Research

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Evolutionary associations between polyploidy, clonal reproduction, and perenniality in the angiosperms

Wendy E. Van Drunen and Brian C. Husband

Department of Integrative Biology, University of Guelph, Guelph, ON N1G 2W1, Canada

Author for correspondence:
Wendy E. Van Drunen
Tel: +1 519 803 5936
Email: wendy.vandrunen@outlook.com

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Summary

- Clonal reproduction is thought to facilitate polyploid establishment in the angiosperms, but the evolutionary relationship between polyploidy and clonality has not been thoroughly tested. A perennial life history may confer many of the same advantages, and the relative importance of clonality versus perenniality is unknown.
- We used phylogenetic comparative analyses of 1751 species to examine associations between polyploidy, clonality, and life history. We test hypotheses of co-evolution by determining the sequence of trait development.
- Polyploidy is associated with both clonality and perenniality across species, and analyses show that clonality can be an important predictor of polyploidy beyond perenniality. Tests of directionality on our full dataset suggest that polyploidy is more likely to promote clonality or perenniality than vice versa, although there are significant differences in patterns of co-evolution among major angiosperm groups.
- Our results suggest that polyploidy and clonal reproduction are evolutionarily associated across the angiosperms, even when perenniality is considered, but we find little evidence at the whole-angiosperm level for the hypothesis that clonality promotes polyploidy. However, variation among different clades indicates that polyploidy and clonality are interacting in diverse ways, likely to be due to the variable roles of clonality in their evolutionary histories.

Introduction

Polyploidy is considered to be a significant driver of diversification and speciation events in the flowering plants (Stebbins, 1950; Otto & Whitton, 2000; Wood *et al.*, 2009), but many of the evolutionary processes that lead to successful polyploids in this group are still poorly understood. One of the major barriers to polyploid evolution is minority cytotype exclusion (MCE; Levin, 1975), in which rare polyploids experience a lack of same-cytotype mates, resulting in a disproportionate number of between-cytotype fertilisations and low fitness (Levin, 1975; Husband, 2000; Otto & Whitton, 2000). The incidence of polyploidy is therefore expected to be closely associated with attributes that counteract the negative fitness effects of MCE and increase the probability of polyploid establishment (Otto & Whitton, 2000; Levin, 2002; Weiss-Schneeweiss *et al.*, 2013). Some of the best known correlates of polyploidy are shifts towards self-fertilisation (Barringer, 2007; Husband *et al.*, 2008), apomixis (Otto & Whitton, 2000; Weiss-Schneeweiss *et al.*, 2013), and perenniality (Stebbins, 1971; Thompson & Lumaret, 1992). Asexuality via clonal reproduction has also long been associated with polyploidy (Gustafsson, 1948; Darlington, 1958; Stebbins, 1971), but despite frequent claims of a link between clonality and polyploidy in the literature (Grant, 1963; Stebbins, 1971; Thompson & Lumaret, 1992; Otto & Whitton, 2000; Weiss-Schneeweiss *et al.*, 2013) this relationship has received comparatively little attention.

Clonal reproduction can be broadly defined as asexual reproduction through vegetative growth (Klimeš *et al.*, 1997; Vallejo-Marín *et al.*, 2010), such that a genetic individual may consist of multiple daughter ramets. It can occur through a wide variety of modes, including rhizomes, stolons, bulbs, and adventitious root buds (Klimešová & De Bello, 2009). There are two pathways through which a direct evolutionary association between clonal reproduction and polyploidy might arise. The first pathway hypothesises that the ability to reproduce clonally predisposes species to yield successful polyploids. Clonal reproduction is expected to decrease the negative effects of MCE on new polyploids throughout the establishment period, as the production of spatially proximate daughter ramets should enhance polyploid fitness by increasing within-cytotype mating via geitonogamy (Husband & Schemske, 1997; Baack, 2005; Husband *et al.*, 2008). The production of clonal offspring itself also contributes to polyploid fitness even in the absence of functional sexual reproduction (Stebbins, 1971; Ramsey & Schemske, 2002; Husband *et al.*, 2008). Here we expect that polyploid species are more likely to be clonal than nonclonal, and that clonality precedes polyploidy in evolutionary history (i.e. the 'Clonality First' hypothesis).

In the second pathway, polyploidy influences the presence or extent of clonal reproduction. Early researchers hypothesised that the origin of a perennial life history and the ability to reproduce clonally could be direct consequences of a whole-genome duplication event (WGD; i.e. polyploidisation; Müntzing, 1936; Gustafsson, 1948). There is currently no evidence that strictly nonclonal diploids have very close clonal polyploid derivatives, though transitions to asexuality via apomixis after WGD are well documented (reviewed in Horandl & Hojsgaard, 2012; Neiman *et al.*, 2014). However, recent studies have shown that WGD can alter the extent of clonal reproduction normally present in a diploid (Van Drunen & Husband, 2018a,2018b). While the phenotypic consequences of WGD can be unpredictable, a feasible null model is that polyploids will have higher clonal reproduction than diploids if more gene copies and new gene interactions translate into more clonal structures being produced (Guo *et al.*, 1996; Ramsey & Schemske, 2002; Osborn *et al.*, 2003; Riddle *et al.*, 2010; Soltis *et al.*, 2016a), or if larger cell sizes increase the size and survival probability of clonal propagules (Stebbins, 1950; Bennett, 1987; Otto & Whitton, 2000; Levin, 2002; Herben *et al.*, 2017). As polyploids are expected to have lower inbreeding depression than diploids (Lande & Schemske, 1985; Husband *et al.*, 2008), they should also be able to tolerate larger clone sizes than diploids (Baldwin & Husband, 2013). Under this second pathway polyploidy promotes the evolution of clonality (i.e. the 'Polyploidy First' hypothesis).

These hypotheses predict a positive association between polyploidy and clonal reproduction, but the extent to which either process is at play in natural populations is unknown. Studies comparing clonal reproduction between ploidy levels within species often do not find that polyploids are more clonal, or more likely to be clonal, than their diploid relatives (e.g. polyploid > diploid: Schlaepfer *et al.*, 2010; Van Drunen & Husband, 2018a; polyploids < diploid: Schulze *et al.*, 2013; Baldwin & Husband, 2013; Hanzl *et al.*, 2014; Martínková *et al.*, 2015; Van Drunen & Husband, 2018b; equivocal: Keeler, 2004). These discrepancies may reflect how different modes and roles of clonality (Klimeš *et al.*, 1997; Vallejo-Marín *et al.*, 2010) can alter the effect of clonal reproduction on polyploid establishment, as well the effect of WGD itself on clonality. We may therefore see different patterns of association between polyploidy and clonal reproduction in different species or groups of species, which may or may not reflect broad patterns across all angiosperms.

