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# A meta-analysis of whole genome duplication and the effects on flowering traits in plants

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**PREMISE OF THE STUDY**: Polyploidy, or whole genome duplication (WGD), is common in plants despite theory suggesting that polyploid establishment is challenging and polyploids should be evolutionarily transitory. There is renewed interest in understanding the mechanisms that could facilitate polyploid establishment and explain their pervasiveness in nature. In particular, premating isolation from their diploid progenitors is suggested to be a crucial factor. To evaluate how changes in assortative mating occur, we need to understand the phenotypic effects of WGD on reproductive traits.

**METHODS**: We used literature surveys and a meta-analysis to assess how WGD affects floral morphology, flowering phenology, and reproductive output in plants. We focused specifically on comparisons of newly generated polyploids (neopolyploids) and their parents to mitigate potential confounding effects of adaptation and drift that may be present in ancient polyploids.

**KEY RESULTS**: The results indicated that across a broad representation of angiosperms, floral morphology traits increased in size, reproductive output decreased, and flowering phenology was unaffected by WGD. Additionally, we found that increased trait variation after WGD was uncommon for the phenotypic traits examined.

**CONCLUSIONS**: Our results suggest that the phenotypic effects on traits important to premating isolation of neopolyploids are small, in general. Changes in flowering phenology, reproductive output, and phenotypic variation resulting from WGD may be less critical in facilitating premating isolation and neopolyploid establishment. However, floral traits for which size is an important component of function (e.g., pollen transfer) could be strongly influenced by WGD.

**KEY WORDS** effect size; floral display; floral evolution; floral phenotype; flower; gigas; minority cytotype exclusion; neopolyploidy; polyploidy.

In plants, the consequences of large-scale genomic modifications can be extensive, and linking these genetic changes to their subsequent phenotype is critical for understanding ecological and evolutionary dynamics (e.g., Otto and Whitton, 2000; Flagel and Wendel, 2010; Soltis et al., 2010). For instance, chromosomal rearrangements, such as inversions, can give rise to polytypic species with differences in life history or reproductive strategies (Lowry and Willis, 2010; Kupper et al., 2016; Tuttle et al., 2016). In addition, genome size can vary greatly within a single species and can correlate with a number of environmental variables such as elevation, altitude, and environmental moisture levels (reviewed by Levin, 2002; Smarda and Bures, 2010), suggesting that genome size may contribute to local adaptation (Levin, 2002). Although these examples highlight how genomic alterations can have far reaching effects on phenotype and evolutionary ecology, we still lack a clear understanding of the link between the immediate effects of genomic alterations and their effect on organismal success.

A key type of large-scale genomic modification that may affect fitness is whole genome duplication (WGD), i.e., polyploidy. WGD is particularly important in the evolution of plants (Adams and Wendel, 2005; Soltis et al., 2009, 2016) as approximately 15% of speciation events in angiosperms (Wood et al., 2009) and nearly a quarter of extant plant taxa are polyploid (Barker et al., 2016). WGD can generate instantaneous reproductive isolation, setting individuals with duplicated genomes on independent evolutionary trajectories from their ancestors (reviewed by Ramsey and

Schemske, 2002; Otto, 2007; Ramsey and Ramsey, 2014). Once a polyploid lineage has evolved, WGD can have immediate effects on gene expression (reviewed and modeled by Chen and Ni, 2006), morphology (reviewed by Ramsey and Schemske, 2002), and also provides duplicated genetic material that can spur the evolution of novel phenotypes ("neosubfunctionalization"; Flagel and Wendel, 2009). These changes in polyploids can have cascading effects on plant ecology and evolution. For example, changes in ploidy level have been implicated in the ability to colonize new habitats (Leitch and Leitch, 2008; Parisod et al., 2010; te Beest et al., 2012), altering how a plant interacts with its abiotic and biotic environments (Maherali et al., 2009; Liu et al., 2011; Ramsey, 2011; Segraves and Anneberg, 2016), and facilitating species diversification (Vamosi and Dickinson, 2006; Soltis et al., 2009).

The ubiquity of polyploidy in plants is intriguing because polyploids are expected to be uncommon and evolutionarily ephemeral caused by frequency-dependent reproductive disadvantages associated with being the minority cytotype when first arising in a population; i.e., the minority cytotype exclusion principle (Levin, 1975). Thus, the answer to why plant polyploidy is so common remains to be explained. One hypothesis is that genome duplication may confer changes in phenotype that allow new polyploids to overcome minority cytotype exclusion by becoming, at least in part, prezygotically isolated from their parental species during initial establishment (Levin, 1975; Husband, 2000). For example, if polyploidy leads to larger flowers, pollinators may be able to detect these differences and either favor or avoid polyploids, leading to assortative mating (Segraves, 2017). Additionally, if polyploids have overall larger structures, they could take longer to develop (Cavalier-Smith, 1978; Ramsey and Ramsey, 2014), potentially resulting in later flowering dates and a shift in flowering phenology. To determine if phenotypic changes associated with WGD could play a role in premating isolation, the first step is to better understand the consequences of genome duplication with respect to phenotypic traits directly related to reproduction.

