

OIKOS

Research article

Competitive ability, neopolyploid establishment and current distribution of a diploid–tetraploid plant complex

Mariana Castro¹✉, Maria Celeste Dias¹, Joao Loureiro¹, Brian C. Husband² and Silvia Castro¹

¹Department of Life Sciences, CFE, Centre for Functional Ecology, University of Coimbra, Calçada Martim de Freitas, Coimbra, Portugal

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

Correspondence: Mariana Castro (mcastro@uc.pt)

Oikos

2023: e09949

doi: [10.1111/oik.09949](https://doi.org/10.1111/oik.09949)

Subject Editor: Kari Segraves

Editor-in-Chief:

Gerlinde B. De Deyn

Accepted 11 June 2023

Polyploidy is a pervasive phenomenon in nature and has significantly contributed to the adaptive evolution of plants. The conditions necessary for the spread of neopolyploids in populations of the diploid progenitor are limited; however, the superior competitive ability of neopolyploids may promote its establishment. Here, we assess the contribution of polyploidisation to the divergence of plant traits affecting competitive response, which could explain the successful establishment and current geographic distribution of polyploids. We conducted an intraspecific competition experiment using diploids, neotetraploids and established tetraploids of *Jasione maritima* var. *maritima* to determine whether cytotypes differ in phenological, growth and physiological traits and competitive response. Cytotypes respond differently under different competition scenarios with implications for cytotype establishment and distribution. Competition impacted all cytotypes, but neotetraploids were least affected by competition, and the tetraploids were the most impacted. Thus, competitive advantage may have contributed to the displacement of diploid populations and colonisation of new areas by neotetraploids but might have been lost afterwards. Evolutionary changes after polyploidisation have also been detected, and tetraploids invested more in belowground biomass, suggesting that root development might also play a role in colonising southernmost locations. Interestingly, diploids and both tetraploids seem to have different life strategies, the first investing in growth while the latter investing in reserves for the next season. Overall, polyploidisation seems to provide immediate changes that confer an advantage under competition that, together with other factors, may have allowed the establishment of neotetraploids.

Keywords: competitive ability, cytotypes, genome duplications, *Jasione maritima* var. *maritima*, neopolyploids, parapatric distribution

Introduction

Polyploidization, i.e. whole genome duplication, is an important mechanism of evolutionary divergence in plants. The factors determining the success of polyploid lineages have long attracted the attention of the scientific community (Soltis et al.



2010, Ramsey and Ramsey 2014, Van de Peer et al. 2017, 2021). Polyploidization often produces significant changes in cell size and functioning that can result in morphological and physiological changes, with substantial ecological and evolutionary consequences (Melaragno et al. 1993, Li et al. 1996, Maherali et al. 2009, Ramsey 2011, Hao et al. 2013, Madlung 2013, Clo and Kolář 2021). In past years, several studies have reported differences between polyploids and their diploid (or lower ploidy) progenitors (Husband and Sabara 2004, Jersáková et al. 2010, Hao et al. 2013, Laport et al. 2016), including changes in growth rates, secondary metabolism, cold tolerance, water relations and/or stress tolerance (Garbutt and Bazzaz 1983, McArthur and Sanderson 1999, Maherali et al. 2009, Schlaepfer et al. 2010, Liu et al. 2011, Coate et al. 2013). These changes can confer an advantage at the initial stages when the new polyploid is in low numbers within the parental population and subjected to frequency-dependent selection (minority cytotypic exclusion, Levin 1975, Husband 2000). The competitive ability of polyploids is often included in theoretical models as an important determinant of their establishment (Fowler and Levin 1984, Rodríguez 1996); however, both intra and interspecific competition have rarely been explored experimentally (Maceira et al. 1993, Collins et al. 2011, Thompson et al. 2015, Čertner et al. 2019).

Polyploidization has been associated with increased competitive ability because of the direct effects on cell size. The increase in cell size generated by genome duplications frequently leads to an overall increase in the size of plant structures such as leaves, flowers and fruits, as well as an increase in the size of stomata (Schepper et al. 2004, Leitch and Leitch 2008, Sun et al. 2009, Allario et al. 2011, Van Laere et al. 2011, Tan et al. 2015), the so-called 'gigas effect' (Stebbins 1971, Masterson 1994, Buggs and Pannell 2007, Ramsey and Ramsey 2014). In addition, heterosis caused by homeologous genomes present in allopolyploids, increased heterozygosity and gene redundancy may shield polyploids from deleterious recessive mutations and confer an advantage to polyploids by increasing plant vigour in comparison with the parental(s) (Adam and Wendel 2005, Comai 2005). Consequently, polyploids are frequently associated with more robust plants, with higher biomass and overall stronger vigour, compared with their lower ploidy progenitors (Müntzing 1936, Smith 1946, Masterson 1994, Levin 2002, Ramsey and Schemske 2002, Ramsey and Ramsey 2014, Clo and Kolář 2021). In natural populations, these consequences may provide increased intraspecific competitive ability, allowing polyploids to out-compete their diploid progenitors (Maceira et al. 1993), or increased interspecific competitive ability, enabling polyploids to grow in denser vegetation (Maceira et al. 1993, Hülber et al. 2009, Ståhlberg 2009, but see Thompson et al. 2015, Čertner et al. 2019), or even in other sub-optimal conditions such as increased aridity and drought conditions (Manzaneda et al. 2012, 2015, Hao et al. 2013).

Differences in competitive ability can shape cytotypic distribution patterns at contact zones. The interactions between

cytotypes (i.e. intraspecific competition) will affect the spatial dynamics at diploid–polyploid contact areas, promoting more stable or more dynamic zones of contact (Petit et al. 1999). For example, superiority of a given cytotypic under intraspecific competition may lead to the displacement of another cytotypic, resulting in transient mixed-ploidy populations, shifting contact zones and promoting the spread of the fittest cytotypic (Maceira et al. 1993, Buggs and Pannell 2007, Collins et al. 2011, Laport et al. 2013). In contrast, similar responses to competition between cytotypes may increase its stable coexistence (Collins et al. 2011), although other factors are expected to be involved (e.g. interspecific competition, fertility and reproductive fitness). Indeed, different responses to interspecific competition might also impact cytotypic success and, for example, tetraploids were shown to be more robust than diploids under interspecific competition in areas where nutrients are not limited (Čertner et al. 2019). Experiments quantifying the competitive ability of different cytotypes thus provide insights into the factors governing successful establishment of polyploid lineages and the factors maintaining current geographical patterns. However, our understanding of the role of both intra- and interspecific competition is limited to a few studies in the literature (Maceira et al. 1993, Collins et al. 2011, Laport et al. 2013, Čertner et al. 2019).

Jasione maritima is a mixed-ploidy species occurring in the dune systems of the north-western Iberian Peninsula (Castro et al. 2020a). *Jasione maritima* var. *maritima* cytotypes have a parapatric distribution (west of Galicia coast, Spain) of pure-ploidy populations, with diploids ($2n=2x=12$ chromosomes) located in the north of Galicia, from Casas da Hermida to Lariño, and tetraploids ($2n=4x=24$) in southern Galicia, from Ventim to La Guarda (Castro et al. 2020a) (Fig. 1). Recent studies of cytogeographical patterns using niche modelling have suggested that diploid and tetraploid niches partially overlap, and, at present, tetraploids occupy their potential environmental niche. In contrast, diploids are restricted to a smaller portion of their potential distribution (Castro et al. 2020a). This pattern suggests that tetraploids have competitively excluded diploids from part of the range, restricting diploids to northern areas where tetraploids are not able to succeed. This hypothesis could be formally tested using intraspecific competition experiments. Furthermore, given that neotetraploids have been successfully synthesised from diploid *J. maritima* (Castro et al. 2018), including them in the comparisons will enable us to disentangle the role of genome duplications per se from the selection processes that operated along the evolutionary history of this polyploid complex (Ramsey 2011, Martin and Husband 2012, Van Drunen and Husband 2018). The introduction of neopolyploids in such comparisons is of major importance when studying the adaptive ecological potential of polyploid complexes [e.g. *Chamerion angustifolium*; Husband et al. 2008, 2016, Maherali et al. 2009, Baldwin and Husband 2011, *Tragopogon* species, Tate et al. 2009; *Heuchera grossulariifolia*, Oswald and Nuismer 2011; *Achillea borealis*, Ramsey 2011; *Vicia cracca*, Pavlíková et al. 2017].