Two previous studies have assessed the relationship between polyploidy and clonal reproduction across species. The earliest was conducted by Gustafsson (1948), who found that polyploids in several temperate weed floras were most commonly clonal perennials versus annuals/biennials or nonclonal perennials. Herben *et al.* (2017) then used a phylogenetic analysis for species from the Central European flora to show that polyploids rely more heavily on clonal vs sexual reproduction compared with diploids. They found that high rates of clonal spread preceded the evolution of polyploidy, providing support for the Clonality First hypothesis. Though both these studies conclude that polyploidy and clonal reproduction are connected, their restricted nature may limit their applicability to the relationship between polyploidy and clonal reproduction in the flowering plants as a whole. Geographic regions contain different assemblages of species and different proportions of angiosperm families, which can vary widely in rates of both polyploidy and clonal reproduction (Klimeš *et al.*, 1997; Wood *et al.*, 2009; Husband *et al.*, 2013). Temperate floras in particular are associated with high rates of both polyploidy and clonal reproduction (Gustafsson, 1948; Stebbins, 1950; Rice *et al.*, 2019) compared with other regions.

A key issue that has not been addressed is that a relationship between polyploidy and clonal reproduction could be falsely attributed to a direct interaction if there is a confounding third trait closely tied to both polyploidy and clonality. One such trait could be life history. Clonal reproduction is tightly correlated with a perennial life history: all clonal plants are perennial (Cook, 1985; Fischer & van Kleunen, 2002; van Kleunen, 2007; Vallejo-Marín *et al.*, 2010), though not all perennials are clonal. Perenniality is also related to the occurrence of polyploidy (Stebbins, 1950; Thompson & Lumaret, 1992; Otto & Whitton, 2000; Rice *et al.*, 2019). Many of the advantages that clonal reproduction would afford polyploids during their establishment could also be accomplished over the long-term through perenniality, such as increasing the chances of within-cytotype mating through longer persistence within populations (Gustafsson, 1948; Stebbins, 1971). If a perennial life history is sufficient to overcome MCE, clonal reproduction may not provide any additional benefit to polyploid evolution potential (i.e. a 'Perenniality First' versus a 'Clonality First' hypothesis), and a positive association between clonality and polyploidy could be a reflection of an underlying relationship between perenniality and polyploidy. A 'Polyploidy First' hypothesis could also be proposed for the evolution of perenniality. Polyploids are generally slower growing than diploids, because of the increased time needed in DNA replication and cell division due to higher chromosome counts (Stebbins, 1950; Levin, 2002), and therefore are often expected to be perennial versus annual. Gustafsson (1948) attempted to determine the effect of perenniality versus clonality on polyploidy, but despite his interpretation that clonality is responsible for increased polyploidy beyond that seen in nonclonal perennials, the numbers reported in his research do not indicate significant differences between these groups (Swedish flora $\chi^2 = 3.67$, $P = 0.056$; Canadian flora $\chi^2 = 2.32$, $P = 0.127$; data on p. 2 of Gustafsson, 1948). Herben *et al.* (2017) did not consider life history in their analyses, and because Gustafsson's work did not take into account phylogenetic relatedness between species the relative importance of clonality versus life history in facilitating polyploid evolution remains undetermined.

We explore the interactions between clonal reproduction, life history, and polyploidy in the angiosperms by performing a phylogenetic comparative analysis for a circum-global data set of 1751 flowering plants. We ask: (1) Is there an evolutionary association between polyploidy and clonal reproduction?; (2) What is the directionality of this association; does clonal reproduction promote the evolutionary transition to polyploidy or vice versa?; (3) Is the association between polyploidy and clonal reproduction consistent across major angiosperm groups with differing

evolutionary histories?, and (4) Can the association between polyploidy and clonal reproduction be explained by an underlying relationship between polyploidy and life history?

Materials and Methods

Data collection

Data on ploidy levels, chromosome counts, and life history were obtained from Kew Garden's Angiosperm DNA C-Value Database (Bennett & Leitch, 2012). The full database was first reduced to all species entries for which the associated primary reference contained both an estimate of DNA content (genome size) and a chromosome count performed on the same tissue resulting in 4476 total entries, which was further reduced by removing identical entries and those without life history information. Species were considered polyploid if the estimated ploidy level was > 2 and diploid otherwise. For species with intraspecific variation in ploidy level, the highest ploidy level achieved was retained in the dataset, so that ploidy measures were reflective of the degree to which polyploidy exists in these species. We characterised polyploidy using three different measures: the incidence of polyploidy (yes/no), estimated ploidy level, and chromosome number (2N). For many polyploid species there is insufficient evidence to conclusively determine their origins so, following previous studies, we did not distinguish between allopolyploids and autopolyploids (Gustafsson, 1948; Herben *et al.*, 2017). Life history information in the C-Value Database was simplified into a binary classification scheme (annual versus perennial), where species listed as biennial were grouped with annual species as in Gustafsson (1948).

The resulting list of 3420 species was used in an extensive search for information on clonal reproduction, using sources ranging from primary literature (*ISI Web of Science*, 1899–2015) to local floras (e.g. Flora of North America) and online databases (e.g. GrassBase, Clonal Plant Database (CLO-PLA)). Species were classified as clonal if explicit references were made to clonal structures or behaviour (e.g. rhizomes, corms, 'creeping'), and nonclonal if no such language was used in species descriptions. To reduce the possibility of false negatives, where possible we used multiple sources per species to confirm their ability, or lack thereof, to reproduce clonally. Types of clonal reproduction were classified based on descriptions for 17 clonal modes in Klimešová & De Bello (2009), and then further organised into four major categories: stolons and rhizomes, bulbs/corms and tubers, root buds and root splitters, and fragmentation/budding (Supporting Information Table S1).

The final dataset (Table S2) contains cytological, clonality, and life history information for 1751 angiosperm species spanning 132 families and 44 orders. Of the species included, 1096 were diploid and 655 were polyploid (Table S3), with ploidy levels ranging from 2 to ×20 and 2N chromosome numbers ranging from 4 to 230. The dataset contains 875 clonal and 876 nonclonal species, 1407 perennial and 344 annual species (Table S3). Phylogenetic relationships between species in the dataset were based on the APG III system (APG III, 2009). A phylogenetic tree was generated using PHYLOMATIC (v3, 2012; Webb & Donoghue, 2005) and the APG III megatree structure (R20120829). Branch lengths were calculated using the branch length adjusting function in PHYLOCOM 4.2 (*bladj*, Webb *et al.*, 2008) and the 176 node ages provided in Wikström *et al.* (2001), producing an ultrametric tree. The full phylogeny used is provided in Notes S1 in Newick format. Hereafter we refer to the dataset compiled for the current study as the 'full' dataset.

