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No influence of water limitation on the outcome of competition between diploid and tetraploid *Chamerion angustifolium* (Onagraceae)

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Summary

1. Polyploid plants often occupy different geographic ranges than their diploid progenitors, but the causes of this segregation are poorly understood. Differential competitive abilities of cytotypes across an environmental gradient could be responsible for these observed geographic range differences.

2. Cytotypes of *Chamerion angustifolium* (Onagraceae) are mostly allopatric, and prior research indicates that tetraploids are more physiologically tolerant of water limitation and occupy drier habitats than diploids. We hypothesized that tetraploids are stronger competitors than diploids in soils where water is limited, which allows them to persist in dry habitats while diploids cannot.

3. We grew both cytotypes together in competition under water-limited and well-watered conditions. We varied both total plant density and the relative frequency of cytotypes among pots, which allowed us to separate the effects of intra-cytotypic and inter-cytotypic competition.

4. Both diploid and tetraploid plants were smaller in the water-limited treatment than in the well-watered treatment. Nevertheless, there were no differences in the relative strength of intra-cytotypic and inter-cytotypic competition experienced by either cytotype across the watering treatments, indicating that diploids and tetraploids had equal competitive abilities in both treatments.

5. *Synthesis.* Competition for limiting resources is often proposed as a mechanism causing ecological and geographic segregation between diploid and polyploid cytotypes. Our results do not support the hypothesis that tetraploid *Chamerion angustifolium* plants are stronger competitors than diploids when water is limited. A differential ability to compete for water is likely not responsible for the observed ecological and geographic segregation between cytotypes in this species. Competition may not be a general mechanism that causes segregation between diploid and polyploid cytotypes in nature.

Key-words: addition series, *Chamerion angustifolium*, competition, determinants of plant community diversity and structure, drought, fireweed, polyploidy, response surface, water stress

Introduction

Whole-genome multiplication, or polyploidy, has arisen frequently throughout the evolutionary history of plants (Soltis & Soltis 1999). Because polyploids are typically reproductively isolated from their diploid progenitors, polyploidy is viewed as an important mechanism of sympatric speciation (Otto & Whitton 2000; Wood *et al.* 2009) and is hypothesized to be a major determinant of the ecological and evolutionary dominance of angiosperms (Soltis & Soltis 1999; Cui *et al.* 2006; Amborella Genome Project 2013). Shifts in ploidy are often accompanied by a suite of immediate phenotypic changes that influence a plant's ecology (Levin 1983). Polyploids typically differ from diploids in a number of morphological traits, such as having increased cell size (Stebbins 1971), which, for example, can lead to higher xylem hydraulic conductance (Maherali, Walden & Husband 2009). The ecological differences between diploids and polyploids can be significant enough to drive ecological segregation between cytotypes (Hao *et al.* 2013; Šmarda *et al.* 2013) and lead to subsequent rapid ecological adaptation (Ramsey 2011).

Comparative studies demonstrate that ecological differences between diploids and polyploids can contribute to differences

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in their geographic ranges (Manzaneda *et al.* 2012; Thompson, Husband & Maherali 2014). Recent studies have utilized spatial analyses to compare the climate envelopes of diploid and polyploid cytotypes [see Glennon, Ritchie & Segraves (2014) and Soltis, Visger & Soltis (2014)]. Though several environmental variables are correlated with differences in cytotype distributions, and these correlations have helped identify plausible ecological mechanisms for maintaining segregation between cytotypes, experimental tests of these hypotheses are rare (Ramsey & Ramsey 2014).

One hypothesized ecological mechanism of cytotype segregation is that diploid and polyploid cytotypes occupy different geographic ranges because cytotypes differ in their ability to compete for limiting resources across an environmental or geographic gradient (Maceira, Jacquard & Lumaret 1993; Sugiyama 1998; te Beest *et al.* 2012; Laport *et al.* 2013; Šmarda *et al*. 2013; Thompson, Husband & Maherali 2014). In *Centaurea stoebe* (Asteraceae), for example, the mostly allopatric distribution of cytotypes is likely because of differences in the outcome of competition across a longitudinal gradient (Collins, Naderi & Mueller-Schaerer 2011). Even though geographic segregation of cytotypes appears to be influenced by competition, the specific environmental factors associated with this spatial variation in competitive ability are rarely identified. Therefore, experimental studies of competition in polyploids that manipulate physiologically relevant environmental conditions are needed to uncover the specific mechanisms that may underlie observed spatial variation in cytotype competitive ability.

Here, we investigated whether the outcome of competition between diploid and tetraploid cytotypes of *Chamerion angustifolium* L. Holub (Onagraceae) is influenced by soil moisture availability, a resource that differentially affects the performance and survival of cytotypes in this species (Maherali, Walden & Husband 2009). Tetraploid *C. angustifolium* plants have wider xylem conduits and therefore higher hydraulic conductance than diploids, which permits them to maintain the same rate of transpiration as diploids at a smaller water potential gradient between the leaf and soil ($\Psi_{leaf} - \Psi_{soil}$). Therefore, when the two cytotypes experience the same Ψ_{soil}, the Ψ_{leaf} in the tetraploid will be less negative than that of the diploid. Because the photosynthetic and stomatal responses to declining Ψ_{leaf} are the same in both cytotypes, the less negative Ψ_{leaf} of the tetraploid means that it will have higher photosynthesis than the diploid at the same Ψ_{soil}. When grown without competitors, the ability of tetraploids to maintain a less negative Ψ_{leaf} than diploids results in longer times to both wilting and senescence when watering ceases (Maherali, Walden & Husband 2009). This difference in performance should translate into more rapid biomass accumulation and a higher competitive effect for tetraploids when both cytotypes are grown together in competition under water limitation.

