American Journal of Botany

NEWS & VIEW

ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

Overcompensation, environmental stress, and the role of endoreduplication

Ken N. Paige[1,2]

Manuscript received 30 March 2018; revision accepted 9 May 2018.

[1] Program in Ecology, Evolution, and Conservation Biology, School of Integrative Biology, University of Illinois, Urbana, IL 61801, USA

[2] Author for correspondence (e-mail: k-paige@life.illinois.edu)

Citation: Paige, K. N. 2018. Overcompensation, environmental stress, and the role of endoreduplication. *American Journal of Botany* 105(7): 1–4.

doi:10.1002/ajb2.1135

Thirty years ago, we provided the first clear evidence of overcompensation, i.e., increased flower, fruit, and seed production following herbivory (Paige and Whitham, 1987; Stowe et al., 2000). While this observation was initially controversial and seemingly paradoxical, evidence for overcompensation has been observed in a wide variety of plant species, providing substantial evidence that overcompensation indeed occurs (e.g., see Paige and Whitham, 1987; Lennartsson, et al. 1997; Siddappaji et al., 2013). However, while the ecology of overcompensation has been studied in considerable depth, its physiological, developmental, and genetic bases have only begun to be elucidated. Here I summarize our findings on the genetic basis of overcompensation and some of the physiological and developmental processes involved. I conclude with a discussion of some of the fundamental issues that need to be addressed to further our understanding of the mechanistic basis of fitness compensation, focusing primarily on the process of endoreduplication.

To begin to understand the genetic basis of overcompensation, we initially used transcriptomic, quantitative trait loci mapping, and candidate gene knockout and complementation studies in *Arabidopsis thaliana* recombinant inbred lines (showing the full range of compensatory responses from under to overcompensation) and their parents, Landsberg *erecta* (an undercompensating genotype) and Columbia-4 (an overcompensating genotype), to uncover genes involved in overcompensation. From these studies, we uncovered one key metabolic pathway—the oxidative pentose phosphate (OPP) pathway—with strong evidence supporting its role in influencing compensation via the action of glucose-6-phosphate dehydrogenase 1 (G6PD1) (Siddappaji et al., 2013). G6PD1 is the central regulatory enzyme in the OPP pathway that plays a key role in plant metabolism generating NADPH and a variety of metabolic intermediates for biosynthetic processes such as resistance to oxidative damage, the production of secondary defensive compounds and the production of ribo- and deoxyribonucleotides (Kruger and von Schaewen, 2003).

We have also shown that following the removal of apical dominance, phenotypically plastic increases in ploidy level via endoreduplication leads to rapid regrowth and an increase in fitness, explaining, in part, the phenomenon of overcompensation in plants (Scholes and Paige, 2011, 2014). Endoreduplication is the replication of the genome without mitosis, which leads to endopolyploidy, an increase in cellular chromosome number (e.g., see Nagl, 1976). Removal of the apical meristem by herbivores eliminates production of the plant hormone auxin, leading to a rapid break in dormancy of axillary buds and subsequent stem elongation (Ishida et al., 2010). High levels of auxin are also known to repress the endocycle, and by contrast, lower levels of auxin trigger an exit from mitotic cycles and an entry into endocycles. Insect leaf-feeding also can trigger endoreduplication by the upregulation of jasmonic acid, which also lowers auxin production (Machado et al., 2013) and can lead to overcompensation in some ecotypes of *Arabidopsis* (J. M. Mesa, University of Illinois, Urbana-Champaign, and K. N. Paige, unpublished data). Thus, there is a direct link between endoreduplication and plant damage.