Three monophyletic clades with high representation in the full dataset were used to perform separate clade-based analyses, following major divisions in the angiosperms as per APG III (2009): the Monocots ($n = 749$), the Rosids (the Superrosids as defined in APGIII; $n = 361$), and the Asterids (the Superasterids; $n = 535$). We used The Plant List (2013; *TPL* function in R package TAXONSTAND, Cayuela *et al.*, 2017) to assign species into the three clades. Each clade varied in polyploid frequency, ranging from almost 50% polyploid species in the Monocots to 22% in the Rosids (Table 1). Clonality was equally variable, with the highest percentage of clonal species in the Monocots (70%) and the lowest in the Rosids (23%; Table 1). Among clonal species, each of the three clades differed in representation of clonal modes (Table S1). Stolons and rhizomes comprised the majority of clonal species, ranging from *c.* 60% to 70% across all taxa and within each clade (Table S1). Bulbs were most prevalent within the Monocots, while root-based clonal modes were relatively more common in the Rosids and Asterids (Table S1). Perennial versus annual life histories were approximately equally represented across all clades, at *c.* 75–85% perennial (Table 1). Total

Table 1 Summary of polyploid occurrence in different clonality and life history states across all taxa and within each clade for the Full dataset, and for the Herben dataset.

					% Polyploidy		% Polyploidy		% Polyploidy		
	Total species	% Polyploid species	% Clonal species	% Perennial species	Nonclonal	Clonal	Annual	Perennial	Annual	Perennial nonclonal	Perennial clonal
All taxa	1751	37.4	50.0	80.4	27.5	47.3	25.9	40.2	25.9	28.8	47.3
Monocots	749	48.1	70.1	86.3	42.4	50.6	48.0	48.1	48.0	37.6	50.6
Rosids	361	22.3	23.3	74.5	18.8	35.7	10.8	26.9	10.8	22.8	35.7
Asterids	535	32.1	38.5	74.9	23.4	46.1	20.1	36.2	20.1	25.6	46.1
Herben dataset	808	54.8	65.8	–	38.4	63.3	–	–	–	–	–

species counts per clade in each polyploidy, clonality, and life history state combination are shown in Table S3.

To compare our data and analyses with previous research we assembled two additional datasets. First, we obtained the list of species with ploidy and clonality data used in Herben *et al.* (2017). We mapped 808 of the 895 species onto the Daphne phylogeny (Durka & Michalski, 2012), but were unable to replicate the procedure for placing the remaining 87 species. Data on ploidy level in this data set is binary (diploid versus polyploid). Clonal reproduction was re-categorised into a binary variable, in which plants with a vegetative reproduction score of 0 or 1 (i.e. species that do not multiply in a garden environment) were classified as nonclonal and those with a score of 2–5 were deemed clonal. The modified Herben *et al.* dataset is available in Table S4, which we refer to as the 'Herben' dataset. The Herben dataset had a very different composition of species from the major clades (Monocots $n = 204$, Rosids $n = 228$, Asterids $n = 332$) compared with our full dataset, and contained a higher proportion of both polyploid and clonal species (55% vs 37%, and 66% vs 50% respectively; Table 1; Fig. 1a,b).

Second, we used the CLO-PLA database (Klimešová *et al.*, 2017) to search for measurements of clonal lateral spread, motivated by Herben *et al.*'s findings that the evolution of polyploidy was strongly related to high rates of clonal spread. Because our data focus on the incidence of clonal reproduction, the patterns seen in Herben *et al.* (2017) could be masked in our dataset if the 'clonal' label obscures different rates of polyploid evolution in species with high or low clonal output. We found data on lateral spread for 264 species from our full dataset, which we transformed into a binary variable with spread less or > 0.05 m yr^{-1} following Herben *et al.* (2017). We refer to this subset as the 'CLO-PLA' data (Table S2).

Data analysis

All analyses were conducted using R (v.3.4.4; R Development Core Team, 2018). To compare the full dataset with both the surveys presented by Gustafsson (1948) and the Herben data, we first evaluated species distributions and frequency of polyploid using nonphylogenetic χ^2 tests on species counts in polyploidy, clonality, and life history state combinations. Analyses were run for all taxa in the full dataset and each of the three major clades (Monocots, Rosids, and Asterids).

Phylogenetic comparative analyses of trait evolution have been under scrutiny for their tendency to produce spurious results, particularly in situations in which a trait arises very few times or sample sizes are low (Maddison & FitzJohn, 2015; Wright *et al.*, 2015; Uyeda *et al.*, 2018; Zenil-Ferguson *et al.*, 2018). Given that polyploidy, clonality and life history are highly labile traits across the angiosperms with many gains and losses (Klimeš *et al.*, 1997; Soltis *et al.*, 2016b), and that all our tests involve > 250 species, we believe that these problems should not overly impact our study. Due to the potential limitations of comparative methods, we chose to follow several recommendations from the