Field observations indicate that tetraploid *C. angustifolium* occupy a drier climatic niche than diploids – the correspondence between physiological performance and the climatic niches of cytotypes suggests that ecological sorting along environmental gradients is responsible for cytotype distribution in this species (Thompson, Husband & Maherali 2014). However, it is unknown whether the observed segregation between *C. angustifolium* cytotypes is influenced by stronger competitive ability of cytotypes in their respective realized niches. Based on differences in their hydraulic conductance and its effects on photosynthetic gas exchange, we predicted that tetraploid plants of *C. angustifolium* would outcompete diploids when water is limited and that the competitive abilities of cytotypes would be more equal when soil is well watered. That is, under water limitation, tetraploids should experience more intra-cytotypic competition than inter-cytotypic competition because of water limitation reducing the relative competitive ability of diploids. In contrast, diploids should experience more inter-cytotypic competition than intra-cytotypic competition because of water limitation increasing the relative competitive ability of tetraploids. Under well-watered conditions, both cytotypes should experience relatively similar amounts of intra-cytotypic and inter-cytotypic competition. If tetraploids are better competitors than diploids under water limitation, then they may be more likely to persist within a plant community located in a dry habitat.

To test these predictions, we grew diploid and tetraploid cytotypes together in competition under both water-limited and well-watered conditions within a common glasshouse environment. We used an experimental design that altered the relative frequencies of each cytotype, as well as the overall density of plants in pots. This design allowed us to isolate the effects of both intra-cytotypic and inter-cytotypic competition and to make specific, directional inferences about the outcome of competition (Inouye 2001). Maceira, Jacquard & Lumaret (1993) used a similar design to examine competitive interactions between diploid and polyploid cytotypes of *Dactylis glomerata* (Poaceae) in a common environment and found strong evidence for the competitive superiority of tetraploids. The present study is the first to use this experimental design to investigate the outcome of competition between diploids and polyploids at different levels of an abiotic resource associated with cytotype physiological tolerances.

Materials and methods

STUDY SYSTEM AND SEED SOURCE

Chamerion angustifolium (Onagraceae), fireweed, is an autopolyploid, herbaceous, perennial plant with diploid ($2n = 2x = 36$), triploid and tetraploid ($2n = 4x = 72$) cytotypes (Mosquin 1967; Husband & Schemske 1998). *Chamerion angustifolium* has a circumpolar distribution (Mosquin 1967); in North America, diploids occur at higher latitudes than tetraploids, and cytotypes occur together along the southern border of the boreal forest and in the Rocky Mountains (Mosquin & Small 1971; Husband & Schemske 1998). In the Rocky Mountains, diploids are adapted to high altitudes and tetraploids are adapted to low altitudes – single-cytotype populations occur at either altitudinal extreme, with mixed-ploidy populations occurring in between (Sabara, Kron & Husband 2013). Throughout the species' North American range, tetraploid populations occur in drier and warmer habitats than diploid populations (Thompson, Husband & Maherali 2014).

In addition to geographic differences, there are several morphological and phenological differences between cytotypes. Diploid plants have higher seed set than tetraploids after hand pollination in the glasshouse and have inherently higher maternal fitness because seed viability does not differ between cytotypes (Burton & Husband 2000). However, in the field, relative seed production is dependent on the cytotype composition of a population (Husband 2000). Diploid plants of *C. angustifolium* reach reproductive maturity before tetraploids (Husband 2000), and tend to be smaller in size (Burton & Husband 2000). The two cytotypes are visited by similar pollinator species, though individual pollinators contribute to prezygotic reproductive isolation by tending to pollinate only a single cytotype within a flight (Husband & Schemske 2000; Kennedy *et al.* 2006). Tetraploid plants are visited by a larger number of pollinators than diploids, which may be due to their larger floral displays (Husband & Schemske 2000).

We obtained all seeds used in the present study from two large collections made in the Rocky Mountains of Canada in 2011 and 2012. We included seeds from 14 populations spanning the species' range in the Rocky Mountains (Table S1 in Supporting information). The cytotypes co-occur in some Rocky Mountain populations – we included seeds from these mixed-ploidy populations, as well as from pure diploid and pure tetraploid populations. In each population, mature fruits and a small amount of leaf tissue were collected from multiple maternal plants. All seeds and leaf tissue were stored in paper coin envelopes and refrigerated at 4°C with desiccant.

