All statistical analyses were performed with SAS (v.9.3; Cary, NC, USA). The effects of genotype, clipping treatment and their interaction were tested on seed yield, seed weight, composite seed production, inflorescence dry biomass and cycle values via ANOVA in PROC GLM. The sequential Bonferroni procedure was used to maintain a family-wise error rate ($\alpha_{\text{family}} = 0.05$) for multiple comparisons among the number of measures assessed for each genotype (Rice 1989). To test for relationships between measured variables, the changes in composite seed production, biomass and cycle value were calculated for each genotype as the percentage difference between the mean of clipped plants relative to the mean of unclipped plants for each measure. Linear regression was performed for all combinations of the changes in composite seed production, biomass and cycle values upon clipping via PROC REG for the ten Col-4 \times Ler-0 genotypes with and without Col-0 and *ILP1-ox*.

Results

The Columbia-4 \times Landsberg erecta family

Of the Col-4 \times Ler-0 family, including the two parental lines, two genotypes increased average seed weight, three decreased seed weight (one of which was Ler-0), and five had no change in seed weight when clipped relative to unclipped (see Appendix S1: Table 1A,

Supporting information, for statistical tests for each genotype). When combined with the seed yield results reported for these genotypes by Scholes *et al.* (2013), five genotypes increased, two decreased, and three experienced no change in composite seed production upon clipping (Fig. 1a, Appendix S1: Table 1A, Supporting information). Further, eight genotypes increased, one decreased, and one experienced no change in biomass upon clipping (Fig. 1b, Appendix S1: Table 1B, Supporting information). Finally, four genotypes increased their ploidy via endoreduplication upon clipping, while six genotypes exhibited no statistical change in ploidy (Fig. 1a,b, Appendix S1: Table 1B, Supporting information). Genotypes did not differ in their rosette ploidy prior to clipping ($F_{9,40} = 0.90$, $P = 0.5332$), indicating no inherent genetic differences in baseline endoreduplication, and thus, any changes in inflorescence endopolyploidy upon clipping can be directly attributed to the clipping treatment. Further, differences reported are truly changes in endopolyploidy because all genotypes exhibited a baseline level of inflorescence endopolyploidy (i.e. cycle values greater than zero for unclipped plants of all genotypes; all $P < 0.0001$) when unclipped, with plastic changes from this level following damage. When considered together, the responses of these genotypes produce significant positive relationships between the changes in endopolyploidy and composite seed production, endopolyploidy and biomass, and biomass and composite seed production upon clipping (all $P < 0.05$; Fig. 1a–c).

Columbia-0 and the endoreduplication-enhanced *ILP1-ox*

Neither Col-0 nor *ILP1-ox* exhibited changes in seed weight following clipping ($t_{259} = 1.92$, $P = 0.0565$ and $t_{259} = -0.20$, $P = 0.8455$, respectively). Compared to unclipped Col-0 control plants, clipped Col-0 plants had significantly lower seed yield, composite seed production and inflorescence dry biomass at senescence (i.e. Col-0 undercompensated; seed yield: $t_{459} = -7.49$, $P < 0.0001$, composite seed production: $t_{459} = -5.56$, $P < 0.0001$, biomass: $t_{459} = -8.00$, $P < 0.0001$; Fig. 2). Col-0 clipped plants also had significantly lower cycle values relative to unclipped plants ($t_{215} = -2.34$, $P < 0.05$; Fig. 2). Clipped *ILP1-ox* plants, however, showed no change in any of these measures when compared to unclipped *ILP1-ox* plants (seed yield: $t_{459} = -0.92$, $P = 0.3589$, composite seed production: $t_{459} = -1.09$, $P = 0.2775$, biomass: $t_{459} = 0.61$, $P = 0.5415$, cycle value: $t_{215} = -0.19$, $P = 0.8462$; Fig. 2). Because there is no significant difference between cycle values of Col-0 and *ILP1-ox* when unclipped ($t_{215} = 1.48$, $P = 0.1403$), the ability of *ILP1-ox* to maintain its