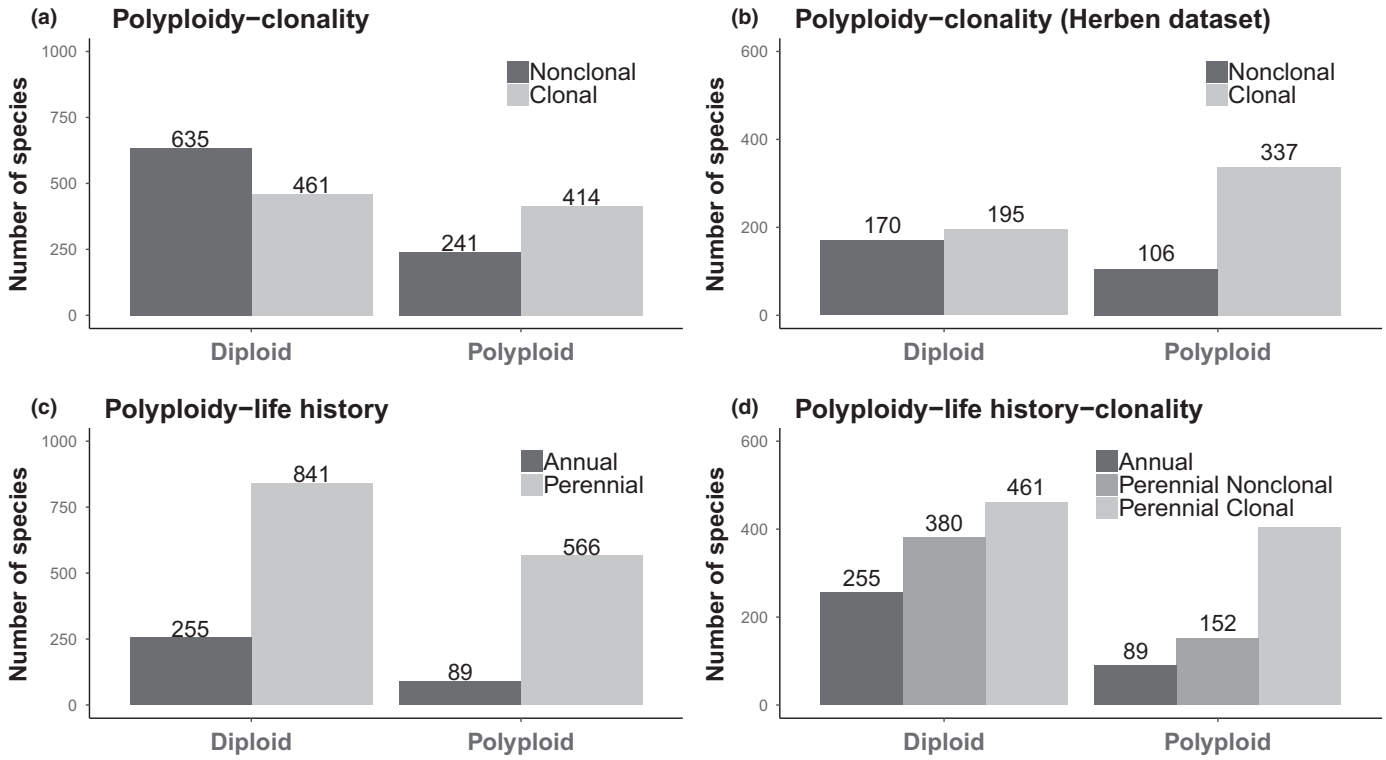

Fig. 1 Species distributions between diploids and polyploids for (a) clonal vs nonclonal species, (b) clonal vs nonclonal species in the Herben *et al*. (2017) dataset, (c) annual vs perennial species, and (d) annual vs perennial nonclonal vs perennial clonal species. See Supporting Information Table S2 for chi-squared analyses performed on the species counts given above columns.

literature to substantiate our findings. Ives & Garland (2014) propose the use of multiple analyses, where agreement between different tests should increase confidence in their conclusions. Here we implement both phylogenetic regression and tests of correlated evolution on our data. Maddison & FitzJohn (2015) suggest splitting phylogenetic datasets into smaller clades and running analyses on these separate parts. If traits of interest are interacting in similar ways in different parts of the whole phylogeny, their correlations likely have a similar mechanistic basis. We run our analyses on the full dataset and each of the three major angiosperm clades individually. Lastly, we perform our analyses in an explicit hypothesis testing framework, in an effort to compare plausible alternative evolutionary hypotheses for correlations between our focal traits (Uyeda *et al.*, 2018).

Phylogenetic regression was used to determine the relationship between the three measures of polyploidy (polyploid – yes/no, estimated ploidy level, chromosome number) and clonality and life history. Analyses were performed over all taxa in the full data set and within each of the three major clades. Also, logistic regression was used for the presence/absence of polyploidy (*phyloglm*; R package PHYLOLM; Ho & Ane, 2014), while least-squares regression was used for the continuous response variables ploidy level and chromosome number (*PGLS*; R package CAPER; Orme *et al.*, 2018). Three regression models, run for all measures of polyploidy, contained the following explanatory variables: (1) clonality, (2) life history, and (3) clonality and life history. Whole model phylogenetic dependence for logistic regressions was assessed via the parameter α as described in Ives & Garland (2010), where smaller values of α correspond to higher phylogenetic contribution. Logistic regression models were bootstrapped over 200 simulated data sets to optimise parameter estimation (Ives & Garland, 2010, 2014). Whole model phylogenetic dependence in the least-squares regressions was estimated using maximum likelihood determined values of Pagel's λ following Revell (2010; Pagel, 1999; Freckleton *et al.*, 2002), where values near 1 indicate high phylogenetic dependence. We note that ploidy level and chromosome number are technically discrete variables and may be more appropriately evaluated through models such as bichrom (Zenil-Ferguson *et al.*, 2017, 2018). However, our PGLS analyses adhered to the assumption of residual normality, suggesting that these variables closely follow a model of Brownian evolution (Martins & Hansen, 1997; Revell, 2010). Therefore, we present the results from the PGLS analyses but note these alternative methods should be explored in future work.

Correlated evolution between polyploidy, clonal reproduction, and life history was evaluated using the *corDISC* function in the R package CORHMM (Beaulieu *et al.*, 2017), which implements Pagel's test of correlated evolution between binary traits (Pagel, 1994). Analyses were run for all taxa in the full data set, and within each of the three major clades. We ran three model types: (1) a four-state model between polyploidy and clonality; (2) a four-state model between polyploidy and life history; and (3) a six-state model between polyploidy, clonality, and life history (noting that Annual-Clonal state combinations do not occur). Overall evidence for trait co-evolution was determined by using log-likelihood ratio (LR) tests between models assuming complete evolutionary independence between traits, and a model assuming co-evolution (LR $df = 4$ for four-state models, $df = 8$ for six-state models). Transition rates (number of transitions per million years) between trait combination states were estimated in the models of dependent evolution. Establishing the directionality of dependent evolution focused on testing four hypotheses: Clonality First, Perenniality First, and Polyploidy First for clonality or perenniality. Hypothesis testing was achieved by setting individual rate pairs equal to each other and then comparing to the appropriate four- or six-state full dependent model (LR $df = 1$ for all tests). For example, to test whether polyploidy evolves more often in clonal taxa (Clonality First) a model in which transition rates to polyploidy along both clonal and nonclonal pathways were constrained as equal ($00 \rightarrow 10 = 01 \rightarrow 11$) was compared via an LR test to the appropriate full dependent model of evolution.

We conducted two additional models of correlated evolution on the Herben and CLO-PLA datasets. First, we reran the four-state model of evolution between polyploidy and clonality using the Herben dataset of 808 species and the Daphne tree structure (Durka & Michalski, 2012). Second, we replicated the analysis between polyploidy and rate of clonal spread performed in Herben *et al.* (2017) using our CLO-PLA matched data subset and the APGIII phylogeny (Notes S1). Hypothesis testing for the directionality of trait evolution proceeded as described above.

Results

Nonphylogenetic χ^2 analyses showed significant differences in species distributions between polyploidy, clonality, and life history combination states for the full dataset across all taxa (Fig. 1a, c,d; Tables 1, S3). Clonal species were more likely than nonclonal species to be polyploid (Fig. 1a; Table 1), as were perennial versus annual species (Fig. 1c; Table 1). When perennial plants were divided into clonal and nonclonal categories, the frequency of polyploidy in annual species was similar to perennial nonclonal species, but was higher for perennial clonal species (Fig. 1d; Table 1). Polyploid frequency in the Herben dataset was higher for both nonclonal and clonal plants compared with the full dataset (Fig. 1a,b; Table 1).