PLOIDY DETERMINATION

To determine the ploidy of the 271 maternal plants from which seed were collected, we used flow cytometry. Approximately 1.5 cm^2 of dried *C. angustifolium* leaf tissue was chopped in 0.7 mL of de Laat buffer (de Laat & Blaas 1984) with 100 μg mL^{-1} propidium iodide, 50 μg mL^{-1} RNAse, 0.25 mmol L^{-1} polyvinylpyrrolidone-40 and 0.1% β-mercaptoethanol substituted for dithiothreitol. Approximately 0.5 cm^2 of fresh tomato (*Solanum lycopersicum* 'Stupické polní rané') leaf tissue was chopped together with each *C. angustifolium* leaf sample as an internal DNA content standard (*S. lycopersicum* DNA content = 1.96 pg $2C^{-1}$; Doležel, Sgorbati & Lucretti 1992). After chopping, we passed the sample through a 30-μm mesh filter, left it undisturbed for 10 min to allow the stain to permeate the nuclei, and then analysed it with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). We used the FL2 detector (585/42 nm) to measure relative fluorescence and the FL2-area (integrated fluorescence) parameter to quantify DNA content. Since tetraploids have twice the DNA content of diploids (Sabara, Kron & Husband 2013), we were able to distinguish the ploidy of maternal plants by visually examining the magnitude of nuclei fluorescence of the focal plant relative to the DNA content standard.

After identifying the ploidy of maternal plants, and thus their seed offspring, we consolidated seeds from screened fruits into separate diploid and tetraploid bulked seed collections. We used bulked seed collections to provide a general representation of each cytotype throughout the sampled section of its range in the Rocky Mountains, which allowed us to generalize the results to each cytotype rather than to specific populations. This consolidation is validated by the lack of ecological differentiation among *C. angustifolium* populations within each cytotype in the Rocky Mountains (Martin & Husband 2013). In addition, the wind-dispersed seeds of *C. angustifolium* travel for hundreds of kilometres in the field (Solbreck & Andersson 1987), and thus even very distant populations have the opportunity to compete with one another. We confirmed the ploidy of bulked seeds by growing 60 plants of each cytotype from these collections, screening with flow cytometry as above, and then quantitatively comparing their DNA content to published values for both cytotypes (see Data S1 in Supporting information).

EXPERIMENTAL DESIGN

To examine the outcome of competition between *C. angustifolium* cytotypes, we grew diploid and tetraploid plants together at different relative frequencies and densities. This specific approach to study the outcome of competition is known as a 'response surface' or 'addition series' design (Law & Watkinson 1987; Cousens 1991; Inouye 2001). Implementing the addition series experimental design allowed us to estimate the relative magnitude of intra-cytotypic competition – the effect of adding a plant of the same cytotype – and inter-cytotypic competition – the effect of adding a plant of the other cytotype. Isolating these two effects is impossible when designs do not vary the frequency of both competitors as well as the overall density (Firbank & Watkinson 1985; Cousens 1991; Jolliffe 2000).

Diploid and tetraploid plants were each planted at densities of two, three or four plants per pot. All paired density combinations of each cytotype were included in the experiment, and each cytotype was grown at these densities without the other (i.e. in monoculture). This resulted in 15 different diploid:tetraploid density combinations (hereafter *competition categories*: 0:2, 2:0, 0:3, 3:0, 0:4, 4:0, 2:2, 2:3, 3:2, 3:3, 2:4, 4:2, 3:4, 4:3, 4:4) spanning all possible densities from two plants per pot to eight plants per pot (Fig. 1). Each of the 15 competition categories was replicated 12 times for a total of 864 plants among 180 pots. Due to a minor error during planting, the experimental design was unbalanced such that 145 pots contained diploids (either in monoculture or in mixture) and 143 pots contained tetraploids. The position of cytotypes in pots relative to one another was consistent for all replicates in a particular competition category to ensure that competitive interactions were similar among replicates. Specifically, we transplanted the plants in a ring approximately 3 cm from the edge of the pot. In mixed-cytotype pots, we ensured that plants of one cytotype were planted adjacent to plants of the other cytotype as frequently as was possible for each competition category.

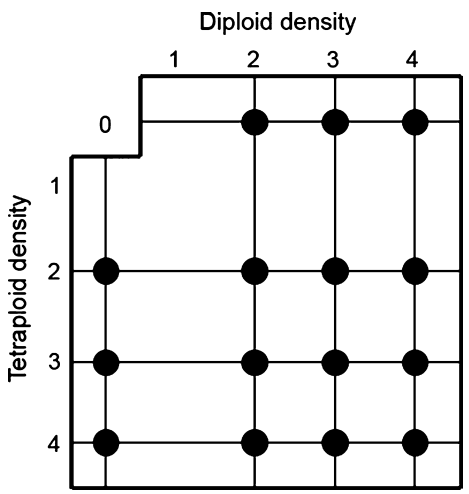

Fig. 1. Diagram of the response surface used in the present study. Closed circles on the surface represent the 15 competition categories.

Plants that did not survive the initial transplanting were replaced within 7 days, and we observed no mortality after this period.