Increasing chromosome number through endoreduplication and therefore gene copy number provides a means of increasing expression of vital genes (e.g., see Bourdon et al., 2012) (such as G6PD1) or genetic pathways that promote rapid regrowth rates following herbivory. G6PD1 feeds compounds into the OPP pathway for nucleotide biosynthesis, by the provision of ribose-5-phosphate, necessary for the significant increase in chromosome number via endoreduplication. The increase in DNA content then feeds back positively on pathways involved in metabolism (e.g., G6PD1) and defense (e.g., glucosinolate production) (see Fig. 1) through increased gene expression (more copies due to increases in endoreduplication following damage; Scholes and Paige, 2014, 2015). An increase in total cellular DNA content through endoreduplication also leads to extensive cell growth via cell expansion (Melaragno et al., 1993). Growth by cell division along with growth by cell expansion through endoreduplication may be faster than growth by cell division alone (Barow, 2006). Rapid growth and development following the removal of apical dominance may be enhanced by maximizing nutrient transport (with fewer plasmodesmata), protein synthesis (with more copies of DNA), and light and water absorption (with larger cell size and storage capacity) (Lee et al., 2009). Importantly, the experimental overexpression of ILP1 (INCREASED LEVEL OF POLYPLOIDY1), an endoreduplication enhancer, increases glucosinolate production and compensation (from undercompensation to equal compensation with a trend toward overcompensation) in a genotype of *A. thaliana* that typically suffers reduced fitness when damaged (Mesa et al., 2017), demonstrating a causal relationship between the process of endoreduplication, fitness compensation, and chemical defense (Fig. 2).