The Rosid and Asterid clades had similar patterns of polyploid frequency as for all taxa; that is, clonal and perennial species tended to contain more polyploid species than nonclonal or annual lineages (Tables 1, S3; Figs S1, S2). By contrast, the monocot clade had a nearly significant difference in polyploid frequency between nonclonal vs clonal species, no difference between annuals vs perennials (Tables 1, S3; Fig. S3), but perennial nonclonal plants were less likely to be polyploid than either annual or perennial clonal species (Tables 1, S3; Fig. S1).

Phylogenetic regression

Polyploid incidence and clonal reproduction were positively associated for all taxa in the full dataset (Model 1; Table 2), a trend that held in each separate clade (Table 2). Analysis of polyploidy and life history similarly showed a significant relationship across

Table 2 Phylogenetic logistic regression of polyploidy vs clonality and life history for all taxa and within the major clades.

	Incidence of polyploidy		
	α (95% CI)	*z*	*P*
Model 1: Polyploidy–Clonality			
All taxa	0.025 (0.019, 0.032)	7.66	**< 0.001**
Monocots	0.074 (0.023, 0.130)	2.37	**0.018**
Rosids	0.055 (0.022, 0.113)	3.11	**0.002**
Asterids	0.015 (0.004, 0.033)	5.09	**< 0.001**
Model 2: Polyploidy–Life History			
All taxa	0.041 (0.033, 0.050)	4.80	**< 0.001**
Monocots	0.056 (0.039, 0.140)	1.88	0.061
Rosids	0.071 (0.037, 0.461)	2.61	**0.009**
Asterids	0.037 (0.024, 0.058)	2.71	**0.006**
Model 3: Polyploidy–Clonality + Life History			
Clonality			
All taxa	0.024 (0.019, 0.032)	6.16	**< 0.001**
Monocots	0.073 (0.022, 0.129)	1.03	0.303
Rosids	0.075 (0.035, 0.175)	2.42	**0.015**
Asterids	0.016 (0.005, 0.036)	4.36	**< 0.001**
Life history			
All taxa	–	0.86	0.392
Monocots	–	1.03	0.301
Rosids	–	1.48	0.140
Asterids	–	0.64	0.516

Phylogenetic dependence is measured by α, and confidence intervals are 95% CI. Logistic regression estimates were bootstrapped over 200 simulated data sets. *P*-values < 0.05 are shown in bold.

all taxa level and within the Rosids and Asterids (Model 2; Table 2), but this relationship was not significant in the Monocots (Table 2). The effect of life history on polyploid incidence disappeared when clonality was also considered for all taxa and within the Rosids and Asterids (Model 3; Table 2). Conversely, the monocot clade showed that neither clonality nor perenniality were significantly related to polyploid incidence (Table 2). Phylogenetic dependence in logistic regression models (α) ranged from 0.015 to 0.075 indicating strong phylogenetic dependence of trait evolution (Ives & Garland, 2010).

PGLS analyses using ploidy level and chromosome number largely corroborated the analyses using polyploid incidence (Table S5). There was a positive relationship between ploidy level and clonality across all taxa and in the Rosid and Asterid clades, but marginally significant in the Monocots (Model 1; Table S5). Chromosome number was positively correlated with clonal reproduction for all taxa and within each of the three clades (Table S5). Life history was associated with ploidy level and chromosome number for all taxa, Monocots and Asterids (Model 2; Table S5), the exception being a marginally significant relationship for chromosome number for the Rosids (Table S5). When clonality and life history are included in the same analyses, both clonality and life history had significant effects on ploidy level and chromosome number across all taxa (Model 3; Table S5). Patterns for Model 3 differed within each clade: the Monocots had only an effect of life history and not clonality, while the Rosids and Asterids showed the opposite (Table S5). Pagel's λ values for ploidy level and chromosome number varied (range 0.180–0.892; Table S5), with the Rosid clade exhibiting consistently lower values than the other clades (Table S5).

Tests of correlated evolution

Pagel's test of correlated evolution indicated that a dependent four-state model of evolution between polyploidy and clonality fit the data significantly better than separate models of independent evolution for all taxa in the full dataset and within each of the clades (Table 3). For all taxa and within the Rosids, there was no evidence for the Clonality First hypothesis (Table 3; Figs 2a, S4 red rates). Rates of polyploid evolution were higher in clonal versus nonclonal lineages for the Monocots and the Asterids, supporting Clonality First in these clades (Table 3; Fig. S4). By contrast, the Polyploidy First hypothesis was supported for all taxa and within the Monocots, but this pattern was not found in the Rosids or Asterids (Table 3; Figs 2a, S4 blue rates).

Patterns of correlated evolution between polyploidy and clonality were opposite for the Herben versus full datasets, and were consistent with the original findings of Herben *et al.* (2017). There was substantial evidence for the Clonality First hypothesis in the Herben data (Table 3, Fig. 2b red rates), and no support for the Polyploidy First hypothesis (Table 3; Fig. 2b blue rates). By contrast, the analysis of lateral clonal spread in the CLO-PLA dataset showed similar results as the full dataset: there was no difference in polyploid evolution between clonal and nonclonal lineages, but a significant difference in the rate of evolution of clonality in polyploid versus diploid lineages (Table 3; Fig. 2c).

The four-state model of polyploidy and life history on the full data showed that dependent models of evolution were a better fit than independent models for all taxa and in the Monocots and Rosids, but not within the Asterids (Table 3). There was no support for the Perenniality First hypothesis for all taxa (Table 3; Fig. 2d purple rates). There were significant differences in polyploid evolution between annual and perennial lineages for the Monocots and Rosids, but only the Rosids showed higher transition rates in perennial taxa (Perenniality First; Table 3; Fig. S4). There was support for the Polyploidy First hypothesis for the evolution of perenniality across all taxa and within the Monocots, but not for the Rosids (Table 3; Figs 2d, S4 yellow rates).

Six-state dependent models between polyploidy, life history, and clonality were favoured over independent models for all taxa and within each clade division (Table 3). Like the four-state models, there was high variability in directionality hypothesis testing. The Clonality First hypothesis was supported within each of the three clade divisions (Table 3; Fig. S4 red rates), but not at the all taxa level (Table 3; Fig. 2e). Testing the Perenniality First hypothesis revealed significantly higher polyploid evolution in perennial nonclonal versus annual lineages for all taxa and for the Rosids and Asterids (Table 3; Figs 2e, S4 purple rates), but the Monocots had higher polyploid evolution in annuals (Table 3; Fig. S4). There was evidence for the Polyploidy First hypothesis for clonality across all taxa and in the Monocots, but not for the

Table 3 Correlated evolution and directionality hypothesis testing for estimated transition rates between polyploidy–clonality combination states, polyploidy–life history combination states, and polyploidy–life history–clonality combination states.