To establish the experiment, we sowed seeds from bulked collections onto 72-plug trays filled with a peat moss and perlite growth medium (Sunshine Mix #4; Sun Gro® Horticulture Ltd., Vancouver, Canada). Four days after sowing, we culled germinated seedlings so one seedling remained per plug. Ten days after sowing, we randomly transplanted seedlings into 4.77-L pots (hereafter the *experimental pots*) (900 Mum Pot 'AZM0900'; ITML® Horticultural Products Inc., Brantford, ON, USA) containing a 70:30 v/v mix of Sunshine Mix #4:Turface® MVP® (Profile Products LLC, Buffalo Grove, IL, USA) in accordance with our competition categories. Experimental pots were established in a glasshouse at the University of Guelph Phytotron (Guelph, Ontario) with 16 h days at 25.5°C and eight hour nights at 20°C. We note that these temperatures were warmer than average temperatures experienced by *C. angustifolium* plants in the Rocky Mountains (Thompson, Husband & Maherali 2014). We applied 100 mL of Plant-Prod Solutions pH reducer 18-9-18 (200 ppm N) liquid fertilizer (Plant Products Co. Ltd., Ancaster, ON, Canada) to each pot during the second, fourth and sixth weeks following transplanting. The growth medium and fertilizer levels used in this study were not representative of what is experienced by natural *C. angustifolium* populations; plants in our experiment likely experienced more nutrient-rich below-ground environments than are available in the field. Watering was withheld the evening before and throughout the day of fertilizer application. We applied several biocontrol agents throughout the experiment, and the quantity of pests on sticky paper did not exceed two per pot per day until the time of harvesting.

The experiment was divided over three temporal blocks separated by 7 days. Each block consisted of 288 plants divided among 60 pots and contained four replicates of each competition category. Blocks were separated spatially within a single glasshouse room, and the position of pots on the glasshouse bench was randomized within each block. We harvested all plants in a block after 10 weeks of growth in experimental pots; this growing period was long enough to allow the majority of plants to complete their vegetative growth and was slightly longer than previous studies examining biomass differences between diploid and tetraploid cytotypes of *C. angustifolium* (e.g. Burton & Husband 2000).

To determine whether the outcome of competition between cytotypes was influenced by water limitation, we imposed a soil moisture treatment with two levels – water-limited and well-watered – on the experimental pots. Plants were allowed to establish in the experimental pots under well-watered conditions for 1 week before we imposed water limitation. Each competition category had half of its replicates within a block randomly allocated to each soil moisture treatment. We irrigated each pot with two pressure compensating angle stakes outfitted with 2 L h^{-1} emitters (Netafim USA™, Fresno, CA, USA). We programed and implemented our automated irrigation using Priva Office software (Priva Group, De Lier, NE) to control #811 solenoid valves (Irritrol Systems®, Bloomington, MN, USA). Each block was equipped with two irrigation lines – one to deliver each treatment. To monitor soil moisture treatments, we measured volumetric water content (VWC) once each week on all pots in the 0:2, 2:0, 2:2 and 4:4 competition categories using a Hydrosense CD620 12 cm soil moisture probe (Campbell Scientific, Edmonton, AB, Canada). While we possess climate data for several populations of *C. angustifolium* in the Rocky Mountains (see Thompson, Husband & Maherali 2014), there are no direct field measurements of soil VWC. Thus, we selected the target VWC levels for our moisture treatments based on those of previous studies that have examined differences in plant traits across ecologically relevant well-watered and water-limited conditions (e.g. Sherrard & Maherali 2006). Pots in the well-watered treatment were watered daily to saturation, and maintained a mean VWC of 44.05 ± 0.43% throughout the 10-week experimental period. In the water limitation treatment, plants were watered to saturation during the first week, and watering was reduced incrementally for the following 2 weeks. Soil moisture in water-limited pots declined until the third week and then stabilized until plants were harvested at a mean VWC of 6.74 ± 0.28%. This soil moisture level was within the permanent wilting point range of many agricultural species (Veihmeyer & Hendrickson 1928). All blocks had identical 10-week watering schedules for each treatment.

PHENOLOGY AND PERFORMANCE MEASUREMENTS

We measured several phenology and performance traits during the 10-week experimental period. For each pot in the 0:2, 2:0, 2:2 and 4:4 competition categories, we made weekly stem length measurements for the tallest plant of each cytotype per pot to monitor growth throughout the experiment (data not shown). We also recorded the date that the first plant of each cytotype flowered in all experimental pots (see Data S1). After 10 weeks of growth in experimental pots, all plants of a common cytotype in a pot were cut at soil level and placed in a large paper bag. We dried plants at 80°C for 72 h, measured the biomass in each bag and calculated the mean biomass per plant in each pot, which we used as an indicator of plant performance.

STATISTICAL ANALYSIS

We compared the performance of cytotypes in response to both competition and the watering treatments. We used ANOVA to determine how above-ground dry biomass differed between cytotypes in response to the addition of competitors and across the watering treatments. We also used ANOVA to compare differences in flowering time between cytotypes in response to water limitation (see Data S1). We used R v3.0.2 for the above analyses (R Development Core Team 2014).