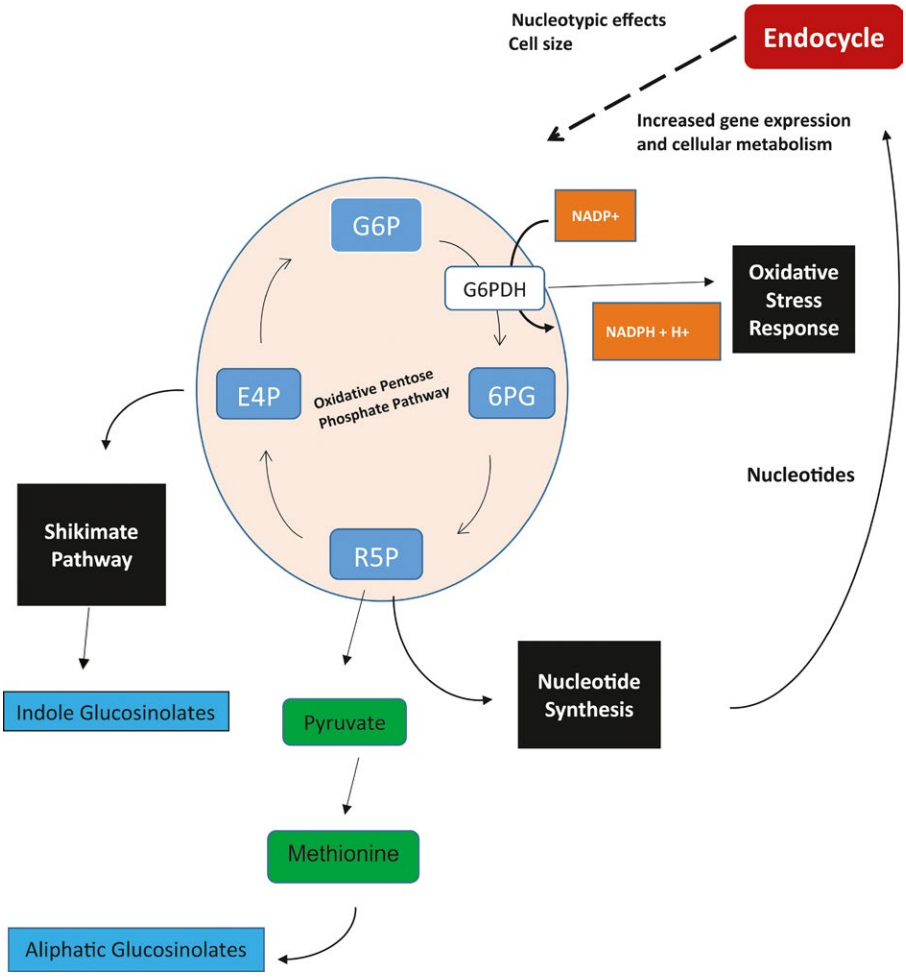

FIGURE 1. Integration of the endocycle with stress-responsive pathways. Shown are the relationships between the oxidative pentose phosphate (OPP) pathway (in tan), the shikimate pathway, and the endocycle. Arrows indicate the production or the movement of compounds from one pathway to another in the direction of the arrow. The broken line between the endocycle and the OPP pathway depicts our model for the positive feedback of endopolyploidy on the expression of OPP pathway genes (namely G6PD1) to increase cell metabolism. Indole glucosinolates are produced by the shikimate pathway, whereas aliphatic glucosinolates are produced by the OPP pathway in combination with the glycolytic pathway and the tricarboxylic acid cycle, producing methionine (in green), an amino acid that is the precursor to aliphatic glucosinolate production. G6P, glucose 6-phosphate; 6PG, 6-phosphogluconate; R5P, ribose 5-phosphate; E4P, erythrose 4-phosphate.

My ultimate goal here is to inspire new studies directed toward a broader understanding of the ecological and evolutionary roles of endoreduplication in plant compensation, focusing on areas that I think will be fruitful for future research.

Initially, one should address whether endoreduplication is a general mechanism for explaining patterns of overcompensation and more generally tolerance (plant genotypes that can sustain tissue loss with little or no decrease in fitness relative to that in the undamaged state). We know that endoreduplication is common in plants, with approximately 90% of herbaceous angiosperms being endopolyploid (Nagl, 1976). We also know that there is genetic variation for compensation/tolerance across numerous plant species. For example, some families exhibit overcompensating tolerance, whereas others express incomplete tolerance (e.g., see Tiffin and Rausher, 1999; Siddappaji et al., 2013). What is unknown is the degree to which plant species are genetically/genotypically *plastic* in terms of the level of endoreduplication following herbivory and more generally how common the association between endoreduplication and overcompensation is. Therefore, there is a need for studies on other species to determine whether there is a positive association between the degree of fitness compensation following herbivory and the level of endopolyploidy, and ultimately, a causal relationship, as we have shown in *A. thaliana*. It would also be of interest to assess the phylogenetic relationships between the degree of compensation/tolerance and endopolyploidy among plant taxa, potentially informing how commonly plasticity in endoployploidy is involved in overcompensation and more generally tolerance.

Endoreduplication appears to occur predominantly among plants adapted to habitats that require fast growth and development,

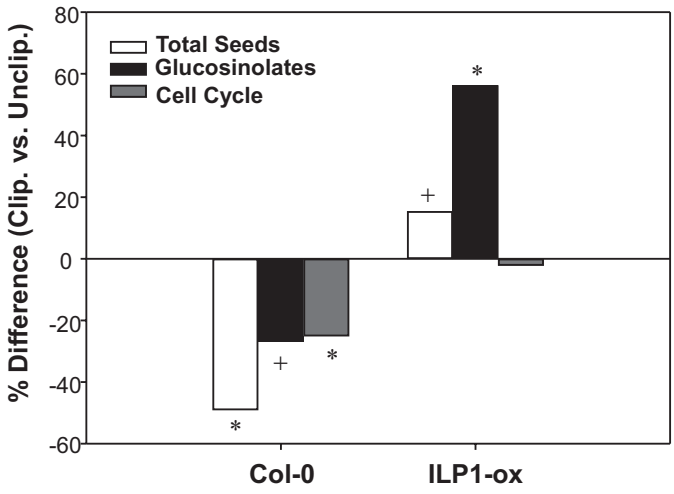

FIGURE 2. Percentage difference between clipped (Clip.) and unclipped (Unclip.) plants for the undercompensating wild-type *Arabidopsis thaliana* Columbia-0 (Col-0) ecotype and an ILP1-ox mutant with 29 copies of the ILP1 gene and a CAM35S promotor region in a Col-0 background. Measures include seeds, glucosinolates, and cell cycle values (endopolyploidy). Genotypic responses range from undercompensation (negative percentage difference values) to overcompensation (positive percentage difference values). Asterisks indicate significance at a familywise error rate of $\alpha = 0.05$. Plus signs indicate marginal ($P < 0.1$) significance. Cell cycle values were generated in a separate study (Scholes and Paige, 2014). Figure from Mesa et al. (2017), *Ecology Special Feature*.