One commonly observed effect of WGD that is seen in polyploids is an increase in size and greater robustness of plant traits, termed the "gigas effect." The gigas effect is thought to be the result of polyploids having greater quantities of DNA, which cause larger cells and translates into larger tissues and organs (Muntzing, 1936; Stebbins, 1971). However, this directional effect on plant phenotype is not the rule (Otto and Whitton, 2000; Vamosi et al., 2007) because there are numerous examples of polyploids having smaller or equal-sized floral organs relative to their diploid counterparts (Segraves and Thompson, 1999; Tables 1 and 2 of Vamosi et al., 2007; Ning et al., 2009; Trojak-Goluch and Skomra, 2013). In addition to changing floral organ size, increases in cell size can also contribute to other phenotypic changes, such as flowering phenology. Larger cells often require more time to divide (Bennett, 1987; Francis et al., 2008), and this might delay flowering. Similar to flower size, this prediction is not always observed (Nuismer and Cunningham, 2005; Thompson and Merg, 2008; Nghiem et al., 2011).

Recent work has identified considerable variation among studies that examine phenotypes before and after WGD (e.g., Vamosi et al., 2007). Some of this variation is probably caused, in part, by examining polyploid-progenitor pairs that have experienced different evolutionary histories. For example, polyploid-progenitor pairs that have been identified in nature have experienced many generations of adaptation prior to study, and thus have had time to obscure the initial phenotypic effects of WGD. Indeed, there is some evidence suggesting that phenotypes can degrade or change in subsequent generations after WGD (Butterfass, 1987; Oswald and Nuismer, 2011; Ramsey, 2011; Husband Baldwin and Sabara, 2016), underscoring the need to study phenotypes of newly formed polyploids. Consequently, to understand if there are predictable, quantitative effects of WGD on plant phenotypes, results of single case studies that compare newly formed polyploids with their parents need to be compiled and analyzed. In particular, we need to understand if polyploidy results in significant shifts in reproductive traits that could play a role in allowing new polyploids to escape minority cytotype exclusion.

Here, we surveyed the literature and performed a meta-analysis to quantify the immediate consequences of WGD on reproductive traits. In our analysis, we included studies that contained data from newly synthesized polyploids to disentangle the effects of genome duplication from subsequent adaptation. This was done to mitigate confounding effects of adaptation and drift, and because the phenotypic effects of genome duplication will be most critical in determining which traits might facilitate reproductive isolation during initial establishment immediately following WGD. Our goals were to (1) determine the effect size and impact on variation that WGD has on plant reproductive traits, (2) identify the traits that are most affected by genome duplication, and (3) test whether phylogenetic history or genome size might help us to better predict changes after WGD.

## METHODS

### Literature search

To find relevant literature that would address our questions, we performed searches in three separate databases using Syracuse University Library's subscription packages. First, we used Web of Science (ISI) to search for the terms (neopoly* or *synthes* or colchicine or oryzalin or trifluralin or nitrous) and (phenoty* or morphol* or phenolo*) and (flower* or floral or pollen or petal) and (plant* or *ploid*) from 1900 to the present. This search returned 234 results. For the second search, we used the database Agricola open to all years with the same search terms as above except it excluded the precursory wildcards because Agricola does not support that search function; this search returned 339 results. Third, we searched JSTOR open to all years, with the search identical to Agricola, but without the term "synthes*" because removing the term reduced the results to a feasible number to examine. This search was open to any content type and filtered by subject types 'Biological Sciences', 'Botany & Plant Science', 'Ecology & Evolutionary Biology', and 'General Science', which returned 2805 results. This initial pool of 3378 search results was further narrowed by including only the subset of articles that indicated in the title or abstract that traits were measured before and after polyploid induction. This narrowed the results to 130 research papers. From this pool, research papers were excluded from subsequent analysis if they did not meet the following conditions: (1) contained extractable, quantitative data on floral phenotype or flowering phenology of both newly formed polyploids and their progenitors, and (2) reported sample sizes, means, and either standard deviation or standard error. In instances when the publication did not include the data necessary to calculate effect sizes, the corresponding author was contacted to request those data or data were extracted

