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Whole-genome duplication decreases clonal stolon production and genet size in the wild strawberry *Fragaria vesca*

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PREMISE OF THE STUDY: Clonal reproduction is often associated with polyploidy and is expected to influence polyploid establishment success, but the immediate effects of whole-genome duplication (WGD) on clonal reproduction in autopolyploids are unknown.

METHODS: We used synthesized neopolyploids to assess the direct effects of WGD on stolon and plantlet production in the wild strawberry *Fragaria vesca* by (1) comparing absolute clonal investment between diploids and neotetraploids under high and low resource conditions in the greenhouse and (2) determining realized clonal plantlet establishment and genet spatial structure using artificial field populations comprising both cytotypes.

KEY RESULTS: Neotetraploids produced fewer stolons and plantlets than diploids at slower weekly rates in the greenhouse when resources were high, resulting in lower total investment in clonal reproduction. Low resources led to smaller reductions in clonal biomass for neotetraploids and less pronounced differences between cytotypes. Comparisons between neotetraploids representing 13 independent WGD events and close diploid relatives revealed considerable variation in the response to polyploidization for some clonal traits. Field populations corroborated greenhouse results; neotetraploid genets were smaller than diploid genets, containing 28% fewer stolons and 46% fewer rooted plantlets.

CONCLUSIONS: WGD significantly decreases the clonal output of neotetraploid *F. vesca*, which is likely attributable to slower whole-plant growth of the neotetraploids than the diploids. In natural populations, smaller neotetraploid genets could decrease the probability of polyploid establishment in this species. However, variation between separate neopolyploid lines emphasizes that the response of clonal investment to WGD may not be uniform across polyploid origins.

KEY WORDS autopolyploidy; clonal reproduction; colchicine; neopolyploids; polyploidy; Rosaceae; synthetic polyploids.

Polyploidy is nearly ubiquitous throughout the angiosperms (Wood et al., 2009) and is widely thought to be significant contributor to speciation and diversification (Otto and Whitton, 2000; Soltis and Soltis, 2016). Increases in ploidy are often accompanied by phenotypic change (Stebbins, 1950; Levin, 2002; Ramsey and Schemske, 2002; Husband et al., 2016), which may arise immediately after WGD due to changes in cell size (Stebbins, 1950; Ramsey and Schemske, 2002; Maherali et al., 2009) or in gene expression associated with gene dosage balance, genome structure, and gene neofunctionalization (Osborn et al., 2003; Parisod et al., 2010; Roulin et al., 2013; Soltis et al., 2016a). Synthetic autopolyploids (i.e., neopolyploids) present an opportunity to study the phenotypic effects of whole-genome duplication (WGD) without the influence of hybridization present in allopolyploids (Martin and Husband, 2012), but studies using neopolyploids from nonmodel systems to evaluate the ecological and evolutionary impacts of polyploidization remain uncommon (Bretagnolle and Lumaret, 1995; Husband et al., 2008, 2016; Maherali et al., 2009; Oswald and Nuismer, 2011; Ramsey, 2011; Martin and Husband, 2012; Baldwin and Husband, 2013). Instantaneous phenotypic shifts in neopolyploids can affect

morphological, physiological, reproductive, and life history traits (Stebbins, 1950; Otto and Whitton, 2000; Ramsey and Schemske, 2002), which can have a significant influence on their early establishment success amid their diploid progenitors (Ramsey, 2011; Husband et al., 2016).

Clonal reproduction has long been associated with polyploidy (Müntzing, 1936; Gustafsson, 1948; Stebbins 1950; Herben et al., 2017), but the immediate effects of WGD on patterns of investment in clonal reproduction and clonal propagule production are unknown. Clonal reproduction is present in 50–70% of angiosperms (Klimeš et al., 1997; Klimešová et al., 2017) and can be broadly defined as asexual reproduction through the propagation of plant parts not involving seeds (Vallejo-Marín et al., 2010), such that a genetic individual (a genet) may consist of multiple, genetically identical, daughter ramets. It can occur through a wide variety of modes (Klimešová et al., 2017), and genets may or may not have persistent physical connections between ramets. The ability to clonally reproduce can confer fitness advantages through the production of asexual offspring (Charpentier, 2002; Vallejo-Marín et al., 2010), the promotion of sexual gamete dispersal (Van Drunen et al., 2015), increased genet survival through spreading risk among multiple ramets and enhanced regeneration following disturbance (Eckert, 2002; Silvertown, 2008), and the exploration and exploitation of local habitat (Hutchings and Wijesinghe, 1997; Liu et al., 2016).

How might WGD immediately affect clonal reproduction in neopolyploids? Early researchers believed that the origin of a perennial life history and the ability to clonally reproduce could be direct consequences of a polyploidization event (Müntzing, 1936; Gustafsson, 1948), but there is currently no evidence that strictly nonclonal diploids have close autopolyploid clonal derivatives. Alternatively, WGD could shift the magnitude of clonal reproduction normally present in a diploid (Stebbins, 1950; Soltis et al., 2016a). Changes in clonal traits in a new polyploid could be due to changes in cell size (Stebbins, 1950; Ramsey and Schemske, 2002), or altered expression of clonal traits due to increased gene dosage or new gene interactions (Guo et al., 1996; Ramsey and Schemske, 2002; Osborn et al., 2003; Riddle et al., 2010; Soltis et al., 2016a). As a null model, we might hypothesize that WGD will lead to higher clonal reproduction in polyploids if more gene copies translates into higher gene expression and more clonal structures being produced, or if cell size increases the size and survival probability of clonal propagules. Altered gene expression and cell size could further modify clonal architecture or the distance between established ramets. The direction of phenotypic change caused by WGD is not always clear, however, and whether independent polyploidization events produce phenotypes that are consistent and predictable is an active area of research (Yu et al., 2010; Oswald and Nuismer, 2011).

Neopolyploids with the ability to reproduce asexually through clonality, at rates similar to or exceeding that in diploid relatives, could experience increased establishment potential over nonclonal neopolyploids. Clonal reproduction is expected to decrease the negative effects of minority cytotype exclusion (MCE; Levin, 1975), a major barrier to polyploid evolution where rare polyploids experience a lack of same-cytotype mates resulting in a disproportionate number of between-cytotype fertilizations, low fitness, and their eventual exclusion from the population (Husband, 2000; Otto and Whitton, 2000). Clonal reproduction may circumvent MCE in three primary ways. First, the production of spatially proximate daughter clones increases the number of prospective same-cytotype mates within a predominantly diploid population (Husband and Schemske, 1997; Husband et al., 2008), thereby increasing the number of opportunities for geitonogamous within-cytotype matings (Charpentier, 2002; Baack, 2005; Vallejo-Marín et al., 2010). Second, clonal reproduction increases the survival of rare neotetraploids through the bottleneck stage of establishment via physical competition and persistence even in the absence of functional sexual reproduction (Stebbins, 1950; Ramsey and Schemske, 2002; Husband et al., 2008). Last, by enabling long-distance spread through clonal propagules (e.g., "guerrilla" type clonal reproduction; Lovett-Doust, 1981), neopolyploids can colonize areas away from their diploid parents, reducing local competition and promoting habitat segregation between cytotypes (Levin, 1975; Fowler and Levin, 1984). For the above reasons, we expect that clonal reproduction will benefit neopolyploid fitness and increase the probability of their successful establishment. If genome duplication acts to immediately increase clonal reproduction in neopolyploids, these advantages could be further multiplied.

The majority of evolutionary or ecological research comparing phenotypic traits between ploidy levels contrast established cytotypes where many generations of selection may have further altered or even erased any immediate effects of polyploidization (Ramsey, 2011; Husband et al., 2016). There are no studies to date that have determined how WGD itself may affect clonal reproduction in neopolyploids, and studies that have compared clonal reproduction between naturally occurring ploidy levels within autopolyploid species have yielded mixed results: some found that established polyploids have increased levels of clonality in comparison to diploids (Bruneau and Anderson, 1988; Hroudová and Zákravský, 1993; Schlaepfer et al., 2010), others found that they are less clonal (Schulze et al., 2013; Baldwin and Husband, 2013; Hanzl et al., 2014; Martínková et al., 2015), and one demonstrated no differences between ploidies (Keeler, 2004).