	Trait dependence		Clonality First				Polyploidy First			
Polyploidy–clonality	χ^2	*P*	χ^2	*P*	00↓10	01↓11	χ^2	*P*	00 → 01	10 → 11
All taxa	102.7	< **0.001**	1.7	0.197	0.014^a	0.019^a	18.0	< **0.001***	0.005^a	0.024^b
Monocots	114.6	< **0.001**	33.7	< **0.001***	0^a	0.021^b	51.1	< **0.001***	0.004^a	0.116^b
Rosids	21.3	< **0.001**	0.8	0.377	0.039^a	0.009^a	0.6	0.457	0.006^a	0.014^a
Asterids	43.9	< **0.001**	26.3	< **0.001***	0.005^a	0.044^b	0.8	0.360	0.008^a	0.004^a
Herben dataset	37.8	< **0.001**	16.6	< **0.001***	0.009^a	0.040^b	0	0.969	0.026^a	0.026^a
CLO-PLA dataset	11.2	**0.024**	0.2	0.682	0.058^a	0.044^a	4.2	**0.040**	0^a	0.045^b

	Trait dependence		Perenniality First				Polyploidy First			
Polyploidy–life history	χ^2	*P*	χ^2	*P*	00↓10	01↓11	χ^2	*P*	00 → 01	10 → 11
All taxa	108.9	< **0.001**	2.7	0.100	0.008^a	0.015^a	5.45	**0.020***	0.031^a	0.081^b
Monocots	59.8	< **0.001**	16.5	< **0.001**	0.219^a	0.011^b	11.0	< **0.001***	0.072^a	0.208^b
Rosids	22.4	< **0.001**	14.3	< **0.001***	0^a	0.049^b	0.1	0.740	0.037^a	0.043^a
Asterids	4.89	0.298	–	–	–	–	–	–	–	–

	Trait dependence		Clonality First				Polyploidy First (clonality)			
Polyploidy–life history–clonality	χ^2	*P*	χ^2	*P*	010↓110	011↓111	χ^2	*P*	010 → 011	110 → 111
All taxa	500.5	< **0.001**	0.7	0.415	0.017^a	0.020^a	43.2	< **0.001***	0.006^a	0.064^b
Monocots	410.8	< **0.001**	37.7	< **0.001***	0^a	0.022^b	124.0	< **0.001***	0.004^a	0.284^b
Rosids	50.8	< **0.001**	5.1	**0.024***	0.030^a	0.285^b	5.0	**0.026**	0.015^a	0^b
Asterids	103.3	< **0.001**	23.8	< **0.001***	0.007^a	0.192^b	1.6	0.205	0.020^a	0.005^a

	Perenniality First				Polyploidy First (life history)			
	χ^2	*P*	000↓100	010↓110	χ^2	*P*	000 → 010	100 → 110
All taxa	19.9	< **0.001***	0.005^a	0.017^b	39.0	< **0.001**	0.045^a	0^b
Monocots	56.5	< **0.001**	1.040^a	0^b	40.0	< **0.001***	0^a	0.314^b
Rosids	12.9	< **0.001***	0^a	0.030^b	0.4	0.561	0.050^a	0^a
Asterids	4.1	**0.041***	0.003^a	0.007^b	0.1	0.740	0.032^a	0^a

Evolutionary dependence between traits is determined via likelihood ratio (LR) testing (χ^2) between independent and dependent models of evolution, where a significant LR indicates that a dependent model of evolution fits the data better than independent models (*df* = 4 for four-state models, *df* = 8 for six-state models). The directionality of dependent evolution between traits is evaluated via explicit hypothesis testing (e.g. 'Clonality First'): appropriate transition rates are set as equal and then compared with the full models of evolution via likelihood ratio testing (*df* = 1), where a significant LR indicates that the transition rates are different from each other. Same or different letters by transition estimates indicate rate equality or asymmetry, respectively, while asterisks by *P*-values denote that the rates differ as expected by the given hypothesis (e.g. evolution of polyploid is higher in clonal vs nonclonal lineages for the Clonality First hypothesis). See the Materials and Methods section, as well as Fig. 2 for more details on hypothesis testing. *P*-values < 0.05 are shown in bold.

Rosids or Asterids (Table 3; Figs 2e, S4 blue rates). The Polyploidy First hypothesis for perenniality was supported only in the Monocots (Table 3; Figs 2e, S4 yellow rates).

Discussion

Asexual reproduction via clonality has become a classic characteristic associated with polyploidy and successful polyploid establishment in the literature, but evidence supporting a relationship between these two traits is incomplete. Here we show that polyploidy and clonal reproduction are positively correlated across the angiosperms. By contrast with widely held hypotheses and previous research, the overall patterns of correlated evolution suggest that polyploidy promotes the evolution of clonal reproduction. When we consider perenniality as a confounding and alternative correlate of polyploidy, the relationships between polyploidy and clonality becomes less clear, particularly between separate angiosperm clades. Our results suggest that clonal reproduction may enhance polyploid evolution beyond that seen in perennial plants but, in some circumstances, may play a redundant role in polyploid evolution for species with a perennial life history. Variability in the evolutionary relationships between polyploidy, clonality, and life history in different angiosperm clades could indicate that the overall associations seen across all