directly from the figures using Plot Digitizer Ver. 2.6.8 (Huwaldt and Steinhorst, 2015). In addition to data collected from database searches, we also obtained data from two unpublished studies that were shared by the authors (L. Comai and H. Wu, University of Washington, unpublished data; L. Porturas et al., Syracuse University, unpublished data). When a study reported data from multiple genotypes within a single species, we collapsed the genotypic data into an average for the species. If a study reported data from multiple varieties, they were treated separately because varieties of a single species often display very different floral traits (e.g., *Brassica oleracea*). In our compiled data set, we included information on the reference, species, ploidy level, chromosome number, mode of genome duplication (auto- or allopolyploid), selection history (natural or artificial selection for agricultural and horticultural plants), the means of polyploidy synthesis (e.g., colchicine), type of traits examined, and trait measurements. 'Selection history' type was assigned subjectively. If the species' floral phenotype or related features, such as fruit, had been subject to a well-known history of artificial selection (e.g., maize, *Brassica oleracea*, *Chrysanthemum*), they were assigned to the agricultural/horticultural selection history type. Otherwise, the species was assigned to the natural selection history type. We collated data on three major trait categories (phenology, size, and reproductive output) that included a variety of trait measurement types (Appendix S1).

### Meta-analyses

We used the R Statistical Software (R Core Team, 2016) to perform our meta-analyses. For all analyses, we used the log response ratio ($\ln RR = \ln(mean_{after\ WGD} / mean_{before\ WGD})$) as the effect size measure to compare trait differences before and after WGD. This was calculated using the 'escalc' function in the R package 'metafor' (Viechtbauer, 2010). We also estimated the coefficient of variation ratio ($\ln CVR = \ln[CV_{after\ WGD} / CV_{before\ WGD}]$), calculated using the 'calc.lnCVR' function provided by Nakagawa et al. (2015) to compare variation in traits before and after WGD.

In our analysis, we first determined whether phylogenetic history and genome size would be important covariates to account for in our models. To do this, we mapped the log response ratio of size-related traits onto the plant phylogeny published by Zanne et al. (2014). Size-related traits were used for this analysis because size traits were expected to increase with WGD, and subsequent analysis verified that there were no differences in the magnitude of the effect of WGD on the different size-related traits. Because many species in our data set were not included in this phylogeny, the phylogeny was trimmed so that the tips represented genera instead of species. We used the 'drop.tip' function from the R package 'phytools' (Revell, 2012). If there was more than one representative species or lnRR measure per genus, the average lnRR was used. The generic name of one species in our database, *Dendranthema nankingense*, was not included in the phylogeny, so the name was replaced by its suggested synonym (*Chrysanthemum indicum*) according to The Plant List database (www.theplantlist.org). We tested for phylogenetic signal in the data using Blomberg's K and Pagel's λ. Tests for nonrandom distribution of the effect size of WGD across the phylogeny were done using the 'phylosig' function from the R package 'phytools' (Revell, 2012), specifying both Blomberg's K and Pagel's λ as output variables. We also determined whether genome size influenced the effect of genome duplication in plants. The C-values were obtained from the Kew Royal Botanical Gardens Plant DNA C-values Database, and we used these data to calculate the Pearson's correlation coefficient between C-values and average effect size (lnRR) of size-related traits for each species.

In the second step of our analysis, we used linear mixed models to estimate the average effect size of WGD on flowering phenology, reproductive output, and flower size. Because we found no evidence of a relationship between effect size and either phylogenetic history or genome size, these variables were excluded from our models. The first model included all of the calculated effect sizes that were assigned to one of three trait categories: 'phenology', 'output', and 'size'. Trait category was used as the fixed effect variable for this model. The random effects variables were (1) the article reference, (2) plant species nested within article reference, and (3) trait category nested within plant species nested within article reference. These were the assigned random effects because some studies measured multiple traits (e.g., flower length, flower width, pollen size) on multiple species. The log response ratio was used as the response variable for the model. The mechanism of polyploid formation (allopolyploid versus autopolyploid) and selection history (horticultural/agricultural versus natural) were analyzed as interactive fixed effects. In a second model, we tested the hypothesis that WGD increases variation in traits by using the same model but substituting the coefficient of variation ratio for the response variable.

Next, we estimated the average effect size of WGD on specific size-related traits: the size of gametes, petals, flowers, and inflorescences. This model included 106 effect sizes, which were grouped into four size trait categories: 'gamete', 'petal', 'flower', and 'inflorescence'. Some size traits were excluded from this data set because there were insufficient sample sizes to calculate reliable estimates. The 'size trait' category was used because the fixed effects variable and the other factors were identical to the models described above. Lastly, we used a similar approach to estimate the average effect size of WGD on the reproductive output of gametes, flowers, and inflorescences. This model included 29 effect sizes that were placed into three reproductive output trait categories: 'gamete', 'flower', and 'inflorescence'. The model was identical to those described above except that no interactions were included because there were too few measurements to calculate reliable estimates when parsed between the interaction categories.