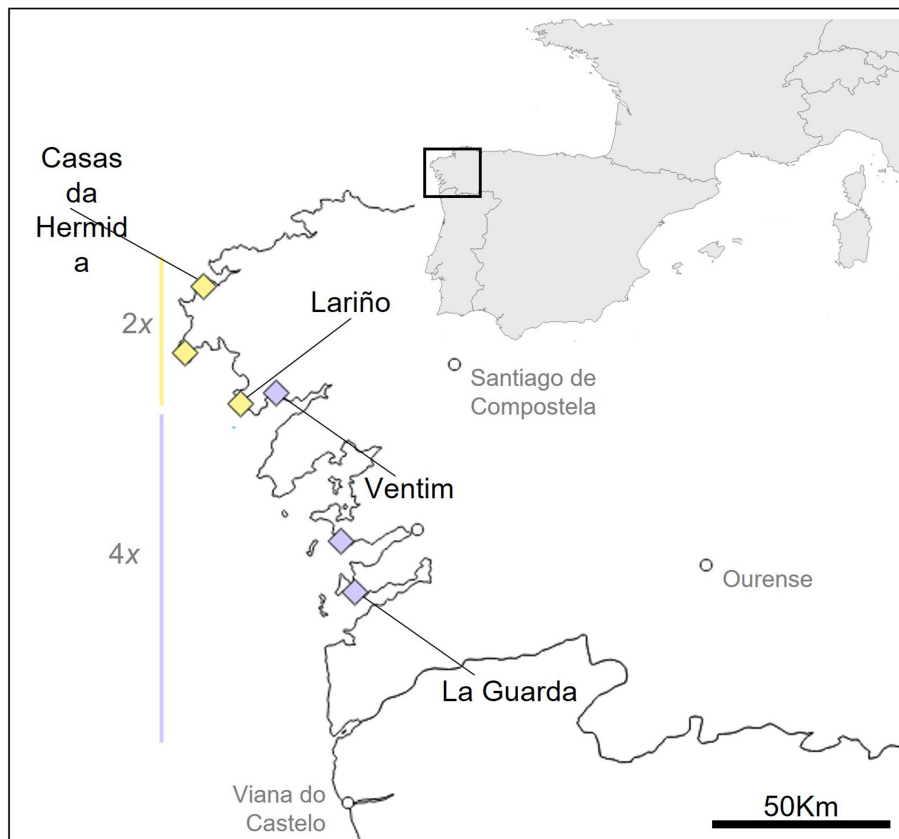


Figure 1. Cytotypes distribution and sampled population locality. Lines correspond to the longitudinal distribution of cytotypes and diamonds to sampled populations. Yellow and blue colours correspond to the areas of diploids and tetraploids, respectively.

Here, we assess the immediate and evolutionary consequences of whole genome duplication for the competitive responses of diploid and tetraploid *J. maritima* var. *maritima* and their relation to current geographical distributions. We quantified and compared the response to intraspecific competition of diploids, established tetraploids and synthetic tetraploids (neotetraploids) in a common environment, with and without intraspecific competition. Neotetraploids are used for the first time in a competition experiment to disentangle the direct impact of genome duplication from its effect on selection after polyploidisation. We posed the following specific questions: 1) does genome duplication increase the competitive ability of newly formed tetraploids compared with diploids? We hypothesise that increased competitive ability, due to genome duplications, has contributed to the successful establishment of newly formed tetraploids and helps to explain the success of tetraploids in nature; 2) does the competitive response of diploids and established tetraploids help to explain the current distribution patterns? We hypothesise that tetraploids have greater competitive ability than diploids, supporting niche modelling results and explaining current distributions.

Material and methods

Plant material

Three cytotypes were included in the experiment: diploids (2x), established tetraploids (4x, hereafter called tetraploids) and synthetic tetraploids (Neo4x, hereafter called neotetraploids). In 2013 and 2014, seeds were collected from three diploid and three tetraploid populations (Table 1, Fig. 1). In each population, infructescences were collected from 40 maternal plants, each separated by at least 4 m. In the laboratory, the fruits were air-dried, and the seeds were removed, cleaned and stored in labelled microtubes.

Synthetic neotetraploid plants were produced from diploid populations by treating seedlings with colchicine (for methodological details, Castro et al. 2018) and confirming their ploidy using flow cytometry (Castro et al. 2018). Each successfully transformed plant was cross-pollinated with multiple randomly chosen neotetraploids from different mother families of the same population to yield an F1 seed generation. Specifically, neotetraploid lines were grown to the flowering stage within an insect-free cage at the Botanic Garden

Table 1. Locality, DNA ploidy level (2x, diploid; 4x, tetraploid) and geographic information of the natural *Jasione maritima* populations. Populations marked with * were consider as populations from contact zone.

Populations	DNA Ploidy level	Longitude	Latitude
Lourido, La Coruña	2x	43°05'12.4"N	9°13'15.9"W
Fisterra, Afora beach, La Coruña	2x	42°54'30.6"N	9°16'23.8"W
Lariño, La Coruña*	2x	42°46'15.7"N	9°07'20.2"W
Ventim, Abelheira, La Coruña*	4x	42°47'57.0"N	9°01'36.7"W
Barbeito, Pontevedra	4x	42°23'58.4"N	8°51'01.8"W
Liméns, Pontevedra	4x	42°15'36.8"N	8°48'49.3"W

of the University of Coimbra. Each inflorescence served as a pollen donor and receiver, and pollinations were performed by gently rubbing the inflorescences. Because inflorescences open gradually, each inflorescence was pollinated on at least three days. Matured infructescences were harvested and air-dried, and seeds were removed, cleaned and stored in labelled microtubes.

Competition experiment

Competitive responses of *J. maritima* cytotypes (i.e. 2x, Neo4x and 4x) were quantified by subjecting plants to two treatments: 1) no competition – one *J. maritima* plant of a given cytotype grown alone and 2) competition – one *J. maritima* plant of a given cytotype (focal plant) grown with another *J. maritima* plant (competitor), for which the competitor plant included all possible cytotype combinations (2x, Neo4x and 4x; Appendix 1). In the competition pots, both plants were used to assess phenological, growth and physiological traits. The no-competition treatment was replicated 15 times per population. The competition treatment was replicated 15 times for each cytotype combination per population except for the same cytotype combination, which was replicated 16 times. Sixteen mother families per selected population were used in every treatment, totalling 342 pots and 549 transplanted seedlings. The experiment was performed in the greenhouse of the Botanic Garden of the University of Coimbra from 2 November 2015 to 30 June 2016. Since germination rates were uniformly high among *J. maritima* cytotypes (mean \pm SE; 2x: $94.9 \pm 0.001\%$ and 4x: $92.4 \pm 0.002\%$, Castro unpubl.), and that mortality after transplantation of five-day old seedling was low (about 1.0%, Castro unpubl.), we started the experiment in the seedling stage. This strategy enabled us to control the cytotype of all the plants involved in the experiment.

Ten days before the start of the experiment, 10 seeds from each of the 16 mother plants per population were placed in individual petri dishes on filter paper moistened with

distilled water and stored at 4°C for five days to synchronise seed germination (Castro et al. 2018). Petri dishes were then transferred to a growth chamber and incubated at 24°C with a 16h:8h (light:dark) photoperiod. After five days, most seeds germinated and produced fully expanded cotyledons. Seedlings were transplanted into 1-l plastic pots (8.6 × 8.6 cm wide and 21.5 cm deep) filled with a 1:1 mixture of commercial soil and sand. All the pots were randomly assigned to a position in the greenhouse bench at the beginning of the experiment and re-randomised four more times during the experiment. All the seedlings that died within the first two weeks after the transplant were replaced and interpreted as losses due to the transplant process. No more seedlings were replaced afterwards. Plants were watered regularly, three times per week in the winter and daily in the spring and summer. The ploidy level of all the plants used in the experiment was confirmed through flow cytometry using the protocol described in Castro et al. (2018).

Plant measurements

Survival was measured monthly after the first month. During spring, each individual was monitored every two days to record the beginning of flowering, expressed as the number of days from the beginning of the experiment to the day when the first flower opened. Eight months after the beginning of the experiment, at the peak of flowering, all the plants were harvested. Of the 547 plants that survived until the end of the experiment, 97 were used for measuring physiological traits (eight plants per treatment and cytotype combination), and 450 were used for measuring growth parameters.

Growth was measured as aboveground biomass and belowground biomass. Whenever possible, aboveground biomass was further divided into vegetative and reproductive parts (peduncles and inflorescences). Roots were washed thoroughly to eliminate soil. Estimating belowground biomass in the competition treatments was difficult as the fine roots of the two plants were often intertwined. Therefore, only the taproot and main roots of each plant were collected. The same procedure was followed with plants growing alone so they could be compared directly. Above- and belowground plant material was dried at 60°C for 48 h and weighed.

For the physiological parameters, we measured starch content, as an indicator of potential changes in life-cycle strategies, and cell membrane leakage, as a biomarker of oxidative damages potentially caused by competition treatment. Fresh leaves were collected in individual aluminium foil envelopes, immediately frozen in liquid nitrogen, and stored at –80°C until carbohydrate quantification. Starch content was extracted from leaf samples and quantified using the anthrone method described in Osaki et al. (1991). To measure cell membrane leakage, two to four fresh leaves, depending on leaf dimensions, were collected, washed with deionised water, placed in closed microtubes with deionised water and incubated overnight on a rotary shaker. Electrical conductivity on the solution was determined two times, one after 24 h (Lt) and another after samples were autoclaved (L0) (samples

were autoclaved at 120°C for 20 min, and the measures were obtained after cooling to 25°C). Cell membrane leakage was then assessed following Lutts et al. (1996), and the electrolyte leakage was calculated as L_t/L_0 and expressed as a percentage.

The response ratio (R , according to Hedges et al. 1999) was used to quantify the magnitude of the competition response and was calculated for every plant growing under competition as follows:

$$R = \ln \frac{\text{treatment}}{\text{control}}$$

where treatment refers to the trait value obtained for the focal plant under competition, and control refers to the mean value of the same trait acquired for the focal plant cytotype without competition. Values closer to zero indicate no response of a given trait to competition. Conversely, values significantly lower or higher than zero indicate a negative or positive response, respectively, of a given trait to competition.