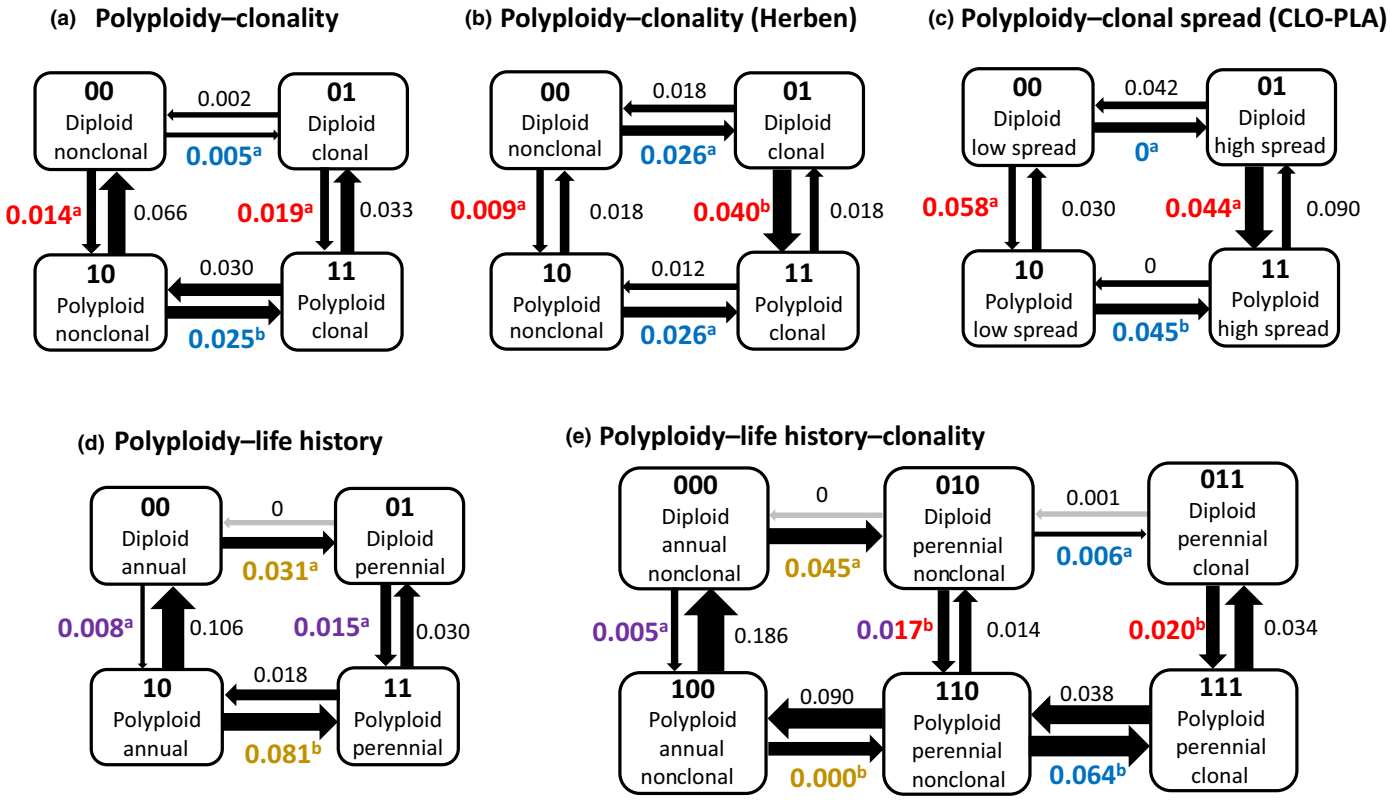

Fig. 2 Transition diagrams of correlated evolution between polyploidy, clonality, and life history combination states. Panels show: (a) dependent evolution between polyploidy and clonality for the full dataset; (b) dependent evolution between polyploidy and clonality in the Herben dataset; (c) dependent evolution between polyploidy and rate of clonal spread (where low spread is < 0.05 m yr^{-1} and high is > 0.05 m yr^{-1}); (d) dependent evolution between polyploidy and life history for the full dataset; and (e) dependent evolution between polyploidy, life history, and clonality. Arrow thickness is proportional to rate magnitude; grey arrows indicate rates that were not significantly different from zero. Contrasting directional hypotheses of evolution are tested by comparing the transition rates in different colours, where matching letter superscripts indicate rate equality, while nonmatching superscripts indicate rate asymmetry. Red, 'Clonality First'; Blue, 'Polyploidy First (clonality)'; Purple, 'Perenniality First'; and Yellow, 'Polyploidy First (life history)'. See the Materials and Methods section, as well as Table 3 for more details on hypothesis testing.

species in our dataset do not accurately reflect the evolutionary processes occurring at finer scales.

A significant relationship between polyploidy and clonal reproduction was supported by all analyses conducted on the full dataset in this study: clonal reproduction was associated with the incidence of polyploidy, higher ploidy levels, and higher chromosome numbers. Though there was unanimous support for a positive correlation, there was no broad-scale evidence for the frequently cited hypothesis that clonal reproduction facilitates the evolution of polyploidy at the whole-angiosperm level (Clonality First; Grant, 1963; Stebbins, 1971; Thompson & Lumaret, 1992; Otto & Whitton, 2000; Husband *et al.*, 2013; Weiss-Schneeweiss *et al.*, 2013; Herben *et al.*, 2017). Instead, results at the whole-angiosperm level indicated that polyploidy is likely to precede the evolution of clonality, in agreement with the Polyploidy First hypothesis. This pattern remained after perenniality was taken into account, showing that, at least at the whole-angiosperm level, a perennial life history does not explain the entire relationship between polyploidy and clonality. The lack of support for the Clonality First hypothesis is contrary to the results of Herben *et al.* (2017)'s phylogenetic analysis of the Central European flora where they found that polyploidy was more likely to arise following the evolution of high rates of clonal spread. The reanalysis of the Herben data we performed, coding clonality as presence/absence, agreed with the original study's outcome: clonality promotes the evolution of polyploidy (Table 3; Fig. 2b). Conversely, when we use a subset of our data with clonal spread measurements (CLO-PLA data) the results agree with those from the full dataset: high clonal spread does not promote polyploidy (Table 3; Fig. 2c). How can we reconcile the conflicting outcomes of these analyses?

One potential explanation are the large differences in representation of polyploidy, clonality, and different angiosperm groups between our dataset and that in Herben *et al.* (2017). The Herben dataset contained much higher proportions of both polyploid and clonal species (Table 1), which could be a sign that both polyploidy and clonality are more likely to be significantly correlated in the Central European flora, whether causally or by chance. This may be a consequence of the temperate nature of their data, where both polyploidy and clonality are expected to be more frequent than in other biomes (Stebbins, 1950; Rice *et al.*, 2019). Our dataset is likely biased towards temperate species as well, due to the availability of cytological data in the literature, though not as severely as previous studies. Hence, either

the limited sampling in Herben *et al.* (2017) means that the polyploidy–clonality relationship was biased, or that, within the region of their study, polyploidy and clonality are interacting in different ways than they are across a broader sampling of angiosperm species. Indeed, the Herben dataset was dominated by Asterid species, a clade in which we did see the Clonality First hypothesis occurring in our own dataset. Differences in species composition between datasets may explain our different conclusions and could indicate that they are not actually in direct conflict. However, it is also worthwhile to note that two of the three measures of clonality in Herben *et al.* (2017) did not support either the Clonality First or Polyploidy First hypotheses.