Estimated average effect sizes were modeled using the 'rma.mv' function. For all models, we tested whether there were significant interactions between factors (Wald-type chi-square tests, $Q_M$). If there was a significant interaction effect, we used Tukey's HSD post-hoc tests to determine whether there were pairwise differences between the levels of the trait categories; significant differences were detected using the function 'ghlt' from the R package 'multcomp' (Torsten Frank and Peter, 2008). All null models are summarized in Appendix S2. We also tested for publication bias with Egger's regression test by including variance as a moderator to our null models. If the studies included in our analysis are not affected by publication bias, then the intercept should not significantly deviate from zero at $\alpha = 0.10$ (Egger et al., 1997).

## RESULTS

### Overview

We had 185 effect-size and variation-size measures from 39 studies and 55 independent WGD events (Appendices S1, S3). Our data set

represented 30 genera across 18 plant families. The vast majority of our measures came from diploid to tetraploid genome duplications (89.2%), and the remaining forms of WGD events were relatively rare (haploid to diploid 4.3%, triploid to hexaploid 4.3%, tetraploid to octaploid 1.6%, octaploid to hexadecaploid 0.5%). Most of the studies induced WGD events using the mitotic inhibitor colchicine (72.4%). The other polyploid induction types included somaclonal variation during embryo culture (8.1%), oryzalin (4.9%), nitrous oxide gas (4.3%), protoplast fusion (1%), trifluralin (0.5%), or were unspecified (8.6%). Egger's regression test identified evidence of publication bias in all three of our data sets because the intercepts were significantly different from zero at α = 0.10: all trait categories ($P$ = 0.072), size trait categories ($P$ = 0.003), and reproductive output trait categories ($P$ = 0.053).

### Phylogenetic history and genome size correlations

We found no evidence of a correlation between the effect of WGD on size traits and evolutionary history (Blomberg's K: 0.297, $P$ = 0.111; Pagel's λ: 0.252, $P$ = 0.441; Appendix S4). We also found no evidence of a correlation between the effect of WGD on size traits and genome size (Pearson's correlation estimate = 0.029, $P$ = 0.937). Thus, subsequent analyses did not correct for phylogenetic history or genome size.

### Overall effect of genome duplication on reproductive output, size, and phenology

There were significant differences in how WGD affected reproductive output, size, and phenology ($Q_M$ = 952.318, df = 2, $P$ < 0.0001). The estimated mean effect size for reproductive output was negative (–0.190 ± 0.078), indicating that WGD reduced the reproductive output of polyploid plants. In contrast, the estimated mean effect size of size-related traits was positive (0.195 ± 0.075), showing that the size of floral traits generally increased following WGD. The estimated mean effect size of phenology (0.010 ± 0.140) did not differ from zero (Fig. 1). We found no evidence for an interaction between these trait categories and the mechanism of polyploid formation ($Q_M$ = 1.430, df = 1, $P$ = 0.232). Reproductive output was dropped from this test for an interaction because the data set had no allopolyploids with that measure. In addition, we found a significant interaction between trait category and selection history ($Q_M$ = 32.961, df = 2, $P$ < 0.0001), but there were no significant differences identified in pairwise comparisons of the two selection history categories for the three traits (Appendix S5).

We were also interested in knowing whether WGD significantly increased trait variation after WGD. Indeed, we found significant differences in how WGD affected variation in reproductive output, size and phenology ($Q_M$ = 5059.650, df = 2, $P$ < 0.0001). There was no significant difference in the mean estimated variation in phenology and size (0.288 ± 0.476 and 0.076 ± 0.280, respectively); however, we did see an increase in variation after WGD for reproductive output (0.974 ± 0.281) (Fig. 2). Similar to the trends observed in effect size, we found no evidence of an interaction between trait category and mechanism of polyploid formation ($Q_M$ = 1.576, df = 1, $P$ = 0.209). Reproductive output was dropped from this test because of a lack of allopolyploids with that measure. Moreover, we did find a significant interaction between trait category and selection history ($Q_M$ = 954.605, df = 2, $P$ < 0.0001). Pairwise comparisons examining differences between the two selection history categories for