We expect that investment in clonal reproduction in diploids and neopolyploids will shift in response to environmental stress in different ways if changes induced by WGD alter the impact of abiotic stressors. Polyploids are thought to have broader ecological tolerances than their diploid relatives, facilitated by favorable changes in morphology, physiology or increased heterozygosity (Stebbins, 1950; Levin, 2002; Soltis et al., 2010; te Beest et al., 2012). Data supporting this hypothesis continue to be scarce (Ramsey and Ramsey, 2014; Soltis et al., 2016b; but see Maherali et al., 2009; Ramsey, 2011; Wei et al., 2018), but we might expect that variation in clonal investment between stressful and benign conditions will be reduced if polyploids are able to maintain similar rates of clonal reproduction in both environments. For example, polyploids with higher drought tolerance than diploids (Levin, 2002; Maherali et al., 2009; Hao et al., 2013) could maintain higher investment in vegetative growth and clonal reproduction under water stress. Similarly, WGD-induced cell enlargement in clonal plants with integrated genets (Vallejo-Marín et al., 2010) may increase the size of transport conduits between ramets and make shunting resources easier, increasing sustainable genet size under low resource conditions (Levin, 2002; Schlaepfer et al., 2010; te Beest et al., 2012).

In this study, we used diploid and synthesized neotetraploid *Fragaria vesca* to investigate the immediate effects of WGD on total investment in clonal growth and realized clonal propagule production. Specifically, we asked: (1) Are neopolyploids more clonal than diploids? (2) Are differences in clonal reproduction between

diploids and neopolyploids maintained under different environmental conditions? (3) Are changes in clonal reproduction consistent among independent WGD events? (4) Does realized clonal propagule production or ramet spatial arrangement differ between neopolyploids and diploids? We used diploid and neotetraploid *F. vesca* in two experiments to address these questions. For addressing questions 1–3, diploids and neotetraploids were grown in the greenhouse with high or low nutrients, and their absolute investment in stolon and plantlet production was monitored over 13 weeks. For question 4, diploids and neotetraploids were planted outside under natural conditions for one full year, over which their realized stolon and plantlet production, genet spatial organization, and allocation to sexual reproduction were assessed.

MATERIALS AND METHODS

Study system

Fragaria vesca is a long-lived, herbaceous, perennial strawberry widely distributed across Europe and North America (Hancock, 1999) and is common in mature forests within the study region (Halton and Wellington Counties, Ontario, Canada). Both auto- and allopolyploids are found throughout the *Fragaria* genus, and though *F. vesca* is solely diploid ($2n = 2x = 14$; Hancock, 1999) in natural populations, it is believed to be the progenitor of the allopolyploids *F. virginiana*, *F. chiloensis*, and *F. iturupensis* (Hancock, 1999; Rousseau-Gueutin et al., 2009; Njuguna et al., 2013), the first two of which gave rise to the cultivated octoploid strawberry *F. ×ananassa*. *Fragaria vesca* reproduces both sexually and asexually through clonal reproduction (Schulze et al., 2012). Seeds are produced on typically bisexual and self-compatible flowers (*F. vesca* subsp. *bracteata* is gynodioecious; Li et al., 2012), and clonal plantlets arise at rooting nodes on aboveground stolons (Hancock, 1999). Plants grow as compressed rosettes, and the axillary buds of crown leaves can develop into either an additional leaf, a stolon, or an inflorescence depending on environmental cues (Darrow, 1966; Hancock, 1999). Within the study region, flowering occurs in early spring and is followed by stolon production that continues into the fall (Darrow, 1966). Plantlets within a genet are physiologically integrated throughout the growing season via connecting stolons, but these linkages can fade over winter (Angevine, 1983; Schulze et al., 2012). *Fragaria vesca* presents a "guerilla" clonal growth strategy (Lovett-Doust, 1981; Charpentier, 2002) and can produce rooting plantlets >1 m from the parent ramet in a single season (Angevine, 1983).

Neotetraploid synthesis and ploidy determination

Seeds were collected from nine *F. vesca* populations in Halton and Wellington Counties (Ontario, Canada) in 2011 (Table 1). Between 5 and 27 maternal plants were sampled from each population, with at least 5 m between samples to reduce the chances of sampling the same clone multiple times (Kwok, 2013). The number of seeds available from each maternal plant sampled varied, and 6–20 seeds were randomly chosen from each maternal source and germinated in December 2013. Seeds were soaked in water at 4°C for 24 h and germinated on moist filter paper in petri dishes for 1 week in a Percival growth cabinet at 24°C with a 12-h photoperiod. Before colchicine application, approximately 10% of the seedlings from each maternal family were reserved for use as untreated diploids for the two experiments in this study.

Seedlings were individually treated with 40 µL of 1.5% (w/v) colchicine when cotyledons began to emerge by applying solution droplets directly on the apical meristem. After 24 h, treated seedlings were washed with deionized water and then transplanted along with the untreated seedlings to pots of Sunshine Mix LA4 (SunGrow, Vancouver, BC, Canada) in the University of Guelph Phytotron. Seedlings were grown under greenhouse conditions (24.5–26.5°C/19.5–20.5°C day/night temperatures, natural day length) for 3 months before using flow cytometry to determine whether colchicine treatment had resulted in successful genome duplication.

The ploidy of surviving colchicine-treated plants was confirmed using a FACSCaliber flow cytometer (BD Bioscience, San Jose, CA, USA) to estimate DNA content. Roughly 1 cm^2 of leaf tissue from the treated plants was finely chopped with a new razor blade alongside an equal amount of leaf tissue from an internal standard (*Verbena officinalis*) in 0.7 mL modified De Laats buffer solution (Kron and Husband, 2009) containing 50 µg/mL propidium iodide (a DNA-selective fluorochrome) and 50 µg/mL RNase. The solution was passed through a 30 µm filter and left to stain for

TABLE 1. Distribution of diploid (2*x*) and neotetraploid ($4x_{neo}$) *Fragraria vesca* used in Experiments 1 and 2 among seed and clone families for each of the nine source populations. Maternal seed families correspond to seeds originating from a single plant sampled in the source population. Clone families refer to the sets of plants generated via plantlet propagation from the same parent. For neotetraploids, this is equal to the number of successful polyploid conversions. Distributions of seed and clone families among plants used within Experiments 1 and 2 can be found in Appendices S2 and S3, respectively.

			Maternal seed families		Clone families		Total plants in Experiment 1		Total plants in Experiment 2	
Population	Longitude	Latitude	2*x*	$4x_{neo}$	2*x*	$4x_{neo}$	2*x*	$4x_{neo}$	2*x*	$4x_{neo}$
BAD	43°28′22.80″N	80°04′48.24″W	8	6	10	8	26	27	8	6
CON	43°29′35.54″N	80°05′13.36″W	1	1	2	1	8	4	3	1
CUR	43°29′57.66″N	79°59′56.27″W	1	-	1	-	-	-	1	-
EDE	43°35′11.70″N	80°08′48.92″W	1	2	2	2	1	4	1	2
ELL	43°31′12.82″N	80°03′22.89″W	1	-	1	-	-	-	1	-
GRL	43°32′59.50″N	80°13′08.43″W	5	2	7	7	8	18	1	8
LIT	43°26′47.12″N	80°14′51.09″W	14	8	16	10	41	31	10	7
SHA	43°29′35.54″N	80°02′29.50″W	3	-	4	-	3	-	3	-
STA	43°32′43.90″N	80°09′22.11″W	7	3	11	7	22	20	7	7
		TOTAL:	41	22	54	35	109	102	35	31