To estimate parameters of intra-cytotypic and inter-cytotypic competition, we used a reciprocal-yield model (modified from Spitters 1983) implemented in SPSS 21 (IBM Corporation 2012). This method uses multiple linear regression equations of the form:

$$1/W_x = b_{x0} + b_i N_x + b_j N_y + N_x N_y + \rho_k \qquad \text{eqn1}$$

where W_x is the mean above-ground dry biomass (i.e. yield) per plant of cytotype x. N_x and N_y are the densities of cytotypes x and y, respectively, and ρ_k is the k^{th} experimental block. We used a $\log_{10}$ transformation to normalize mean above-ground dry biomass. The intercept, b_{x0}, is an estimate of the reciprocal biomass of isolated plants. The strength of intra-cytotypic competition is estimated by the coefficient, b_i, and the strength of inter-cytotypic competition is estimated by b_j; these coefficients represent the ability of plants to suppress competitors (i.e. competitive effect), and are comparable to many traditional indices of plant competition (Weigelt & Jolliffe 2003). Because the reciprocal-yield model (eqn 1) implements the reciprocal of the biomass of individual plants as the response variable, larger values indicate that individual plants are smaller. The model assesses the competitive ability of cytotypes by considering changes in the biomass of individual plants in response to the addition of

competitors. Thus, large plants will not be regarded as better competitors than smaller plants (as theorized by Grime (1973)) if they are more negatively affected by the addition of competitors than smaller plants are. Models were constructed separately for each cytotype and in each watering treatment for a total of four models (2*x* – water-limited, 2*x* – well-watered, 4*x* – water-limited and 4*x* – well-watered).

To compare the strength of intra-cytotypic and inter-cytotypic competition, we calculated the *t*-test statistic (sometimes called the *partial F-statistic*) between partial regression coefficients within each model using a method from Zar (2009):

$$t = \frac{b_i - b_j}{\sqrt{s^2_{b_i} + s^2_{b_j} + 2s^2_{Y_{x,y}} c_{x,y}}}; df = n - m - 1 \qquad \text{eqn2}$$

where b_i and b_j are parameters of intra-cytotypic and inter-cytotypic competition, s^2_{bj} and s^2_{bj} are estimates of the standard error of the intra-cytotypic and inter-cytotypic competition coefficients, $s^2_{Y_{x,y}}$ is the residual mean square, $c_{x,y}$ is the correlation coefficient between the densities of cytotypes x and y, n is the pooled number of data points in the two regressions and m is the number of independent factors in the regression model. Statistically significant *t*-test statistics for this test indicate that the effects of intra-cytotypic and inter-cytotypic competition on plant biomass are not equal. For example, if regression parameters (from eqn 1) were such that intra-cytotypic competition (b_i) was larger in magnitude than inter-cytotypic competition (b_j), and the *t*-test statistic (from eqn 2) was statistically significant, this would indicate that intra-cytotypic competition has a larger effect than inter-cytotypic competition on reducing plant biomass for the cytotype being modelled.

To determine whether the regression models for a cytotype were different across soil moisture treatments, we calculated *F*-test statistics using a method from Zar (2009):

$$F = \frac{\mathrm{SS}_t - \mathrm{SS}_p}{(m+1)(k-1)} \div \frac{\mathrm{SS}_p}{\mathrm{DF}_p}; df = (m+1)(k-1), \mathrm{DF}_p \qquad \text{eqn3}$$

where SS_t is the total residual sum of squares calculated from consolidating data from both treatments, SS_p is the pooled residual sum of squares, k is the number of regression equations being compared, m is as above and DF_p is the pooled degrees of freedom. A statistically significant *F*-test indicates that a cytotype responded differently to the addition of competitors in the two watering treatments (i.e. the regression planes are not identical across both watering treatments).

Results

Above-ground dry biomass differed between cytotypes and was negatively impacted by both increasing plant density and water limitation. The biomass of individual plants decreased as the density of plants within pots increased for both diploid and tetraploid cytotypes averaged across watering treatments (diploid $F_{1,143} = 94.28$, $P < 0.001$; tetraploid $F_{1,141} = 64.18$, $P < 0.001$) (Fig. 2a). The cytotype × density interaction term was not statistically significant ($F_{6,284} = 1.581$, $P = 0.210$). Tetraploid plants were 22% larger than diploid plants in the well-watered treatment ($F_{1,143} = 5.064$, $P = 0.026$), but there was no difference in biomass between the cytotypes in the water-limited treatment ($F_{1,143} = 0.271$, $P = 0.603$) (Fig. 2b). Across all competition categories, water-limited diploids were 44% smaller than well-watered diploids ($F_{1,143} = 49.02$, $P < 0.001$), and water-limited tetraploids were 52% smaller than well-watered tetraploids ($F_{1,141} = 55.76$, $P < 0.001$) (Fig. 2b). The stronger negative effect of water limitation on tetraploids relative to diploids was likely responsible for a nearly significant cytotype × watering treatment interaction term ($F_{1,284} = 3.317$, $P = 0.0696$). In addition to biomass, the watering treatments also affected cytotype phenology (see Data S1).

The outcome of competition was the same in both watering treatments (Fig. 3). All intra-cytotypic and inter-cytotypic competition parameters were highly significant, and statistically significant r^2 values were all >0.7, indicating that the reciprocal-yield model (eqn 1) fit the data well (Table 1). The negative intercept terms for all models (Table 1) reflect the $\log_{10}$ data transformation applied to the reciprocal biomass. In any case, extrapolation beyond the densities present in our experiment should be done with caution (see Spitters 1983). In the well-watered treatment, the strength of intra-cytotypic and inter-cytotypic competition were not significantly different for both diploid and tetraploid cytotypes (Table 1). In the water-limited treatment, there were also no differences in the relative magnitude of intra-cytotypic and inter-cytotypic competition between cytotypes. *F*-test statistics calculated using eqn 3 were highly significant for both cytotypes (diploid $F_{2,135} = 48.428$, $P < 0.001$; tetraploid $F_{2,133} = 93.279$, $P < 0.001$), indicating that the intensity of competitive interactions experienced by cytotypes differed across watering treatments. Specifically, the biomass of individual plants was more negatively affected by the addition of competitors in the water-limited treatment than it was in the well-watered treatment.