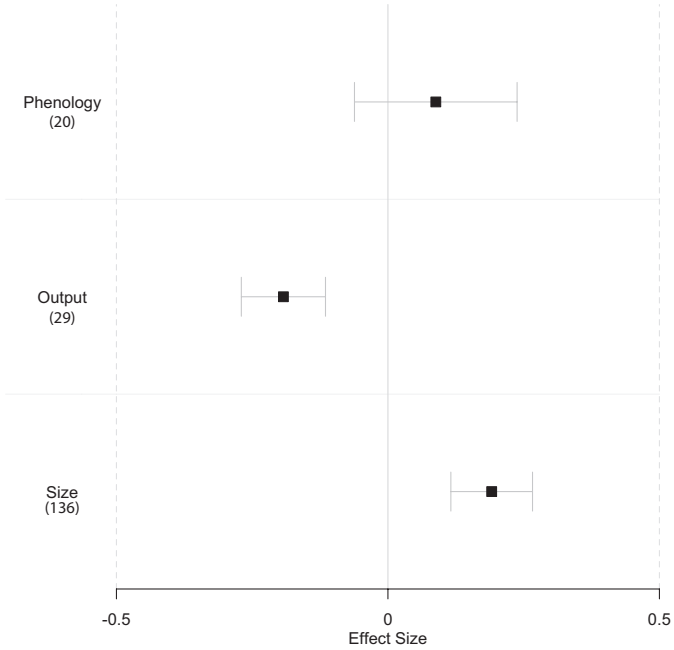


**FIGURE 1.** The average estimated effect size of whole genome duplication on phenology, reproductive output, and size-related traits. Values are coefficient estimates of log response ratios and their corresponding 95% confidence intervals. If the confidence interval includes zero, the estimate is not statistically different from zero. Number of effect size measures are in parentheses following the trait identifier.

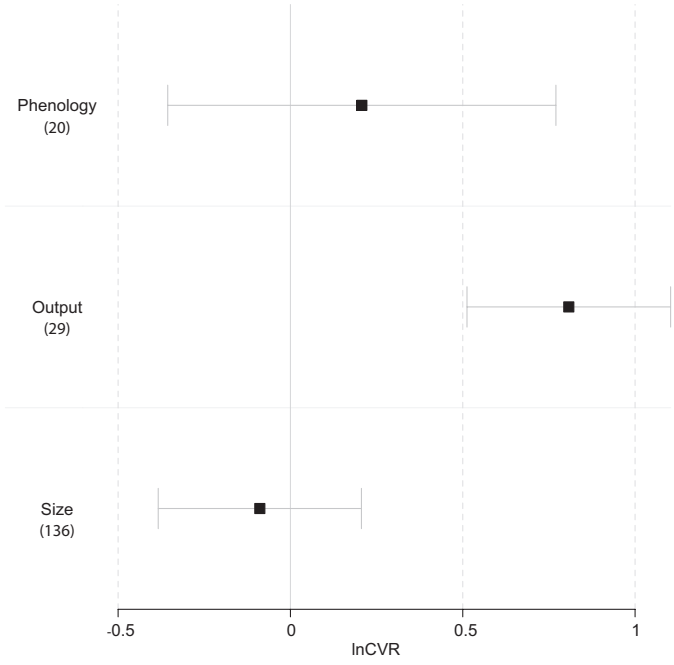


**FIGURE 2.** The average estimated effect on the amount of trait variation in response to whole genome duplication in phenology, reproductive output, and size related traits. Values are coefficient estimates of the log coefficient of variation ratios (lnCVR) and the corresponding 95% confidence intervals. If the confidence interval passes through zero, the estimate is not statistically different from zero. Number of effect size measures are in parentheses following the trait identifier.

the three traits showed that reproductive output was significantly different (Tukey's HSD post-hoc test, $P < 0.0001$, Appendix S6).

### Effect of genome duplication on specific size traits

Although we found an overall significant increase in the size-related traits after WGD (Appendix S5), we found no significant differences in the magnitude of effect size when comparing across gametes, petals, flowers, and inflorescences ($Q_M = 1.920$, df = 3, $P = 0.590$) (Fig. 3). There was also no evidence of an interaction between the size traits and mechanism of polyploid formation ($Q_M = 2.274$, df = 1, $P = 0.132$); gamete and inflorescence data were excluded from this test because the data set lacked allopolyploids with either of those measures. Finally, we found no interaction between size and selection history category ($Q_M = 0.731$, df = 2, $P = 0.694$). We excluded inflorescence from this test because there was only one effect size measure of a natural inflorescence.

When we tested for overall changes in variation after WGD, we found no significant effect on variation in size. However, when we tested for changes in variation after WGD within the size-related traits, we found that WGD affects variation in size differently among gametes, petals, flowers, and inflorescences ($Q_M = 21.657$, df = 3, $P < 0.0001$) (Appendix S7). We also found no evidence of an interaction between the size traits and mode of genome duplication ($Q_M = 0.171$, df = 1, $P = 0.680$), or between size traits and selection history category ($Q_M = 0.517$, df = 2, $P = 0.772$). We excluded gamete and inflorescences from the test for interactions with mode of genome duplication because there were no allopolyploids with either of those measures in our data set, and for similar reasons, we also excluded inflorescence from the test for interactions with selection history category.