20 min. Relative fluorescence intensity of nuclei was measured using the FL2 detector (585/42 nm bandpass filter), and DNA content was quantified with FL2-area (integrated fluorescence). Ploidy was determined by estimating the relative fluorescence of nuclei for each sample compared to the internal standard using CellQuest software (BD Bioscience). Sample fluorescence peaks violating quality thresholds (confidence value [CV] > 8%, nuclei count < 500) were run twice and excluded if the peak was not identifiable as diploid or tetraploid. Fluorescence peaks are generally clearly recognizable for *F. vesca*, as the distribution of mean fluorescence values for diploids, tetraploids, and the internal standard *V. officinalis* are non-overlapping. Colchicine-treated plants that had double the relative fluorescence of diploid *F. vesca* (2C DNA content 0.25 pg; Kwok, 2013) were considered neotetraploids. Mean fluorescence values, peak nuclei counts, and CVs for the 35 successfully converted neotetraploids relative to that of the internal standard can be found in Appendix S1 (see Supplemental Data with this article). The relative fluorescence values for all successfully converted neotetraploid individuals tested fell within 10% of the mean fluorescence value (1.538 ± 10% = 1.383–1.692). Plants that did not convert (i.e., had fluorescence peaks within the diploid range only) or were chimeric with peaks at both diploid and tetraploid positions were excluded from experiments. In all experiments described below, neotetraploids were compared to untreated diploids because previous research comparing vegetative traits between colchicine-treated and untreated diploids in *F. vesca* showed that no residual effects of colchicine remained after 8 weeks of growth (Kwok, 2013).

Colchicine treatment resulted in a total of 35 independent polyploidization events, derived from diploids in 22 maternal seed families from six populations (Table 1). Clone families were generated from successfully converted neotetraploids and untreated diploids by propagating plantlets borne on stolons. The number of seed and clone families that contributed plants to either Experiment 1 or Experiment 2 or both are listed in Table 1. (Seed and clone families for each experiment individually are shown in Appendices S2 and S3.) To ensure that plantlets produced by a neotetraploid parent plant retained the original ploidy level, the DNA content of a subset of plantlets from each clone family (3–5 plantlets/clone family) was determined using the methods above. Ploidy was also confirmed for a subset of each untreated diploid clone family (1–2 plantlets/clone family). The relative fluorescence values for all individuals tested fell within 10% of the mean fluorescence values for diploid (0.775 ± 10% = 0.697–0.852, N = 101) or neotetraploid clones (1.542 ± 10% = 1.38 –1.697, N = 167). Fluorescence means, peak nuclei counts, and CVs of the diploid and neotetraploid clones tested are listed in Appendix S1.

Experiment 1: Stolon and plantlet production under different nutrient treatments

Established diploid and neotetraploid plants were grown in a common greenhouse beginning in the summer of 2014 to compare clonal reproduction between ploidies and two nutrient treatments. Plantlets were given an establishment period of 2 months in the greenhouse before undergoing a pre-experiment vernalisation treatment in May 2014 at 4°C and total darkness for 4 weeks to induce natural growth cycles (Heide et al., 2013; Dalton et al., 2013). In this experiment, an effort was made to match the maternal seed sources for diploid and neotetraploid clone families, such that each neotetraploid clone family had a diploid clone family counterpart that originated from the same maternal seed family (Appendix S2). If a diploid from the same maternal source was not available, the neotetraploid clone family was matched with a diploid clone family derived from the same population. Due to mortality after the vernalization treatment, not all clone families contributed an equal number of plants, and not all clone families had a different ploidy counterpart. Additionally, a small number of plants flowered during the duration of Experiment 1 (17 diploids and 8 neotetraploids), and these plants were excluded to avoid bias introduced by inflorescence/stolon meristem trade-offs, or resource trade-offs between sexual and clonal reproduction. Table 1 shows the total numbers of diploid and neotetraploid plants used in Experiment 1, while Appendix S2 lists their seed and clone family distributions.

In August 2014, plants were placed in randomized positions in the greenhouse (24.5–26.5°C/19.5–20.5°C day/night temperatures, natural day length) for the 3-month duration of Experiment 1. Four clones from each available diploid and neotetraploid clone family were randomly assigned to a high or low nutrient treatment (2 clones/treatment level; total counts in Appendix S2). Plants in the high nutrient treatment were given 50 mL of 200 ppm N 18:9:18 fertilizer (Plant Prod: pH Reducer; Ancaster, Ontario, Canada) each week, while plants in the low nutrient treatment were given 50 mL fertilizer bimonthly. All plants were watered twice weekly in addition to scheduled fertilizer application to ensure they did not experience drought stress.

Stolon and plantlet production was recorded weekly for each individual over a total of 13 weeks. A stolon node was characterized as a full plantlet if it had at least one fully expanded trifoliate leaf. On the 13th week of the experiment, five clonal traits were assessed for each individual plant: total number of stolons produced, total number of plantlets produced, clonal dry biomass (g; stolons and plantlets dried at 65°C for 72 h before weighing), mean stolon diameter (mm; between the main plant and the primary plantlet for each stolon), and longest stolon length (cm). Final vegetative size was calculated as the number of fully expanded leaves multiplied by the width of the central leaflet (cm; Dalton et al., 2013) and used as a covariate in analyses.

All statistical analyses were performed in R (ver. 3.4.0; R Core Team, 2017). In all tests, plant traits were transformed if necessary to achieve model assumptions of normality (Table 2). Linear mixed models were used to determine the effects of ploidy and nutrient treatment on the five clonal traits listed above. Models were performed using the lmer function (lme4; Bates et al., 2015). Vegetative size was included as a covariate in all models to control for the influence of plant size, while population, maternal seed family nested in population, and clone family nested in seed family were used as random grouping variables. Significance was determined via likelihood ratio tests (LRTs) between full and reduced models, and differences between ploidies and treatments levels were determined via Tukey's honestly significance difference (HSD) post-hoc tests. Linear mixed models were further used to determine the influence of ploidy and nutrient treatment on the average weekly rates of stolon and plantlet production and on the weeks of peak stolon and plantlet production (i.e., the week of highest production for each individual). To assess the influence of plant size on clonal traits and determine whether plant size had divergent influences on clonality between ploidies and treatment levels, we performed linear regressions between the predicted response values for each clonal trait from the linear mixed models and plant size.

TABLE 2. Linear mixed models for the five clonal characteristics and clonal phenology from Experiment 1. Transformations (TF) were performed on some variables to improve normality (SR = $\sqrt[2]{x}$, CR = $\sqrt[3]{x}$, FR = $\sqrt[4]{x}$, LOG = $\log_{10}$). Test statistics from mixed models are shown as $\underline{X}^2$ values from likelihood ratio tests (LRTs) between full and reduced models. Interaction significance in models for the five main clonal traits using the family-balanced data set, which contains 13 maternal seed families with both diploid and neotetraploid plants in each nutrient treatment, were determined by LRTs against full base models. The complete model output for the family-balanced data set is available in Appendix S8. Brackets by X^2 values for random effects are percent total variance components. Asterisks indicate significance levels (*P < 0.05, **P < 0.01, ***P < 0.001).