Statistical analyses

Generalised linear models (GLMs) were used to explore variations in phenological, growth and physiological traits. Competition treatment, cytotype of focal plant and competition treatment*cytotype were used as explanatory variables; response variables included: flowering time (days) phenology, aboveground vegetative biomass (g), aboveground reproductive biomass (g), total belowground biomass (g), aboveground biomass (g), total biomass (g), starch content (mg mg^{-1} of fresh leaf) and cell membrane leakage (percentage). A Poisson distribution with a log link function was used to model phenology (given in number of days), and a Gaussian distribution with the identity link function was used for biomass traits, starch content and cell membrane leakage. Outlier values were inspected and excluded from the analyses. The population of origin was initially used as a random factor using mixed models; however, it was ultimately removed due to low variance compared to residuals (Bolker et al. 2009).

GLMs were also used to explore differences in competition response of phenological, growth and physiological traits between cytotypes. Cytotype of focal plant and competitor biomass were defined as explanatory variables, except for physiological traits for which both focal plant and competitor were used for physiologic analyses and, thus, biomass was unavailable. Including biomass enable us to assess the contribution of competitor size in the competitive response of the focal plant. Competition responses of all the traits studied were defined as response variables, and a Gaussian distribution with the identity link function was used to model responses. As mentioned above, the population of origin was initially used as a random factor but subsequently removed (Bolker et al. 2009).

Model validation was performed by visually inspecting the residuals for heteroscedasticity and normality (Zuur et al. 2009). All analyses were performed in R ver. 3.6.1 (www.r-project.org), using the packages 'car' for type-III analysis of

variance (Fox and Weisberg 2019), 'lme4' for GLMs and generalised linear mixed models (Bates et al. 2015), 'lsmeans' for least-squares means (Lenth 2016) and 'multcomp' for multiple comparisons after type-III analysis of variance (Hothorn et al. 2017). Plots were designed in the 'ggplot2' R package (www.r-project.org, Wickham et al. 2016).

Results

Effect of competition on cytotype phenological, growth and physiological variation

Significant differences were observed between competition treatments, cytotypes and their interaction for all traits analysed (except cytotype in starch content and cytotype \times treatment interaction in phenology; Supporting information). Overall, plants under competition had lower biomass (Fig. 2A–C, Appendix 2A–B) and flowered later (significant for 2x and 4x; Appendix 2C) than when growing alone, regardless of cytotype. Plants under competition also had higher starch content (significant for 2x and Neo4x; Fig. 2D) and lower cell membrane leakage (significant for 4x and Neo4x; Fig. 2E) than plants growing alone.

The effect of the competition treatments differed among cytotypes. When growing alone (white boxes in Fig. 2, Appendix 2), diploids produced significantly higher vegetative and aboveground biomass than neotetraploids and tetraploids. At the same time, no significant differences were observed for reproductive biomass (Fig. 2A–B, Appendix 2A). Tetraploids produced significantly higher belowground biomass and had lower phenological values (i.e. start flower earlier) than diploids and neotetraploids (Fig. 2C, Appendix 2C). Overall, diploids had the highest total biomass, neotetraploids the lowest and tetraploids had intermediate values, not differing from the other cytotypes (Appendix 2B). Finally, diploids had significantly lower starch content and cell membrane leakage when growing alone than neotetraploids and tetraploids (Fig. 2D–E).

When growing under competition (grey boxes in Fig. 2), diploids and neotetraploids did not differ statistically for all traits analysed, except cell membrane leakage, for which neotetraploids were similar to tetraploids and significantly higher than diploids (Fig. 2, Appendix 2). Diploids and neotetraploids had significantly higher vegetative, reproductive, aboveground and total biomass, as well as higher starch content and phenological values (i.e. start flower later) than tetraploids. Tetraploids had significantly higher belowground biomass than diploids and neotetraploids (Fig. 2, Appendix 2).

Cytotype responses under competition

The competition response ratio differed significantly from zero (i.e. competition impacted significantly) for all cytotypes across all traits analysed, except for starch content in tetraploids (Supporting information). The impact of competition was negative for all biomass traits and positive for

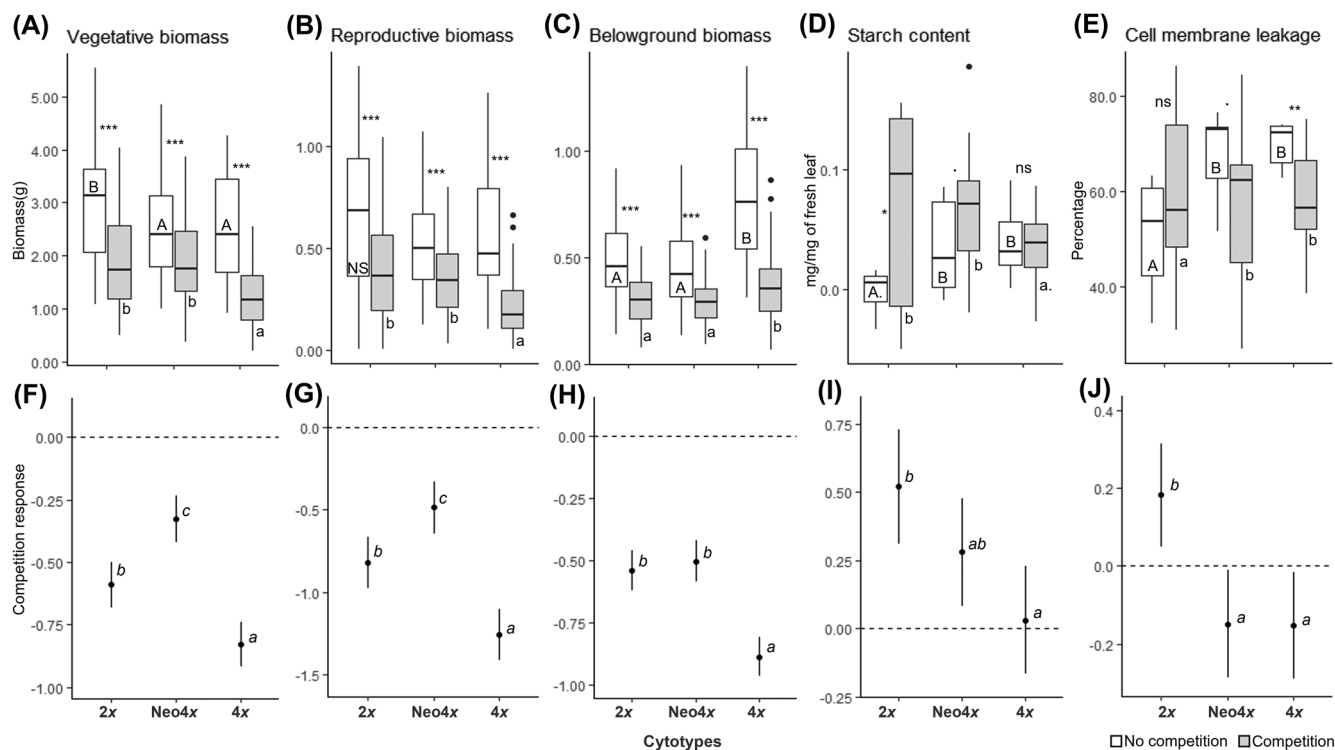


Figure 2. Boxplot for diploid (2x), neotetraploid (Neo4x) and tetraploid (4x) focal plants of *Jasion maritima* grown in different competitive environments based on growth and physiological traits. Competitive environments comprise growth in the absence of a neighbour (no competition, white boxes) and growth with a neighbour (grey boxes); dot plots represent the mean and upper and lower limits of competition response for each cytotpe. Five variables are presented: Vegetative biomass, Reproductive biomass, Belowground biomass, Starch content and Cell membrane leakage. Upper case letters indicate differences ($p < 0.10$) between cytotypes when grown alone (white boxes); lower case letters indicate differences between cytotypes grown with a neighbour (grey boxes); and italic letters represent differences between the competition response (dots); the absence of letters reveals nonsignificant differences between cytotypes at $p < 0.10$. Differences between plants growing alone and under competition (white versus grey boxes) are denoted as $0.10 < p < 0.05$; * $0.01 < p < 0.05$ ** $0.01 < p < 0.001$ and *** $p < 0.001$.

starch content and phenology, while cell membrane leakage increased in diploids and decreased in neotetraploids and tetraploids (Fig. 2F–J, Appendix 2D–F).

Significant differences were observed among cytotypes for all the trait response ratios analysed, and competitor biomass was positively related to reproduction biomass and total aboveground biomass of focal plants (Supporting information, Appendix 3). Neotetraploids were less negatively impacted by competition (i.e. presented significantly higher negative response ratios) for vegetative, reproductive, aboveground and total biomass response ratios than the other cytotypes. At the same time, tetraploids had significantly lower ratio values (i.e. were the most impacted), and diploids presented intermediate values significantly different from the other cytotypes (Fig. 2F–G, Appendix 2D–E). Tetraploids also had significantly lower negative competition response ratios for belowground biomasses than diploids and neotetraploids that were similarly impacted (Fig. 2H). For starch content, a gradient was observed with diploids having significantly higher and positive response ratios, tetraploids considerably lower values (not different from zero; Supporting information), and neotetraploids having intermediate values not significantly different from the other cytotypes (Fig. 2I).