### Effect of genome duplication on specific reproductive output traits

Although we found an overall significant decrease in reproductive output after WGD (Fig. 2), there was no significant difference in the magnitude of effect size when comparing across gametes, flowers, and inflorescences ($Q_M = 1.677$, df = 2, $P = 0.432$) (Fig. 4). When we tested for overall changes in variation after WGD, there was a significant increase in variation in reproductive output related traits. However, when we examined just the reproductive traits, we found that WGD did not affect variation in reproductive output differently among gametes, flowers, and inflorescences ($Q_M = 0.544$, df = 2, $P = 0.762$), and the average estimated effect on the amount of trait variation after WGD in these traits did not differ significantly from zero.

## DISCUSSION

The ubiquity of polyploidy in plants is interesting because theoretical predictions suggest that polyploids should rarely be able to successfully establish in natural populations (Levin, 1983; Fowler and Levin, 1984, 2016; Felber, 1991; Baack, 2005). Better understanding the phenotypes resulting from WGD can help us understand which traits might play key ecological roles during establishment in the critical generations immediately following polyploidization (Segraves, 2017). This study is the first to use meta-analytical approaches to assess how WGD affects floral traits in the generations immediately following genome duplication. Using data available in the literature, we examined how size of floral traits, reproductive output, and phenology are affected by WGD. This study builds on the previous work of Vamosi et al. (2007) by including additional

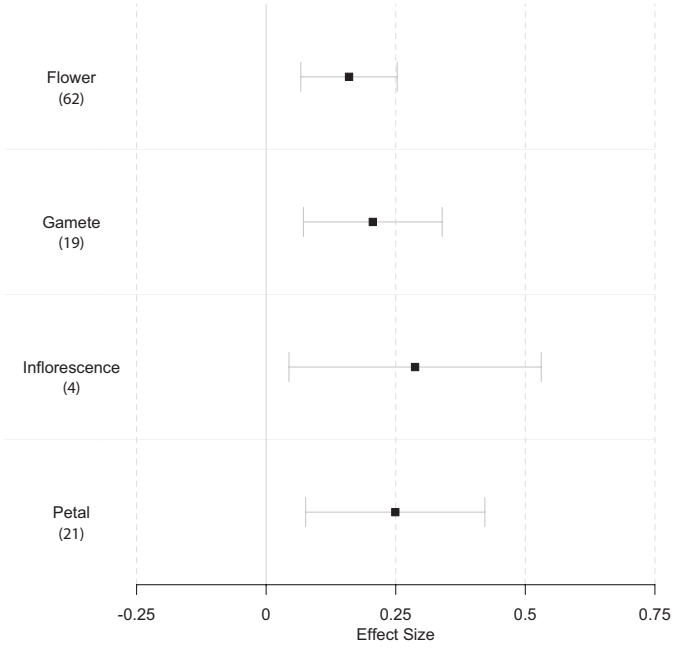


**FIGURE 3.** The average estimated effect size of whole genome duplication on size-related traits. Values are coefficient estimates of log response ratios and their corresponding 95% confidence intervals. Number of effect size measures are in parentheses following the trait identifier.

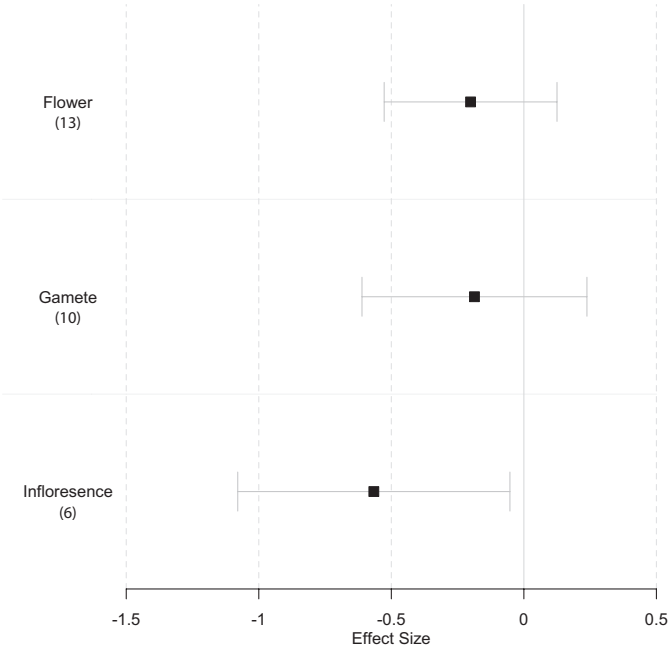


**FIGURE 4.** The average estimated effect size of whole genome duplication on the reproductive output traits. Values are coefficient estimates of log response ratios and their corresponding 95% confidence intervals. Number of effect size measures are in parentheses following the trait identifier.

studies, focusing specifically on newly formed polyploids, and by estimating the effect sizes of WGD. This approach allows us to determine how genome duplication effects floral phenotypes and phenology and to identify key traits affected by WGD.