Clonal traits	Stolons (TF: SR)	Plantlets (TF: SR)	Clonal biomass (g) (TF: SR)	Longest stolon (cm)	Stolon diameter (mm) (TF: SR)
Fixed effects					
Ploidy	11.49**	45.62***	18.23***	5.02	56.70***
Nutrient	72.99***	52.88***	135.19***	6.88*	8.43*
Ploidy × nutrient	0.10	1.41	6.25*	0.59	8.09**
Plant size	51.54***	0.68	0.73	3.25	5.01*
Random effects					
Population	0.11 (0%)	0 (0%)	4.08* (17.53%)	0 (0%)	0 (0%)
Seed family[Population]	0.35 (3.79%)	4.47* (21.70%)	2.77 (11.89%)	2.39 (7.56%)	9.86** (9.60%)
Clone family[Seed family[Population]]	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3.38 (14.59%)
Family-Balanced Data set					
Ploidy × seed family	37.30*	27.97	25.33	47.97**	52.63***
Ploidy × nutrient × seed family	74.74**	54.04	62.42	71.47*	106.33***

Phenology of stolon and plantlet production	Mean stolon production (week^{-1}) (TF: LOG)	Highest stolon production week (TF: CR)	Mean plantlet production (week^{-1}) (TF: LOG)	Highest plantlet production week
Fixed effects				
Ploidy	14.17***	4.23	50.66***	18.33***
Nutrient	108.96***	4.74	67.45***	35.79***
Ploidy × nutrient	4.42*	0.23	1.31	18.18***
Plant size	56.16***	16.65***	0.03	8.52**
Random effects				
Population	0.83 (0%)	0 (0%)	0.14 (0%)	0.28 (0.01%)
Seed family[Population]	2.64 (12.03%)	0.88 (0%)	8.12** (24.58%)	1.66 (0.72%)
Clone family[Seed family[Population]]	0 (0%)	1.33 (9.67%)	0 (0%)	5.06* (20.36%)

Additionally, we evaluated the differences in clonal reproduction between the four categories (2x, high and low nutrient; 4x_{neo}, high and low nutrient) with respect to the five clonal traits plus final vegetative size using a multivariate analysis of variance test (MANOVA), which did not include random effects. Pillai's trace was used as a test statistic, as a robust alternative to Wilk's lambda. An accompanying linear discriminant analysis (LDA; candisc package; Friendly and Fox, 2016) was used to visualize individual trait contributions to group differences in canonical space.

A second data set (total N = 128; hereafter the "family-balanced" data set) containing 13 maternal seed families with representatives from both ploidies in each nutrient treatment (2x, high and low nutrients; 4x_{neo}, high and low nutrients) was used to assess variability in trait shifts between diploids and neotetraploids resulting from 13 separate genome duplication events. To determine whether maternal seed families differed in their responses to whole-genome duplication, linear mixed models of the form described above (including nested random effects and variable transformations, Table 2) were performed on the family-balanced data set for the five main clonal and vegetative response traits, and then compared using LRTs to models including an interaction term between ploidy and maternal seed family. We further modified the original analyses to assess whether the response to nutrient treatment between diploids and neotetraploids differed between maternal seed families by comparing base models to models including a three-way interaction term (ploidy × nutrient × seed family).

Experiment 2: Artificial field populations

In May 2015, 35 diploids and 31 neotetraploids (Table 1, seed and clone families listed in Appendix S3) were randomly transplanted directly into the soil in the centers of adjacent 1 m^2 grids at a freshly tilled plot located at the Guelph Turfgrass Institute (43°54′74.58″N, 80°21′34.26″W). Plants were clones of the originally converted plants, initiated ~4 weeks before transplanting. No supplemental watering or fertilization was applied for the duration of the experiment. After 3 months, clonal reproduction via stolon and plantlet production was evaluated by spatially mapping all established plantlets within the study plot using a Leica Disto laser mapper (Leica Geosystems, Norcross, GA, USA). Plantlets were not mapped if they had not yet successfully rooted in the ground. Due to the nature of clonal reproduction in *F. vesca* (i.e., aboveground stolons), established plantlets were easily assigned to genets by following stolon connections. Using the mapping data, we calculated six measures of clonal investment for each genet: number of stolons initiated per parent plant, total number of plantlets, number of plantlets per stolon originating from the parent plant, longest stolon (m), mean internode length between plantlets (m), and the total sum of internodes (m).

The spatial attributes of genets and plantlets were calculated from the mapping data, resulting in four variables of interest: genet area (m^2), genet shape, plantlet density (m^{-2}), and the number of branching points per stolon for each genet. Genet area was estimated

by minimum convex polygons (MCPs) using the R packages sp (Pebesma and Bivand, 2005) and adehabitatHR (Calenge, 2006). The MCPs encompassed 100% of the plantlets in a given genet. The genet shape parameter was defined as the ratio of the area of a circle with a radius of half the longest axis to MCP area, such that higher ratios correspond to elongated elliptical genets and ratios close to 1 indicate that the genet is more circularly shaped. The number of branching points per stolon was included as a measure of local vs. longer-distance clonal foraging strategy and defined as the number of plantlets in the genet that initiated more than one stolon divided by the number of stolons initiated by the genet's parent plant.

Investment in sexual reproduction was evaluated in June 2016. During an overwintering period, stolons between plantlets break down so that connections are not always apparent; therefore, we made the assumption that all plants within a certain distance from a parent plant were part of the same genet. A circle of radius 27 cm was chosen, where an optimum of 92% of plantlets within the circle were part of the parent plant's genet according to the maps produced in the previous fall. Increasing or decreasing the radius did not improve genet fidelity (91% at 20 cm and 87% at 40 cm). Within each circle, the numbers of inflorescences and flowers were counted for each plantlet and used as a proxy for the sexual effort of the whole genet. The number of flowers per plantlet was defined as the number of currently open flowers + the number of flowers that had already senesced at the time of measurement (unopened buds were not included). When flowering began to ebb approximately 2 weeks after the initial assessment of sexual reproduction, the total number of inflorescences and flowers for the parent plant in each genet was counted. The number of flowers on the parent plant that had aborted fruit production (small, black, withered receptacles) was also recorded. Finally, the number of leaves for each parent plant was counted to determine whether meristem allocation between inflorescences, stolons, and leaves differed between diploids and neotetraploids.

The effects of ploidy on clonal, spatial, and sexual traits and on meristem allocation were determined using linear mixed models in R (ver. 3.4.0; R Development Core Team, 2017) using the lmer function (lme4; Bates et al., 2015). In all tests, response traits were transformed if necessary to achieve model assumptions of normality (Table 3), and the population source of the focal parent plant was included as a random effect in the models. Significance was determined via likelihood ratio tests (LRTs) between full and reduced models.

RESULTS

Stolon and plantlet production under different nutrient treatments

Ploidy level and nutrient treatment were significant contributors to differences between plants for most clonal traits measured (Table 2; see Appendix S4 for raw means). The numbers of stolons and plantlets produced per plant were significantly affected by both ploidy and treatment (Table 2). Diploids produced more stolons and plantlets than neotetraploids in the high nutrient treatment (24% and 50% more respectively; Appendix S4), while in the low nutrient treatments, the ploidies produced similar numbers of stolons based on Tukey's HSD post-hoc tests but again differed in their total plantlet production (Fig. 1A, B). Plantlet production for neotetraploids in the high nutrient treatment was equivalent to that of diploids in low nutrient conditions. Clonal biomass was highest for diploids under high nutrients with no difference between ploidies in low nutrients (Fig. 1C), leading to a weak interaction between ploidy and nutrient treatment (Table 2). The longest stolon per plant was not affected by ploidy, but stolons were generally longer for plants in the low nutrient treatment (Table 2, Fig. 1D). Mean average stolon diameter was largely influenced by ploidy level, with neotetraploids having stolons with wider diameter than diploids (Table 2, Fig. 1E). Plant

TABLE 3. Linear mixed models testing the effect of ploidy on clonal, spatial, and sexual traits in Experiment 2. Test statistics from mixed models for the effect of ploidy and the random effect population on each response variable are shown as likelihood ratio tests (LRTs). Percentages in brackets following population LRT X^2 indicate the percentage total variance explained by population. Transformations (TF) were performed on some variables to improve normality (SR – $\sqrt[2]{x}$, LOG – $\log_{10}$). Asterisks indicate significance levels (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