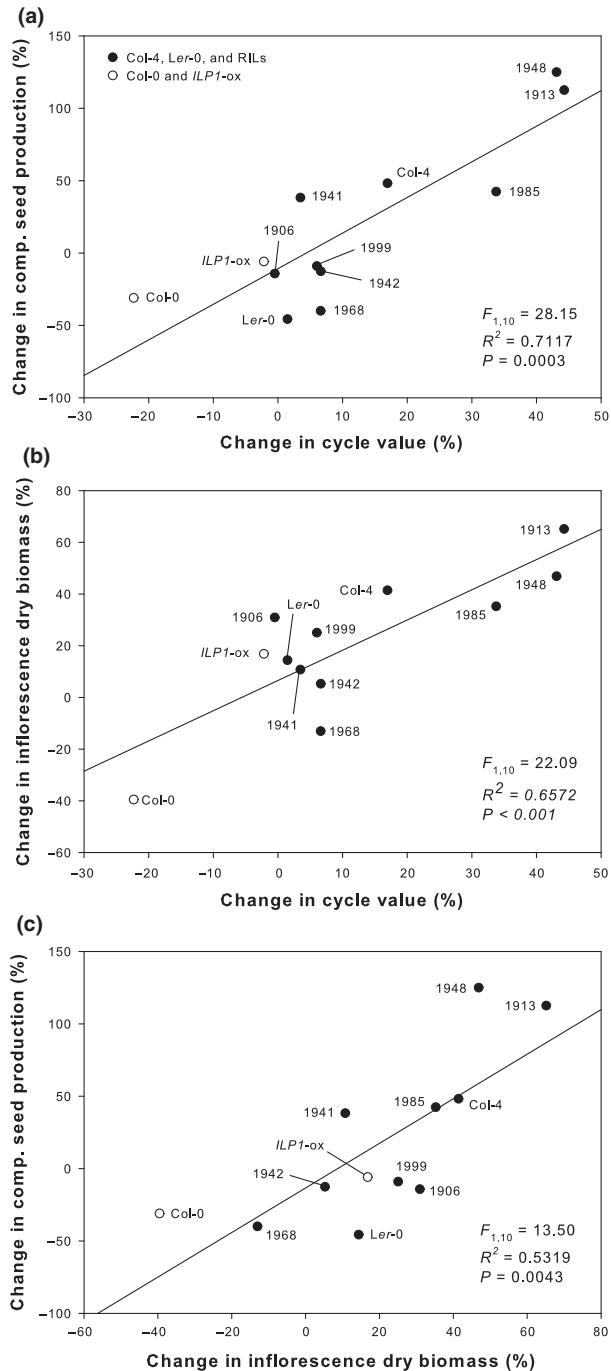


Fig. 1 Regressions of phenotypic measures. Regression of the percentage change in (a) composite seed production and (b) inflorescence dry biomass vs. the percentage change in cycle value, and (c) composite seed production vs. inflorescence dry biomass across Columbia-4, Landsberg *erecta*, the eight RILs, Columbia-0 and *ILP1-ox*. Recombinant inbred lines are labelled as an abbreviation of their full designation (CS####). All relationships are significant ($\alpha = 0.05$). Col-4 × Ler-0 family alone: (a) $F_{1,8} = 28.98$, $P = 0.0007$, $R^2 = 0.7566$; (b) $F_{1,8} = 10.54$, $P = 0.0118$, $R^2 = 0.5146$; (c) $F_{1,8} = 14.75$, $P = 0.0049$, $R^2 = 0.6044$.

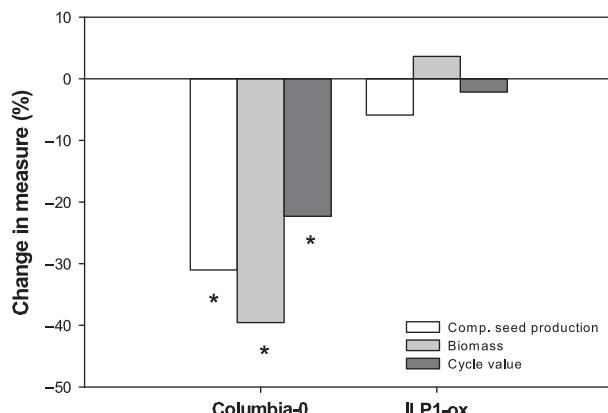


Fig. 2 Percentage change in phenotypic measures of clipped Columbia-0 wild type and *ILP1*-ox mutants relative to unclipped controls. Measures include composite seed production (white bars), inflorescence dry biomass (light grey bars) and cycle value (dark grey bars). Asterisks (*) indicate a significant ($\alpha = 0.05$) difference in clipped plants relative to unclipped plants for the respective measure.

endopolyploidy following damage appears to underlie its ability to also maintain seed production and biomass while Col-0 suffers fitness losses. The compensatory responses of these two genotypes fall within the spectrum of responses observed for the Col-4 \times Ler-0 family (Fig. 1a–c).

Discussion

Scholes & Paige (2011) provided the first evidence that endoreduplication is correlated with fitness compensation following apical damage by their assessment of the processes in two genotypes of *Arabidopsis thaliana*: Columbia-4 and Landsberg *erecta*. Scholes *et al.* (2013) then reported an initial relationship between the response of ploidy and seed yield to damage in the Columbia-4 \times Landsberg *erecta* family of genotypes. Here, we extend their findings by assessing more comprehensive measures of fitness and biomass production and provide two lines of evidence that endoreduplication is causally involved in the compensatory response of apically damaged plants. First, recombination of the Columbia-4 and Landsberg *erecta* genotypes should break up the association of the endoreduplication and fitness compensation genetic pathways if there is no direct interaction between them, yet fitness and biomass measures remain correlated with endoreduplication among their offspring. Second, and most compellingly, the experimental manipulation of the endoreduplication pathway changes the compensatory ability of the control genotype as predicted if endoreduplication directly influences the regrowth and fitness of apically damaged plants. In total, these results demonstrate that a novel

mechanism, plasticity in cellular ploidy via endoreduplication, underlies the ability of these plant genotypes not only to mitigate apical damage but for some genotypes to even benefit from being damaged.