For cell membrane leakage, diploids had significantly higher and positive values than neotetraploids, and tetraploids had low negative response ratios (Fig. 2J). Finally, tetraploids had significantly higher positive response ratios for phenology, neotetraploids had significantly lower values, and diploids had intermediate values that were not significantly different from the other cytotypes (Appendix 2F).

Discussion

Our results show that cytotypes respond differently under different intraspecific competition scenarios with implications for neotetraploid establishment and current cytotpe distribution. Under optimal conditions, diploids invested more in biomass (except for belowground biomass) and less in starch accumulation. At the same time, genome duplications produced smaller plants with a high capacity to accumulate starch and high cell membrane leakage, a pattern conserved over the evolutionary history. Competition reduced plant growth for all cytotypes, with diploids and neotetraploids presenting similar biomass investments and tetraploid plants being globally smaller while maintaining

a higher investment in belowground biomass. The effect of competition was also observed in the physiological traits, with plants under competition having higher starch content and lower cell membrane leakage than plants growing alone. Finally, neotetraploids were less impacted by competition (competition response ratios closer to zero), and tetraploids tend to be more impacted by competition than diploids.

Immediate effects of genome duplication

Genome duplications impacted the development and competition response of neotetraploids, as previously proposed by other studies, although the processes seem complex. On the one hand, without competition, neotetraploids were more similar to established tetraploids (except for belowground biomass) and were smaller overall than diploids. This trend is unexpected as genome duplications are usually associated with the 'gigas effect' (Stebbins 1971, Buggs and Pannell 2007, Hoya et al. 2007, Ramsey and Ramsey 2014) and because, in previous studies, diploid *J. maritima* had lower biomasses (Castro et al. 2023a) and higher stomatal density with lower sizes than neotetraploids (Siopa et al. 2020). Also, the lack of consistency between the biomass results obtained here and in Castro et al. (2023a) is likely related to the length of the experiment. Here, the experiment was conducted until flowering (for eight months), while in Castro et al. (2023a) the experiment lasted for three and a half months. Thus, it is likely that the effect of having bigger body sizes in neotetraploids is lost in latter stages, not because cells reduce their size but because they eventually multiply in slower rate. The lack of clear 'gigas effects' has been observed in a few polyploid complexes, only (Segraves and Thompson 1999, Vamosi et al. 2007, Ning et al. 2009, Trojak-Goluch and Skomra 2013) and might be caused by developmental tradeoffs. Increased cell sizes come with a cost in mitotic cell cycle that needs more energy (Cavalier-Smith 2005) and more time (Bennet 1977, Francis et al. 2008) for the DNA replication with expected consequences in plant sizes. These consequences might be even more impactful under stressful conditions such as dunes systems where resources (e.g. water and nutrients) are usually limited. Therefore, dune plants, such as *J. maritima*, may have evolved to efficiently manage available resources and this may impact the responses after genome duplications in the neotetraploids. Additionally, one cannot rule out the possibility of transgenerational effects of colchicine (Münzbergová 2017), despite that in some cases, the effects dissipated after the first generation (Husband et al. 2016). On the other hand, in the presence of a competitor plant, neotetraploids performed more similarly to diploids and had higher values than established tetraploids for phenological and most growth traits.

When analysing the response to intraspecific competition, neopolyploid plants were less impacted by competition than diploids, which were also less impacted than established tetraploids for most traits. This is probably the consequence of genome duplications that promoted a differentiated phenotype that allows neotetraploids to compete for fewer

resources. The lower impact of competition may have provided an advantage to neotetraploids under competition with their diploid parentals at the initial stages of neopolyploid emergence (Husband 2000, Levin 2002, Treier et al. 2009, Schlaepfer et al. 2010, te Beest et al. 2011, Hahn et al. 2012, Rey et al. 2017). Our results thus support the theoretical models proposing a competitive advantage for polyploidy (Rodríguez 1996), using for the first time neotetraploids in a controlled competition experiment. The few available experimental studies using diploids and tetraploids under direct competition showed contrasting results, from the dominance of polyploids (*Dactylis glomerata*, Maceira et al. 1993, *Centaurea stoebe*, Collin et al. 2011) to the lack of differences between cytotypes (e.g. *Ranunculus adoneus*, Baack and Stanton 2005, *Aster amellus*, Münzbergová 2007, *Senecio carniolicus*, Hülber et al. 2011, *Allium oleraceum*, Fialová and Duchoslav 2014, *C. angustifolium*, Thompson et al. 2015). In *J. maritima*, intraspecific competitive advantage may have further contributed to the displacement of diploid populations and colonisation of new areas by tetraploids as observed in *C. stoebe* (Collins et al. 2011).

While competition has been suggested as a key factor determining polyploid success (Fowler and Levin 1984, Rodríguez 1996, Levin 2002), polyploid establishment and spread is frequently determined by the combination of multiple factors (e.g. higher tolerance to salt, Chao et al. 2013, higher tolerance to drought stress, Ruiz et al. 2016; higher resistance to pathogens, Wang et al. 2018). Neopolyploid fertility and breeding system (Oswald and Nuismer 2011, Castro et al. 2020b, Siopa et al. 2020) is one such factor and directly influences population dynamics (Levin 1975, Husband 2004). Polyploidization is frequently associated with meiotic abnormalities and reduced gamete viability leading to low fertility, reducing the chances of establishment (Ramsey and Schemske 2002, Comai 2005, Doyle and Coate 2019, Clo and Kolář 2021). Indeed, likely due to these abnormalities, Siopa et al. (2020) observed a reduced reproductive fitness (as fruit and seed production) of the neotetraploid *J. maritima* after outcrossing compared with diploids, hampering the probabilities for successful establishment. However, genome duplications in *J. maritima* also resulted in leaky self-incompatibility, enabling the production of offspring by neotetraploids, mitigating the disadvantage under minority cytotype scenarios (Siopa et al. 2020). The observations by Siopa et al. (2020) combined with the results obtained here support the assumption that increased competitive ability and selfing combined might have played an important role in *J. maritima* neopolyploid establishment.

Differences among cytotypes were also observed in physiological traits and could be linked with different plant growth strategies in subsequent life-cycle stages. Under favourable conditions, diploids invest in plant growth, accumulating less starch than neotetraploids (and established tetraploids), while under stressful conditions such as competition, growth decreases, and starch accumulation increases. In contrast, neotetraploids invest similarly in starch accumulation regardless of the competition

treatment. This pattern is maintained in established tetraploids suggesting that this strategy could have been advantageous for the success of neotetraploids. *Jasione maritima* is a perennial dune plant that persists during the winter in the form of small rosettes produced in autumn (Sales and Hedge 2001, Serrano-Peréz 2021) after the energy-demanding reproduction period. Thus, having more reserves might be particularly advantageous in subsequent life-cycle stages. Having a higher amount of starch as an energetic reserve could enable neotetraploids to re-direct to a higher production of rosettes, which constitutes an advantage in the following year. Indeed, studies over longer time scales or measuring long-term persistence traits revealed differences between cytotypes in growth strategies that affect plant performance (Levin 2002, Collins et al. 2011, Eliášová and Münzbergová 2017). For example, tetraploids of *C. stoebe* produced a greater number of rosettes than their diploid counterparts, regardless of the competition regime, suggesting significant shifts in the life cycle between cytotypes (Collins et al. 2011). Diploids and tetraploids of *Vicia cracca* presented different strategies over two years. While diploids had higher fitness than tetraploids in the first year, tetraploids grew faster in the second year, minimising the differences between cytotypes (Eliášová and Münzbergová 2017). Thus, better performance in traits associated with long-term persistence may allow neotetraploids to establish and out-compete diploids (Collins et al. 2011, Thébault et al. 2011), despite their initial numerical disadvantage. Studies over extended periods are needed to assess the fitness advantage of long-term persistence traits in *J. maritima*.

An interesting pattern was also observed for cell membrane permeability, used here as a biomarker of oxidative damage (cell membrane electrolyte, Demidchik et al. 2014) to assess plant stress under competition. Although the basal cell membrane permeability level was higher in neotetraploids (and tetraploids) than in diploids, under competition, neotetraploids (and tetraploids) reduced oxidative damage. In contrast, in diploids, the cell membrane leakage increased. Considering that low oxidative status correlates with a high antioxidant response (Dias et al. 2018), our data suggest that competition may lead to increased defence capacity in neotetraploids (and tetraploids). Similar patterns were observed when comparing diploids and tetraploids of *Arabidopsis thaliana* under stressful conditions (del Pozo and Ramirez-Parra 2014), suggesting that polyploidisation affected the expression of genes involved in stress response, which in turn provided a flexible and rapid response of tetraploids to external/internal stimuli. Indeed, whole genome duplication is a very stressful event itself and may trigger up-regulation of stress response genes (e.g. *J. maritima* neotetraploids tend to behave as stressed plants, even without competition, e.g. late flowering), which in turn provide a general stress-acclimatization effect; these responses can be, later on, maintained or selected in tetraploids (Bomblies et al. 2020), enabling their spread and establishment in harsher environments. Polyploidization has been described to promote the antioxidant capacity, in part due to an up-regulation of genes related to the antioxidant

system, reactive oxygen species (ROS) scavenging function and ROS signalling processes, making tetraploids more tolerant, especially under stress conditions (del Pozo and Ramirez-Parra 2015, Tan et al. 2015, Kong et al. 2017). For example, tetraploids of *Dioscorea zingiberensis* presented lower levels of ROS (superoxide anions and hydrogen peroxide) and membrane injuries (cell membrane permeability and lipid peroxidation) associated with higher antioxidant enzyme activity (Zhang et al. 2010). Our results suggest that in *J. maritima*, genome duplications might be responsible for increased antioxidant response, being this trait selected over the evolution; however, we could not establish a direct link between this and the other measured traits.