		Mean ± SE		ANOVA	
Genet traits	TF	2x ($N = 35$)	$4x_{neo}$ ($N = 31$)	Ploidy LRT X^2	Population LRT X^2
Clonal reproduction					
Stolons	LOG	11.77 ± 0.56	8.58 ± 0.46	18.27 ***	0 (0%)
Plantlets	LOG	61.09 ± 3.89	33.23 ± 2.65	27.41***	0 (0%)
Plantlets per stolon	-	5.13 ± 0.28	3.70 ± 0.21	14.89***	0 (0%)
Longest stolon (m)	LOG	1.01 ± 0.04	0.83 ± 0.03	12.02***	0 (0%)
Mean internode length (m)	LOG	0.15 ± 0.01	0.17 ± 0.01	7.18**	0 (0%)
Internode sum (m)	-	8.87 ± 0.52	5.28 ± 0.40	24.39***	0 (0%)
Spatial layout					
Genet area (m^2)	-	1.00 ± 0.06	0.64 ± 0.05	18.42***	0 (0%)
Genet shape	LOG	1.85 ± 0.07	2.33 ± 0.23	4.77*	0 (0%)
Plantlet density (m^{-2})	SR	63.63 ± 3.49	55.74 ± 3.51	2.44	0.02 (8.7%)
Branching points per stolon	SR	1.07 ± 0.09	0.65 ± 0.07	12.56***	0 (0%)
Sexual reproduction					
Genet flowers		74.09 ± 4.05	35.71 ± 3.35	39.0***	0 (0%)
Genet inflorescences	-	26.06 ± 1.17	14.09 ± 1.11	39.7***	0 (0%)
Parent plant flowers	SR	21.15 ± 1.86	14.28 ± 1.82	4.60*	2.03 (26.9%)
Parent plant inflorescences	SR	4.00 ± 0.35	2.50 ± 0.38	8.58**	0.58 (19.8%)
Parent flower abortion rate	SR	0.15 ± 0.47	0.24 ± 0.52	2.81	0 (0%)

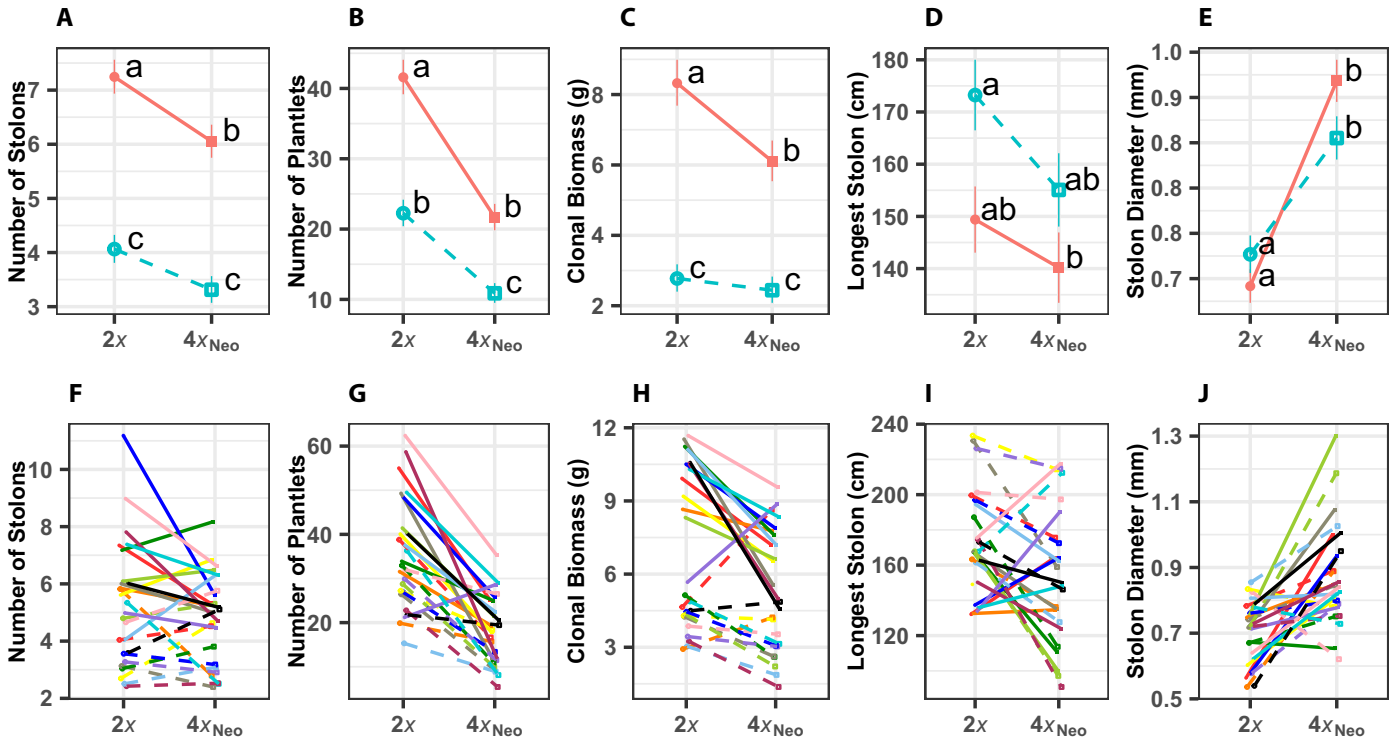

FIGURE 1. Effects of ploidy on clonal traits in *F. vesca*. (A–E) Detail of the differences in the five clonal characteristics measured in Experiment 1 across ploidies (circles: 2*x*, squares: $4x_{neo}$) and nutrient treatments (red solid lines: high, blue dashed lines: low). Values are adjusted least-square mean values (±SE), where letters indicate significant differences as determined by Tukey's HSD post-hoc tests on the linear mixed models detailed in Table 2. (F–J) show the least-square means for the five clonal traits for each of the 13 maternal seed families in the family-balanced data set (colored lines) (solid lines: high nutrient treatment, dashed lines: low nutrient treatment), representing the two-way interaction between ploidy and seed family and the three-way interaction between ploidy, nutrient treatment, and seed family (Table 2).

size was a significant predictor for only two of the five clonal traits measured (Table 2), both the number of stolons and stolon diameter increased with plant size (linear regressions in Appendices S5 and S6). For all clonal traits, the 95% confidence intervals of the estimated slopes overlapped, indicating that the influence of plant size on each trait was consistent across ploidy levels and nutrient treatments (Appendix S6).

The MANOVA for the five clonal traits plus plant size revealed significant differences both between each ploidy level and between high and low nutrient treatments (ploidy: Pillai's trace = 0.583, $P < 0.001$; nutrient: Pillai's trace = 0.754, $P < 0.001$). The interaction term between ploidy and nutrient treatment was also significant (Pillai's trace = 0.087, $P < 0.05$). Linear discriminant analysis resulted in two canonical axes with eigenvalues accounting for >99% of the variation between ploidy and nutrient groups. The first canonical axis primarily separated the high and low nutrient treatments, while the second emphasized differences between diploids and neotetraploids (Fig. 2). The canonical distance between low and high nutrient treatment group centroids for neotetraploids was smaller than the distance between treatment groups for diploids (3.02 ± 0.34 vs. 3.88 ± 0.49 canonical units, means ± 95% CI), reflecting the interaction between ploidy and nutrient treatment in the MANOVA analysis.

Analysis of the family-balanced data set containing the 13 maternal seed families with representatives in each ploidy and treatment group showed considerable variation in trait shifts between seed families as a result of whole genome duplication (Table 2, Fig. 1F–J; see Appendix S7 for an expanded version of Fig. 1F–J). Maternal seed family nested in population explained the highest percentage of variance of the random effects for both the full and family-balanced data set, ranging from ~4–22% across response variables (Table 2; Appendix S8). Plantlet number and clonal biomass had consistent trait differences between diploids and neotetraploids for all maternal seed families (ploidy × seed family, $P > 0.05$; Table 2, Fig. 1G and H), and these differences were relatively uniform between high and low nutrient treatments; neotetraploids produced fewer plantlets and had lower clonal biomass (ploidy × nutrient × seed family, $P > 0.05$; Table 2). However, stolon number, longest stolon, and stolon diameter had significant interactions between ploidy and maternal seed family and between ploidy, nutrient treatment, and maternal seed family (Table 2, Fig. 1F, I, J; plots per seed family in Appendix S7), indicating variability across seed families in their response to whole-genome duplication. The significance levels of ploidy, nutrient treatment, and the ploidy × nutrient treatment interaction for the family-balanced data set were overall similar to the results from the full data set (Appendix S8).