for example, those completing their life cycles before canopy closure (Barow and Meister, 2003). We suggest that plasticity in ploidy through endoreduplication is also adaptive in that it allows rapid regrowth and development following herbivory (particularly when 95% of the aboveground biomass is removed by herbivores in some plant species; see e.g., Paige and Whitham, 1987; Lennartsson et al., 1997), ultimately increasing plant fitness. However, given that studies to date have focused primarily on *Arabidopsis thaliana* (e.g., Scholes and Paige, 2014), studies on other plant species that overcompensate will be necessary to see whether the phenomenon is a general one. Such studies should incorporate relative plant growth rates through time, number of cell divisions, and cell sizes in addition to endopolyploid measures and measures of fitness.

Although there is genetic variation in endoreduplication/endopolyploidy within and among plants, the causes of such variation are unknown. The tremendous allelic diversity in cell cycle regulators (cyclins and cyclin-dependent kinases) may be the key in explaining such variation in endopolyploidy (Nieuwland et al., 2007; Scholes and Paige, 2015). In addition, one might expect allelic variation within genes in the OPP pathway to affect the level of endoreduplication and hence the degree of fitness compensation. For example, a mutation that decreases the expression of G6PD1 could limit nucleotide synthesis and perhaps the degree of endopolyploidy. Furthermore, the molecular mechanisms specifying the number of endocycles within a cell are also unknown.

From an evolutionary perspective, we know that there is heritable genetic variation for endopolyploid levels (e.g., see Siddappaji et al., 2013; Neiman et al., 2017); however, the role of natural selection in maintaining variation in endopolyploidy within and across taxa and among cells and tissues needs to be experimentally addressed. Specifically, can endopolyploidy and its associated induction threshold evolve via natural selection (Neiman et al., 2017) in relation to differences among herbivores and herbivory (such as the predictability, frequency, and timing of herbivory) and what are the associated molecular changes or targets of selection?

We also do not know whether there are material costs of endopolyploidy in regards to phosphorus and nitrogen availability for endoreduplication (Scholes and Paige, 2015; Neiman et al., 2017). Plants growing in high-resource conditions are assumed to be the ones best able to compensate for herbivory. However, just the opposite has been found for dicotyledonous plants exhibiting patterns of overcompensation, with most occurring in resource-poor conditions. Thus, the relationships among nutrient content (primarily phosphorus and nitrogen), the degree of endoreduplication, and the phenomenon of overcompensation are unclear and need to be experimentally explored. There may also be costs of endopolyploidy associated with the creation of new cells (e.g., see Neiman et al., 2017). Once a cell begins to endoreduplicate, it cannot return to the mitotic cycle to divide, an essential repair mechanism. Such costs have not been directly assessed.

It is also noteworthy that endoreduplication may serve as a generalized response mechanism for mitigating stress by plastically increasing endopolyploidy in response to a host of environmental factors, other than herbivory, including high light/UV, low temperature, water stress, heavy metals, salt, wounding, pathogens (e.g., fungi, nematodes), and symbiotic biotrophs (e.g., rhizobial bacteria, mycorrhizal fungi), among others (see Scholes and Paige [2015] for a review). The integration of the endocycle in the stress response pathway (the OPP and shikimate) provides increases in transcriptional output, metabolism, stress-mitigating compounds, and nucleotypic effects necessary to mitigate a variety of environmental stressors (Scholes and Paige, 2015). Thus, plasticity in cellular ploidy may be important in the response to environmental stress by providing a mechanism for organisms to fine-tune themselves to their local environment via control at the level of individual cells. Nonetheless, with limited exception, few experimental studies have been directed toward the role of endoreduplication in ecological, evolutionary, or environmental contexts, clearly opening new avenues for inquiry.

Overall, it is clear that an understanding of the ecological and evolutionary roles of endoreduplication in fitness compensation and more generally environmental stress will require broad surveys analyzing examples of endopolyploidy across plant taxa, cell types, cellular characteristics, and environments. Targeted assessments manipulating environmental factors and/or the endocycle itself will undoubtedly strengthen our understanding of the nature and impact of endopolyploidy beyond the correlational and observational data that currently pervades the literature (Scholes and Paige, 2015). Of course, the ultimate goal is to understand how endoreduplication affects ecological function and the adaptive potential of natural populations (Neiman et al., 2017).