In concert with broad expectations (Stebbins, 1971; Levin, 2002), we found that on average, WGD increased the size of floral traits. The gigas effect has long been recognized as a consequence of WGD despite some reports of WGD imparting no differences or decreases in size. The data here support the longstanding belief that, in general, neopolyploids experience the gigas effect and suggest that the plants that experience no size increase after WGD are in the minority. Our estimates suggest that floral traits increase in size 20–25% as a direct result of WGD. On the other hand, we observed a general decrease in reproductive output measures by 20–25% (Fig. 1). An increase in size traits coupled with a decrease in reproductive output suggest that WGD may cause differences in resource allocation to reproductive structures (Segraves and Thompson, 1999). In addition to examining the overall effect of WGD on these general categories of traits, we also dissected size and reproductive output into individual traits. This analysis found that WGD had consistent effects on the traits within their respective categories. For example, the magnitude of the effect of WGD on the increase in size of gametes was not significantly different from the magnitude of the effect on petals, flowers, and inflorescences (Fig. 3). This is surprising because we expected the largest effect to be seen in gametes because they are single cells as opposed to the other anatomical structures, which are aggregates of cells and different tissue types. We had predicted that these larger organs could have smaller effect sizes if fewer but larger cells were used to compose those structures. Similar to size traits, the magnitude of the effect that WGD had on reproductive output was not different between pollen, flowers, and inflorescences (Fig. 4). Together, the results suggest that WGD has a consistent effect on floral morphology and reproductive output traits.

In contrast to changes in morphology and reproductive output, there was no evidence that WGD results in delayed flowering phenology. This was surprising given that longer mitotic division times of polyploid cells are predicted to translate into later or longer flowering periods (Ramsey and Schemske, 2002). Moreover, some of the seminal ecological studies of polyploidy that document flowering phenology of polyploids and their diploid progenitors have identified later flowering phenology in polyploids (Segraves and Thompson, 1999; Husband and Sabara, 2003; Jersáková et al., 2010; Oswald and Nuismer, 2011; Ramsey, 2011; Roccaforte Russo and Pilson, 2015). Our data trend towards this expectation; however, the effect size was not significantly different from zero, suggesting that flowering phenology of polyploids is, on average, similar to that of their parents.

Another general expectation of polyploids is that they will likely exhibit greater variability in traits due to increased or fixed heterozygosity, or phenotypic and genomic instability in the generations following WGD (Soltis and Soltis, 1995; Comai et al., 2000; Otto and Whitton, 2000; Ramsey and Schemske, 2002). If this were the case, greater trait variability could likely be a beneficial artifact of WGD, because selection could act on a wide variety of phenotypes during critical establishment periods, allowing faster adaptation to the environment. Interestingly, we did not find a general trend of greater variation in traits after genome duplication. We did detect increased variation in reproductive output; however, when we examined the individual traits within the reproductive output model (gametes, flowers, and inflorescences), we found that these individual categories did not explain the overall increased variation seen in reproductive output. This suggests that none of these categories of reproductive output measures are strongly affected by WGD, but taken together, the changes lead to increased overall variation. The only traits that showed significantly increased variation after WGD were the size of inflorescences (Appendix S7) and reproductive output traits from non-horticultural/agricultural species (Appendix S6). Because so few traits had greater variation after WGD, increased variation in phenotype may be a less common consequence of polyploidy than previously expected.

In addition to investigating variation after WGD, we were also interested in determining whether phylogenetic history or genome size might reliably predict how WGD affects floral phenotype. We expected evolutionary history to correlate with the magnitude of the effect of WGD because developmental or genetic constraints on reproductive development could be shared within clades and create similar responses to WGD. However, there was no evidence of phylogenetic signal in the effect that WGD had on size-related traits (Appendix S4). Similarly, we also predicted that genome size might correlate with the effect of genome duplication. We know there is a strong relationship between cell size and genome size (Beaulieu et al., 2008), so we expected that doubling the genomic content of a plant with a large C-value would generate a stronger response than doubling the genomic content of a plant with a small C-value. Nonetheless, we did not detect a correlation between genome size and the magnitude of the effect of WGD on size-related traits. We found no evidence that evolutionary history or genome size correlated with the magnitude of effect of WGD. This, in combination with the poor predictive power of our interactive effects (mode of genome duplication and selection history type), suggests that the processes dictating the effect of WGD on reproductive traits are dynamic and not easily predictable.