The phenology patterns for stolon and plantlet growth show that production rates were similar until approximately the 5th week of growth (Fig. 3A), at which point production for the diploids in the high nutrient treatment began to pull away from the other groups. Average weekly stolon production was highest in the diploid high nutrient group, followed by the neotetraploids under high nutrients,

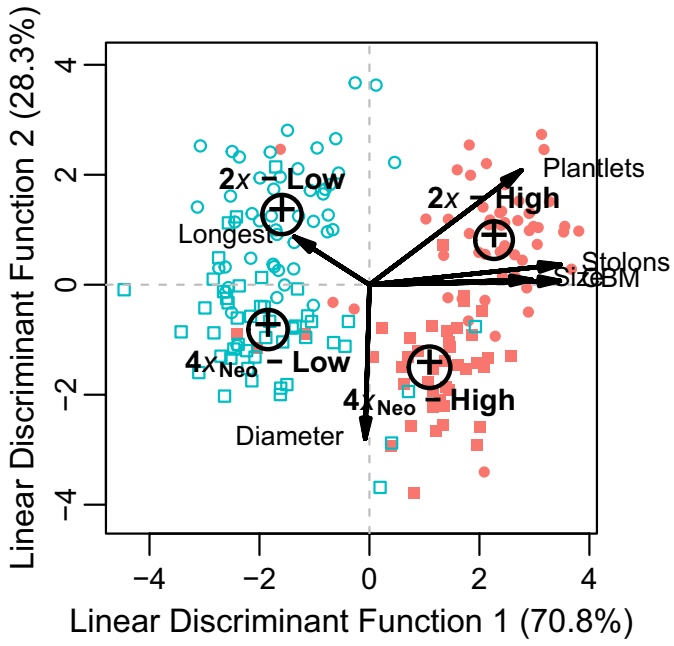

FIGURE 2. Canonical plot of the linear discriminant analysis of the five clonal characteristics measured in Experiment 1 (labels: Stolon = stolon number; Plantlets = plantlet number; CBM = clonal biomass; Longest = longest stolon; Diameter = stolon diameter) and vegetative plant size (= Size) as an additional covariate. Symbols and colors differentiate the four categories of plants (circles: 2*x*; squares: $4x_{neo}$; red: high nutrients; blue: low nutrients). Crosses show group centroid means surrounded by 95% confidence circles. Percentages on axis labels indicate the variation explained by each discriminant function. Canonical vectors are scaled by a factor of 4 for plotting.

with no differences between diploids and neotetraploids in low nutrients according to Tukey's HSD post-hoc tests (Table 2, Fig. 3B; see Appendix S4 for raw means). Weekly plantlet production showed that diploids under high nutrients had the highest plantlet production rates, while the low nutrient diploids were comparable to the high nutrient neotetraploids and the neotetraploids in the low nutrient treatment had the lowest production rate (Table 2, Fig. 3B). The peak week for stolon production occurred slightly earlier for diploids compared to neotetraploids (Fig. 3C), but neither ploidy nor nutrient treatment differences were significant (Table 2). Peak plantlet production timing was affected by both ploidy and nutrient treatment (Table 2), and plants in the low nutrient groups tended to peak later than those under high nutrients (Fig. 3C). Peak plantlet production for neotetraploids was later than diploids for the high nutrient groups, but the pattern was reversed under low nutrients (Fig. 3C). Plant size was a significant positive predictor for average stolons production per week, and the highest production week for both stolons and plantlets (Appendices S6 and S9). Like the clonal traits above, the 95% confidence intervals of the estimated slopes overlapped, indicating that the influence of plant size on each phenological trait was consistent across ploidy levels and nutrient treatments (Appendix S6).

Artificial field populations

The analysis of clonal characteristics measured on the genets in Experiment 2 reiterates the results found in Experiment 1: diploids are more clonal than neotetraploids. Mapping resulted in a total of 3168 plantlets; 2138 plantlets were produced by diploid parent plants, while 1030 were produced by neotetraploid parent plants. Diploid genets contained more stolons and plantlets than neotetraploid genets (28% and 46% more respectively; Table 3) and had a higher mean number of plantlets per stolon (Table 3). Diploid genets also had a higher maximum stolon length than neotetraploids (Table 3). Neotetraploids and diploids had similar mean internode lengths (Table 3), with neotetraploids having slightly longer internodes. The total sum of internodes for each genet (i.e., the sum of the lengths of all stolons produced) was significantly higher in diploids than neotetraploids (41% higher; Table 3).

The sizes, shapes, and the spatial arrangements of plantlets of genets were affected by ploidy level. Diploid genets covered 56% more area than neotetraploid genets (Table 3) and were more circularly shaped (lower shape values; Table 3). Plantlet density was 37% higher in diploid vs. neotetraploid genets (Table 3). Stolons in diploid genets had almost two times the number of branching points as stolons in neotetraploid genets (Table 3).

The number of flowers and inflorescences produced per genet were both significantly higher for diploid genets (52% and 46% higher than neotetraploid genets, Table 3). The parent plants themselves showed the same pattern, with diploids producing 33% more flowers and 37% inflorescences than neotetraploids (Table 3). Fruit abortion rates for both ploidies were similar (Table 3). While absolute values of inflorescence, stolon, and leaf production were all higher in diploid parent plants (inflorescences and stolons: see Table 3; leaves: $2x = 12.14 \pm 0.61$, $4x_{neo} = 9.19 \pm 0.53$, ploidy LRT $X^2 = 12.12$, $P < 0.001$), the proportion of meristems allocated to these three structures per parent plant did not differ between diploids and neotetraploids. Both ploidies allocated the smallest proportion of meristems to inflorescence production ($2x = 0.14 \pm 0.01$, $4x_{neo} = 0.11 \pm 0.02$; ploidy LRT $X^2 = 1.95$, $P = 0.163$), and equal proportions of meristems to both stolons ($2x = 0.43 \pm 0.01$, $4x_{neo} = 0.44 \pm 0.02$; ploidy LRT $X^2 = 0.01$, $P = 0.973$) and leaves ($2x = 0.43 \pm 0.01$, $4x_{neo} = 0.45 \pm 0.01$; Ploidy LRT $X^2 = 1.68$, $P = 0.195$).

DISCUSSION

Clonal reproduction is hypothesized to increase with ploidy and contribute to the establishment success of polyploid cytotypes, but little is known about the direct effects of genome duplication on patterns of clonal reproduction in newly formed polyploids. For autopolyploids that arise in otherwise diploid populations, shifts in clonal reproduction could greatly impact their ability to spread and persist in sympatry with their progenitors in both the early stages of their evolution and during naturalization. Here we found that synthetic neotetraploid *F. vesca* produces fewer total stolons and plantlets than diploid plants in a common greenhouse environment. Notably, phenotypic shifts varied among independent polyploidization events in three of five clonal traits, indicating that the phenotypic outcome of WGD on clonal reproduction can be variable. Clonal biomass differences between low and high nutrient treatments were smaller in neotetraploids vs. diploids, providing weak support for the hypothesis that investment in clonal reproduction in neotetraploids may be less affected by nutrient stress. In field populations, low clonal output by neotetraploids translated into small, sparse genets with fewer established ramets than diploid genets. While the conclusion that neotetraploid *F. vesca* are