Evolutionary changes after polyploidisation

Evolutionary changes after polyploidisation have also been detected, with tetraploids investing more in belowground biomass and being negatively impacted by competition. For example, a higher biomass allocation to belowground structures has been reported for tetraploid *Solidago gigantea* (Schaefer et al. 2010) and tetraploid *C. stoebe* (Collins et al. 2011) in comparison with diploid individuals. In some cases, it has been related to increased competitive ability (Gaudet and Keddy 1988, Aerts et al. 1991); however, this was not observed in *J. maritima*, at least under optimal conditions. Instead, root development might have played an important role in colonising southernmost locations. The species is distributed along a latitudinal gradient, with northern diploid populations marked by steeper slopes, lower mean summer temperatures and denser vegetation cover. In comparison, tetraploids colonise areas with lower slopes and sites facing S-SW, characterised by hotter and drier environments and harbour sparser vegetation cover than diploid sites (Castro et al. 2020a). On the one hand, a well-developed root might enable tetraploid plants to explore water reserves in deeper soil layers. Thus, it could have been particularly relevant in colonising southern and drier locations where tetraploids currently occur (Castro et al. 2020a). On the other hand, the competitive response might not have been a key advantage during the colonisation of southern locations with sparser dune vegetation as tetraploids lost their competitive response and the ability to cope with competition seems to have been lost. Our results support that a developed root system might have been selected after genome duplication. This trait may have allowed tetraploids to establish and persist in habitats not favourable to its diploid progenitor, expanding the range of *J. maritima* southwards. Environmental gradients and adaptation to drier environments have been shown in other polyploid complexes, in which polyploids were able to colonise drier habitats (Levin 2002, te Beest et al. 2011, Manzaneda et al. 2015, Rey et al. 2017). For example, the tetraploid *Brachypodium hybridum* presented a drought-escape strategy, having higher performance and colonising drier places than the diploid parental *Brachypodium distachyon*, which grows in more humid environments (Manzaneda et al. 2012, 2015).

Current distribution and contact zone dynamics

Our results support the current cytotype distribution but suggest a dynamic contact zone. *Jasione maritima* exhibits a parapatric distribution, with diploids in the north and tetraploids in the south of the species range (Castro et al. 2020a). Niche modelling studies suggested that tetraploids occupy their potential environmental niche, while diploids are restricted to a smaller area in the north compared with their potential distribution (Castro et al. 2020a). Here we observe more competitive diploid populations in the north (in locations with denser vegetation, suggesting a possible increased performance also under interspecific competition scenarios) and tetraploids with well-developed root systems in drier and warmer southern areas. The superiority of a given cytotype is expected to generate dynamic contact zones and the displacement of the unfit cytotype over time (Maceira et al. 1993, Petit et al. 1999, Collins et al. 2011). For example, tetraploids of *C. stoebe* revealed an intraspecific competitive superiority in western Europe, where they are dominant, suggesting that they have led to the competitive exclusion of diploids in this area (Treier et al. 2009). In *J. maritima*, while the tetraploids seem to occupy their potential environmental niche currently, diploids potentially could expand further south over the tetraploid distribution area colonising suitable areas (Castro et al. 2020a) and taking advantage of their superior intraspecific competitive ability (results herein), supporting the existence of a moving contact zone. Experiments such as reciprocal transplants and drought tolerance experiments under intra and interspecific competition are needed to test all the hypotheses proposed above.

Concluding remarks

The performance of *J. maritima* var. *maritima* cytotypes is differentiated and context-dependent: diploids growing without intraspecific competition are the biggest, neotetraploids are the least affected by competition and established tetraploids are the best adapted to dry environments. Additionally, the biggest investment in growth by diploids and the highest reserve accumulation by both tetraploids suggests different life strategies. The highest biomass of diploids (excluding belowground biomass) and intermediate competitive capacity may promote diploids persistence in northern areas with higher vegetative cover, while the highest competitive ability of neotetraploids resulting from polyploidisation, was probably one of the mechanisms involved in their establishment and allowed the minority cytotype exclusion overcome. After the establishment, tetraploids genotypes with higher investment in belowground biomass were selected replacing the more competitive to colonised dry and southernmost areas. More studies such as reciprocal transplants, drought tolerance experiments and intra and interspecific competition experiments are needed to test these hypotheses.

Funding – This work was funded by the Integrated Program of Scientific Research and Technological Development CULTIVAR (CENTRO-01-0145-FEDER-000020), co-financed by the Regional Operational Programme Centro 2020, Portugal 2020 and European Union, through the European Fund for Regional Development (ERDF). The Portuguese Foundation for Science and Technology (FCT – Fundação para a Ciência e a Tecnologia, I.P.) financed the work of MC through the fellowship SFRH/BD/89617/2012 and SC through the Scientific Employment Stimulus 2021.02697.CEECIND.

Author contributions

Mariana Castro: Conceptualization (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review and editing (equal). **Maria Celeste Dias:** Investigation (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Joao Loureiro:** Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (lead); Supervision (equal); Visualization (equal); Writing – review and editing (equal). **Brian C. Husband:** Supervision (equal); Validation (equal); Visualization (equal); Writing – review and editing (equal). **Silvia Castro:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Supervision (equal); Validation (lead); Visualization (lead); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.wm37pvmt2> (Castro et al. 2023b).

Supporting information

The Supporting information associated with this article is available with the online version.

References

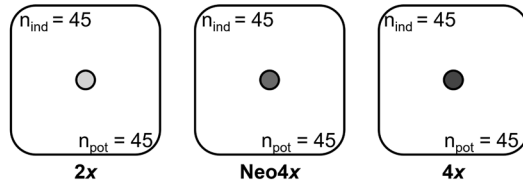
- Adam, S. K. L. and Wendel, J. F. 2005. Novel patterns of gene expression in polyploidy. – *Trends Genet.* 21: 539–543.
- Aerts, R., Boot, R. G. A. and Van der Aart, P. J. M. 1991. The relation between above-and belowground biomass allocation patterns and competitive ability. – *Oecologia* 87: 551–559.
- Allario, T., Brumos, J., Colmenero-Flores, J. M., Tadeo, F., Froelicher, Y., Talon, M., Navarro, L., Ollitrault, P. and Morillon, R. 2011. Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. – *J. Exp. Bot.* 62: 2507–2519.
- Baack, E. J. and Stanton, M. L. 2005. Ecological factors influencing tetraploid speciation in snow buttercups (*Ranunculus adonis*): niche differentiation and tetraploid establishment. – *Evolution* 59: 1936–1944.