Discussion

Many polyploid plants occupy a different geographic range than their diploid progenitors (Martin & Husband 2009), and recent comparative studies suggest that ecological differences between cytotypes and sorting along environmental gradients contribute to these range differences (e.g. Manzaneda *et al.* 2012; Laport *et al.* 2013; Thompson, Husband & Maherali 2014). These comparative studies have generated a number of hypotheses about the cause of cytotype segregation, but many of these hypotheses have not been tested experimentally (Ramsey & Ramsey 2014). In the present study, we conducted an experiment to test the hypothesis that diploid and tetraploid plants of *C. angustifolium* occupy different geographic ranges because of differences in their competitive abilities under water limitation. Our prediction that tetraploid cytotypes should be stronger competitors than diploid cytotypes was derived from previous physiological studies which showed that tetraploids have stronger performance than diploids under water limitation (Maherali, Walden & Husband 2009).

Our results do not support the prediction that tetraploids of *C. angustifolium* would be stronger competitors than diploids under water limitation. Although both cytotypes experienced equal intra-cytotypic and inter-cytotypic competition in the

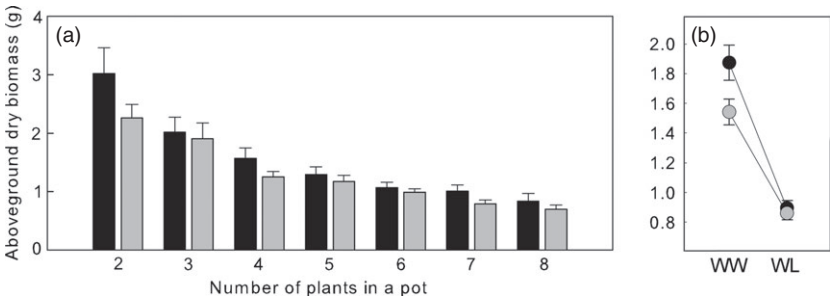

Fig. 2. Response of diploid (grey bars and circles) and tetraploid (black bars and circles) plants of *Chamerion angustifolium* to the addition of competitors and across watering treatments. (a) The above-ground dry biomass of individual plants decreased as the number of plants in a pot increased. (b) The biomass of individual plants was higher in the well-watered (*WW* symbols) treatment than in the water-limited (*WL* symbols) treatment.

well-watered treatment, this balance did not change in the water-limited treatment as predicted. Thus, the outcome of competition did not differ between the well-watered and water-limited treatments (Table 1) (Fig. 3). Diploid and tetraploid *C. angustifolium* were competitively equivalent in both watering treatments, even though tetraploids were significantly larger than diploids in the well-watered treatment. Previous experiments examining plants of *C. angustifolium* under non-stressed conditions have also reported that diploids were smaller than tetraploids (e.g. Burton & Husband 2000; Maherali, Walden & Husband 2009), which suggests that the well-watered treatment functioned properly as a control. Accordingly, concluding that tetraploids are competitively superior under well-watered conditions based on their larger biomass would have been incorrect. The addition of an individual plant, regardless of its cytotype, to a pot in either watering treatment had the same effect for suppressing the other plants growing in that pot. This indicates that both cytotypes were equally competitive in our experiment despite the significant physiological differences between them.

It is unlikely that the absence of tetraploid competitive superiority over diploids in water-limited conditions was due to ineffective treatments. Relative to the well-watered treatment, the biomass of individual plants was reduced by 49% in the water-limited treatment when averaged across both cytotypes (Fig. 2b), indicating that the water-limited treatment negatively affected plant performance as intended. The biomass of tetraploids was more negatively impacted by the imposition of water limitation than that of diploids (Fig. 2b). This result was surprising given the previous observations that tetraploid *C. angustifolium* are more tolerant of water limitation than diploids (Maherali, Walden & Husband 2009). It may be that competition compromises the ability of tetraploid *C. angustifolium* to withstand water limitation. Unfortunately, we did not grow plants in isolation during the present experiment and cannot explore this hypothesis more fully. The highly significant *F*-tests calculated with eqn 3 provide further support that the water-limited treatment was effective – specifically, the above-ground dry biomass of individual plants was less negatively affected by the addition of competitors in the well-watered treatment than it was in the water-limited treatment. In addition, the above-ground dry biomass of individual plants decreased with increasing plant density (Fig. 2a), which indicates that the conditions of our experiment were suitable for causing competitive intensity to increase with the addition of competing plants, even at the lowest densities. The large, significant r^2 values and highly significant parameter estimates for all four models (Table 1) indicate that the reciprocal-yield model was an appropriate choice for our data analysis.