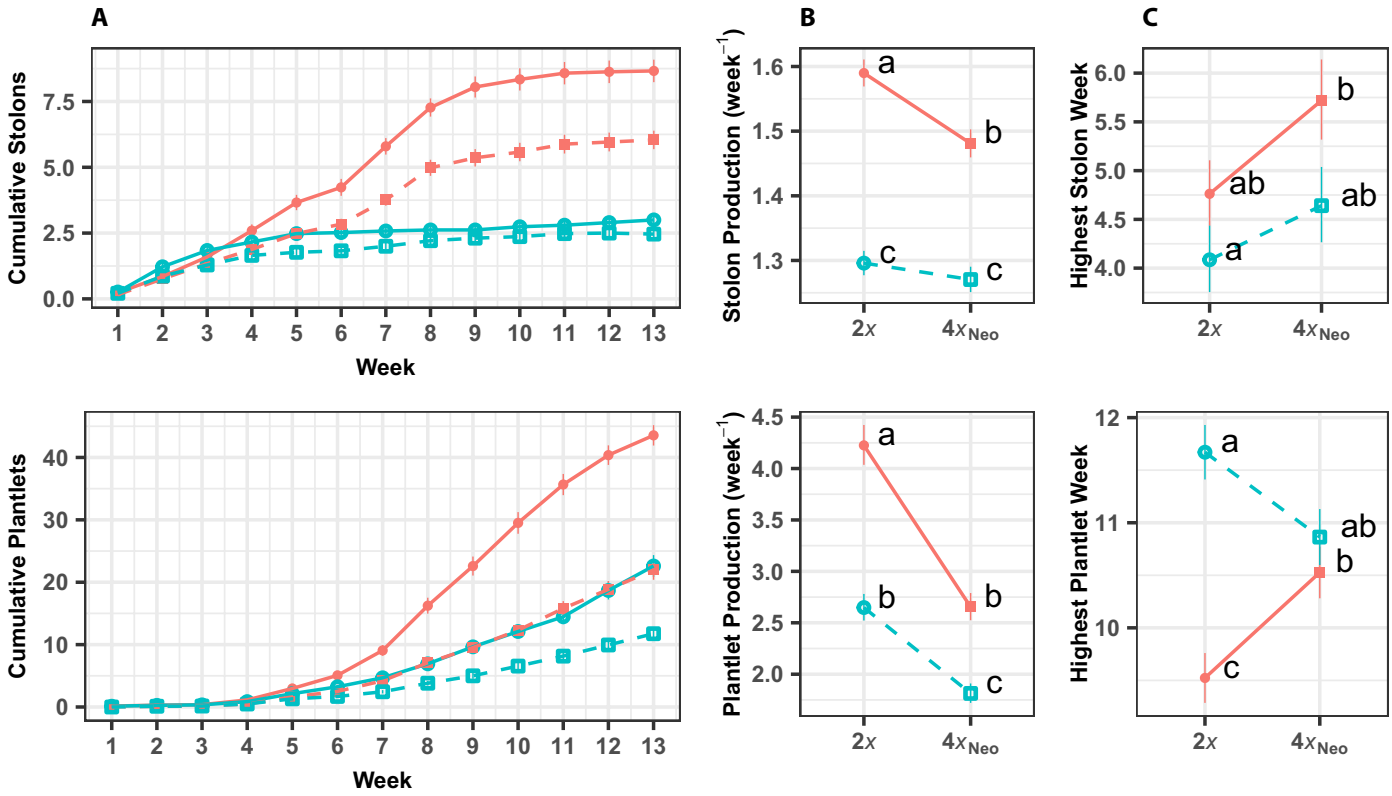

FIGURE 3. Effects of ploidy and nutrient treatment on the phenology of stolon and plantlet production in Experiment 1 (circles: 2*x*; squares: $4x_{neo}$; red solid lines: high nutrients; blue dashed lines: low nutrients). (A) Cumulative stolon and plantlet production over 13 weeks of growth in the greenhouse; (B) average weekly stolon and plantlet production values; and (C) the weeks of peak stolon and plantlet production. Values are adjusted least-square mean values (±SE) where letters indicate significant differences as determined by Tukey's HSD post-hoc tests on the linear mixed models detailed in Table 2.

less clonal than their diploid parents is contrary to expectations, it highlights our lack of knowledge of the phenotypic consequences of WGD in neopolyploids and of the processes leading to phenotypic discrepancies between early and extant polyploids, which have likely undergone divergence through selection. Moreover, if high levels of clonal reproduction give neopolyploids an establishment advantage, limited clonality in neotetraploid *F. vesca* could explain the absence of naturally occurring autopolyploids in this species.

Direct effects of WGD on clonal reproduction

The neotetraploids used in this study were synthesized using colchicine, a cytokinesis inhibitor that results in cells with double the DNA content when applied to plant tissue (Caperta et al., 2006). The application of chemicals to induce WGD has the potential to create lasting side effects affecting growth, morphology, and reproduction in new polyploids. Kwok (2013) investigated the long-term effects of colchicine in *F. vesca* by comparing phenotypic differences (e.g., number of leaves, basal width, plant height, leaf length, days to flower, and number of flowers) between unexposed diploids (N = 147) and diploids that were exposed to colchicine but did not convert to neopolyploids (N = 15). Nonparametric tests for unbalanced samples revealed that exposed diploids had slight developmental delays, but after 8 weeks no significant phenotypic differences between exposed and unexposed plants remained. Similar studies between treated and untreated diploids in *Chamerion angustifolium* and *Arabidopsis thaliana* also show weak long-term effects of colchicine (Husband et al., 2016; A. Green and B. C. Husband, University of Guelph, unpublished data). It is difficult to be certain that there were no strong long-term effects of colchicine in the neotetraploids used in our study without also including treated but unconverted diploids in experiments, which were rare due to the high mortality of treated plants. However, the neotetraploid *F. vesca* used in our experiments were clones of the originally converted neotetraploids, propagated approximately 3 months after colchicine exposure, and were themselves grown for an additional 2 months before experiment commencement. Due to the lack of long-term effects in previous research and the ample acclimatization period, lingering colchicine effects are expected to be minimal, and the differences observed here between diploids and neopolyploids can be directly attributed to genome duplication.

We find that neotetraploid *F. vesca* invested less in clonal reproduction than diploids, resulting in smaller genets with reduced stolon production and fewer total clonal plantlets. A WGD event may have induced immediate changes in clonality by altering the genetic factors determining stolon production and plantlet growth. The environmental cues and number of genes involved in initiating stolons and inflorescences differ between diploid *F. vesca* (Brown and Wareing, 1965; Albani et al., 2004) and allo-octoploid *F.* ×*ananassa* (Gaston et al., 2013), but whether these genetic differences can be attributed to polyploidization, hybridization, or artificial selection is unknown. Bud determination resulting in stolons, leaves, or

inflorescences in *F. vesca* is primarily influenced by temperature and photoperiod, which alter hormone production (e.g., gibberellins; Guttridge and Thompson, 1964; Heide et al., 2013; Tenreira et al., 2017) and the expression of the genes deciding meristem identity (Heide et al., 2013). Genome duplication provides abundant opportunity for changes in gene expression via increased gene dosage (Guo et al., 1996; Riddle et al., 2010; Blomme et al., 2014) or modifications to epigenetic relationships (Osborn et al., 2003; Parisod et al., 2010; Roulin et al., 2013). Both processes could affect the expression of genes controlling axillary meristem determination (Cheng et al., 2015; Dai et al., 2015) and result in either fewer stolons being initiated overall or in an increase in meristems developing into inflorescences and leaves at the expense of stolon production.

The artificial field populations revealed no differences in proportional meristem allocation between cytotypes; rather, neotetraploids had fewer stolons, fewer inflorescences, and fewer leaves than diploids. While genetic changes could contribute to this shift, the limited number of each structure suggests that WGD results in delayed whole-plant growth in neotetraploids. In *F. vesca*, the number of stolons, inflorescences and leaves produced by individuals experiencing similar environmental conditions is mainly dependent on plant size (Darrow, 1966; Chabot, 1978; Hancock, 1999). If neotetraploids have slower growth and meristem development they could produce fewer stolons during the same time period than diploids. Polyploids have generally slower growth in comparison to diploids (Stebbins, 1950; Bennett, 1987; Levin, 2002), as copying larger amounts of nuclear DNA into bigger cells contributes to longer cell cycles and ultimately slower whole organism development (Stebbins, 1950; Bennett, 1987; Beaulieu et al., 2008; Blomme et al., 2014; Zhou et al., 2015). In this study, final vegetative plant size in the greenhouse (number of fully expanded leaves × the width of the central leaflet) was significantly lower in neotetraploids for both nutrient treatments (mean ± SE: low nutrients: $2x$ = 80.25 ± 4.97 cm, $4x_{neo}$ = 64.57 ± 3.84; high nutrients: $2x$ = 41.56 ± 1.82, $4x_{neo}$ = 38.43 ± 1.56; ploidy LRT X^2 = 7.49, P = 0.024). There was also evidence that stolon and plantlet production rates were delayed in neotetraploids (Fig. 3, Appendix S4), supporting the idea that slower development in neotetraploid *F. vesca* leads to lower investment in clonal reproduction.