The direct association of endopolyploidy and fitness compensation following apical damage is most clearly demonstrated by the genetic manipulation of the endoreduplication pathway. By overexpressing *ILP1*, a positive regulator of endoreduplication, we experimentally increased the ability of the Columbia-0 genotype to generate endopolyploidy following apical damage. This improved Col-0's ability to compensate for seed and biomass production, changing its performance from undercompensation to equal compensation for these measures following damage. Recall also that Col-0 and *ILP1*-ox did not differ in their cycle values when unclipped. This demonstrates that any differences in endopolyploidy among unclipped and clipped plants for these genotypes at senescence occurred explicitly during their regrowth following the damage treatment. Ultimately, the enhanced ability of the *ILP1*-ox mutant to endoreduplicate during regrowth saved the Col-0 background from undercompensation, demonstrating the substantial impact of endoreduplication on the mitigation of the detrimental effects of apical damage.

Further, the fact that the positive relationship between endoreduplication and fitness compensation of Columbia-4 and Landsberg *erecta* is retained in their offspring suggests that endoreduplication directly influences the regrowth of these genotypes and may be generalizable to the Columbia family. If the correlation reported by Scholes & Paige (2011) in the parents was spurious due to chance linkage of traits, genetic recombination of the parental genotypes during their crossing should break up any chance association between the genes regulating each process separately. Retaining a positive association in the offspring despite recombination, both initially by Scholes *et al.* (2013) and more comprehensively here, suggests that the action of one set of genes is dependent on the other. The endoreduplication pathway therefore appears to be directly integrated within the framework of rapid regrowth and fitness compensation following damage of these genotypes. Further, because the parental lines are homozygous at all genetic loci, the recombinant inbred offspring have at most two alleles per locus among them, although most loci are probably fixed among parental lines due to their common ancestry from the Laibach Landsberg population (The Nottingham Arabidopsis Stock Centre, <http://www.arabidopsis.info>). Despite starting with relatively limited genetic variability, estimates of nuclear DNA content and fitness measures of the Col-4 \times Ler-0 offspring show wide variation around the parental values (Fig. 1a,b). This

suggests, as expected, that fitness and cell cycle regulation are polygenic traits, and the bidirectional transgression for both traits indicates that the parental genotypes are composed of alleles that increase and decrease the values of these traits from the parental means (e.g. although the *Ler-0* genotype undercompensates, it contains alleles that promote overcompensation, evident by the offspring genotypes with greater overcompensation than the *Col-4* genotype; Fig. 1a,b; see also Siddappaji *et al.* 2013).

Given these results, how might endoreduplication be directly influencing the regrowth and fitness of damaged plants? While the role of endoreduplication in compensation has only recently been investigated, the generalized role of endoreduplication in the rapid production of plant tissue has been described in some detail (Nagl 1976, 1978; Barow & Meister 2003; Barow 2006). Plant growth primarily occurs via two processes: cell division and cell expansion (Jacobs 1997). While proliferative cell divisions increase plant size through greater cell number, cell expansion can increase size via two distinct mechanisms. In most plant tissues, particularly in those species that do not endoreduplicate systemically, cell expansion is achieved by auxin-mediated water importation into the cells' vacuoles (Hager *et al.* 1971; Rayle & Cleland 1992). In the absence of adequate water, as for many drought-adapted succulents (de Rocher *et al.* 1990), or in plants that endoreduplicate systemically and lack apical auxin production, like our clipped *A. thaliana* plants (Galbraith *et al.* 1991), endoreduplication can provide an efficient means of increasing cellular volume to facilitate growth. Upon clipping, there is a significant relationship between the change in endopolyploidy and the change in biomass of the genotypes studied here (Fig. 1b), supporting the notion that biomass regeneration during the shortened growth period was accomplished in part by endoreduplication. Increased ploidy has been linked to accelerated growth in other systems as well, including dark-grown seedlings of *Glycine max*, *Pisum sativum*, *A. thaliana* and others (van Oostveldt & van Parijs 1975; Galli 1988; Gendreau *et al.* 1998; Barow 2006), where apical auxin is nearly absent and selection for rapid hypocotyl elongation is strong (Bhalerao *et al.* 2002; Halliday *et al.* 2009). In fact, ploidy is positively correlated with hypocotyl and root elongation in dark-grown *ILP1-ox*, the plasticity-enhanced endoreduplication mutant studied here; conversely, hypocotyls and roots of an *ILP1* knockout mutant are unusually short (Yoshizumi *et al.* 2006). The results of Yoshizumi *et al.* (2006) and others therefore lend support that the endoreduplication pathway directly influences the production of biomass and thus contributes to the compensatory response as our results suggest.