- Baldwin, S. J. and Husband, B. C. 2011. Genome duplication and the evolution of conspecific pollen precedence. – *Proc. R. Soc. B* 278: 2011–2017.
- Bates, D., Maechler, M., Bolker, B. and Walker, S. 2015. lme4: linear mixed-effects models using Eigen and S4. – <http://CRAN.R-project.org/package=lme4>
- Bennett, M. D. 1977. The time and duration of meiosis. – *Phil. Trans. R. Soc. B* 277: 201–226.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. and White, J. S. S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – *Trends Ecol. Evol.* 24: 127–135.
- Bombliès, K. 2020. When everything changes at once: finding a new normal after genome duplication. – *Proc. R. Soc. B* 287: 20202154.
- Buggs, R. J. and Pannell, J. R. 2007. Ecological differentiation and diploid superiority across a moving ploidy contact zone. – *Evolution* 61: 125–140.
- Castro, M., Castro, S. and Loureiro, J. 2018. Production of synthetic tetraploids in the dune species *Jasione maritima*. – *Web Ecol.* 18: 129–141.
- Castro, M., Loureiro, J., Figueiredo, A., Serrano, M., Husband, B. C. and Castro, S. 2020a. Different patterns of ecological divergence between two tetraploids and their diploid counterpart in a parapatric linear coastal distribution polyploid complex. – *Front. Plant Sci.* 11: 315.
- Castro, M., Loureiro, J., Husband, B. C. and Castro, S. 2020b. The role of multiple reproductive barriers: strong post-pollination interactions govern cytotype isolation in a tetraploid-octoploid contact zone. – *Ann. Bot.* 126: 991–1003.
- Castro, H., Dias, M. C., Castro, M., Loureiro, J. and Castro, S. 2023a. Impact of genome duplications in drought tolerance and distribution of the diploid-tetraploid *Jasione maritima*. – *Front. Plant Sci.* 14: 1–15.
- Castro, M., Dias, M. C., Loureiro, J., Husband, B. C. and Castro, S. 2023b. Data from: Competitive ability, neopolyploid establishment and current distribution of a diploid-tetraploid plant complex. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.wm37pvmt2>.
- Cavalier-Smith, T. 2005. Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. – *Ann. Bot.* 95: 147–175.
- Čertner, M., Sudová, R., Weiser, M., Suda, J. and Kolář, F. 2019. Ploidy-altered phenotype interacts with local environment and may enhance polyploid establishment in *Knautia serpentinicola* (Caprifoliaceae). – *New Phytol.* 221: 1117–1127.
- Chao, D.-Y., Dilkes, B., Luo, H., Douglas, A., Yakubova, E., Lahner, B. and Salt, D. E. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. – *Science* 341: 658–659.
- Clo, J. and Kolář, F. 2021. Short-and long-term consequences of genome doubling: a meta-analysis. – *Am. J. Bot.* 108: 2315–2322.
- Coate, J. E., Powell, A. F., Owens, T. G. and Doyle, J. J. 2013. Transgressive physiological and transcriptomic responses to light stress in allopolyploid *Glycine dolichocarpa* (Leguminosae). – *Heredity* 110: 160–170.
- Collins, A. R., Naderi, R. and Mueller-Schaerer, H. 2011. Competition between cytotypes changes across a longitudinal gradient in *Centaurea stoebe* (Asteraceae). – *Am. J. Bot.* 98: 1935–1942.
- Comai, L. 2005. The advantages and disadvantages of being polyploid. – *Nat. Rev. Genet.* 6: 836–846.
- del Pozo, J. C. and Ramirez-Parra, E. 2014. Deciphering the molecular bases for drought tolerance in *Arabidopsis* autotetraploids. – *Plant Cell Environ.* 37: 2722–2737.
- del Pozo, J. C. and Ramirez-Parra, E. 2015. Whole genome duplications in plants: an overview from *Arabidopsis*. – *J. Exp. Bot.* 66: 6991–7003.
- Demidchik, V., Straltsova, D., Medvedev, S. S., Pozhvanov, G. A., Sokolik, A. and Yurin, V. 2014. Stress-induced electrolyte leakage: the role of K⁺ permeable channels and involvement in programmed cell death and metabolic adjustment. – *J. Exp. Bot.* 65: 1259–1270.
- Dias, M. C., Correia, S., Serôdio, J., Silva, A. M. S., Freitas, H. and Santos, C. 2018. Chlorophyll fluorescence and oxidative stress endpoints to discriminate olive cultivars tolerance to drought and heat episodes. – *Sci. Horticult.* 231: 31–35.
- Doyle, J. J. and Coate, J. E. 2019. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. – *Int. J. Plant Sci.* 180: 1–52.
- Elišáková, A. and Münzbergová, Z. 2017. Factors influencing distribution and local coexistence of diploids and tetraploids of *Vicia cracca*: inferences from a common garden experiment. – *J. Plant Res.* 130: 677–687.
- Fialová, M. and Duchoslav, M. 2014. Response to competition of bulbous geophyte *Allium oleraceum* differing in ploidy level. – *Plant Biol.* 16: 186–196.
- Fowler, N. L. and Levin, D. A. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. – *Am. Nat.* 124: 703–711.
- Fox, J. and Weisberg, S. 2019. An R companion to applied regression, 3rd edn. – Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- Francis, D., Davies, M. S. and Barlow, P. W. 2008. A strong nucleotypic effect on the cell cycle regardless of ploidy level. – *Ann. Bot.* 101: 747–757.
- Garbutt, K. and Bazzaz, F. A. 1983. Leaf demography, flower production and biomass of diploid and tetraploid populations of *Phlox drummondii* Hook. on a soil moisture gradient. – *New Phytol.* 93: 129–141.
- Gaudet, C. L. and Keddy, P. A. 1988. A comparative approach to predicting competitive ability from plant traits. – *Nature* 334: 242–243.
- Hahn, M. A., Buckley, Y. M. and Müller-Schärer, H. 2012. Increased population growth rate in invasive polyploid *Centaurea stoebe* in a common garden. – *Ecol. Lett.* 15: 947–954.
- Hao, G. Y., Lucero, M. E., Sanderson, S. C., Zacharias, E. H. and Holbrook, N. M. 2013. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related tradeoffs in *Atriplex canescens* (Chenopodiaceae). – *New Phytol.* 197: 970–978.
- Hedges, L. V., Gurevitch, J. and Curtis, P. S. 1999. The meta-analysis of response ratios in experimental ecology. – *Ecology* 80: 1150–1156.
- Hothorn, T., Bretz, F., Westfall, P. and Heiberger, R. M. 2017. Multcomp: simultaneous inference for general linear hypotheses. – <http://CRAN.Rproject.org/package=multcomp>.
- Hoya, A., Shibaie, H., Morita, T. and Ito, M. 2007. Germination characteristics of native Japanese dandelion autopolyploids and their putative diploid parent species. – *J. Plant Res.* 120: 139–147.
- Hülber, K., Sonnleitner, M., Flatscher, R., Berger, A., Dobrovsky, R., Niessner, S., Nigl, T., Schneeweiss, G. M., Kubešová, M., Rauchová, J., Suda, J. and Schönswetter, P. 2009. Ecological segregation drives fine-scale cytotype distribution of *Senecio carniolicus* in the Eastern Alps. – *Preslia* 81: 309–319.