Variability between angiosperm clades

While analyses of large datasets are valuable because they can provide information on angiosperm-wide trends they likely mask the ecological and evolutionary processes behind trait correlations with polyploidy at lower levels of organisation (Maddison & FitzJohn, 2015; Soltis *et al.*, 2016b). Although there was often strong evidence for correlated evolution between polyploidy, clonality, and perenniality within each of our clades, there was considerable variability in the directionality of co-evolution. Clonal reproduction is a highly diverse trait with many different modes and functions (Klimeš *et al.*, 1997; Vallejo-Marín *et al.*, 2010), and variability in polyploidy–clonality interactions at the family, genus, species, or individual level could influence patterns seen within each angiosperm group. Clonal reproduction through different clonal modes results in variable spatial patterns of ramets (Table S1; Lovett-Doust, 1981), which will influence the ratio of within- and between-cytotype mating opportunities for polyploids and their ability to overcome MCE (Levin, 1975; Husband & Schemske, 1997; Baldwin & Husband, 2013), and ultimately will affect whether the Clonality First hypothesis is a viable pathway towards polyploid evolution. We might expect that clumped ramets would result in the most within-cytotype mating, and we do find that the clade with the highest frequency of bulb-type clonal reproduction does support the Clonality First hypothesis across all species (Monocots, Table 3). The Monocots also contain the caespitose grasses, which here are categorised as rhizomes, but which form tightly packed tussocks (Klimeš *et al.*, 1997). However, spatial patterns of ramet growth are complicated by the fact that each clonal mode represents a continuum; rhizomes can be short versus long, and corms or bulbs can result in longer distance dispersal in aquatic vs terrestrial species (Klimeš *et al.*, 1997; Vallejo-Marín *et al.*, 2010; Eckert *et al.*, 2016). Interestingly, the Asterids also show consistent support for the Clonality First hypothesis but are characterised by a high percentage of clonal modes that are 'guerilla' type (Table S1). It is likely that different clonal modes can promote polyploidy under different circumstances, and a range of evolutionary processes could contribute to the patterns we see both within each clade and across our dataset.

If we consider the Polyploidy First hypothesis for the evolution of clonality, differences between clades could reflect variation in the effect of WGD on clonal mode types (Table S1), which are associated with different morphological and physiological traits (Klimeš *et al.*, 1997; Klimešová & De Bello, 2009). The effects of WGD on clonal organs that are used in resource storage (e.g. bulbs, corms) could be very unlike the effects of WGD on clonal structures meant for high lateral spread and foraging (e.g. stolons, rhizomes). Besides the overall analyses, the Monocots were the only clade to consistently show strong evidence for the Polyploidy First hypothesis and, as discussed above, the types of clonality in this group may be structurally and functionally different than those in the Rosids or Asterids. Little is currently known about the effects of WGD on clonality, but two recent studies show contrasting effects of WGD on clonal reproduction via stolons in *Fragaria vesca* versus root buds in *Chamerion angustifolium* (Van Drunen & Husband, 2018a,b). More research on the impact of WGD on clonal reproduction is needed before we can confidently address the role of this pathway in associations between polyploidy–clonality.

Clonal reproduction versus perennial life history

Studies examining evolutionary correlates of polyploidy rarely consider additional confounding traits that could influence the relationship of polyploidy to their particular trait of interest (Ramsey & Ramsey, 2014). This highlights a potential problem with studying traits correlated with polyploidy, stemming from the well known, but often overlooked, difficulties in implying causation from relationships between carefully chosen traits (Freckleton, 2009; Maddison & FitzJohn, 2015). We find that perenniality and polyploidy also share a significant relationship across our dataset, so is polyploidy truly evolutionarily associated with clonality, or with perenniality? Are there advantages to be gained from clonal reproduction beyond those achieved by just being perennial? Across our full dataset, the answer to these questions appears to be yes: phylogenetic regressions across all taxa supported a relationship between polyploid incidence and clonality even when life history was taken into account (Tables 2, S5).

Perenniality appears to be a fairly weak contributor to polyploid evolution compared with clonality in the Asterids in particular, but the opposite may be true for the Rosids. The Rosids contained the lowest occurrence of both polyploidy and clonality, and our results show that correlated evolution between polyploidy and perenniality was generally stronger in this group than between polyploidy and clonality, likely indicating that the relationship between clonality and polyploidy may not always be completely separate from a relationship between perenniality and polyploidy in this clade. In groups with low amounts of clonality we might expect that perenniality confers many of the same advantages as clonality to new polyploids in overcoming MCE, as perenniality also increases persistence and the probability of same-cytotype matings (Stebbins, 1950, 1971). The Monocots, again, tended to differ from the other clades; analyses showed that sometimes neither clonality nor perenniality were significantly associated with some measures of polyploidy. The differences between the Monocots and other angiosperms remains to be thoroughly tested but could potentially be related to the direct link between clonality and perenniality in some monocot species

(e.g. tussock forming grasses; Klimeš *et al.*, 1997), which may muddy the relationships between clonality and life history in this group.

A worthwhile next step would be to examine how clonal reproduction could provide further advantages over perenniality, such as increasing the rapidity at which polyploids become established in natural populations. Theoretical studies would be useful in exploring the specific roles clonality versus perenniality play in polyploid establishment. Simulations or models looking at the rates and timing of successful polyploid establishment in diploid populations in clonal versus nonclonal perennials would be particularly insightful.

Conclusions

Overall, this study confirms that the evolution of polyploidy and clonal reproduction are positively associated in the angiosperms. However, the nature of this relationship and its link to perenniality is variable between major angiosperm groups, suggesting that interactions between these two traits may be complex and dependent on the evolutionary history and differing roles of clonality in different species and clades. It seems probable that the effects of clonal reproduction on polyploid evolution, and vice versa, can be dynamic and distinctive between and within species (Van Drunen & Husband, 2018a,b). Until further studies are conducted on smaller scales it will be difficult to make sweeping conclusions about the nature of the polyploidy–clonality relationship at the whole-angiosperm level. Like many aspects of polyploid research, more ecological and evolutionary studies are needed to produce a more complete view of the role of clonal reproduction in polyploid evolution (Soltis *et al.*, 2010; Soltis *et al.*, 2016b). Future studies should focus on how polyploid establishment is influenced by clonality versus life history, and how different modes of clonality affect, and are affected by, polyploidisation. Furthermore, given the wide-spread nature and diverse evolutionary histories of both polyploid and clonal plants, better understanding how these interactions could change under different ecological, environmental, and genetic circumstances will also be valuable.

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Author contributions

WEVD and BCH planned and designed the research. WEVD collected the data, and performed data analysis. WEVD and BCH interpreted the data and wrote the manuscript.

ORCID

Brian C. Husband https://orcid.org/0000-0002-1675-9420
Wendy E. Van Drunen https://orcid.org/0000-0003-1093-6665

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Species distributions among polyploidy, clonal, and life history character states in the Rosids.

Fig. S2 Species distributions among polyploidy, clonal, and life history character states in the Asterids.

Fig. S3 Species distributions among polyploidy, clonal, and life history character states in the Monocots.

Fig. S4 Transition diagrams of correlated evolution between polyploidy and clonality for each major clade.

Notes S1 Phylogeny for the full dataset in Newick format.

Table S1 Modes of clonal reproduction represented in the dataset and their distributions among species.

Table S2 Full dataset containing cytological, clonality, and life history information for the current study.

Table S3 Distribution of species among polyploidy, clonality, and life history combination states.

Table S4 Data for reanalysis from Herben *et al.* (2017).

Table S5 Phylogenetic regressions for ploidy level and chromosome number against clonality and life history.

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