Once plant regrowth has been stimulated, endoreduplication may be particularly important in those tissues directly involved in endosperm development and seed filling given the significant relationship between endoreduplication and compensation for composite seed production (our measure of fitness that reflects both seed number and quality; Fig. 1a). Endoreduplication impacts endosperm development and function in numerous ways, including providing increased cell volume for nutrient storage, enhancing rates of nutrient transport and serving as a nutritive process itself – endoreduplication of the endosperm provides a substantial resource of nucleotides, proteins and their nitrogen- and phosphorus-rich derivatives to the embryo for development (Engelen-Eigles *et al.* 2001; Barow 2006; Lee *et al.* 2009). Rates of endoreduplication are particularly high in suspensor cells, which connect the developing embryo to the surrounding endosperm (Barow 2006). The suspensor serves as the conduit through which nutrients must pass for transfer to the embryo; endoreduplication enhances the rate of long-distance nutrient and water transfer by providing greater volume for efficient intracellular transport and fewer cell-to-cell transitions through narrow plasmodesmata that slow transport (Barow 2006). While we did not directly assess the outcomes of endoreduplication in seed production (e.g. seed germination rates and seedling survival), our seed weight and seed yield results suggest that enhanced endoreduplication may impact the fitness compensation of damaged plants via the benefits it provides specialized cell types, such as these, that are directly involved in seed production.

Because the genotypes examined here with the greatest compensation for seed yield also have the greatest compensation for biomass (Fig. 1c), endoreduplication, a process that is involved in both traits, is probably a component of plants' generalized response to apical damage. Endoreduplication's role may in part be via its link to the OPPP (Kruger & von Schaewen 2003; Siddappaji *et al.* 2013). The OPPP is composed of a series of carbohydrate metabolism reactions that support generalized metabolism, but also stress metabolism as part of the oxidative stress response pathway that supplies intermediates for the production of alkaloids, flavonoids, glucosinolates and other secondary defence compounds (Herrmann & Weaver 1999; Scharte *et al.* 2009). The OPPP is initially catalysed by G6PD1, an enzyme whose production following clipping is positively correlated with the differing compensatory responses of *A. thaliana* *Col-4* and *Ler-0* (over- and undercompensation, respectively; Siddappaji *et al.* 2013). The OPPP also supplies ribose-5-phosphate for nucleotide synthesis, which generates the tremendous quantity of nucleotides necessary for increases in ploidy during regrowth

(Kruger & von Schaewen 2003). Because the removal of apical auxin production by herbivory stimulates endoreduplication (Ishida *et al.* 2010), we suggest that genotypes plastically capable of increasing ploidy during regrowth may also be those that can best stimulate the OPPP by increasing the expression of *G6PD1* and other OPPP regulators, in turn inducing defensive chemistry, remediating oxidative damage, increasing carbohydrate metabolism and further increasing endoreduplication in a positive feedback loop (Scholes *et al.* 2013; Siddappaji *et al.* 2013).

Our results here collectively demonstrate that increasing ploidy via endoreduplication directly facilitates growth and fitness compensation of *A. thaliana* following apical damage. To better understand the integration of endoreduplication regulators with metabolic pathways such as the OPPP, full-transcriptome sequencing of damaged and undamaged plants has recently been performed and should help identify the important genes and pathways that contribute to a plant's response to apical damage. Endoreduplication and compensation are both phenomena that occur widely among plants, yet their interaction has only recently been the subject of initial investigation. Given the results of this study and the various roles that endoreduplication plays in plant growth, development and fitness, endoreduplication appears to be an important, although largely overlooked, mechanism that provides plants a plastic level of cellular optimization to maximize organismal fitness upon damage, and in some cases even to overcompensate.

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- D.R.S. and K.N.P. designed the research and wrote the paper, and D.R.S. performed the research and analysed the data.
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- ### Data accessibility
- Fitness and cycle value data of the *A. thaliana* accessions examined are archived in Dryad <http://dx.doi.org/10.5061/dryad.2sp38>. Genetic information of the genotypes is accessible via The Arabidopsis Information Resource website at <http://www.arabidopsis.org>.
- ### Supporting information
- Additional supporting information may be found in the online version of this article.
- Appendix S1** Statistical comparisons of unclipped and clipped plants of each genotype for seed weight, seed yield, composite seed production, inflorescence dry biomass, and cycle value measures.