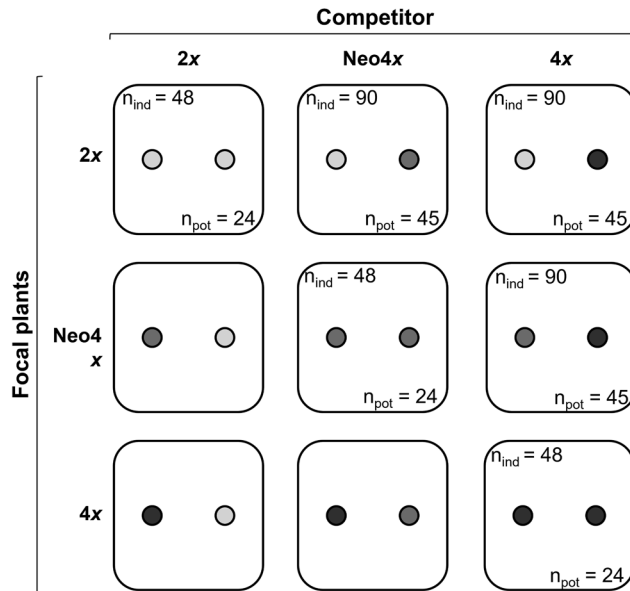
- Hülber, K., Berger, A., Gilli, C., Hofbauer, M., Patek, M. and Schneeweiss, G. M. 2011. No evidence for a role of competitive capabilities of adults in causing habitat segregation of diploid and hexaploid *Senecio carniolicus* (Asteraceae). – *Alpine Bot.* 121: 123–127.
- Husband, B. C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. – *Proc. R. Soc. B* 267: 217–223.
- Husband, B. C. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. – *Biol. J. Linn. Soc.* 82: 537–546.
- Husband, B. C. and Sabara, H. A. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). – *New Phytol.* 161: 703–713.
- Husband, B. C., Ozimec, B., Martin, S. L. and Pollock, L. 2008. Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. – *Int. J. Plant Sci.* 169: 195–206.
- Husband, B. C., Baldwin, S. J. and Sabara, H. A. 2016. Direct vs indirect effects of whole-genome duplication on prezygotic isolation in *Chamerion angustifolium*: implications for rapid speciation. – *Am. J. Bot.* 103: 1259–1271.
- Jersáková, J., Castro, S., Sonk, N., Milchreit, K., Schödelbauerová, I., Tolasch, T. and Dötterl, S. 2010. Absence of pollinator-mediated pre-mating barriers in mixed-ploidy populations of *Gymnadenia conopsea* s.l. (Orchidaceae). – *Evol. Ecol.* 24: 1199–1218.
- Kong, D., Li, Y., Bai, M., Deng, Y., Liang, G. and Wu, H. 2017. A comparative study of the dynamic accumulation of polyphenol components and the changes in their antioxidant activities in diploid and tetraploid *Lonicera japonica*. – *Plant Physiol. Biochem.* 112: 87–96.
- Laport, R. G., Hatem, L., Minckley, R. L. and Ramsey, J. 2013. Ecological niche modeling implicates climatic adaptation, competitive exclusion, and niche conservatism among *Larrea tridentata* cytotypes in North American deserts. – *J. Torrey Bot. Soc.* 140: 349–363.
- Laport, R. G., Minckley, R. L. and Ramsey, J. 2016. Ecological distributions, phenological isolation, and genetic structure in sympatric and parapatric populations of the *Larrea tridentata* polyploid complex. – *Am. J. Bot.* 103: 1358–1374.
- Leitch, A. R. and Leitch, I. J. 2008. Genomic plasticity and the diversity of polyploid plants. – *Science* 320: 481–483.
- Lenth, R. V. 2016. Least-squares means: the R package lsmmeans. – *J. Stat. Softw.* 69: 1–33. <https://cran.r-project.org/package=lsmmeans>
- Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. – *Taxon* 24: 35–43.
- Levin, D. A. 2002. The role of chromosomal change in plant evolution. – Oxford Univ. Press.
- Li, W. L., Berlyn, G. P. and Ashton, P. M. S. 1996. Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). – *Am. J. Bot.* 83: 15–20.
- Liu, S., Chen, S., Chen, Y., Guan, Z., Yin, D. and Chen, F. 2011. In vitro induced tetraploid of *Dendranthema nankingense* (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. – *Sci. Horticul.* 127: 411–419.
- Lutts, S., Kinet, J. M. and Bouharmont, J. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. – *Ann. Bot.* 78: 389–398.
- Maceira, N. O., Jacquard, P. and Lumaret, R. 1993. Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. – *New Phytol.* 124: 321–328.
- Madlung, A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with new tools. – *Heredity* 110: 99–104.
- Maherali, H., Walden, A. E. and Husband, B. C. 2009. Genome duplication and the evolution of physiological responses to water stress. – *New Phytol.* 184: 721–731.
- Manzaneda, A. J., Rey, P. J., Bastida, J. M., Weiss-Lehman, C., Raskin, E. and Mitchell-Olds, T. 2012. Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). – *New Phytol.* 193: 797–805.
- Manzaneda, A. J., Rey, P. J., Anderson, J. T., Raskin, E., Weiss-Lehman, C. and Mitchell-Olds, T. 2015. Natural variation, differentiation, and genetic trade-offs of ecophysiological traits in response to water limitation in *Brachypodium distachyon* and its descendent allotetraploid *B. hybridum* (Poaceae). – *Evolution* 69: 2689–2704.
- Martin, S. L. and Husband, B. C. 2012. Whole genome duplication affects evolvability of flowering time in an autotetraploid plant. – *PLoS One* 7: e44784.
- Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. – *Science* 264: 421–424.
- McArthur, E. D. and Sanderson, S. C. 1999. Cytogeography and chromosome evolution of subgenus *Tridentatae* of *Artemisia* (Asteraceae). – *Am. J. Bot.* 86: 1754–1775.
- Melaragno, J. E., Mehrotra, B. and Coleman, A. W. 1993. Relationship between endopolyploidy and cell size in epidermal tissue of *Arabidopsis*. – *Plant Cell* 5: 1661–1668.
- Müntzing, A. 1936. The evolutionary significance of autopolyploidy. – *Hereditas* 21: 363–378.
- Münzbergová, Z. 2007. No effect of ploidy level in plant response to competition in a common garden experiment. – *Biol. J. Linn. Soc.* 92: 211–219.
- Münzbergová, Z. 2017. Colchicine application significantly affects plant performance in the second generation of synthetic polyploids and its effects vary between populations. – *Ann. Bot.* 120: 329–339.
- Ning, G. G., Shi, X. P., Hu, H. R., Yan, Y. and Bao, M. Z. 2009. Development of a range of polyploid lines in petunia hybrida and the relationship of ploidy with the single-/double-flower trait. – *HortScience* 44: 250–255.
- Osaki, M., Shinano, T. and Tadano, T. 1991. Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation in field crops. – *Soil Sci. Plant Nutr.* 37: 117–128.
- Oswald, B. P. and Nuismer, S. L. 2011. Neopolyploidy and diversification in *Heuchera grossulariifolia*. – *Evolution* 65: 1667–1679.
- Pavliková, Z., Paštová, L. and Münzbergová, Z. 2017. Synthetic polyploids in *Vicia cracca*: methodology, effects on plant performance and aneuploidy. – *Plant Syst. Evol.* 303: 827–839.
- Petit, C., Bretagnolle, F. and Felber, F. 1999. Evolutionary consequences of diploid–polyploid hybrid zones in wild species. – *Trends Ecol. Evol.* 14: 306–311.
- Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. – *Proc. Natl Acad. Sci.* 108: 7096–7101
- Ramsey, J. and Schemske, D. W. 2002. Neopolyploidy in flowering plants. – *Annu. Rev. Ecol. Syst.* 33: 589–639.
- Ramsey, J. and Ramsey, T. S. 2014. Ecological studies of polyploidy in the 100 years following its discovery. – *Phil. Trans. R. Soc. B* 369: 20130352.

- Rey, P. J., Manzaneda, A. J. and Alcántara, J. M. 2017. The interplay between aridity and competition determines colonization ability, exclusion and ecological segregation in the heteroploid *Brachypodium distachyon* species complex. – *New Phytol.* 215: 85–96.
- Rodríguez, D. J. 1996. Model for the establishment of polyploidy in plants: viable but infertile hybrids, iteroparity, and demographic stochasticity. – *J. Theor. Biol.* 180: 189–196.
- Ruiz, M., Quiñones, A., Martínez-Cuenca, M.-R., Aleza, P., Morillon, R., Navarro, L., Primo-Millo, E. and Martínez-Alcántara, B. 2016. Tetraploidy enhances the ability to exclude chloride from leaves in *Carrizo citrange* seedlings. – *J. Plant Physiol.* 205: 1–10.
- Sales, F. and Hedge, I. C. 2001. Campanulaceae. – In: Castroviejo, S., Paiva, J., Sales, F., Hedge, I. C., Aedo, C., Aldasoro, J. J., Herrero, A. and Velayos, M. (eds), *Flora Iberica. Plantas vasculares de la Península Ibérica e Islas Baleares*, vol. XIV. Real Jardín Botánico, C.S.I.C.
- Schepper, D. S., Leus, L., Eeckhaut, T., Van Bockstaele, E., Debergh, P. and De Loose, M. 2004. Somatic polyploid petals: regeneration offers new roads for breeding Belgian pot azaleas. – *Plant Cell Tissue Organ Cult.* 76: 183–188.
- Schlaepfer, D. R., Edwards, P. J. and Billeter, R. 2010. Why only tetraploid *Solidago gigantea* (Asteraceae) became invasive: a common garden comparison of ploidy levels. – *Oecologia* 163: 661–673.
- Segraves, K. A. and Thompson, J. N. 1999. Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. – *Evolution* 53: 1114–1127.
- Serrano-Pérez, L. M. 2001. *Jasione L. (Campanulaceae)*. Biogeographic history, evolutionary relationships, species delimitation, and nomenclatural revision. – Univ. de Santiago de Compostela.
- Siopa, C., Dias, M. C., Castro, M., Loureiro, J. and Castro, S., 2020. Is selfing a reproductive assurance promoting polyploid establishment? Reduced fitness, leaky self-incompatibility and lower inbreeding depression in neotetraploids. – *Am. J. Bot.* 107: 526–538.
- Smith, L. 1946. A comparison of the effects of heat and X-rays on dormant seeds of cereals, with special reference to polyploidy. – *J. Agric. Res.* 73: 137–158.
- Soltis, D. E., Buggs, R. J., Doyle, J. J. and Soltis, P. S. 2010. What we still don't know about polyploidy. – *Taxon* 59: 1387–1403.
- Ståhlberg, D. 2009. Habitat differentiation, hybridization and gene flow patterns in mixed populations of diploid and autotetraploid *Dactylorhiza maculata* s.l. (Orchidaceae). – *Evol. Ecol.* 23: 295–328.
- Stebbins, G. L. 1971. *Chromosomal evolution in higher plants*. – Addison Wesley.
- Sun, Q., Sun, H., Li, L. and Bell, R. L. 2009. In vitro colchicine-induced polyploid plantlet production and regeneration from leaf explants of the diploid pear (*Pyrus communis* L.) cultivar, 'Fertility'. – *J. Horticult. Sci. Biotechnol.* 84: 548–552.
- Tan, F. Q., Tu, H., Liang, W. J., Long, J. M., Wu, X. M., Zhang, H. Y. and Guo, W. W. 2015. Comparative metabolic and transcriptional analysis of a doubled diploid and its diploid citrus rootstock (*C. junos* cv. Ziyang xiangcheng) suggests its potential value for stress resistance improvement. – *BMC Plant Biol.* 15: 89.
- Tate, J. A., Symonds, V. V., Doust, A. N., Buggs, R. J., Mavrodiev, E., Majure, L. C., Soltis, P. S. and Soltis, D. E. 2009. Synthetic polyploids of *Tragopogon miscellus* and *T. mirus* (Asteraceae): 60 Years after Ownbey's discovery. – *Am. J. Bot.* 96: 979–988.
- te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubešová, M. and Pyšek, P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. – *Ann. Bot.* 109: 19–45.
- Thébault, A., Gillet, F., Müller-Schärer, H. and Buttler, A. 2011. Polyploidy and invasion success: trait trade-offs in native and introduced cytotypes of two Asteraceae species. – *Plant Ecol.* 212: 315–325.
- Thompson, K. A., Husband, B. C. and Maherali, H. 2015. No influence of water limitation on the outcome of competition between diploid and tetraploid *Chamerion angustifolium* (Onagraceae). – *J. Ecol.* 103: 733–741.
- Treier, U. A., Broennimann, O., Normand, S., Guisan, A., Schaffner, U., Steinger, T. and Müller-Schärer, H. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. – *Ecology* 90: 1366–1377.
- Trojak-Goluch, A. and Skomra, U. 2013. Artificially induced polyploidization in *Humulus lupulus* L. and its effect on morphological and chemical traits. – *Breed. Sci.* 63: 393–399.
- Vamosi, J. C., Goring, S. J., Kennedy, B. F., Mayberry, R. J., Moray, C. M., Neame, L. A., Tunbridge, N. D. and Elle, E. 2007. Pollination, floral display, and the ecological correlates of polyploidy. – *Funct. Ecosyst. Commun.* 1: 1–9.
- Van de Peer, Y., Mizrachi, E. and Marchal, K. 2017. The evolutionary significance of polyploidy. – *Nat. Rev. Genet.* 18: 411.
- Van de Peer, Y., Ashman, T.-L., Soltis, P. S. and Soltis, D. E. 2021. Polyploidy: an evolutionary and ecological force in stressful times. – *Plant Cell* 33: 11–26.
- Van Drunen, W. E. and Husband, B. C. 2018. Immediate vs evolutionary consequences of polyploidy on clonal reproduction in an autopolyploid plant. – *Ann. Bot.* 122: 195–205.
- Van Laere, K., França, S. C., Vansteenkiste, H., Van Huylenbroeck, J., Steppe, K. and Van Labeke, M. C. 2011. Influence of ploidy level on morphology, growth and drought susceptibility in *Spathiphyllum wallisii*. – *Acta Physiol. Plantarum* 33: 1149–1156.
- Wang, W., He, Y., Cao, Z. and Deng, Z. 2018. Induction of tetraploids in *Impatiens walleriana* and characterization of their changes in morphology and resistance to downy mildew. – *HortScience* 53: 925–931.
- Wickham, H., Chang, W. and Wickham, M. H. 2016. Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics. – Version 2: 1–189.
- Zhang, X. Y., Hu, C. G. and Yao, J. L. 2010. Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defense system and increased heat tolerance. – *J. Plant Physiol.* 167: 88–94.
- Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M. 2009. Mixed effects models and extensions in ecology with R. – In: Gail, M., Krickeberg, K., Samet, J., Tsiatis, A. and Wong, W. (eds), *Statistics for biology and health*. Springer Science and Business Media.