Using a meta-analysis framework to synthesize the literature on the phenotypic effects of WGD is not without its limitations. One caveat of this approach is that the data are restricted to the subset of studies that contain the minimal information required for the analysis. For this reason, we excluded more than 25 studies because they did not report the data necessary to calculate effect sizes. As a result, some of our estimates are made using very few effect sizes (e.g., inflorescences in Figures 3 and 4). Although we report our best estimates given the available data in the literature, the power to detect reliable estimates using so few effect sizes is low, and therefore those estimates should be viewed only as provisional until more studies measuring the effects of WGD on floral traits are published and can be included in meta-analysis. In addition, there were too few samples within some categories so these factors were excluded from some of the models (e.g., mode of polyploid formation). This also meant that some interactions between traits and predictors, such as mechanism of polyploid formation and selection history, could not be tested. Despite our extensive literature searches in multiple databases, we only identified 55 WGD events that contained the data needed for analysis. Overall, we concur with the opinion of Vamosi et al. (2007) that there remains surprisingly little information available regarding flower phenotypes and polyploidy. Further, the majority of systems in our analysis were autopolyploid; more documentation of the effects of WGD on allopolyploid phenotypes would greatly help us understand the differences between these types of polyploids. An additional caveat to consider is that publication bias may inflate the significance of patterns observed in the data. Indeed, the results indicated that publication bias exists in our data, so the true effects of WGD could be smaller than our estimates indicate.

Despite these caveats, this study represents a comprehensive analysis examining the direct effects of genome duplication on the floral phenotype. Together, the results suggest that neopolyploids face a severe reproductive disadvantage as compared to their parents of lower ploidy level. Our findings show that polyploids experience reduced reproductive output and flower at the same time as their parental species. Although polyploids can have substantial changes in floral morphological traits, these shifts would need to substantially modify patterns of assortative mating. Therefore, we believe one area in need of more research would be to examine how the increased size of floral traits may affect prezygotic barriers. Questions that remain to be answered include: How does the increased size of pollen cells after WGD affect the interactions between pollen and pistil? Do pollinators respond directly to the increased size of floral displays? If interactions that are important to reproduction are altered by WGD, does it facilitate reproductive isolation of neopolyploids from diploids? Thus far, the limited experimental evidence on reproductive isolating mechanisms in neopolyploids suggests that immediate changes of WGD are responsible for only a small amount of reproductive isolation (Husband Baldwin and Sabara, 2016). Another informative avenue of research could be to examine the effect of WGD on other traits that may facilitate reproductive isolation, such as aspects of dispersal or self-fertilization. This would greatly complement the results of this study by forming a more complete picture of which phenotypes, if any, are driving premating isolation of neopolyploids from their diploid progenitors. Ultimately, if we are to understand the perplexing ubiquity of polyploids, linking these phenotypic effects to their ecological roles in the generations following genome duplication is critical.

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## AUTHOR CONTRIBUTIONS

All authors conceived and planned this project and contributed to data acquisition. L.D.P. conducted the analysis and wrote the manuscript with assistance from K.A.S. and D.M.A. All authors discussed the results and provided comments on the manuscript.

## DATA ACCESSIBILITY

The full data set used in this study is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.qm6v6v1 (Porturas et al., 2018).

Additional databases accessed in this study include Web of Science: webofknowledge.com, Agricola: agricola.nal.usda.gov, JSTOR: jstor.org, Royal Botanic Gardens, Kew: Plant DNA C-values database: http://data.kew.org/cvalues, and The Plant List database: theplantlist.org.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**APPENDIX S1.** Traits included in final data set, including all the measures collected from the literature that fell within the three main categories.

**APPENDIX S2.** List of null models used in the meta-analysis.

**APPENDIX S3.** References used to generate the data set.

**APPENDIX S4.** Phylogenetic relationship of the effect size of whole genome duplication on size- related traits across 27 genera.

**APPENDIX S5.** The average estimated effect size of whole genome duplication on phenology, reproductive output, and size-related traits by their selection history (agricultural/horticultural or natural).

**APPENDIX S6.** The average estimated effect on the amount of trait variation in response to whole genome duplication in phenology, reproductive output, and size-related traits by their selection history (agricultural/horticultural or natural).

**APPENDIX S7.** The average estimated effect on variation in response to whole genome duplication in size-related traits.

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