Although many studies have investigated competitive interactions between diploid and polyploid cytotypes, none have experimentally manipulated ecologically relevant factors that can influence the outcome of competition. The majority of studies investigating competition in polyploids are observational, in that they invoke no specific treatments akin to the competition categories used here (see review of studies by Levin (2002)). In addition, the few experimental tests that do exist typically do not vary both the relative frequencies of cytotypes and the total planting densities (e.g. Münzbergová 2007; Fialová & Duchoslav 2014). To our knowledge, the only exception to this latter point is the 2-year study conducted by Maceira, Jacquard & Lumaret (1993), who found that tetraploid plants of *Dactylis glomerata* (Poaceae) were stronger competitors than diploids in a common garden, possibly because of higher allelic diversity or seedling vigour of tetraploids relative to diploids (Maceira, Jacquard & Lumaret 1993). By implementing the response surface experimental design, we were able to quantitatively test specific predictions about changes in the magnitude of intra-cytotypic and inter-cytotypic competition from the 'perspective' of both cytotypes. The lack of experimental studies similar to the present study makes it difficult to determine how general our findings are. We note, however, that our results are consistent with the most recent studies investigating competition between diploids and polyploids, which have consistently found no difference in competitive ability between cytotypes (e.g. Baack & Stanton 2005; Münzbergová 2007; Fialová & Duchoslav 2014).

Even though we detected no difference in the outcome of competition between cytotypes in this study, it is possible for competition to occur differently at other life stages. For example, competitive interactions between cytotypes may be different at the seed or seedling stage than at the juvenile or adult stages because of differences in seed viability or seedling

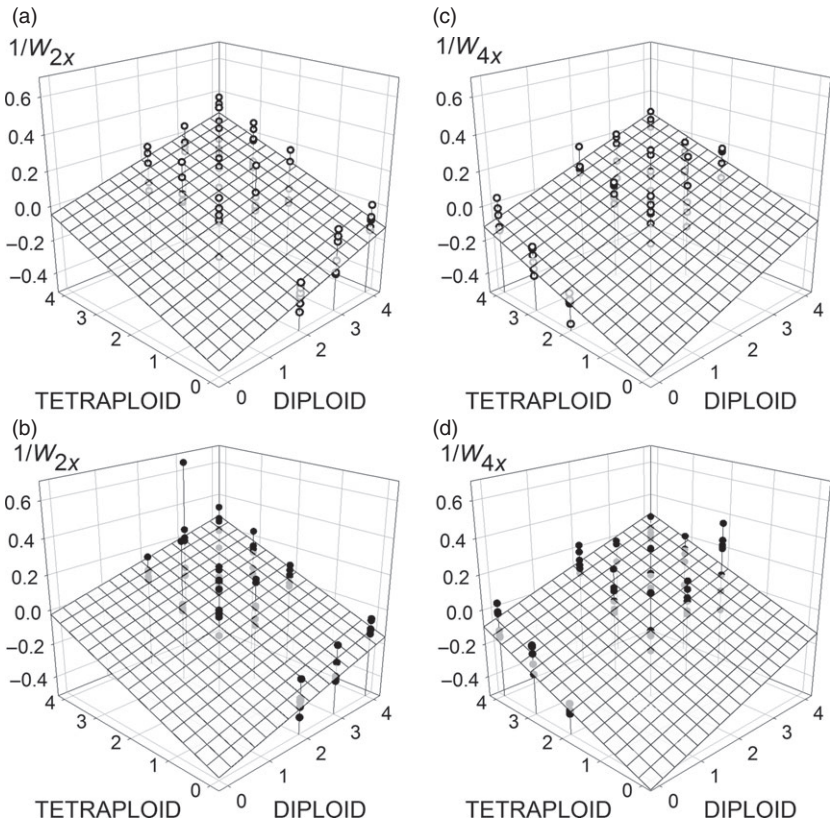

Fig. 3. Multiple regression planes demonstrating the effect of cytotype planting density on plant performance for both diploid ($1/W_{2x}$; a, b) and tetraploid ($1/W_{4x}$; c, d) cytotypes of *Chamerion angustifolium* across both water-limited (open circles) and well-watered (closed circles) treatments. Regression planes were fit to the residual $\log_{10}$-transformed reciprocal above-ground dry biomass of individual plants after removing the effect of experimental block. Because the response variable is the reciprocal of biomass, large values on the z-axis are indicative of small individual plants. Negative values on this axis indicate that the $\log_{10}$-transformed reciprocal biomass of individual plants was lower than 1 g. No interaction terms were used to generate the regression planes depicted above, but were included in the statistical analysis. Values on the *x*- and *y*-axes are the planting densities of diploid and tetraploid cytotypes.

Table 1. Parameters of reciprocal-yield model (eqn 1) for $\log_{10}$ above-ground dry biomass for diploid ($2x$) and tetraploid ($4x$) plants of *Chamerion angustifolium* in both water-limited and well-watered treatments. The *t*-value column shows the test statistic calculated between the intra-cytotypic and inter-cytotypic partial regression coefficients using eqn 2; significant *t*-values would indicate that one cytotype was a superior competitor. The adjusted r^2 column indicates the degree to which each reciprocal-yield model fit the data

Response variable	Watering treatment	*N*	Intercept	Intra-cytotypic competition	Inter-cytotypic competition	*t*-value	Adj. r^2
			α_{2x0}	α_{2x2x}	α_{2x4x}		
$\log_{10}(1/W_{2x})$	Water-limited	73	−0.389*	0.106*	0.139*	0.304 (n.s.)	0.785*
	Well watered	72	−0.663*	0.085*	0.121*	0.256 (n.s.)	0.736*
			α_{4x0}	α_{4x4x}	α_{4x2x}		
$\log_{10}(1/W_{4x})$	Water-limited	70	−0.592*	0.136*	0.165*	0.262 (n.s.)	0.810*
	Well watered	73	−1.038*	0.163*	0.173*	0.079 (n.s.)	0.816*