Comparing rates of clonal reproduction between *Fragaria* species with different ploidy levels has yielded variable results, and interpreting the direct effects of WGD is often clouded by hybridization and generations of artificial or natural selection. Bringhurst and Khan (1963) compared stolon production between *F. vesca*, octoploid *F. chiloensis*, and their sterile pentaploid hybrid and found that *F. vesca* produced twice the number of stolons as *F. chiloensis*, while the hybrid had the highest stolon production overall. Conversely, Schulze et al. (2013) found that pentaploid hybrids of *F. vesca* and *F. ×ananassa* had significantly delayed stolon production compared to *F. vesca*. In other species, studies on naturally occurring cytotypes similarly offer no consensus as to how ploidy level influences clonal reproduction (e.g., Keeler, 2004; Schlaepfer et al., 2010; Baldwin and Husband, 2013; Hanzl et al., 2014). However, since no studies to date have measured clonal reproduction in synthetic neopolyploids, nothing is known about the immediate effects of WGD on clonality. Clonal reproduction likely has extensive ecological consequences for polyploids (Stebbins, 1950; Herben et al., 2017), and for newly arisen polyploids the competitive, persistence, and mating advantages of clonality could make or break their survival potential very early in their evolutionary history. Decreased clonal reproduction and smaller, sparser genets in neotetraploid *F. vesca* could greatly impact the probability that they will survive within a population of clonally aggressive diploids (Bringhurst and Khan, 1963; Angevine, 1983; Schulze et al., 2013). Indeed, *F. vesca* has no naturally occurring autotetraploid cytotype, which could be a result of a fitness disadvantage due to WGD induced reductions in clonal reproduction.

Phenotypic variability in response to WGD

Predicting polyploid phenotypes has proven to be particularly challenging, and few large-scale patterns are consistent across the angiosperms (Soltis et al., 2016b). The impact of WGD and ploidy level on morphology, physiology, or reproduction is often variable between different species (Stebbins, 1950; Maherali et al., 2009; Oswald and Nuismer, 2011), and very little is known about the consistency of phenotypic shifts across repeated autopolyploidization events within nonmodel or noncrop species (Soltis et al., 2010, 2016b; Weiss-Schneeweiss et al., 2013; Husband et al., 2016). Most studies using synthetic neo-autopolyploids have as few as one, and up to ~30 independent polyploid lines (e.g., Raina et al., 1994; Stupar et al., 2007; Maherali et al., 2009; Yu et al., 2010; Ramsey, 2011), but few have compared phenotypic changes in plants across these different WGD events (but see Martin and Husband, 2012; Oswald and Nuismer, 2011; Husband et al., 2016). Our results show that the effect of WGD on three of five clonal traits differed among 13 independent neotetraploid lines. Notably, polyploid lines had either lower, higher, or equal stolon production and overall clonal biomass compared to their diploid counterparts (Fig. 1F, Appendix S7). Some new polyploids could therefore be more clonally successful than others, as clonal output impacts genet size, ability to spread locally and compete with diploids, and overall establishment potential. Future studies repeated across and within different species will reveal whether the effects of WGD on clonal reproduction are fundamentally variable or have repeatable trends.

Effect of nutrient stress in diploids vs. neotetraploids

Low resource conditions led to decreased investment in clonal reproduction for both cytotypes. For some traits, including clonal biomass, the differences between neotetraploids in low vs. high nutrient treatments were smaller than for diploids (Fig. 1C, Table 2), a result echoed by the LDA combining all five clonal characteristics (Fig. 2). Plantlet production in diploids under low nutrients peaked later than diploids in the high treatment, but neotetraploids did not experience any lag in production (Fig. 3, 14% vs. 0% lag at 13 weeks). These results provide some weak support that neopolyploid *F. vesca* may be better able to handle nutrient stress than diploids. Polyploids have been credited with broader ecological tolerances than diploids (Stebbins, 1950; Levin, 2002), though studies have yet to reveal widespread across-species patterns in the ecological changes associated with polyploidy (te Beest et al., 2012; Ramsey and Ramsey, 2014; Soltis et al., 2016b). Physiological alterations wrought by increases in cell size can enhance polyploid performance under drought stress, high salinity, or low temperatures (Levin, 2002; Maherali et al., 2009; te Beest et al., 2012; Luo et al., 2017; Oustric et al., 2017; Wei et al., 2018), and gene buffering or polysomic inheritance can confer higher fitness, plasticity, and rates of adaptation in novel or stressful conditions (Otto and Whitton, 2000; Parisod et al., 2010; Wei et al., 2018).

The evidence that polyploids have increased tolerance to nutrient poor conditions is largely species-specific; some studies show that polyploids outperform diploids (Levin, 2002; Schlaepfer et al., 2010; Wei et al., 2018), while others do not (Grewell et al., 2016; Guignard et al., 2016). Studies comparing the responses of different cytotypes to contrasting environmental conditions are infrequent in general (Soltis et al., 2010, 2016b; te Beest et al., 2012), and there is even less research on how the immediate physiological changes of WGD may affect reactions to nutrient stress (Maherali et al., 2009; te Beest et al., 2012; Soltis et al., 2016b). However, hydraulic conductivity and xylem conduit size can increase with ploidy (Zhao et al., 2005; Maherali et al., 2009), possibly allowing polyploids to take up and transport water and nutrients more efficiently than diploids (Cacco et al., 1976; Levin, 2002). While we collected no data concerning structure or function of the root system in neotetraploid *F. vesca*, we did find that stolon diameter was increased with ploidy. Increased stolon diameter could affect transport conduit sizes (Darrow, 1966), which may allow neotetraploids with integrated ramets to maintain a higher investment in clonal reproduction by facilitating the movement of photosynthate and water to developing plantlets. Further study into how the physiological effects of WGD in *F. vesca* can affect phenotype and investment in clonal reproduction will be needed to conclusively determine whether neotetraploids have an advantage over diploids in low resource environments.

CONCLUSIONS

Our data suggest that WGD results in lower clonal investment for neotetraploid *F. vesca*. However, the effect of WGD on clonal reproduction could be highly dependent on genetic background, and whether decreased clonal reproduction in neopolyploids is a general trend is far from certain. In *F. vesca* neopolyploids, low clonality and limited spread could contribute to an inability to successfully establish alongside diploid plants. Conversely, the effects of WGD on clonal root bud production in *Chamerion angustifolium* reveal that root bud production is actually increased in neotetraploids, resulting in a very different narrative for polyploid establishment in that species (Van Drunen and Husband, 2018). To determine whether WGD has a generally positive or negative effect on clonal reproduction, further research is needed using neopolyploids in species with different clonal modes and strategies. Moreover, though the idea that clonal reproduction enhances polyploid establishment success is widely held, there has been no experimental investigation into the mechanisms behind this hypothesis. Finally, studies that integrate the effects of WGD on phenotype, ecology, physiology, and genetics are sorely lacking (Soltis et al., 2016b), but will be indispensable in providing a fuller understanding of how the direct effects of polyploidization can shape the evolution and success of polyploids in the angiosperms.

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AUTHOR CONTRIBUTIONS

W.E.V.D. and B.C.H. planned and designed the research. W.E.V.D. completed experiments, collected data, and performed data analysis. W.E.V.D. and B.C.H interpreted the data and wrote the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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