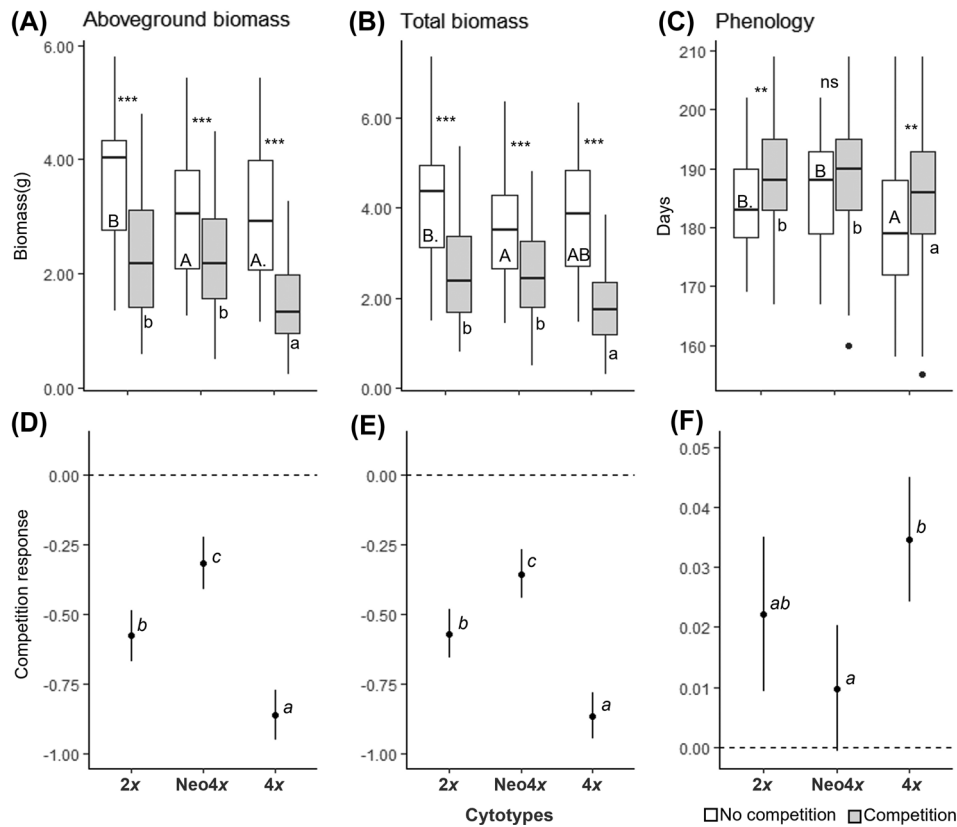
- **No competition**



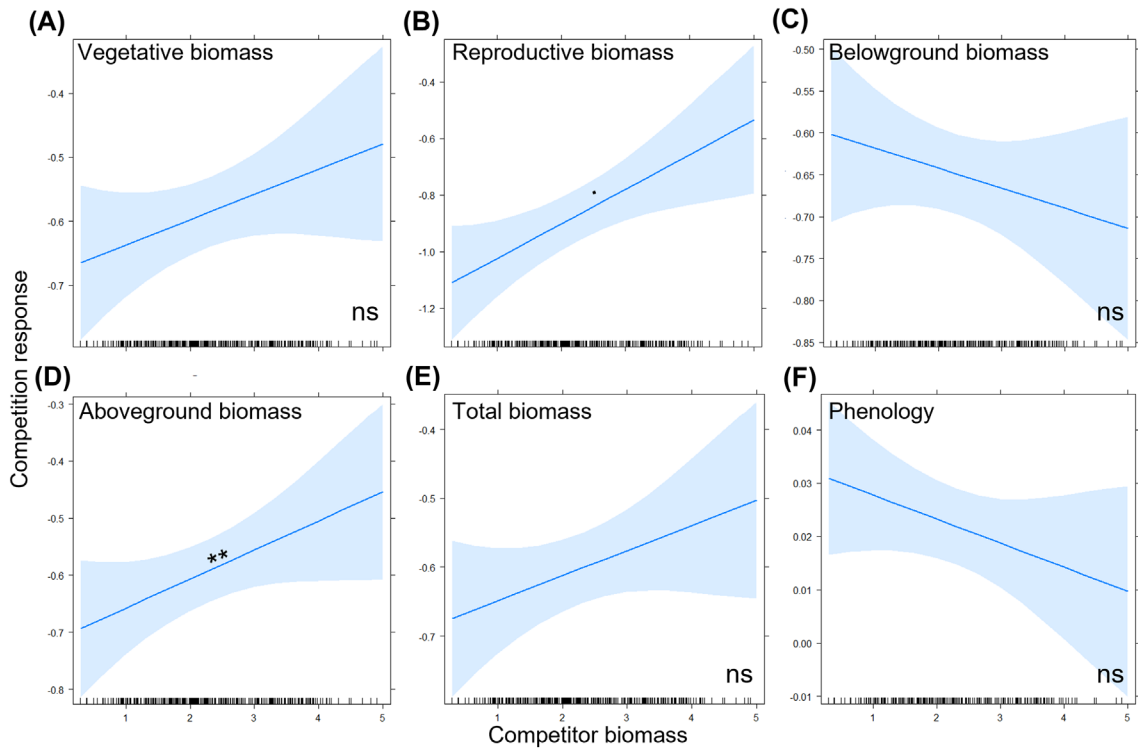
- **Competition**



Appendix 1. Design of the competition experiment. Twelve treatments were included: cytotypes (2x, diploids; Neo4x, neotetraploids; 4x, tetraploids) growing alone (no competition) or with another plant (competition). In the competition treatment, each focal plant was grown with a different competitor: competition with a diploid plant (2x + 2x, Neo4x + 2x and 4x + 2x), competition with a neotetraploid plant (2x + Neo4x, 4x + Neo4x and Neo4x + Neo4x), or competition with a tetraploid plant (2x + 4x, 4x + 4x and Neo4x + 4x). The number of individuals and pots (n_{ind} and n_{pot} , respectively) is given for each treatment.



Appendix 2. Boxplot for diploid (2x), neotetraploid (Neo4x) and tetraploid (4x) focal plants of *Jasione maritima* grown in different competitive environments based on growth and physiological traits. Competitive environments comprise growth in the absence of a neighbour (no competition, white boxes) and growth with a neighbour (grey boxes); dot plots represent the mean and upper and lower limits of competition response for each cyotype. Three variables are presented: Aboveground biomass, Total biomass, and Phenology. Upper case letters indicate differences ($p < 0.10$) between cyotypes when grown alone (white boxes); lower case letters indicate differences between cyotypes grown with a neighbour (grey boxes); and italic letters represent differences between the competition response (dots); the absence of letters reveals nonsignificant differences between cyotypes at $p < 0.05$. Differences between plants growing alone and under competition (white vs. grey boxes) are denoted as $0.10 < p < 0.05$; *, $0.01 < p < 0.05$; ** $0.01 < p < 0.001$ and *** $p < 0.001$.



Appendix 3. Effect of competition biomass on competition response on biomass (A) vegetative, (B) reproductive, (C) belowground, (D) above and (E) total and phenology. Statistically significant correlations are denoted as $0.10 < p < 0.05$; *, $0.01 < p < 0.05$; ** $0.01 < p < 0.001$ and *** $p < 0.001$. Nonsignificant differences are denoted as ns.