*$P < 0.001$.

growth rate (e.g. Bretagnolle, Thompson & Lumaret 1995), or drought tolerance (e.g. Sher & Marshall 2003). If this were the case in *C. angustifolium*, the use of transplanted juvenile plants in our experiment could have precluded our ability to detect this difference. One limitation of our glasshouse study is that plants were not exposed to natural pollination and did not produce seeds. The length of the experimental period was too short to measure reproduction in all plants; diploids flowered earlier than tetraploids, and only half of tetraploids flowered before the end of the experiment (see Data S1). Earlier flowering in diploid plants has been observed previously in *C. angustifolium* and thus is not a result of our experimental conditions (Husband & Schemske 1998; Husband 2000). Diploid and tetraploid *C. angustifolium* have equal proportions of viable seed (Burton & Husband 2000), at least under moist conditions, so it is unlikely that this factor influenced the outcome of competition in our study (as in Bouharmont & Mace (1972)). However, germination rates may differ between cytotypes under water-limited conditions, and further experiments will be necessary to determine whether such differences exist in *C. angustifolium.* We note that previous field reciprocal transplant experiments with *C. angustifolium* show that differential cytotype mortality occurred in juveniles but not in seedlings (Martin & Husband 2013), which supports our use of juvenile plant transplants for testing hypotheses about the outcome of competition.

The outcome of competition could also be influenced by differential mortality between the cytotypes in response to

water limitation (Firbank & Watkinson 1985). We could not have detected this possible outcome because no plants died during our experiment. Previous research found that tetraploid *C. angustifolium* take 30% longer to wilt and 28% longer to die than diploids after the cessation of watering (Maherali, Walden & Husband 2009), and so it is possible that the earlier mortality of diploids could make them competitively inferior under levels of water stress more extreme than those implemented in the present study. Future studies could evaluate competitive outcomes between cytotypes at different life stages or under more extreme moisture limitation.

Differential competition in dry habitats is unlikely to be the mechanism maintaining the observed allopatry between diploid and tetraploid cytotypes of *C. angustifolium* in the field. As a result, both cytotypes likely have an equal competitive effect on each other, as well as on other co-occurring plant species across both wet and water-limited habitats in the field. In this respect, our findings disagree with Grime's (1977) C-S-R life-history model, where competition is expected to be weak in stressful environments when compared to resource rich, stable environments. We found that competition (i.e. the negative effect of adding individual plants) was stronger under water-limited then well-watered conditions. Similarly, the competitive equivalency of tetraploids and diploids under both watering treatments implies that there was no trade-off between stress tolerance and competitive ability. If ecological sorting of cytotypes across moisture gradients is not influenced by competition, then fitness-related differences in physiological and phenological performance between cytotypes may be sufficient to explain differences in distribution. For example, Martin & Husband (2013) conducted a reciprocal transplant experiment in the Rocky Mountains using *C. angustifolium*, and found that cytotype fitness varied with elevation, with diploids having highest fitness at high altitudes and tetraploids at low altitudes (Martin & Husband 2013).

In our experiment, we used seeds collected from populations in the Rocky Mountains, where the cytotypes co-occur (Husband & Schemske 1998), but we acknowledge that these populations may not be representative of the entire range of *C. angustifolium*. While we found no difference in competitive ability between cytotypes in our study, competitive interactions may influence their respective distributions outside of the Rocky Mountains. Relative to tetraploids, diploids are dominant in colder environments (Thompson, Husband & Maherali 2014), and it is possible that they are better competitors than tetraploids in cold habitats because their narrow xylem conduits allow them to resist freezing-induced cavitation (Davis, Sperry & Hacke 1999; Maherali, Walden & Husband 2009). Similarly, the earlier flowering of diploids relative to tetraploids could allow them – but not tetraploids – to complete their life cycle in cold habitats characterized by a shortened growing season (Thompson, Husband & Maherali 2014). Future research could investigate whether temperature or growing season length influence competitive ability in *C. angustifolium* cytotypes.

In conclusion, our findings indicate that the stronger performance of tetraploids relative to diploids when grown individually in pots under water stress does not translate into a stronger competitive ability under water limitation. This finding highlights the importance of experimentally testing hypotheses about competition derived from studies of individual performance. Given that superior competitive ability is often hypothesized to be a mechanism of cytotype segregation in nature (te Beest *et al.* 2012), further experiments of the type described here are necessary to determine whether competition is a general mechanism responsible for frequent observations of cytotype segregation in nature.

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Data accessibility

Data collected from the experiment associated with the present article have been archived with the Dryad Digital Repository: doi:10.5061/dryad.2q840 (Thompson *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and Methods: Ploidy confirmation of bulked seed collections; phenology results..

Figure S1. Histogram of DNA content from flow cytometry analysis of 120 *Chamerion angustifolium* populations grown from the bulk seed collections used in the experiment.

Figure S2. Reproductive trait responses of diploid and tetraploid plants of *Chamerion angustifolium* across watering treatments.

Table S1. Summary of the source populations for seed of *Chamerion angustifolium* used in the experiment.