ACKNOWLEDGEMENTS

Thanks to Pam Diggle for the invitation to write this essay and for her constructive comments that helped improve this manuscript. I also thank two anonymous reviewers for their help in improving this manuscript. Research herein was supported by the National Science Foundation (DEB-1146085) to K.N.P.

LITERATURE CITED

Barow, M. 2006. Endopolyploidy in seed plants. *BioEssays* 28: 271–281.

Barow, M., and A. Meister. 2003. Endopolyploidy in seed plants is differently correlated systematics, organ, life strategy, and size. *Plant, Cell and Environment* 26: 571–584.

Bourdon, M., J. Pirrello, C. Cheniclet, O. Coriton, M. Bourge, S. Brown, A. Moïse, et al. 2012. Evidence for karyoplasmic homeostasis during endoreduplication and a ploidy-dependent increase in gene transcription during tomato fruit growth. *Development* 139: 3817–3826.

Ishida, T., S. Adachi, M. Yoshimura, K. Shimizu, M. Umeda, and K. Sugimoto. 2010. Auxin modulates the transition from the mitotic cycle to the endocycle in *Arabidopsis*. *Development* 137: 63–71.

Kruger, N. J., and A. von Schaewen. 2003. The oxidative pentose phosphate pathway: structure and organisation. *Current Opinions in Plant Biology* 6: 236–246.

Lee, H. O., J. M. Davidson, and R. J. Duronio. 2009. Endoreplication: ploidy with purpose. *Genes and Development* 23: 2461–2477.

Lennartsson, T., P. Nilsson, and J. Tuomi. 1997. Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). *American Naturalist* 149: 1147–1155.

Machado, R. A. R., A. P. Ferrieri, C. A. M. Robert, G. Glauser, M. Kallenbach, I. T. Baldwin, and M. Erb. 2013. Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytologist* 200: 1234–1246.

Melaragno, J. E., B. Mehrotra, and A. W. Coleman. 1993. Relationship between endopolyploidy and cell size in epidermal tissue of Arabidopsis. *Plant Cell* 5: 1661–1668.

Mesa, J. M., D. R. Scholes, J. Juvik, and K. N. Paige. 2017. Molecular constraints on resistance–tolerance trade-offs. *Ecology Special Feature* 98: 2528–2537.

Nagl, W. 1976. Endoreduplication and polyteny understood as evolutionary strategies. *Nature* 261: 614–615.

Neiman, M., M. J. Beaton, D. O. Hessen, P. D. Jeyasingh, and L. J. Weider. 2017. Endopolyploidy as a potential driver of animal ecology and evolution. *Biological Reviews* 92: 234–247.

Nieuwland, J., M. Menges, and J.A.H. Murray. 2007. The plant cyclins. *In* D. Inzé [ed.], Annual plant reviews vol. 32, Cell cycle control and plant development, 31–61. Wiley-Blackwell, Oxford, UK.

Paige, K. N., and T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist* 129: 407–416.

Scholes, D. R., and K. N. Paige. 2011. Chromosomal plasticity: mitigating the impacts of herbivory. *Ecology* 92: 1691–1698.

Scholes, D. R., and K. N. Paige. 2014. Plasticity in ploidy underlies plant fitness compensation to herbivore damage. *Molecular Ecology* 23: 4862–4870.

Scholes, D. R., and K. N. Paige. 2015. Plasticity in ploidy: a generalized stress response. *Trends in Plant Science* 20: 165–175.

Siddappaji, M. H., D. R. Scholes, and K. N. Paige. 2013. Overcompensation in response to herbivory in *Arabidopsis thaliana*: the role of glucose-6-phosphate dehydrogenase and the oxidative pentose-phosphate pathway. *Genetics* 195: 589–598.

Stowe, K. A., R. J. Marquis, C. G. Hochwender, and E. L. Simms. 2000. The evolutionary ecology of tolerance to consumer damage. *Annual Review of Ecology and Systematics* 31: 565–595.

Tiffin, P., and M. D. Rausher. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. *American Naturalist* 154: 700–716.